An electroplated copper–silver alloy as antibacterial coating on stainless steel

Transfer and growth of pathogenic microorganisms must be prevented in many areas such as the clinical sector. One element of transfer is the adhesion of pathogens to different surfaces and the purpose of the present study was to develop and investigate the antibacterial efficacy of stainless steel electroplated with a copper-silver alloy with the aim of developing antibacterial surfaces for the medical and health care sector. The microstructural characterization showed a porous microstructure of electroplated copper-silver coating and a homogeneous alloy with presence of interstitial silver. The copper-silver alloy coating showed active corrosion behavior in chloride-containing environments. ICP-MS measurements revealed a selective and localized dissolution of copper ions in wet conditions due to its galvanic coupling with silver. No live bacteria adhered to the copper-silver surfaces when exposed to suspensions of S. aureus and E. coli at a level of $10^8$CFU/ml whereas $10^4$CFU/cm² adhered after 24h on the stainless steel controls. In addition, the Cu-Ag alloy caused a significant reduction of bacteria in the suspensions. The coating was superior in its antibacterial activity as compared to pure copper and silver electroplated surfaces. Therefore, the results showed that the electroplated copper-silver coating represents an effective and potentially economically feasible way of limiting surface spreading of pathogens.
Complete Genome Sequence of *Shewanella* sp. WE21, a Rare Isolate with Multiple Novel Large Genomic Islands

We present here the whole-genome sequence of *Shewanella* sp. WE21, an unusual omega-3 fatty acid-producing bacterium isolated from the gastrointestinal tract of the freshwater fish *Sander vitreus* (walleye). This genome contains a number of unique, large genomic islands with genes not present in other *Shewanella* bacteria.

**General information**

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Organisations: Department of Biotechnology and Biomedicine, Bacterial Ecophysiology and Biotechnology, University of Copenhagen, University of Wisconsin

Authors: Castillo, D. (Ekstern), Gram, L. (Intern), Dailey, F. E. (Ekstern)

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Effect of TDA-producing Phaeobacter inhibens on the fish pathogen Vibrio anguillarum in non-axenic algae and copepod systems

The expanding aquaculture industry plays an important role in feeding the growing human population and with the expansion, sustainable bacterial disease control, such as probiotics, becomes increasingly important. Tropodithietic acid (TDA)-producing Phaeobacter spp. can protect live feed, for example rotifers and Artemia as well as larvae of turbot and cod against pathogenic vibrios. Here, we show that the emerging live feed, copepods, is unaffected by colonization of the fish pathogen Vibrio anguillarum, making them potential infection vectors. However, TDA-producing Phaeobacter inhibens was able to significantly inhibit V. anguillarum in non-axenic cultures of copepod Acartia tonsa and the copepod feed Rhodomonas salina. Vibrio grew to 10^6 CFU ml⁻¹ and 10^7 CFU ml⁻¹ in copepod and R. salina cultures, respectively. However, vibrio counts remained at the inoculum level (10^4 CFU ml⁻¹) when P. inhibens was also added. We further developed a semi-strain-specific qPCR for V. anguillarum to detect and quantify the pathogen in non-axenic systems. In conclusion, P. inhibens efficiently inhibits the fish larval pathogen V. anguillarum in the emerging live feed, copepods, supporting its use as a probiotic in aquaculture. Furthermore, qPCR provides an effective method for detecting vibrio pathogens in complex non-axenic live feed systems.
Exploring the Effect of Phage Therapy in Preventing Vibrio anguillarum Infections in Cod and Turbot Larvae

The aquaculture industry is suffering from losses associated with bacterial infections by opportunistic pathogens. Vibrio anguillarum is one of the most important pathogens, causing vibriosis in fish and shellfish cultures leading to high mortalities and economic losses. Bacterial resistance to antibiotics and inefficient vaccination at the larval stage of fish emphasizes the need for novel approaches, and phage therapy for controlling Vibrio pathogens has gained interest in the past few years. In this study, we examined the potential of the broad-host-range phage KVP40 to control four different V. anguillarum strains in Atlantic cod (Gadus morhua L.) and turbot (Scophthalmus maximus L.) larvae. We examined larval mortality and abundance of bacteria and phages. Phage KVP40 was able to reduce and/or delay the mortality of the cod and turbot larvae challenged with V. anguillarum. However, growth of other pathogenic bacteria naturally occurring on the fish eggs prior to our experiment caused mortality of the larvae in the unchallenged control groups. Interestingly, the broad-spectrum phage KVP40 was able to reduce mortality in these groups, compared to the nonchallenge control groups not treated with phage KVP40, demonstrating that the phage could also reduce mortality imposed by the background population of pathogens. Overall, phage-mediated reduction in mortality of cod and turbot larvae in experimental challenge assays with V. anguillarum pathogens suggested that application of broad-host-range phages can reduce Vibrio-induced mortality in turbot and cod larvae, emphasizing that phage therapy is a promising alternative to traditional treatment of vibriosis in marine aquaculture.

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Authors: Rørbo, N. (Ekstern), Rønneseth, A. (Ekstern), Kalatzis, P. G. (Ekstern), Rasmussen, B. B. (Intern), Engell-Sørensen, K. (Ekstern), Kleppen, H. P. (Ekstern), Wergeland, H. I. (Ekstern), Gram, L. (Intern), Middelboe, M. (Ekstern)
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Genome Sequences of Shewanella baltica and Shewanella morhuae Strains Isolated from the Gastrointestinal Tract of Freshwater Fish

We present here the genome sequences of Shewanella baltica strain CW2 and Shewanella morhuae strain CW7, isolated from the gastrointestinal tract of Salvelinus namaycush (lean lake trout) and Coregonus clupeaformis (whitefish), respectively. These genome sequences provide insights into the niche adaptation of these specific species in freshwater systems.

Phylogenetic distribution of roseobacticides in the *Roseobacter* group and their effect on microalgae

The *Roseobacter*-group species *Phaeobacter inhibens* produces the antibacterial tropodithietic acid (TDA) and the algaecidal roseobacticides with both compound classes sharing part of the same biosynthetic pathway. The purpose of this study was to investigate the production of roseobacticides more broadly in TDA-producing roseobacters and to compare the effect of producers and non-producers on microalgae. Of 33 roseobacters analyzed, roseobacticide production was a unique feature of TDA-producing *P. inhibens*, *P. gallaeciensis* and *P. piscinae* strains. One TDA-producing *Ruegeria* and *Pseudovibrio* did not produce roseobacticides. Addition of roseobacticide-containing bacterial extracts affected the growth of the microalgae *Rhodomonas salina*, *Thalassiosira pseudonana* and *Emiliania huxleyi*, while growth of *Tetraselmis suecica* was unaffected. During co-cultivation, growth of *E. huxleyi* was initially stimulated by the roseobacticide producer DSM 17395, while the subsequent decline in algal cell numbers during senescence was enhanced. Strain 27-4 that does not produce roseobacticides had no effect on algal growth. Both bacterial strains, DSM...
17395 and 27-4, grew during co-cultivation presumably utilizing algal exudates. Furthermore, TDA-producing roseobacters have potential as probiotics in marine larviculture and it is promising that the live feed *Tetraselmis* was unaffected by roseobacticides-containing extracts.

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**Bacterial resistance and susceptibility to antimicrobial peptides and peptidomimetics**

Bacterial resistance to conventional antibiotics has become a global challenge and there is an urgent need for new and alternative compounds. Antimicrobial peptides (AMPs) are under investigation as novel antibiotics. These are part of the immune defense of all living organisms; hence, they represent a valid candidate both for their antibacterial activity and for their immunomodulation features. However, these compounds have several disadvantages once administered in vivo. The shortcomings have led to extensive attempts of improving their features with rational synthetic design. Peptidomimetics are one class of such synthetic modified peptides. The purpose of this PhD project was to determine the antibacterial spectrum and potential use of synthetic antimicrobial peptides and peptidomimetics. Another key investigation has been the experimental development of resistance to these novel antibacterial agents. We investigated the antibacterial effect of selected peptidomimetics in an in vitro environment using human blood plasma and mouse serum. We hypothesized that the activity of peptidomimetics was hampered by the presence of blood fluids. However, the antibacterial activity was enhanced in the presence of human blood plasma and not in the presence of human blood serum.
ntsor clotting factors present in plasma but not in serum were causing the enhanced effect of peptidomimetics. Interestingly, incubation with heated blood matrices decreased the activity of the compounds, indicating that complement components were involved. We conclude that complement system and other factors present in human blood plasma interact synergistically with membrane active compounds such as AMPs. As a result, the concentration of peptidomimetics and peptidomimetics needed for inhibition was lower than predicted from standard antimicrobial susceptibility testing. Unfortunately, bacteria can easily adapt to AMPs in laboratory settings and we found (Manuscript 2) that Escherichia coli through a daptiveweight experiment. We hypothesized that the evolution of resistance to these combinations would be slower than with single compounds. However, the lineages exposed to P9-4 (alone or in combination) were the slowest adapting as compared to the other treatments. We suggest that the AMPPP9-4 could be considered a promising candidate for future application in clinical settings, because of its slow resistance development rate.

Using whole-genome sequencing, we investigated the genetic basis of resistance to the adapted lineages and derived clones. Deletions in genes encoding for the enzyme CDP-glycerophospho transferase were the most common variants, indicating that a common mechanism of mutation was involved in the development of resistance. The deletion of the enzyme was lethal in a wild-type strain, and we therefore hypothesized that a potential mechanism of resistance is a loss of surface charge in the cell wall. In Manuscript 3, we investigated the stability of the evolved resistance in the absence of the compound. Several clones retained resistance after cultivation in the absence of the compound. Genomic analyses demonstrated that deletions in the gene encoding for the enzyme CDP-glycerophospho transferase were responsible for the resistance. Thus, this enzyme may be involved in the mechanism of resistance. Cross-resistance was common in the adapted lineages, and we therefore determined whether the adapted resistance was due to an altered susceptibility or to the presence of the compound. The resistant clones had altered susceptibility to other microbial compounds. The resistant clones were more resistant to the compounds in intracellular assays. However, the same clones were susceptible to the wild-type when exposed to membrane-active compounds with specific features such as lipidation, incorporation of D-amino acids, and presence of IR motifs. Thus, the concern that AMP-resistant clones may be a threat to our immunity may be overstated.

In conclusion, this PhD project supports the belief that bacteria hold the potential to develop resistance to new antimicrobial agents. Nevertheless, strategies to circumvent resistance exist and must be pursued.

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Biotechnological Applications of the Roseobacter Clade
The multitude of distinct niches that prevail in the marine environment has facilitated the development of very diverse marine microorganisms. This diversity is, naturally, reflected in their biochemistry and secondary metabolites and, hence, marine microbes represent a virtually untapped source of new bioactive compounds. The Roseobacter clade of marine α-proteobacteria represents some of the most abundant organisms in the marine environment and they may constitute as much as 20–30% of the prokaryotic community during algal blooms. Often, they exhibit traits suggestive of a lifestyle in close association with phytoplankton; including traits related to surface colonization, iron scavenging, and the production of bioactive secondary metabolites. Despite the fact that relatively few bioactive compounds have been identified in the α-proteobacteria, the roseobacters are known to produce compounds capable of stimulating algae growth, i.e. auxins, and algaecidal compounds, i.e. the roseobacticides. In addition, the roseobacters can produce a range of antibacterial products, such as the small tropolone compound tropodithetic acid (TDA) and the nonribosomal peptide indigoidine. TDA targets a broad spectrum of Gram-positive and Gram-negative bacteria in which resistance towards the compound does not arise easily. Mining the genomes of roseobacters also reveal that they are likely capable of producing other compounds than hitherto observed by classical bio-assay guided fractionation, since the genomes contain genes/gene clusters probably encoding unknown bioactive secondary metabolites. Therefore, bacteria of the Roseobacter clade may
serve as potential sources of novel bioactive compounds, including novel antibiotics, which is of paramount importance in the battle against antibiotic resistant pathogenic bacteria. The discovery of new antibiotic compounds is not the only means by which we can counter the spread of antibiotic resistance. Development of sustainable alternatives to the application of antibiotics in agri- and aquaculture may be equally important. Attributable to their inherent properties, the roseobacters may be such an alternative in the aquaculture industry. Especially at the younger stages in larviculture, disease outbreaks caused by fish pathogenic microorganisms may lead to mortality rates of 100 % when antibiotic treatment is not initiated. Adding roseobacters as probiotics is promising as fish larvae challenged with fish pathogens of the genus Vibrio exhibit survival rates similar to, or better than, unchallenged larvae when roseobacter probionts are added. Thus, the Roseobacter clade is a promising source of new bioactive compounds and a possible sustainable alternative to the prophylactic administration of antibiotics in fish rearing.

**General information**

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Authors: Bentzon-Tilia, M. (Intern), Gram, L. (Intern)
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**Comparative assessment of Vibrio virulence in marine fish larvae**

Vibrionaceae infections are a major obstacle for marine larviculture; however, little is known about virulence differences of Vibrio strains. The virulence of Vibrio strains, mostly isolated from vibriosis outbreaks in farmed fish, was tested in larval challenge trials with cod (Gadus morhua), turbot (Scophthalmus maximus) and halibut (Hippoglossus hippoglossus) using a multiwell dish assays with single-egg/larvae cultures. The strains differed significantly in virulence as some caused a high mortality of larva reaching 100% mortality after a few days, while others had no or only marginal effects on survival. Some Vibrio strains were pathogenic in all of the larva species, while some caused disease only in one of the species. Twenty-nine of the Vibrio anguillarum strains increased the mortality of larvae from at least one fish species; however, pathogenicity of the strains differed markedly. Other Vibrio species had no or less pronounced effects on larval mortalities. Iron uptake has been related to V. anguillarum virulence; however, the presence or absence of the plasmid pJM1 encoding anguibactin did not correlate with virulence. The genomes of V. anguillarum were compared (D. Castillo, P.W. D'Alvise, M. Middelboe & L. Gram, unpublished data) and most of the high-virulent strains had acquired virulence genes from other pathogenic Vibrio.

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Authors: Rønneseth, A. (Ekstern), Castillo, D. (Ekstern), D'Alvise, P. (Intern), Tønnesen, Ø. (Ekstern), Haugland, G. (Ekstern), Grotkjær, T. (Intern), Engell-Sørensen, K. (Ekstern), Nørremark, L. (Ekstern), Bergh, Ø. (Ekstern), Wergeland, H. I. (Ekstern), Gram, L. (Intern)
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Comparative Genome Analyses of Vibrio anguillarum Strains Reveal a Link with Pathogenicity Traits

Vibrio anguillarum is a marine bacterium that can cause vibriosis in many fish and shellfish species, leading to high mortalities and economic losses in aquaculture. Although putative virulence factors have been identified, the mechanism of pathogenesis of V. anguillarum is not fully understood. Here, we analyzed whole-genome sequences of a collection of V. anguillarum strains and compared them to virulence of the strains as determined in larval challenge assays. Previously identified virulence factors were globally distributed among the strains, with some genetic diversity. However, the pan-genome revealed that six out of nine high-virulence strains possessed a unique accessory genome that was attributed to pathogenic genomic islands, prophage-like elements, virulence factors, and a new set of gene clusters involved in biosynthesis, modification, and transport of polysaccharides. In contrast, V. anguillarum strains that were medium to nonvirulent had a high degree of genomic homogeneity. Finally, we found that a phylogeny based on the core genomes clustered the strains with moderate to no virulence, while six out of nine high-virulence strains represented phylogenetically separate clusters. Hence, we suggest a link between genotype and virulence characteristics of Vibrio anguillarum, which can be used to unravel the molecular evolution of V. anguillarum and can also be important from survey and diagnostic perspectives.
Importance: Comparative genome analysis of strains of a pathogenic bacterial species can be a powerful tool to discover acquisition of mobile genetic elements related to virulence. Here, we compared 28 V. anguillarum strains that differed in virulence in fish larval models. By pan-genome analyses, we found that six of nine highly virulent strains had a unique core and accessory genome. In contrast, V. anguillarum strains that were medium to nonvirulent had low genomic diversity. Integration of genomic and phenotypic features provides insights into the evolution of V. anguillarum and can also be important for survey and diagnostic purposes.
Effects of gelling agent and extracellular signaling molecules on the culturability of marine bacteria

Only 1% of marine bacteria are currently culturable using standard laboratory procedures and this is a major obstacle for our understanding of the biology of marine microorganisms and for the discovery of novel microbial natural products. Therefore, the purpose of the present study was to investigate if improved cultivation conditions, including the use of an alternative gelling agent, and supplementation with signaling molecules, could improve the culturability of bacteria from seawater. Substituting agar with gellan gum improved viable counts 3–40-fold, depending on medium composition and incubation conditions, with a maximum of 6.6% culturability relative to direct cell counts. Through V4 amplicon sequencing we found that culturable diversity was also affected by a change in gelling agent, facilitating the growth of orders not culturable on agar-based substrates. Community analyses showed that communities grown on gellan gum substrates were significantly different from communities grown on agar, and that they covered a larger fraction of the seawater.
community. Other factors, such as incubation temperature and time, had less obvious effects on viable counts and culturable diversity. Supplementation with acyl homoserine lactones (AHLs) did not have a positive effect on total viable counts and no strong effect on culturable diversity. However, low concentrations of AHLs did increase the relative abundance of Sphingobacteria. Hence, with alternative growth substrates it is possible to significantly increase the number and diversity of cultured marine bacteria.

**General information**

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Organisations: Department of Systems Biology, Department of Biotechnology and Biomedicine, DTU Metabolomics Core, Bacterial Ecophysiology and Biotechnology
Authors: Rygaard, A. M. (Intern), Schmidt Thøgersen, M. (Intern), Nielsen, K. F. (Intern), Gram, L. (Intern), Bentzon-Tilia, M. (Intern)
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ISI indexed (2013): ISI indexed yes
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Scopus rating (2008): SJR 2.156 SNIP 1.572
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Streptomycetes are known for their ability to produce a range of different secondary metabolites, including antibiotics, immunosuppressive, anti-fungals, and anti-cancer compounds. Of these compounds, antibiotics play an important role in the clinics for treatment of both mild and severe bacterial infections. However, with the rise of multi-resistant pathogens, the demand for new antibiotics or derivatives of old ones, with improved properties, is now higher than ever. Recent efforts in genome sequencing and mining have revealed a so far untapped potential of streptomycetes and related actinomycetes as evident from so-called “silent” biosynthetic gene clusters, whose products remain undetectable under standard laboratory conditions. These clusters harbour all information necessary for production of potentially novel bioactive compounds, and hence provide high priority candidates for engineering to activate their production. With this knowledge, the need for better molecular tools to harness the potential of the gifted microorganisms is now greater than ever. One such molecular tool, which has truly revolutionised the field of genome engineering, is the CRISPR-Cas9 genome engineering system. In this thesis, the CRISPR-Cas9 system for genome engineering of actinomycetes was expanded for future applications in a high-throughput semi-automatic setting. First, a toolbox and workflow for construction of CRISPR plasmids, for a range of different engineering purposes was developed, including the computational prediction of suitable 20 bp protospacers for the single guide RNAs and a USER-cloning method for construction of the CRISPR plasmids. Additional improvement to the system was achieved through the development of an optimised USER assembly workflow for cheaper and faster plasmid construction. The workflow was verified by manual knock-down of two biosynthetic gene clusters in model organism Streptomyces coelicolor A3(2), which confirmed the applicability of the system. A second part of the thesis was devoted to engineering of Streptomyces collinus Tü 365, which is a known producer of the narrow-spectrum antibiotic kirromycin. While there exists several studies addressing the PKS scaffold biosynthesis of kirromycin, knowledge about the supply of the precursor ethylmalonyl-CoA and most of the tailoring reactions remained scarce. In this thesis, the role of the gene kirN, believed to be involved in precursor supply, and the six genes kirM, kirHV1, kirHV, kirHV1, kirOLi and kirOII, all predicted to be involved in tailoring reactions, were investigated by gene inactivations, complementations, and characterisation of the biosynthetic products of the generated mutants. Within our studies, four novel kirromycin derivatives were generated and characterised. Our investigations allowed for closing some of the missing gaps in the biosynthesis of kirromycin, along with providing us with a toolbox of new mutants, which produce derivatives of the original compound. These derivatives could serve as scaffolds for future bioderivatization efforts. This thesis lays the groundwork for future engineering of streptomycetes to improve secondary metabolite production. For the USER-CRISPR-Cas9 platform, the next logical step will be to implement the workflow in a robotic setting. Furthermore, the mutants of S. collinus Tü 365 will be included in a derivatization platform to produce new kirromycin analogues with improved pharmacokinetic properties.
FurIOS: a web-based tool for identification of Vibrionaceae species using the fur gene

Gene based methods for identification of species from the Vibrionaceae family have been developed during the last decades to address the limitations of the commonly used 16S rRNA gene phylogeny. Recently, we found that the ferric-uptake regulator gene (fur) can be used as a single identification marker providing species discrimination, consistent with multi-locus sequencing analyses and whole genome phylogenies. To allow for broader and easy use of this marker, we have developed an online prediction service that allows the identification of Vibrionaceae species based on their fur-sequence. The input is a DNA sequence that can be uploaded on the web service; the output is a table containing the strain identifier, e-value, and percentage of identity for each of the matches with rows colored in green for hits with high probability of being the same species. The service is available on the web at: http://www.cbs.dtu.dk/services/furIOS-1.0/.

The fur-sequences can be derived either from genome sequences or from PCR-amplification of the genomic region encoding the fur gene. We have used 191 strains identified as Vibrionaceae based on 16S rRNA gene sequence to test the PCR method and the web service on a large dataset. We were able to classify 171 of 191 strains at the species level and 20 strains remained unclassified. Furthermore, the fur phylogenetics and subsequent in silico DNA-DNA hybridization demonstrated that two strains (ATCC 33789 and ZS-139) previously identified as Vibrio splendidus are more closely related to V. tasmaniensis and V. cyclitrophicus, respectively. FurIOS is an easy-to-use online service that allows the identification of bacteria from the Vibrionaceae family at the species level using the fur gene as a single marker. Its simplistic design and straightforward pipeline makes it suitable for any research environment, from academia to industry.

Introduction
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Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 1.571 SNIP 1.179 CiteScore 3.32
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BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.507 SNIP 1.146 CiteScore 3.38
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.487 SNIP 1.125 CiteScore 3.4
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.433 SNIP 1.034
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.474 SNIP 0.964
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.398 SNIP 0.979
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.388 SNIP 0.949
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.304 SNIP 0.834
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Relations
Projects:
FurIOS: a web-based tool for identification of Vibrionaceae species using the fur gene
Publication: Research - peer-review › Journal article – Annual report year: 2017
Whole genome sequencing is increasingly used in epidemiology, e.g. for tracing outbreaks of foodborne diseases. This requires a deep understanding of pathogen emergence, persistence, and genomic diversity along the food production chain including in food processing plants. We sequenced the genomes of 80 isolates of Listeria monocytogenes sampled from Danish food processing plants over a timespan of 20 years, and analyzed the sequences together with 10 publicly available reference genomes to advance our understanding of the genotypic diversity of L. monocytogenes. Except for three persisting sequence types (ST) based on Multi Locus Sequence Typing (MLST) being ST7, ST8 and ST121, long-term persistence of clonal groups was limited, and new clones were introduced continuously, potentially from raw materials. No particular gene could be linked to the persistence phenotype. Using time-based phylogenetic analyses of the persistent STs, we estimate the L. monocytogenes evolutionary rate to be 0.18-0.35 SNPs/year, suggesting that the persistent STs emerged approximately 100 years ago, which correlates with the onset of industrialization and globalization of the food market.
Global occurrence and heterogeneity of the Roseobacter-clade species Ruegeria mobilis

Tropodithietic acid (TDA)-producing Ruegeria mobilis strains of the Roseobacter clade have primarily been isolated from marine aquaculture and have probiotic potential due to inhibition of fish pathogens. We hypothesized that TDA producers with additional novel features are present in the oceanic environment. We isolated 42 TDA-producing R. mobilis strains during a global marine research cruise. While highly similar on the 16S ribosomal RNA gene level (99–100% identity), the strains separated into four sub-clusters in a multilocus sequence analysis. They were further differentiated to the strain level by average nucleotide identity using pairwise genome comparison. The four sub-clusters could not be associated with a specific environmental niche, however, correlated with the pattern of sub-typing using co-isolated phages, the number of prophages in the genomes and the distribution in ocean provinces. Major genomic differences within the sub-clusters include prophages and toxin-antitoxin systems. In general, the genome of R. mobilis revealed adaptation to a particle-associated life style and querying TARA ocean data confirmed that R. mobilis is more abundant in the particle-associated fraction than in the free-living fraction occurring in 40% and 6% of the samples, respectively. Our data and the TARA data, although lacking sufficient data from the polar regions, demonstrate that R. mobilis is a globally distributed marine bacterial species found primarily in the upper open oceans. It has preserved key phenotypic behaviors such as the production of TDA, but contains diverse sub-clusters, which could provide new capabilities for utilization in aquaculture.
Growth on Chitin Impacts the Transcriptome and Metabolite Profiles of Antibiotic-Producing Vibrio coralliilyticus S2052 and Photobacterium galatheae S2753

Members of the Vibrionaceae family are often associated with chitin-containing organisms, and they are thought to play a major role in chitin degradation. The purpose of the present study was to determine how chitin affects the transcriptome and metabolome of two bioactive Vibrionaceae strains, Vibrio coralliilyticus and Photobacterium galatheae. We focused on chitin degradation genes and secondary metabolites based on the assumption that these molecules in nature confer an advantage to the producer. Growth on chitin caused upregulation of genes related to chitin metabolism and of genes potentially involved in host colonization and/or infection. The expression of genes involved in secondary metabolism was also significantly affected by growth on chitin, in one case being 34-fold upregulated. This was reflected in the metabolome, where the antibiotics andrimid and holomycin were produced in larger amounts on chitin. Other polyketide synthase/nonribosomal peptide synthetase (PKS-NRPS) clusters in P. galatheae were also strongly upregulated on chitin. Collectively, this suggests that both V. coralliilyticus and P. galatheae have a specific lifestyle for growth on chitin and that their secondary metabolites likely play a crucial role during chitin colonization. IMPORTANCE The bacterial family Vibrionaceae (vibrios) is considered a major player in the degradation of chitin, the most abundant polymer in the marine environment; however, the majority of studies on the topic have focused on a small number of Vibrio species. In this study, we analyzed the genomes of two vibrios to assess their genetic potential for the degradation of chitin. We then used transcriptomics and metabolomics to demonstrate that chitin strongly affects these vibrios at both the transcriptional and metabolic levels. We observed a strong increase in production of secondary metabolites, suggesting an ecological role for these molecules during chitin colonization in the marine environment.
Listeria monocytogenes incidence changes and diversity in some Brazilian dairy industries and retail products

Listeria monocytogenes can cause listeriosis, a severe foodborne disease. In Brazil, despite very few reported cases of listeriosis, the pathogen has been repeatedly isolated from dairies. This has led the government to implement specific legislation to reduce the hazard. Here, we determined the incidence of L. monocytogenes in five dairies and retail products in the Southeast and Midwest regions of Brazil over eight months. Of 437 samples, three samples (0.7%) from retail and only one sample (0.2%) from the dairies were positive for L. monocytogenes. Thus, the contamination rate was significantly reduced as compared to previous studies. MultiLocus Sequence Typing (MLST) was used to determine if contamination was caused by new or persistent clones leading to the first MLST profile of L. monocytogenes from the Brazilian dairy industry. The processing environment isolate is of concern being a sequence-type (ST) 2, belonging to the lineage I responsible for the majority of listeriosis outbreaks. Also, ST3 and ST8 found in commercialized cheese have previously been reported in outbreaks. Despite the lower incidence, dairy products still pose a potential health risk and the occurrence of L. monocytogenes in dairies and retail products emphasize the need for continuous surveillance of this pathogen in the Brazilian dairy industry. (C) 2017 Elsevier Ltd. All rights reserved.

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Scopus rating (2016): CiteScore 4.31 SJR 1.723 SNIP 1.675
Phaeobacter piscinae sp. nov., a species of the Roseobacter group and potential aquaculture probiont

Four heterotrophic, antimicrobial, motile, marine bacterial strains, 27-4T, 8-1, M6-4.2 and S26, were isolated from aquaculture units in Spain, Denmark and Greece. All four strains produced the antibiotic compound tropodithietic acid, which is a key molecule in their antagonism against fish pathogenic bacteria. Cells of the strains were Gram-reaction-negative, rod-shaped and formed star-shaped aggregates in liquid culture and brown-coloured colonies on marine agar. The predominant cellular fatty acids were C18:1ω7c, C16:0, C11 methyl C18:1ω7c and C16:0 2-OH, and the polar lipids comprised phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an aminolipid, a phospholipid and an unidentified lipid. The strains grew optimally at 31-33°C. Growth was observed at a salt concentration between 0.5 and 5-6% NaCl with an optimum at 2-3%. The pH range for growth of the strains was from pH 6 to 8-8.5 with an optimum at pH 7. Based on 16S rRNA gene sequence analysis, the strains are affiliated with the genus Phaeobacter.
The genome sequences of the strains have a DNA G+C content of 60.1% and share an average nucleotide identity (ANI) of more than 95%. The four strains are distinct from the type strains of the closely related species Phaeobactergallaeciensis and Phaeobacterinhibens based on an ANI of 90.5-91.7 and 89.6-90.4%, respectively, and an in silico DNA-DNA hybridization relatedness of 43.9-46.9 and 39.8-41.9%, respectively. On the basis of phylogenetic analyses as well as phenotypic and chemotaxonomic properties, the isolates are considered to represent a novel species, for which the name Phaeobacter piscinae sp. nov. is proposed. The type strain is 27-4T (=DSM 103509T=LMG 29708T).

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Scopus rating (2010): SJR 1.056 SNIP 1.195
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.955 SNIP 1.251
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.068 SNIP 1.344
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.103 SNIP 1.585
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.394 SNIP 1.554
A new siderophore containing a 4,5-dihydroimidazole moiety was isolated from Pseudoalteromonas piscicida S2040 together with myxochelins A and B, alteramide A and its cycloaddition product, and bromo- and dibromoalterochromides. The structure of pseudochelin A was established by spectroscopic techniques including 2D NMR and MS/MS fragmentation data. In bioassays selected fractions of the crude extract of S2040 inhibited the opportunistic pathogen Pseudomonas aeruginosa. Pseudochelin A displayed siderophore activity in the chrome azurol S assay at concentrations higher than 50 μM, and showed weak activity against the fungus Aspergillus fumigatus, but did not display antibacterial, anti-inflammatory or anticonvulsant activity.
Staphylococcus aureus in Some Brazilian Dairy Industries: Changes of Contamination and Diversity

Staphylococcus aureus, a major food-poisoning pathogen, is a common contaminant in dairy industries worldwide, including in Brazil. We determined the occurrence of S. aureus in five dairies in Brazil over 8 months. Of 421 samples, 31 (7.4%) were positive for S. aureus and prevalence varied from 0 to 63.3% between dairies. Sixty-six isolates from the 31 samples were typed by Multi-Locus Sequence Typing to determine if these isolates were persistent or continuously reintroduced. Seven known sequence types (STs), ST1, ST5, ST30, ST97, ST126, ST188 and ST398, and four new ST were identified, ST3531, ST3540, ST3562 and ST3534. Clonal complex (CC) 1 (including the four new ST), known as an epidemic clone, was the dominant CC. However, there were no indications of persistence of particular ST. The resistance toward 11 antibiotic compounds was assessed. Twelve profiles were generated with 75.8% of strains being sensitive to all antibiotic classes and no Methicillin-resistant S. aureus (MRSA) strains were found. The enterotoxin-encoding genes involved in food-poisoning, e.g., sea, sed, see, and seg were targeted by PCR. The two toxin-encoding genes, sed and see, were not detected. Only three strains (4.5%) harbored seg and two of these also harbored sea. Despite the isolates being Methicillin-sensitive S. aureus (MSSA), the presence of CC1 clones in the processing environment, including some harboring enterotoxin encoding genes, is of concern and hygiene must have high priority to reduce contamination.
The aquaculture microbiome at the centre of business creation

Editorial: The microbiome as a source of new enterprises and job creation

General information
State: Published
Organisations: Department of Biotechnology and Biomedicine, Bacterial Ecophysiology and Biotechnology, Lallemand SAS
Authors: Dittmann, K. K. (Intern), Rasmussen, B. B. (Intern), Castex, M. (Ekstern), Gram, L. (Intern), Bentzon-Tilia, M. (Intern)
Pages: 1279-1282
The Influence of the Toxin/Antitoxin mazEF on Growth and Survival of Listeria monocytogenes under Stress

A major factor in the resilience of *Listeria monocytogenes* is the alternative sigma factor B (σB). Type II Toxin/Antitoxin (TA) systems are also known to have a role in the bacterial stress response upon activation via the ClpP or Lon proteases. Directly upstream of the σB operon in *L. monocytogenes* is the TA system mazEF, which can cleave mRNA at UACMU sites. In this study, we showed that the mazEF TA locus does not affect the level of persister formation during treatment with antibiotics in lethal doses, but exerts different effects according to the sub-inhibitory stress added. Growth of a ΔmazEF mutant was enhanced relative to the wildtype in the presence of sub-inhibitory norfloxacin and at 42 °C, but was decreased when challenged with ampicillin and gentamicin. In contrast to studies in *Staphylococcus aureus*, we found that the mazEF locus did not affect transcription of genes within the σB operon, but MazEF affected the expression of the σB-dependent genes *opuCA* and *lmo0880*, with a 0.22 and 0.05 fold change, respectively, compared to the wildtype under sub-inhibitory norfloxacin conditions. How exactly this system operates remains an open question, however, our data indicates it is not analogous to the system of *S. aureus*, suggesting a novel mode of action for MazEF in *L. monocytogenes*. 
Trajectories and Drivers of Genome Evolution in Surface-Associated Marine Phaeobacter

The extent of genome divergence and the evolutionary events leading to speciation of marine bacteria have mostly been studied for (locally) abundant, free-living groups. The genus Phaeobacter is found on different marine surfaces, seems to occupy geographically disjunct habitats, and is involved in different biotic interactions, and was therefore targeted in the present study. The analysis of the chromosomes of 32 closely related but geographically spread Phaeobacter strains revealed an exceptionally large, highly syntenic core genome. The flexible gene pool is constantly but slightly expanding across all Phaeobacter lineages. The horizontally transferred genes mostly originated from bacteria of the Roseobacter group and horizontal transfer most likely was mediated by gene transfer agents. No evidence for geographic isolation and habitat specificity of the different phylogenomic Phaeobacter clades was detected based on the sources of isolation. In contrast, the functional gene repertoire and physiological traits of different phylogenomic Phaeobacter clades were sufficiently distinct to suggest an adaptation to an associated lifestyle with algae, to additional nutrient sources, or toxic heavy metals. Our study reveals that the evolutionary trajectories of surface-associated marine bacteria can differ significantly from free-living marine bacteria or marine generalists.
Advancing Functional Metagenomics using Synthetic Biology from Soil to Sequence

Facing a growing and aging world population, mankind is in need for new drug molecules and ways to produce nutrients. Instead of using chemical synthesis, drugs and nutrients can be produced in a sustainable way by modified bacteria. Most of those interesting molecules are already produced by billions of bacteria in the environment. Unfortunately it is difficult to grow the majority of the bacteria in a laboratory and it is therefore not possible to use their capabilities directly. However, bacteria store the information on how to produce molecules in their DNA. Using a method known as ‘functional metagenomics’, the DNA of these bacteria can be recovered from the environment and used by host-bacteria which can be grown in a lab. This allows us to make use of the capabilities of the billions of bacteria that are present in the environment without actually growing them but by making use of their DNA instead. In my thesis I describe methods to find DNA fragments in the environment that are of interest for the biotech industry. We found for example DNA fragments in
cow manure that can enhance the production of the nutrient lysine. I also describe a method to discover potential anti-viral molecules by making use of DNA from microorganisms found in soil. In this thesis I describe the application of the relatively new nanopore sequencing technology to read the DNA, which was obtained using the previous mentioned methods. Our deFUME webserver makes it easier for researchers to investigate the DNA afterwards.

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Authors: van der Helm, E. (Intern), Sommer, M. O. A. (Intern), Gram, L. (Intern)
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An Integrated Metabolomic and Genomic Mining Workflow to Uncover the Biosynthetic Potential of Bacteria
Microorganisms are a rich source of bioactives; however, chemical identification is a major bottleneck. Strategies that can prioritize the most prolific microbial strains and novel compounds are of great interest. Here, we present an integrated approach to evaluate the biosynthetic richness in bacteria and mine the associated chemical diversity. Thirteen strains closely related to Pseudoalteromonas luteoviolacea isolated from all over the Earth were analyzed using an untargeted metabolomics strategy, and metabolomic profiles were correlated with whole-genome sequences of the strains. We found considerable diversity: only 2% of the chemical features and 7% of the biosynthetic genes were common to all strains, while 30% of all features and 24% of the genes were unique to single strains. The list of chemical features was reduced to 50 discriminating features using a genetic algorithm and support vector machines. Features were dereplicated by tandem mass spectrometry (MS/MS) networking to identify molecular families of the same biosynthetic origin, and the associated pathways were probed using comparative genomics. Most of the discriminating features were related to antibacterial compounds, including the thiomarinols that were reported from P. luteoviolacea here for the first time. By comparative genomics, we identified the biosynthetic cluster responsible for the production of the antibiotic indolmycin, which could not be predicted with standard methods. In conclusion, we present an efficient, integrative strategy for elucidating the chemical richness of a given set of bacteria and link the chemistry to biosynthetic genes.

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Antimicrobial Tolerance in Listeria monocytogenes: Persister cells and the survival of the slowest

There are two ways in which bacteria survive killing by antibiotics. The most well-known, is antibiotic resistance, which results from the acquisition of a resistance gene or mutation that allows bacteria to grow and divide in the presence of antibiotic concentrations that would normally kill other bacteria. Antibiotic tolerance on the other hand, is the ability of bacteria to survive (but not grow) prolonged exposure to concentrations that should normally kill them. The predominant mechanism underlying tolerance is the so-called persister cell, a small subpopulation of dormant like cells that are completely refractory to antibiotics due to the inactivity of cellular processes. Persister cells have been linked to treatment failures in several bacterial infections including Mycobacterium tuberculosis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.

Preceding the start of this PhD project, Listeria monocytogenes was observed to form these antibiotic tolerant persister cells. L. monocytogenes is a Gram-positive, foodborne pathogen that causes listeriosis, a rare, but often lethal disease, even with antibiotic treatment. It typically affects pregnant women, neonates, the elderly and the immunocompromised, and can cause mortality rates of up to 34%. In addition to the human host, this resilient organism has evolved to persevere in several environments, such as the soil and food processing environments, where it can cope with and even grow at temperatures between -0.4 and 45˚C or a pH range of between 4.6 and 9.6, and survive NaCl concentrations as high as 40%.

Given its resilience, as well as the industrial and clinical implications, we felt that L. monocytogenes would make a good model to study antibiotic tolerance and sought to further investigate persister cells and their underlying mechanisms in this organism. The body of work over the course of this PhD study has been organized into three manuscripts, which are summarized below.

The first manuscript sought to link the Small Colony Variant (SCV) to persister cells in L. monocytogenes. SCV cells of bacteria are a slow growing phenotype that can result from specific defects in the electron transport chain. We speculated that a stable L. monocytogenes heme deficient SCV would show a similar tolerance towards a broad range of antibiotics that persister cells do and found that apart from the macrolide erythromycin, the SCV exhibited increased tolerance towards all tested antibiotics. Furthermore, we found that the SCV of L. monocytogenes could tolerate the reactive oxygen species produced in the macrophage significantly better than the wildtype, both of which suggest that SCV of L. monocytogenes may be clinically relevant.

The two remaining manuscripts investigated the role of chromosomally encoded Toxin/Antitoxin (TA) systems in the ability of L. monocytogenes to tolerate and respond to different stress factors, including antibiotics. TA systems typically consist of a stable toxic protein that acts on some bacterial process, resulting in stasis, and a less stable antitoxin that binds and neutralizes the toxin. In the past few decades they have emerged as one of the primary mechanisms underlying the formation of persister cells.

In L. monocytogenes, the alternative sigma factor sigma B (σB) is responsible for the transcription of many stress and virulence genes, and transcriptomic studies indicate that the mazEF TA system is also part of the σB operon. In the second manuscript, we found that while the mazEF TA locus does not affect the level of persister formation during treatment with antibiotics in lethal doses, it does exert an effect on the expression of σB dependent genes, as well as the growth of this organism under stress.

Lastly, we looked deeper into the genome of L. monocytogenes for additional TA systems and, in addition to the two previously predicted TA systems, detected four loci with type II TA genomic features. However, due to unforeseen challenges we were unable to further characterize them and will employ fluorescence activated cell sorting in the future to both experimentally verify these putative TA systems and elucidate the conditions under which they are induced.
Biogeography and environmental genomics of the Roseobacter-affiliated pelagic CHAB-I-5 lineage

The identification and functional characterization of microbial communities remains a prevailing topic in microbial oceanography as information on environmentally relevant pelagic prokaryotes is still limited. The Roseobacter group, an abundant lineage of marine Alphaproteobacteria, can constitute large proportions of the bacterioplankton. Roseobacters also occur associated with eukaryotic organisms and possess streamlined as well as larger genomes from 2.2 to >5 Mbp. Here, we show that one pelagic cluster of this group, CHAB-I-5, occurs globally from tropical to polar regions and accounts for up to 22% of the active North Sea bacterioplankton in the summer. The first sequenced genome of a CHAB-I-5 organism comprises 3.6 Mbp and exhibits features of an oligotrophic lifestyle. In a metatranscriptome of North Sea surface waters, 98% of the encoded genes were present, and genes encoding various ABC transporters, glutamate synthase and CO oxidation were particularly upregulated. Phylogenetic gene content analyses of 41 genomes of the Roseobacter group revealed a unique cluster of pelagic organisms distinct from other lineages of this group, highlighting the adaptation to life in nutrient-depleted environments.
Improved in vitro evaluation of novel antimicrobials: potential synergy between human plasma and antibacterial peptidomimetics, AMPs and antibiotics against human pathogenic bacteria

Stable peptidomimetics mimicking natural antimicrobial peptides (AMPs) have emerged as a promising class of potential novel antibiotics. In the present study, we aimed at determining whether the antibacterial activity of two α-peptide/β-peptoid peptidomimetics against a range of bacterial pathogens was affected by conditions mimicking in vivo settings. Their activity was enhanced to an unexpected degree in the presence of human blood plasma for thirteen pathogenic Gram-positive and Gram-negative bacteria. MIC values typically decreased 2- to 16-fold in the presence of a human
plasma concentration that alone did not damage the cell membrane. Hence, MIC and MBC data collected in these settings appear to represent a more appropriate basis for in vivo experiments preceding clinical trials. In fact, concentrations of peptidomimetics and peptide antibiotics (e.g. polymyxin B) required for in vivo treatments might be lower than traditionally deduced from MICs determined in laboratory media. Thus, antibiotics previously considered too toxic could be developed into usable last-resort drugs, due to ensuing lowered risk of side effects. In contrast, the activity of the compounds was significantly decreased in heat-inactivated plasma. We hypothesize that synergistic interactions with complement proteins and/or clotting factors most likely are involved.

**General information**

State: Published

Organisations: Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, Department of Micro- and Nanotechnology, Center for Electron Nanoscopy, University of Copenhagen, University of Southern Denmark

Authors: Citterio, L. (Intern), Franzyk, H. (Ekstern), Palarasah, Y. (Ekstern), Andersen, T. E. (Ekstern), Mateiu, R. V. (Intern), Gram, L. (Intern)

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Scopus rating (2010): SJR 1.333 SNIP 0.841

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Web of Science (2008): Indexed yes

Scopus rating (2007): SJR 1.146 SNIP 0.885

Scopus rating (2006): SJR 1.418 SNIP 0.967
Influence of Iron on Production of the Antibacterial Compound Tropodithietic Acid and Its Noninhibitory Analog in Phaeobacter inhibens

Tropodithietic acid (TDA) is an antibacterial compound produced by some Phaeobacter and Ruegeria spp. of the Roseobacter clade. TDA production is studied in marine broth or agar since antibacterial activity in other media is not observed. The purpose of this study was to determine how TDA production is influenced by substrate components. High concentrations of ferric citrate, as present in marine broth, or other iron sources were required for production of antibacterially active TDA. However, when supernatants of noninhibitory, low-iron cultures of Phaeobacter inhibens were acidified, antibacterial activity was detected in a bioassay. The absence of TDA in nonacidified cultures and the presence of TDA in acidified cultures were verified by liquid chromatography-high-resolution mass spectrometry. A noninhibitory TDA analog (pre-TDA) was produced by P. inhibens, Ruegeria mobilis F1926, and Phaeobacter sp. strain 27-4 under low-iron concentrations and was instantaneously converted to TDA when pH was lowered. Production of TDA in the presence of Fe^{3+} coincides with formation of a dark brown substance, which could be precipitated by acid addition. From this brown pigment TDA could be liberated slowly with aqueous ammonia, and both direct-infusion mass spectrometry and elemental analysis indicated a [Fe^{III}(TDA)_2] complex. The pigment could also be produced by precipitation of pure TDA with FeCl_3. Our results raise questions about how biologically active TDA is produced in natural marine settings where iron is typically limited and whether the affinity of TDA to iron points to a physiological or ecological function of TDA other than as an antibacterial compound.
Influence of Niche-Specific Nutrients on Secondary Metabolism in Vibrionaceae

Many factors, such as the substrate and the growth phase, influence biosynthesis of secondary metabolites in microorganisms. Therefore, it is crucial to consider these factors when establishing a bioprospecting strategy. Mimicking the conditions of the natural environment has been suggested as a means of inducing or influencing microbial secondary metabolite production. The purpose of the present study was to determine how the bioactivity of Vibrionaceae was influenced by carbon sources typical of their natural environment. We determined how mannose and chitin, compared to...
glucose, influenced the antibacterial activity of a collection of Vibrionaceae strains isolated because of their ability to produce antibacterial compounds but that in subsequent screenings seemed to have lost this ability. The numbers of bioactive isolates were 2- and 3.5-fold higher when strains were grown on mannose and chitin, respectively, than on glucose. As secondary metabolites are typically produced during late growth, potential producers were also allowed 1 to 2 days of growth before exposure to the pathogen. This strategy led to a 3-fold increase in the number of bioactive strains on glucose and an 8-fold increase on both chitin and mannose. We selected two bioactive strains belonging to species for which antibacterial activity had not previously been identified. Using ultrahigh-performance liquid chromatography-high-resolution mass spectrometry and bioassay-guided fractionation, we found that the siderophore fluvibactin was responsible for the antibacterial activity of Vibrio furnissii and Vibrio fluvialis. These results suggest a role of chitin in the regulation of secondary metabolism in vibrios and demonstrate that considering bacterial ecophysiology during development of screening strategies will facilitate bioprospecting. A challenge in microbial natural product discovery is the elicitation of the biosynthetic gene clusters that are silent when microorganisms are grown under standard laboratory conditions. We hypothesized that, since the clusters are not lost during proliferation in the natural niche of the microorganisms, they must, under such conditions, be functional. Here, we demonstrate that an ecology-based approach in which the producer organism is allowed a temporal advantage and where growth conditions are mimicking the natural niche remarkably increases the number of Vibrionaceae strains producing antibacterial compounds.

**General information**

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Authors: Giubergia, S. (Intern), Phippen, C. (Intern), Gottfredsen, C. H. (Intern), Nielsen, K. F. (Intern), Gram, L. (Intern)
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Molluscan shellfish can cause foodborne diseases and here we investigated if addition of Vibrio-antagonising bacteria could reduce Vibrio vulnificus in model oyster systems and prevent its establishment in live animals. Phaeobacter inhibens, which produces an antibacterial compound, tropodithietic acid (TDA), inhibited V. vulnificus as did pure TDA (MIC of 1-3.9 μM). P. inhibens DSM 17395 (at 10⁵ cfu/ml) eradicated 10⁵ cfu/ml V. vulnificus CMCP6 (a rifampicin resistant variant) from a co-culture oyster model system (oyster juice) whereas the pathogen grew to 10⁷ cfu/ml when co-cultured with a TDA negative Phaeobacter mutant. P. inhibens grew well in oyster juice to 10⁸ CFU/ml and sterile filtered samples from these cultures were inhibitory to Vibrio spp. P. inhibens established itself in live European flat oysters (Ostrea edulis) and remained at 10⁵ cfu/g for five days. However, the presence of P. inhibens could not prevent subsequently added V. vulnificus from entering the live animals, likely because of too low levels of the biocontrol strain. Whilst the oyster model studies provided indication that P. inhibens DSM 17395 could be a good candidate as biocontrol agent against V. vulnificus further optimization is needed in the actual animal rearing situation.

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Authors: Porsby, C. H. (Intern), Gram, L. (Intern)
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Phaeobacter inhibens as probiotic bacteria in non-axenic Artemia and algae cultures

The growing aquaculture industry is in need for non-antibiotic based disease control strategies to reduce risk of bacteria developing and spreading antibiotic resistance. We have previously, in axenic model systems of live larval feed, demonstrated that bacteria from the Roseobacter clade can antagonize fish pathogens such as Vibrio anguillarum and Vibrio harveyi and that they can reduce larval mortality in challenge trials. However, in the aquaculture production, a natural microbiota is present at all stages and may affect the efficacy of the probiotic bacteria. The purpose of the present study was to determine if marine roseobacters in non-axenic systems were capable of antagonizing fish pathogenic vibrios. We added a controlled background microbiota of four bacterial strains to axenic Artemia and algae (Dunaliella) and these bacteria had a marginal but significant reducing effect on inoculated Vibrio anguillarum that grew to $10^7$ in control samples but to a level 1–2 log lower in samples with background microbiota. The addition of the Roseobacter-clade bacteria, Phaeobacter inhibens, caused a significant reduction in growth of the pathogen that reached levels 3–4 log lower than in the control. In non-axenic natural Artemia and algae (Tetraselmis) received from an aquaculture unit, Vibrio anguillarum grew to $10^7$ CFU/ml but only reached $10^4$ CFU/ml when P. inhibens was also added. P. inhibens was added at a concentration $10^6$ CFU/ml in all systems and remained at this concentration at the end of the study, irrespective of the background microbiota. We therefore conclude that P. inhibens are indeed promising as probiotic bacteria in marine larval culture where it in natural live feed can suppress fish larval pathogens.
Isolation of TDA-producing *Phaeobacter* strains from sea bass larval rearing units and their probiotic effect against pathogenic *Vibrio* spp. in *Artemia* cultures

Fish-pathogenic *Vibrio* can cause large-scale crashes in marine larval rearing units and, since the use of antibiotics can result in bacterial antibiotic resistance, new strategies for disease prevention are needed. *Roseobacter*-clade bacteria from turbot larval rearing facilities can antagonize *Vibrio anguillarum* and reduce mortality in *V. anguillarum*-infected cod and turbot larvae. In this study, it was demonstrated that antagonistic *Roseobacter*-clade bacteria could be isolated from sea bass larval rearing units. In addition, it was shown that they not only antagonized *V. anguillarum* but also *V. harveyi*, which is the major bacterial pathogen in crustaceans and Mediterranean sea bass larvae cultures. Concomitantly, they significantly improved survival of *V. harveyi*-infected brine shrimp. 16S rRNA gene sequence homology identified the antagonists as *Phaeobacter* spp., and *in silico* DNA-DNA hybridization indicated that they could belong to a new species. The genomes contained genes involved in synthesis of the antibacterial compound tropodithietic acid (TDA), and its production was confirmed by UHPLC-TOFMS. The new *Phaeobacter* colonized live feed (*Artemia* cultures) and reduced *Vibrio* counts significantly, since they reached only $10^4$ CFU mL$^{-1}$, as opposed to $10^8$ CFU mL$^{-1}$ in non-*Phaeobacter* treated controls. Survival of *V. anguillarum*-challenged *Artemia* nauplii was enhanced by the presence of wild type *Phaeobacter* compared to challenged control cultures (89±1.0% vs 8±3.2%). In conclusion, TDA-producing *Phaeobacter* isolated from Mediterranean marine larviculture are promising probiotic bacteria against pathogenic *Vibrio* in crustacean live-feed cultures for marine fish larvae.

**General information**

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Organisations: Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, Metabolomics Platform, Selonda
Vibrio anguillarum Is Genetically and Phenotypically Unaffected by Long-Term Continuous Exposure to the Antibacterial Compound Tropolithietic Acid

Minimizing the use of antibiotics in the food production chain is essential for limiting the development and spread of antibiotic-resistant bacteria. One alternative intervention strategy is the use of probiotic bacteria, and bacteria of the marine Roseobacter clade are capable of antagonizing fish-pathogenic vibrios in fish larvae and live feed cultures for fish larvae. The antibacterial compound tropodithietic acid (TDA), an antiporter that disrupts the proton motive force, is key in the antibacterial activity of several roseobacters. Introducing probiotics on a larger scale requires understanding of any potential side effects of long-term exposure of the pathogen to the probionts or any compounds they produce. Here we exposed the fish pathogen Vibrio anguillarum to TDA for several hundred generations in an adaptive evolution experiment. No tolerance or resistance arose during the 90 days of exposure, and whole-genome sequencing of TDA-exposed lineages and clones revealed few mutational changes, compared to lineages grown without TDA. Amino acid-changing mutations were found in two to six different genes per clone; however, no mutations appeared unique to the TDA-exposed lineages or clones. None of the virulence genes of V. anguillarum was affected, and infectivity assays using fish cell lines indicated that the TDA-exposed lineages and clones were less invasive than the wild-type strain. Thus, long-term TDA exposure does not appear to result in TDA resistance and the physiology of V. anguillarum appears unaffected, supporting the application of TDA-producing roseobacters as probiotics in aquaculture. It is important to limit the use of antibiotics in our food production, to reduce the risk of bacteria developing antibiotic resistance. We showed previously that marine bacteria of the Roseobacter clade can prevent or reduce bacterial diseases in fish larvae, acting as probiotics. Roseobacters produce the antimicrobial compound tropodithietic acid (TDA), and we were concerned regarding whether long-term exposure to this compound could induce resistance or affect the disease-causing ability of the fish pathogen. Therefore, we exposed the fish pathogen Vibrio anguillarum to increasing TDA concentrations over 3 months. We did not see the development of any resistance to TDA, and subsequent infection assays revealed that none of the TDA-exposed clones had increased virulence toward fish cells. Hence, this study supports the use of roseobacters as a non-risk-based disease control measure in aquaculture.
Marine Bacterial Genomics: an ocean of opportunity

For decades, terrestrial microorganisms have been used as sources of countless enzymes and chemical compounds that have been produced by pharmaceutical and biotech companies and used by mankind. There is a need for new chemical compounds, including antibiotics, new enzymatic activities and new microorganisms to be used as cell factories for production. Therefore exploitation of new microbial niches and use of different strategies is an opportunity to boost discoveries. Even though scientists have started to explore several habitats other than the terrestrial ones, the marine
environment stands out as a hitherto under-explored niche. This thesis work uses high-throughput sequencing technologies on a collection of marine bacteria established during the Galathea 3 expedition, with the purpose of unraveling new biodiversity and new bioactivities. Several tools were used for genomic analysis in order to better understand the potential harbored in marine bacteria. The work presented makes use of whole genome sequencing of marine bacteria to prove that the genetic repertoire for secondary metabolite production harbored in these bacteria is far larger than anticipated; to identify and develop a new phylogenetic marker for the identification of members of the Vibrionaceae family, which led to the identification of two new species using this straightforward pipeline; to discovery of new cytochrome P450 enzymes to be used in biotechnology; and to a thorough study of the marine genus Photobacterium, by means of comparative genomics. In conclusion, this PhD thesis has contributed to our understanding of the marine microbial environment by studying genomic information of several marine bacteria, expanding the number of marine species taxonomically described, providing identification tools for further marine species documentation and pointing to these organisms as a very promising resource for further bioprospecting.
Monitoring and managing microbes in aquaculture - Towards a sustainable industry
Microorganisms are of great importance to aquaculture where they occur naturally, and can be added artificially, fulfilling different roles. They recycle nutrients, degrade organic matter and, occasionally, they infect and kill the fish, their larvae or the live feed. Also, some microorganisms may protect fish and larvae against disease. Hence, monitoring and manipulating the microbial communities in aquaculture environments hold great potential; both in terms of assessing and improving water quality, but also in terms of controlling the development of microbial infections. Using microbial communities to monitor water quality and to efficiently carry out ecosystem services within the aquaculture systems may only be a few years away. Initially, however, we need to thoroughly understand the microbiomes of both healthy and diseased aquaculture systems, and we need to determine how to successfully manipulate and engineer these microbiomes. Similarly, we can reduce the need to apply antibiotics in aquaculture through manipulation of the microbiome, i.e. by the use of probiotic bacteria. Recent studies have demonstrated that fish pathogenic bacteria in live feed can be controlled by probiotics and that mortality of infected fish larvae can be reduced significantly by probiotic bacteria. However, the successful management of the aquaculture microbiota is currently hampered by our lack of knowledge of relevant microbial interactions and the overall ecology of these systems.
Production of the Bioactive Compounds Violacein and Indolmycin Is Conditional in a maeA Mutant of *Pseudoalteromonas luteoviolacea* S4054 Lacking the Malic Enzyme

It has previously been reported that some strains of the marine bacterium *Pseudoalteromonas luteoviolacea* produce the purple bioactive pigment violacein as well as the antibiotic compound indolmycin, hitherto only found in *Streptomyces*. The purpose of the present study was to determine the relative role of each of these two compounds as antibacterial compounds in *P. luteoviolacea* S4054. Using Tn10 transposon mutagenesis, a mutant strain that was significantly reduced in violacein production in mannose-containing substrates was created. Full genome analyses revealed that the viol biosynthetic gene cluster was not interrupted by the transposon; instead the insertion was located to the maeA gene encoding the malic enzyme. Supernatant of the mutant strain inhibited *Vibrio anguillarum* and *Staphylococcus aureus* in well diffusion assays and in MIC assays at the same level as the wild type strain. The mutant strain killed *V. anguillarum* in co-culture experiments as efficiently as the wild type. Using UHPLC-UV/Vis analyses, we quantified violacein and indolmycin, and the mutant strain only produced 7-10% the amount of violacein compared to the wild type strain. In contrast, the amount of indolmycin produced by the mutant strain was about 300% that of the wild type. Since inhibition of *V. anguillarum* and *S. aureus* by the mutant strain was similar to that of the wild type, it is concluded that violacein is not the major antibacterial compound in *P. luteoviolacea*. We furthermore propose that production of violacein and indolmycin may be metabolically linked and that yet unidentified antibacterial compound(s) may be play a role in the antibacterial activity of *P. luteoviolacea*.

General information

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Reclassification of Alteromonas fuliginea (Romanenko et al. 1995) as Pseudoalteromonas fuliginea comb. nov. and emended description

A new aerobic marine bacterium, strain S3431, was isolated from swab samples of an unidentified polychaete near Canal Concepción, Chile. This strain was thought to represent a new taxon within the Pseudoalteromonas genus. Although DNA-DNA reassociation values showed less than 70% genomic DNA relatedness to established Pseudoalteromonas type strains, it had 78% DNA-DNA homology with Alteromonas fuliginea DSM 15748 (= KMM 216) (Romanenko et al., 1994). A. fuliginea has later been considered a heterotypic synonym of Pseudoalteromonas citrea (Ivanova et al., 1998). Therefore we here studied the relatedness between strains S3431, A. fuliginea DSM 15748 and the type strain P. citrea LMG 12323T. We found that physiological traits and genomic information are shared at a high level by strains S3431 and DSM 15748, but not between these and P. citrea LMG 12323T. We found only approximately 20% DNA-DNA homology between the type strain of P. citrea LMG 12323T and strains S3431 and DSM 15748. Based on the available phylogenetic and phenotypic data, reclassification of Alteromonas fuliginea DSM15748 (Romanenko et al., 1994) → Pseudoalteromonas citrea (Ivanova et al., 1998) as Pseudoalteromonas fuliginea is proposed, and S3431 should be assigned to this new species. The name Pseudoalteromonas fuliginea is proposed and the type strain is KMM 216 T = DSM15748 T = CIP105339 T.

General information
State: Published
Organisations: Department of Systems Biology, Novo Nordisk Foundation Center for Biosustainability, Bacterial Ecophysiology and Biotechnology, Aarhus University
Authors: Machado, H. (Intern), Vynne, N. G. (Intern), Christiansen, G. (Ekstern), Gram, L. (Intern)
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Roseobacter-clade bacteria as probiotics in marine larvaculture

Disease caused by fish pathogenic bacteria can cause large scale crashes in marine fish larval rearing units. One of the biggest challenges for aquaculture is the management of these bacterial outbreaks. Vaccines can be admitted to fish but only the juvenile and the adult fish because they need to have a mature immune system. This means that the larvae of the fish, until they are 2-3 weeks old are more prone to bacterial infections. A short term solution is antibiotics but this leaves way for the selection for antibiotic resistance among the pathogenic bacteria, which again can be transferred to human pathogens. Alternatives are therefore needed and one could be the use of probiotic bacteria. Marine bacteria from the Roseobacter clade (Phaeobacter inhibens) have shown great potential as probiotic bacteria, and we have hypothesized that they could be used to antagonize pathogenic fish and crustacean bacteria in the environment of the larvae. The purpose of the present PhD study was to determine if antagonistic Roseobacter clade bacteria occurred in marine aquaculture units. The study would determine their clonal relationship and elucidate the mechanisms by which these potential probiotic bacteria affect the fish pathogens. The efficiency as probiotics was studied in cultures of microalgae and live feed organisms in both axenic and none axenic cultures. Chemical, genetic and bioinformatic tools were used to identify and quantify the production of the antibiotic compound and then compare reference strains from the Roseobacter clade. Long-term exposure of the fish pathogen to the antimicrobial compound produced by Phaeobacter was done in order to determine risk of resistance and possible genetic and phenotypic effects on the pathogen. The study showed that some of the isolates from the Greek rearing unit could only be identified via 16S rRNA gene sequencing as Phaeobacter sp. following guidelines from a previously published study. Using whole genome sequencing and in silico DNA-DNA hybridization gave an indication of a new Phaeobacter species. Two such strains together with Phaeobacter inhibens DSM 17395 were chosen for further study. The study demonstrated that in axenic live feed cultures, the potentially new species of Phaeobacter were able to antagonize both the fish pathogen Vibrio anguillarum with up to four logarithmic units and the crustacean pathogen Vibrio harveyi with up to two logarithmic units. This corresponded well with results from reference strains and previous studies. To confirm the in vivo mechanism of action of the antibacterial compound tropodithietic acid (TDA) a defective mutant was included in the study. The mutant was significantly less efficient at antagonizing the pathogen indicating that TDA production is the major contribution to the probiotic action. To further elucidate the probiotic potential of Phaeobacter inhibens, the probiont were added to cultures of Artemia and Duniella tertiolectra with four added bacterial strains representing aquaculture background microbiota. The Phaeobacter inhibens were able to colonize both cultures and still antagonize the pathogen with up to four logarithmic units. Lastly, the efficiency of the probiont was tested in completely non-axenic cultures of either Artemia or Tetraselmis suecica received from an aquaculture unit. The cultures were inoculated with the pathogen Vibrio anguillarum. The pathogen was reduced with up to three logarithmic units compared to the control. The antibacterial compound tropodithietic acid (TDA), an antiporter that disrupts the proton motive force, is key in the antibacterial activity of several roseobacters. Introducing probiotics at a larger scale requires understanding of any potential side effects of long term exposure of the pathogen to the probionts or any compounds they produce. We here exposed the fish pathogen, Vibrio anguillarum, to TDA for several hundred generations in an adaptive evolution experiment. No tolerance or resistance arose during the 90 days of exposure and whole genome sequencing of TDA-exposed lineages and clones revealed few mutational changes as compared to lineages grown without presence of TDA. Amino acid changing mutations were found in two to six different genes per clone, however, no mutations appeared unique to the TDAexposed lineages or clones. None of the virulence genes of V. anguillarum were affected and infectivity assays using fish cell lines indicated that the TDA- exposed lineages and clones were less invasive than the wild type. Thus, long term TDA exposure does not appear to result in TDA resistance and the physiology of V. anguillarum appears unaffected, supporting the application of TDA-producing roseobacters as probiotics in aquaculture. In summary, this study demonstrates that Phaeobacter inhibens and newly isolated Phaeobacter sp. strains can be used as probiotics against fish and crustacean pathogens both in a short term effect to reduce the concentrations, but more importantly also as in a long term effect as no resistance is seen in the pathogen after continuous exposure of the antibiotic compound TDA. All strains of Phaeobacter sp. were able to colonize cultures of Artemia and microalgae whether it be axenic, defined non-axenic or completely non-axenic.
Sublethal Concentrations of Antibiotics Cause Shift to Anaerobic Metabolism in Listeria monocytogenes and Induce Phenotypes Linked to Antibiotic Tolerance

The human pathogenic bacterium Listeria monocytogenes is exposed to antibiotics both during clinical treatment and in its saprophytic lifestyle. As one of the keys to successful treatment is continued antibiotic sensitivity, the purpose of this study was to determine if exposure to sublethal antibiotic concentrations would affect the bacterial physiology and induce antibiotic tolerance. Transcriptomic analyses demonstrated that each of the four antibiotics tested caused an antibiotic-specific gene expression pattern related to mode-of-action of the particular antibiotic. All four antibiotics caused the same changes in expression of several metabolic genes indicating a shift from aerobic to anaerobic metabolism and higher ethanol production. A mutant in the bifunctional acetaldehyde-CoA/alcohol dehydrogenase encoded by Imo1634 did not have altered antibiotic tolerance. However, a mutant in Imo1179 (eutE) encoding an aldehyde oxidoreductase where rerouting caused increased ethanol production was tolerant to three of four antibiotics tested. This shift in metabolism could be a survival strategy in response to antibiotics to avoid generation of ROS production from respiration by oxidation of NADH through ethanol production. The monocin locus encoding a cryptic prophage was induced by co-trimoxazole and repressed by ampicillin and gentamicin, and this correlated with an observed antibiotic-dependent biofilm formation. A monocin mutant (Delta lmaDCBA) had increased biofilm formation when exposed to increasing concentration of co-trimoxazole similar to the wild type, but was more tolerant to killing by co-trimoxazole and ampicillin. Thus, sublethal concentrations of antibiotics caused metabolic and physiological changes indicating that the organism is preparing to withstand lethal antibiotic concentrations.

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The Small Colony Variant of *Listeria monocytogenes* Is More Tolerant to Antibiotics and Has Altered Survival in RAW 264.7 Murine Macrophages

Small Colony Variant (SCV) cells of bacteria are a slow-growing phenotype that result from specific defects in the electron transport chain. They form pinpoint colonies on agar plates and have a variety of phenotypic characteristics, such as altered carbon metabolism, decreased toxin and lytic enzyme production, aminoglycoside resistance, and increased intracellular persistence. They are clinically relevant in *Staphylococcus aureus* and *Pseudomonas aeruginosa*, serving as a reservoir for recurrent or prolonged infections. Here, we found that a SCV mutant in the foodborne pathogen *Listeria monocytogenes* (strain SCV E18), similar to the high persister mutant phenotype, survived significantly better than the wild type when exposed over a 48-h period to concentrations above Minimal Inhibitory Concentration for most tested antibiotics. SCV E18 survived more poorly than the wildtype in unactivated RAW264.7 macrophage cells, presumably because of its reduced listeriolysin O expression, however, it survived better in reactive oxygen species producing, phorbol 12-myristate 13-acetate-activated macrophages. Although SCV E18 was sensitive to oxygen as it entered the stationary phase, it was significantly more tolerant to H$_2$O$_2$ than the wild type, which may result from a shift in metabolism, however, further investigation is needed to resolve this. SCV E18 is a spontaneous mutant with a point mutation in the *hemA* gene. A wild type copy of *hemA* was complemented on plasmid pSOG30222, which restored the wild type phenotype. The results reported here suggest that the SCV of *L. monocytogenes* could be of clinical importance and highlight a need for adequate clinical screening for this phenotype, as it could affect antibiotic treatment outcomes.

**General information**

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Wet STEM in SEM for Morphological Characterization of Novel Bacterial Species: Vibrio galatheae and Photobacterium galatheae

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Adaptive Laboratory Evolution Of Escherichia Coli Reveals Arduous Resistance Development To A Combination Of Three Novel Antimicrobial Compounds And To The Short Amp P9-4
Antimicrobial peptides (AMPs) were for long considered as promising new antimicrobials since resistance was not expected. However, adaptive evolution experiments have demonstrated that bacteria may indeed develop resistance also to AMPs. However, we and others hypothesize that the risk of resistance development decreases when two or more compounds are combined as compared to single-drug treatments. The purpose of this study was to determine if resistance could develop in Escherichia coli ATCC 25922 to the pep tidomimetic HF-1002 2 and the AMPs novicidin and P9-4. The mentioned compounds were applied alone and in a combination of three in an adaptive evolution approach. All
the lineages exposed to HF-1002 and three out of four lineages exposed to novicidin adapted to 32 x MIC, after approximately 350 generations. Conversely, only one out of four lineages exposed to the combination reached adaptation to 32 x MIC. This shows that resistance to novicidin and HF-1002, administered alone, developed more easily than it occurred in lineages exposed to the combination of three drugs. This result further supports combinatorial treatment as a way to circumvent resistance development. Surprisingly, none of the lineages exposed to P9-4 was adapted to 32 x MIC. This indicates that this short-length antimicrobial peptide may be a promising candidate for further optimization for future application in clinical settings.

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Amphibian antimicrobial peptide fallaxin analogue FL9 affects virulence gene expression and DNA replication in Staphylococcus aureus
The rapid rise in antibiotic-resistant pathogens is causing increased health concerns, and consequently there is an urgent need for novel antimicrobial agents. Antimicrobial peptides (AMPs), which have been isolated from a wide range of organisms, represent a very promising class of novel antimicrobials. In the present study, the analogue FL9, based on the amphibian AMP fallaxin, was studied to elucidate its mode of action and antibacterial activity against the human pathogen Staphylococcus aureus. Our data showed that FL9 may have a dual mode of action against S. aureus. At concentrations around the MIC, FL9 bound DNA, inhibited DNA synthesis and induced the SOS DNA damage response, whereas at concentrations above the MIC the interaction between S. aureus and FL9 led to membrane disruption. The antibacterial activity of the peptide was maintained over a wide range of NaCl and MgCl2 concentrations and at alkaline pH, while it was compromised by acidic pH and exposure to serum. Furthermore, at subinhibitory concentrations of FL9, S. aureus responded by increasing the expression of two major virulence factor genes, namely the regulatory rnaIII and hla, encoding α-haemolysin. In addition, the S. aureus-encoded natural tolerance mechanisms included peptide cleavage and the addition of positive charge to the cell surface, both of which minimized the antimicrobial activity of FL9. Our results add new information about FL9 and its effect on S. aureus, which may aid in the future development of analogues with improved therapeutic potential.

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A single exposure to a sublethal pediocin concentration initiates a resistance-associated temporal cell envelope and general stress response in *Listeria monocytogenes*

*Listeria monocytogenes* can cause the potentially fatal food-borne disease listeriosis, and the use of bacteriocin-producing lactic acid bacteria to control *L. monocytogenes* holds great promise. However, development of bacteriocin resistance is a potential challenge and the purpose of this study was to determine if exposure to sublethal concentrations of pediocin-containing *Lactobacillus plantarum* WHE 92 supernatant could prime *L. monocytogenes* for resistance. By transcriptomic analysis, we found two, 55 and 539 genes differentially expressed after 10, 60 and 180 min of exposure to *L. plantarum* WHE 92 supernatant as compared to control exposures. We observed temporal expression changes in genes regulated by the two component system LisRK and the alternative sigma factors SigB and SigL. Additionally, several genes involved in bacteriocin resistance were induced. ΔlisR, ΔsigB and ΔsigL mutants were all more resistant than wild types to *L. plantarum* WHE 92 supernatant. LisRK, SigB and SigL regulation and genes associated with resistance are involved in the
temporal adaptive response to pediocin and all three regulatory systems affect pediocin resistance. Thus, a single exposure to a sublethal pediocin concentration initiates a response pointing to resistance and indicates that further research exploring the link between adaptive responses and resistance is needed.
Chitin Degradation In Marine Bacteria

Introduction: Chitin is the most abundant polymer in the marine environment and the second most abundant in nature. Chitin does not accumulate on the ocean floor, because of microbial breakdown. Chitin degrading bacteria could have potential in the utilization of chitin as a renewable carbon and nitrogen source in the fermentation industry.

Methods: Here, whole genome sequenced marine bacteria were screened for chitin degradation using phenotypic and in silico analyses.

Results: The in silico analyses revealed the presence of three to nine chitinases in each strain, however, the number of chitinases did not correlate to chitin degrading abilities on chitin agar. Two glycosyl hydrolase (GH) groups of chitinases were identified: GH18 and GH19. Interestingly, all strains had genes coding for GH19 chitinases, which for a long time were believed to be present only in higher plants. Differences in genes related to chitin degradation were found between Vibrionaceae and Pseudoalteromonaceae families. The sensor kinase, ChiS, which regulates around 50 genes, was found in all Vibrionaceae but not in any of the strains from the Pseudoalteromonaceae family indicating that the latter has a different chitin regulatory system.

Conclusions: This study has provided insight into the ecology of chitin degradation in marine bacteria. It also served as a basis for choosing a more efficient chitin degrading production strain e.g. for the use of chitin waste for large-scale fermentations.
Continuous Exposure Of Vibrio Anguillarum To Tropodithietic Acid: Genetic Changes And Influence On Virulence

Introduction: The fish pathogen Vibrio anguillarum is a major problem in aquaculture causing Vibriosis. Bacteria of the Roseobacter clade can antagonize pathogenic vibrios in cultures in live feed such as microalgae, rotifers and Artemia, as well as in fish larvae. Therefore, roseobacters could be promising as probiotics in fish rearing. Production of the antibacterial compound tropodithietic acid (TDA) by roseobacters is key in the antagonism of vibrios. However, the effects of continuous exposure to TDA on V. anguillarum remain unknown. The purpose of this study was to investigate how prolonged TDA exposure affects V. anguillarum focusing on the development of resistance towards TDA and changes in virulence.

Methods: Seven lineages of V. anguillarum were exposed to increasing TDA concentrations over 300-400 generations and were subsequently genome sequenced. Virulence of the lineages is currently being tested in fish cell infection trials.

Results: Following exposure, four lineages reached 1.75 x wild-type MIC and three reached 1.5 x wild-type MIC. Genome sequencing revealed no major changes in the genomes of the lineages. The only virulence-related gene affected was fliM, encoding a flagella motor switch protein. However, mutations in this gene were observed in non-exposed controls as well.

Conclusions: In conclusion, TDA resistance does not appear to develop, and the virulence genes of V. anguillarum are unaffected by TDA exposure, supporting the application of TDA-producing roseobacters as probiotics in aquaculture.

Draft Genome Sequence of Vibrio parahaemolyticus VH3, Isolated from an Aquaculture Environment in Greece

Vibrio parahaemolyticus is an important foodborne pathogen responsible for gastroenteritis outbreaks globally. It has also been identified as an important pathogen in aquatic organisms. Here, we report a draft genome sequence of V. parahaemolyticus, strain VH3, isolated from farmed juvenile greater amberjack, Seriola dumerili, in Greece.
Draft Genome Sequences of Vibrio alginolyticus Strains V1 and V2, Opportunistic Marine Pathogens

We announce the draft genome sequences of Vibrio alginolyticus strains V1 and V2, isolated from juvenile Sparus aurata and Dentex dentex, respectively, during outbreaks of vibriosis. The genome sequences are 5,257,950 bp with a G+C content of 44.5% for V. alginolyticus V1 and 5,068,299 bp with a G+C content of 44.8% for strain V2. These genomes provide further insights into the putative virulence factors, prophage carriage, and evolution of this opportunistic marine pathogen.

General information
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Draft Genome Sequences of the Fish Pathogen Vibrio harveyi Strains VH2 and VH5

Vibrio harveyi is an important marine pathogen that is responsible for vibriosis outbreaks in cultured fish and invertebrates worldwide. Here, we announce the draft genome sequences of V. harveyi strains VH2 and VH5, isolated from farmed juvenile Seriola dumerili during outbreaks of vibriosis in Crete, Greece.

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Exploring Marine Environments To Unravel Tolerance Mechanisms To Relevant Compounds

Production of biofuels and chemicals using microorganisms has been a research driver in the last decades. The approach started with the engineering of metabolic pathways for production of compounds of interest, but it was soon realized that tolerance to the compounds being produced was one of the major bottlenecks of this approach. Since then, tolerance engineering of microbial cell factories along with metabolic pathway engineering has been one of the main research focuses. Microorganisms with natural tolerance to relevant compounds, such as ρ-coumaric, glutaric and isobutyric acids were isolated from Iranian sediment samples. This was achieved by replica plating the strains on plates containing high concentrations of the mentioned compounds. Thirty-two samples were analyzed and 96 strains isolated. Isolates with high tolerance were grown in presence of high concentrations of the compounds of interest, HPLC analyses were performed in order to distinguish between compound-degrading and tolerant bacteria. This led to the identification of seven tolerant and non-degrading isolates, the most interesting ones belonging to the genera Bacillus and Pseudomonas. These will be studied using genomic and transcriptomic approaches to identify the tolerance mechanisms used. Exploring new ecological niches, as contaminated marine environments allows the identification of naturally tolerant bacteria to the compounds of interest and most likely to the discovery of new mechanisms of tolerance.

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Genome mining reveals unlocked bioactive potential of marine Gram-negative bacteria

Background: Antibiotic resistance in bacteria spreads quickly, overtaking the pace at which new compounds are discovered and this emphasizes the immediate need to discover new compounds for control of infectious diseases. Terrestrial bacteria have for decades been investigated as a source of bioactive compounds leading to successful applications in pharmaceutical and biotech industries. Marine bacteria have so far not been exploited to the same extent; however, they are believed to harbor a multitude of novel bioactive chemistry. To explore this potential, genomes of 21 marine Alpha- and Gammaproteobacteria collected during the Galathea 3 expedition were sequenced and mined for natural product encoding gene clusters. Results: Independently of genome size, bacteria of all tested genera carried a large number of clusters encoding different potential bioactivities, especially within the Vibrionaceae and Pseudoalteromonadaeae families. A very high potential was identified in pigmented pseudoalteromonads with up to 20 clusters in a single strain, mostly NRPSs and NRPS-PKS hybrids. Furthermore, regulatory elements in bioactivity-related pathways including chitin metabolism, quorum sensing and iron scavenging systems were investigated both in silico and in vitro. Genes with siderophore function were identified in 50% of the strains, however, all but one harboured the ferric-uptake-regulator gene. Genes encoding the synthetases of acylated homoserine lactones were found in Roseobacter-clade bacteria, but not in the Vibrionaceae strains and only in one Pseudoalteromonas strains. The understanding and manipulation of these elements can help in the discovery and production of new compounds never identified under regular laboratory cultivation conditions. High chitinolytic potential was demonstrated and verified for Vibrio and Pseudoalteromonas species that commonly live in close association with eukaryotic organisms in the environment. Chitin regulation by the ChiS histidine-kinase seems to be a general trait of the Vibrionaceae family, however it is absent in the Pseudomonadaceae. Hence, the degree to which chitin influences secondary metabolism in marine bacteria is not known. Conclusions: Utilizing the rapidly developing sequencing technologies and software tools in combination with phenotypic in vitro assays, we demonstrated the high bioactive potential of marine bacteria in an efficient, straightforward manner - an approach that will facilitate natural product discovery in the future.

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Photobacterium galatheae sp. nov., a bioactive bacterium isolated from a mussel in the Solomon Sea

A novel, Gram-negative marine bacterium, S2753\(^{\top}\), was isolated from a mussel of the Solomon Sea, Solomon Islands. Analysis of the 16S rRNA gene sequence and whole genome sequence data placed strain S2753\(^{\top}\) in the genus *Photobacterium* with the closest relative being *Photobacterium halotolerans* DSM 18316\(^{\top}\) (97.7\% 16S rRNA gene similarity). Strain S2753\(^{\top}\) was able to grow from 15 to 40 °C and in NaCl concentrations of 0.5 to 9\% (w/v). The predominant fatty acids were 16:1ω7c/16:1ω6c (27.9\%), 16:0 (22.1\%) and 18:1ω7c/8:1ω6c (21.4\%). The genomic DNA G+C mol content was 49.5mol\%. Based on the phylogenetic, chemotaxonomic and phenotypic differences, strain S2753\(^{\top}\) is considered to represent a novel species of the genus *Photobacterium*. Furthermore, whole genome sequence analysis comparing S2753\(^{\top}\) and type-strains of closely related species of the genus *Photobacterium* also demonstrated that the strain is genomically distinct enough to be considered a novel species. The name *Photobacterium galatheae* is proposed and the type-strain is S2753\(^{\top}\) (=LMG 28894\(^{\top}\) =DSM 100496\(^{\top}\)).
Vibrio galatheae sp. nov., a novel member of the Vibrionaceae family isolated from the Solomon Sea.

Based on genetic, chemotaxonomic and phenotypic characteristics, a novel species belonging to the genus *Vibrio* is described. The facultative anaerobic strain S2757^T^ was isolated from a mussel collected in the Solomon Sea (Solomon Islands). Phylogenetic analyses based on sequences of 16S rRNA and fur genes indicated the affiliation of the strain to a new species. This observation was supported by a multilocus sequence analysis (MLSA) including sequences of the housekeeping genes 16S rRNA, gyrB, pyrH, recA and topA. In silico DNA-DNA hybridization (DDH) and Average Nucleotide Identity (ANI) values comparing the genomic sequence of strain S2757^T^ with those of closely related type strains were lower than 23 and 82%, respectively. The DNA G+C content of the strain was 45.3 mol%. Phenotypic and chemotaxonomic analyses clearly differentiated the strain from other *Vibrio* species. Hence, strain S2757^T^ should be considered a novel species in the genus *Vibrio*. The name *Vibrio galatheae* sp. nov. is proposed, with S2757^T^ (= DSM 100497^T^ = LMG 28895^T^) as the type strain.
Mimicking Seawater For Culturing Marine Bacteria

Only about 1% of marine bacteria have been brought into culture using traditional techniques. The purpose of this study was to investigate if mimicking the natural bacterial environment can increase culturability. We used marine substrates containing defined algal polymers or gellan gum as solidifying agents, and enumerated bacteria from seawater and algal exudates. We tested if culturability could be influenced by addition of quorum sensing signals (AHLs). All plates were incubated at 15°C. Bacterial counts (CFU/g) from algal exudates from brown algae were highest on media containing algal polymers. In general, bacteria isolated from algal exudates preferred more rich media than bacteria isolated from seawater. Overall, culturability ranged from 0.01 to 0.8% as compared to total cell count. Substitution of agar with gellan gum increased the culturability of seawater bacteria approximately 100-fold; from $8.5 \times 10^1$ CFU/ml to $5.2 \times 10^3$ CFU/ml, whereas addition of AHLs did not improve culturability on any of the media. The substitution of agar with gellan gum shows great promise for increasing culturability of marine bacteria, and further studies are ongoing. The AHLs used in this study were selected based on a previous study determining the most common AHLs produced by marine strains of the Vibrionaceae family. However, their effect on culturability could not be fully explained, so also here further studies are being carried out.

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Multilocus Sequence Typing And Antibiotic Resistance Of Staphylococcus Aureus Isolated From The Brazilian Dairy Industry

*Staphylococcus aureus* is a common cause of food poisoning due to enterotoxin production. This is particularly an issue in the dairy industry, where *S. aureus* can contaminate the product e.g. from raw milk or the handlers. In Brazil, soft cheese is mainly produced in small dairy plants where good hygiene practices can be limited. The aim of this study was to determine if Brazilian dairy plants were contaminated by *S. aureus*, and if any clones were persistent. Four dairy plants were sampled during 8 months (398 samples in total). *S. aureus* (n=66) was found in all the dairy plants but the contamination rate varied between the processing plants. Multilocus Sequence Typing was used to type and assess potential persisting sequence types (ST). Seven known STs (ST1, ST5, ST30, ST97, ST126, ST188, ST398) were identified. Three new STs were identified and they all belong to clonal complex (CC) 1, which was the dominant CC in the investigated dairy plants. However, there were no indications of re-occurring (persistent) STs in the plants. The potential health risk of the isolates was assessed by antibiotic resistance and hemolytic activity screening. Resistance levels were low, and all of the isolates were presumptive methicillin-sensitive *S. aureus*. All of the isolates expressed hemolytic activity. The frequent isolation of CC1 strains in Brazilian dairy plants indicates, despite antibiotic sensitivity, a potential health risk to the human consumer.

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Phaeobacter inhibens as Probiotic Bacteria in Non-Axenic Artemia and Algae Cultures

Bacterial diseases are a major constraint in aquaculture, especially in larviculture. Antibiotics that can control pathogens should be avoided due to risk of antibiotic resistance. We have shown in axenic systems of live larval feed that marine Roseobacter clade bacteria can antagonize fish pathogens and improve survival of fish larvae. Both pathogens and probiotics are likely affected by the natural microbiota, and the purpose of this study was to determine if the probiotics would be effective in non-axenic systems. The growth and interaction of pathogen (Vibrio anguillarum) and probiotics (Phaeobacter inhibens) were studied in an Artemia and a Dunaliella tertiolecta challenge setup, and a controlled microbiota of four bacteria isolated from aquaculture was added. P. inhibens grew well in Artemia and D. tertiolecta cultures, also with a background microbiota. V. anguillarum was decreased markedly (up to four log units) by P. inhibens irrespective in presence of background microbiota. In aquaculture, the live feed is a well-known potential entry and propagation point for the fish pathogens and, hence adding the probiont at this stage would be a logical stage of introduction. This study demonstrates that probiotic bacteria can be introduced at the stage of live feed and have a pathogen reducing effect in both an Artemia and a D. tertiolecta challenge setup. This can potentially limit the subsequent use of antibiotics for control of pathogenic bacteria.

Phaeobacter inhibens from the Roseobacter clade has an environmental niche as a surface colonizer in harbors

Phaeobacter inhibens belongs to the marine Roseobacter clade and is important as a carbon and sulfur metabolizer, a biofilm former and producer of the antibiotic tropodithietic acid (TDA). The majority of cultured strains have been isolated from marine aquaculture sites, however, their niche in the environment is to date unknown. Here, we report on the repeated isolation of Phaeobacter inhibens strains from a marine environment (harbors) not related to aquaculture. Based on phenotype and 16S rRNA gene sequence similarity, a total of 64 P. inhibens strains were identified from 35 samples (eukaryotic organisms or biofilms on inert surfaces) in Jyllinge Harbor during late summer and autumn, but not during winter and spring in 2009, 2011, and 2012. P. inhibens strains were also isolated from biofilms at three other Danish harbors (in 2012), but not from the surrounding seawater. Ten of the 14 samples from which P. inhibens was cultured contained bryozoans. DNA was extracted (in 2012) from 55 out of 74 Jyllinge Harbor samples, and 35 were positive for Phaeobacter using a genus-specific PCR. P. inhibens strains were isolated from nine of these samples. DNA and RNA were isolated from 13 random samples and used for amplification of 16S rRNA. P. inhibens was detected in five of these samples, all of which were biofilm samples, by pyrotag-sequencing at a prevalence of 0.02–0.68% of the prokaryotic community. The results indicated that P. inhibens had a niche in biofilms of fouled surfaces in harbor areas and that the population followed a seasonal fluctuation.
Sublethal Concentrations Of Antibiotics Cause Shift To Anaerobic Metabolism In Listeria Monocytogenes And Induce Phenotypes Linked To Antibiotic Tolerance

Introduction: The foodborne pathogen Listeria monocytogenes can cause the severe infection listeriosis, which have up to 20-30% mortality, but if discovered in time, it can be treated with antibiotics. Most antibiotics are bacteriostatic against L. monocytogenes. This could be due to the coexistence with antibiotic-producing organisms during its saprophytic lifestyle. To determine if tolerance could be induced or potentially after virulence, we investigated the transcriptome after exposure to sublethal antibiotic concentrations. Results: Four antibiotics caused induction of the alcohol dehydrogenase gene Lmo1634 and repression of alsA and lmo1992, which are involved in acetoin production leading to more ethanol and less acetoin production. This shift in central metabolism indicates a shift from aerobic to anaerobic metabolism, that could reduce oxidative stress and be a survival strategy in response to antibiotics. We investigated the antibiotic tolerance of a Δlmo1634 mutant, however; it was comparable with the wild-type in a killing assay. L. monocytogenes encodes a second alcohol dehydrogenase Lmo1179, which potentially could cause a redundant pathway and this is under further investigation. The concentration of acetoin and ethanol are also currently under investigation. Conclusions: Consistent with other studies, we hypothesize that L. monocytogenes when exposed to antibiotics alters its metabolism from aerobic to anaerobic metabolism, and this could prepare the organism to withstand lethal concentrations of antibiotics.

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The emergence of Vibrio pathogens in Europe: ecology, evolution, and pathogenesis (Paris, 11-12th March 2015)

Global change has caused a worldwide increase in reports of Vibrio-associated diseases with ecosystem-wide impacts on humans and marine animals. In Europe, higher prevalence of human infections followed regional climatic trends with outbreaks occurring during episodes of unusually warm weather. Similar patterns were also observed in Vibrio-associated diseases affecting marine organisms such as fish, bivalves and corals. Basic knowledge is still lacking on the ecology and evolutionary biology of these bacteria as well as on their virulence mechanisms. Current limitations in experimental systems to study infection and the lack of diagnostic tools still prevent a better understanding of Vibrio emergence. A major challenge is to foster cooperation between fundamental and applied research in order to investigate the consequences of pathogen emergence in natural Vibrio populations and answer federative questions that meet societal needs. Here we report the proceedings of the first European workshop dedicated to these specific goals of the Vibrio research community by connecting current knowledge to societal issues related to ocean health and food security.

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The fur Gene as a New Phylogenetic Marker for Vibrionaceae Species Identification

Microbial taxonomy is essential in all areas of microbial science. The 16S rRNA gene sequence is one of the main phylogenetic species markers; however, it does not provide discrimination in the family Vibrionaceae, where other molecular techniques allow better interspecies resolution. Although multilocus sequence analysis (MLSA) has been used successfully in the identification of Vibrio species, the technique has several limitations. They include the fact that several locus amplifications and sequencing have to be performed, which still sometimes lead to doubtful identifications. Using an in silico approach based on genomes from 103 Vibrionaceae strains, we demonstrate here the high resolution of the fur gene in the identification of Vibrio species and its usefulness as a phylogenetic marker. The fur gene showed within-species similarity higher than 95%, and the relationships inferred from its use were in agreement with those observed for 16S rRNA analysis and MLSA. Furthermore, we developed a fur PCR sequencing-based method that allowed identification of Vibrio species. The discovery of the phylogenetic power of the fur gene and the development of a PCR method that can be used in amplification and sequencing of the gene are of general interest whether for use alone or together with the previously suggested loci in an MLSA.

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The Small Colony Variant Of Listeria Monocytogenes Is More Tolerant To Antibiotics And Grows Better Within Caco-2 Epithelial Cells Than The Wild Type

Introduction: Small Colony Variants (SCV) of bacteria are a slow growing phenotype with a pinpoint colony morphology and several specific characteristics. In several pathogens they have been linked to recurrent and chronic infections. SCV of Listeria monocytogenes can be generated when exposed to sublethal concentration of triclosan, and in this study, we characterized their tolerance to antibiotics and ability to invade and survive in host cells. Results: Complementation assays showed that SCV E18 phenotype is caused by a mutation in the heme biosynthesis pathway. Although no difference in MIC, the SCV E18 survived significantly better than the wild type N53-1 (one and three log₁₀ higher CFU/ml) when exposed to super-MIC concentrations of most tested antibiotics, indicating a persister-like phenotype of the SCV. While SCV E18 displayed sensitivity towards oxygen, it was significantly more tolerant of 20mM H₂O₂ as compared to the wild type, with 6.3 log₁₀ CFU/ml and 3.7 log₁₀ CFU/ml, respectively. The SCV E18 had lower survival rate in unactivated macrophages, however, it was able to survive and multiply to almost 100-fold higher CFU/ml than the wild type in CaCo-2 epithelial cells. Conclusions: This study is the first to demonstrate that the persister-like SCV phenotype of L. monocytogenes potentially could complicate treatment by causing an increase in tolerance towards most of the clinically relevant antibiotics, while also enabling the bacteria to persist in the protected intracellular environment.

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Virulence grading of vibrio spp. strains by experimental challenge of atlantic halibut (hippiglossus hippoglossus l.), atlantic cod (gadus morhua l.) and turbot (scophthalmus maximus l.) yolk sack larv ae

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Authors: Rønneseth, A. (Ekstern), D’Alvise, P. (Intern), Tønnesen, Ø. (Ekstern), Haugland, G. T. (Ekstern), Grotkjær, T. (Intern), Engel-Sørensen, K. (Ekstern), Narremark, L. (Ekstern), Gram, L. (Intern), Bergh, Ø. (Ekstern), Wergeland, H. I. (Ekstern)
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Biofilm formation and antibiotic production in *Ruegeria mobilis* are influenced by intracellular concentrations of cyclic dimeric guanosinmonophosphate

In many species of the marine Roseobacter clade, periods of attached life, in association with phytoplankton or particles, are interspersed with planktonic phases. The purpose of this study was to determine whether shifts between motile and sessile life in the globally abundant Roseobacter clade species *Ruegeria mobilis* are associated with intracellular concentrations of the signal compound cyclic dimeric guanosinmonophosphate (c-di-GMP), which in bacteria regulates transitions between motile and sessile life stages. Genes for diguanylate cyclases and phosphodiesterases, which are involved in c-di-GMP signalling, were found in the genome of *R. mobilis* strain F1926. Ion pair chromatography-tandem mass spectrometry revealed 20-fold higher c-di-GMP concentrations per cell in biofilm-containing cultures than in planktonic cells. An introduced diguanylate cyclase gene increased c-di-GMP and enhanced biofilm formation and production of the potent antibiotic tropodithietic acid (TDA). An introduced phosphodiesterase gene decreased c-di-GMP and reduced biofilm formation and TDA production. *tdaC*, a key gene for TDA biosynthesis, was expressed only in attached or biofilm-forming cells, and expression was induced immediately after initial attachment. In conclusion, c-di-GMP signalling controls biofilm formation and biofilm-associated traits in *R. mobilis* and, as suggested by presence of GGDEF and EAL domain protein genes, also in other Roseobacter clade species.

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Authors: D'Alvise, P. (Intern), Magdenoska, O. (Intern), Melchiorsen, J. (Intern), Nielsen, K. F. (Intern), Gram, L. (Intern)
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Web of Science (2015): Indexed yes
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Biofilm formation is not a prerequisite for production of the antibacterial compound tropodithietic acid in Phaeobacter inhibens DSM17395

Aims
The goal of this study was to investigate if biofilm formation on population level is a physiological requirement for antagonism in Phaeobacter inhibens DSM17395, since the antibiotic compound tropodithietic acid (TDA) is produced by several Roseobacter clade species during growth as multicellular aggregates or biofilms at the air–liquid interface and is induced on single cell level upon attachment.

Methods and Results
A mutant library was created by Tn5 transposon insertion and 22 TDA-positive (brown) mutants with decreased biofilm formation or adhesion, and eight TDA-negative (white) mutants with increased biofilm formation or adhesion were selected. None of the selected biofilm-overproducing white mutants showed any antibiotic activity, while all brown mutants with reduced or disabled biofilm formation produced the antibacterial compound. Sequencing analysis indicated that genes that are likely involved in EPS/LPS production, motility and chemotaxis, and redox regulation play a role in biofilm formation and/or adhesion in P. inhibens DSM17395.

Conclusions
Cell aggregation and biofilm formation are not physiological prerequisites for TDA production.

Significance and Impact of the Study
This study contributes to the understanding of TDA production in P. inhibens, which has great potential as a probiotic in marine larviculture.

General information
State: Published
Organisations: National Food Institute, Department of Systems Biology, Bacterial Ecophysiology and Biotechnology
Authors: Prol Garcia, M. J. (Intern), D'Alvise, P. (Intern), Rygaard, A. M. (Intern), Gram, L. (Intern)
Pages: 1592-1600
Characterisation of Non-Autoinducing Tropodithietic Acid (TDA) Production from Marine Sponge Pseudovibrio Species.

The search for new antimicrobial compounds has gained added momentum in recent years, paralleled by the exponential rise in resistance to most known classes of current antibiotics. While modifications of existing drugs have brought some limited clinical success, there remains a critical need for new classes of antimicrobial compound to which key clinical
Pathogens will be naive. This has provided the context and impetus to marine biodiscovery programmes that seek to isolate and characterize new activities from the aquatic ecosystem. One new antibiotic to emerge from these initiatives is the antibacterial compound tropodithietic acid (TDA). The aim of this study was to provide insight into the bioactivity of and the factors governing the production of TDA in marine Pseudovibrio isolates from a collection of marine sponges. The TDA produced by these Pseudovibrio isolates exhibited potent antimicrobial activity against a broad spectrum of clinical pathogens, while TDA tolerance was frequent in non-TDA producing marine isolates. Comparative genomics analysis suggested a high degree of conservation among the tda biosynthetic clusters while expression studies revealed coordinated regulation of TDA synthesis upon transition from log to stationary phase growth, which was not induced by TDA itself or by the presence of the C10-acyl homoserine lactone quorum sensing signal molecule.
Comparative Genomic and Metabolomic Analysis of Twelve Strains of Pseudoalteromonas luteoviolacea

Draft Genome Sequence of Hoeflea sp. Strain BAL378, a Potential Producer of Bioactive Compounds

General information
State: Published
Organisations: Department of Systems Biology, Natural Product Chemistry, Metabolic Signaling and Regulation, Bacterial Ecophysiology and Biotechnology, University of California, San Diego
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Draft Genome Sequence of Hoeflea sp. Strain BAL378, a Potential Producer of Bioactive Compounds
Some phytoplankton-associated marine bacteria produce bioactive compounds. Members of the genus Hoeflea may be examples of such bacteria; however, data describing their metabolisms are scarce. Here, we report the draft genome sequence of Hoeflea sp. strain BAL378, a putative producer of bacteriocins, polyketides, and auxins, as demonstrated by genome mining.

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Organisations: Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, University of Copenhagen
Authors: Bentzon-Tilia, M. (Intern), Riemann, L. (Ekstern), Gram, L. (Intern)
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Draft Genome Sequence of *Photobacterium halotolerans* S2753, Producer of Bioactive Secondary Metabolites

We report here the whole draft genome sequence of marine isolate *Photobacterium halotolerans* S2753, which produces the known antibiotic holomycin and also ngercheumics and solonamides A and B, which interfere with virulence of methicillin-resistant *Staphylococcus aureus* strains by interacting with the quorum-sensing system.

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**Global and Phylogenetic Distribution of Quorum Sensing Signals, Acyl Homoserine Lactones, in the Family of Vibrionaceae**

Bacterial quorum sensing (QS) and the corresponding signals, acyl homoserine lactones (AHLs), were first described for a luminescent Vibrio species. Since then, detailed knowledge has been gained on the functional level of QS; however, the abundance of AHLs in the family of Vibrionaceae in the environment has remained unclear. Three hundred and one Vibrionaceae strains were collected on a global research cruise and the prevalence and profile of AHL signals in this global collection were determined. AHLs were detected in 32 of the 301 strains using Agrobacterium tumefaciens and Chromobacterium violaceum reporter strains. Ethyl acetate extracts of the cultures were analysed by ultra-high performance liquid chromatography-high resolution mass spectrometry (MS) with automated tandem MS confirmation for AHLs. N-(3-hydroxy-hexanoyl) (OH-C6) and N-(3-hydroxy-decanoyl) (OH-C10) homoserine lactones were the most common AHLs found in 17 and 12 strains, respectively. Several strains produced a diversity of different AHLs, including N-heptanoyl (C7) HL. AHL-producing Vibrionaceae were found in polar, temperate and tropical waters. The AHL profiles correlated with strain phylogeny based on gene sequence homology, however not with geographical location. In conclusion, a wide range of AHL signals are produced by a number of clades in the Vibrionaceae family and these results will allow future investigations of inter- and intra-species interactions within this cosmopolitan family of marine bacteria.
Guanidino groups greatly enhance the action of antimicrobial peptidomimetics against bacterial cytoplasmic membranes. Antimicrobial peptides or their synthetic mimics are a promising class of potential new antibiotics. Herein we assess the effect of the type of cationic side chain (i.e., guanidino vs. amino groups) on the membrane perturbing mechanism of antimicrobial α-peptide-β-peptoid chimeras. Langmuir monolayers composed of 1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol (DPPG) were used to model cytoplasmic membranes of both Gram-positive and Gram-negative bacteria, while lipopolysaccharide Kdo2-lipid A monolayers were mimicking the outer membrane of Gram-negative species. We report the results of the measurements using an array of techniques, including high-resolution synchrotron surface X-ray scattering, epifluorescence microscopy, and in vitro antimicrobial activity to study the molecular mechanisms of peptidomimetic interaction with bacterial membranes. We found guanidino group-containing chimeras to exhibit greater disruptive activity on DPPG monolayers than the amino group-containing analogues. However, this effect was not observed for lipopolysaccharide monolayers where the difference was negligible. Furthermore, the addition of the nitrobenzoxadiazole fluorophore did not reduce the insertion activity of these antimicrobials into both model membrane systems examined, which may be useful for future cellular localization studies.
Reducing antibiotic use in marine larviculture by probiotics

Aquaculture is the fastest growing agricultural industry providing healthy food for mankind. In addition, culture of high valued marine fish, crustacean and mollusk species is financially attractive. However, diseases at the larval stages constitute a major bottleneck and cause economic losses to the industry. Vaccines are not effective at the larval stages and antibiotics are used for disease control, although there are serious concerns about development of bacterial antibiotic resistance and its transfer to human pathogenic bacteria. There is a strong need for development of non-antibiotic disease control strategies, especially at the larval stages. The objective of our work is to reduce the need for antibiotics in marine larviculture by developing probiotic strategies; probiotics being defined by WHO as "live microbial cultures that exert a beneficial effect on the host". Rearing of marine larvae is difficult, as the larvae require live feed (Artemia, rotifers, copepods) which also requires live feed (algae). Larval pathogens can be introduced from the live feed and thereby work on applying our probiont strategy for pathogen control not only at the larval stage, but also in live feed cultures, hence targeting pathogen control at the very beginning of the production chain in an aprophylactic strategy. We collaborate with several aquaculture industries rearing turbot, sea-bass, sea-bream, oysters and flounder. We have at these sites isolated bacteria that are capable of antagonising fish larvae pathogens and that are not detrimental to the fish larvae. At all sites, bacteria belonging to the marine Roseobacter clade have been isolated as strong pathogen-antagonising bacteria. We have demonstrated that these bacteria can antagonise pathogens (Vibrio anguillarum and Vibrio harveyi) in live feeds (algae, rotifers, Artemia) and that they have a dramatic and significant disease-reducing effect in turbot and cod larvae challenged with pathogenic Vibrio. We are elucidating the mechanisms by which the probionts exert their effect, and have by mutagenesis identified tropodithietic acid (TDA) as an important molecule in the pathogen-antagonism. However, other molecules and mechanisms are likely also involved. Understanding the spectrum of mechanisms of action is important to determine where and how the probionts should be applied and also in determining potential side effects that could arise for the probiotic bacteria. Other studies have focused on fish pathogens and it has been suggested that introducing lactic acid bacteria that are used as human probiotics (and have GRAS status) could be a way forward. However, we believe that re-introducing (or boosting) a potential probiotic bacterium already present in the fish larvae feed and rearing environments is likely a more successful strategy than introducing a foreign bacterium to this environment. Indeed, our results on disease prevention in model systems have been very convincing.

General information
Solonamide B Inhibits Quorum Sensing and Reduces Staphylococcus aureus Mediated Killing of Human Neutrophils

Methicillin-resistant Staphylococcus aureus (MRSA) continues to be a serious human pathogen, and particularly the spread of community associated (CA)-MRSA strains such as USA300 is a concern, as these strains can cause severe infections in otherwise healthy adults. Recently, we reported that a cyclodepsipeptide termed Solonamide B isolated from the marine bacterium, Photobacterium halotolerans strongly reduces expression of RNAIII, the effector molecule of the agr quorum sensing system. Here we show that Solonamide B interferes with the binding of S. aureus autoinducing peptides (AIPs) to sensor histidine kinase, AgrC, of the agr two-component system. The hypervirulence of USA300 has been linked to increased expression of central virulence factors like α-hemolysin and the phenol soluble modulins (PSMs). Importantly, in strain USA300 Solonamide B dramatically reduced the activity of α-hemolysin and the transcription of psma encoding...
PSMs with an 80% reduction in toxicity of supernatants towards human neutrophils and rabbit erythrocytes. To our knowledge this is the first report of a compound produced naturally by a Gram-negative marine bacterium that interferes with agr and affects both RNAIII and AgrA controlled virulence gene expression in S. aureus.

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Organisations: Department of Systems Biology, Natural Product Chemistry, Bacterial Ecophysiology and Biotechnology, New York University School of Medicine, University of Copenhagen
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- Scopus rating (2014): SJR 1.559 SNIP 1.148 CiteScore 3.54
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- Web of Science (2012): Indexed yes
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- Web of Science (2010): Indexed yes
- BFI (2009): BFI-level 1
- Scopus rating (2009): SJR 2.614 SNIP 1.046
- Web of Science (2009): Indexed yes
- BFI (2008): BFI-level 1
- Scopus rating (2008): SJR 2.506 SNIP 1.006
- Web of Science (2008): Indexed yes
- Scopus rating (2007): SJR 1.379 SNIP 0.537
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Synthesis and bioactivity of analogues of the marine antibiotic tropodithietic acid

Tropodithietic acid (TDA) is a structurally unique sulfur-containing antibiotic from the Roseobacter clade bacterium Phaeobacter inhibens DSM 17395 and a few other related species. We have synthesised several structural analogues of TDA and used them in bioactivity tests against Staphylococcus aureus and Vibrio anguillarum for a structure-activity relationship (SAR) study, revealing that the sulfur-free analogue of TDA, tropone-2-carboxylic acid, has an antibiotic activity that is even stronger than the bioactivity of the natural product. The synthesis of this compound and of several analogues is presented and the bioactivity of the synthetic compounds is discussed.

General information

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Organisations: Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, Rheinische Friedrich-Wilhelms-Universität Bonn
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  BFI (2010): BFI-level 1
  Scopus rating (2010): SJR 0.472 SNIP 0.328
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Toxicity of Bioactive and Probiotic Marine Bacteria and Their Secondary Metabolites in Artemia sp. and Caenorhabditis elegans as Eukaryotic Model Organisms

We have previously reported that some strains belonging to the marine Actinobacteria class, the Pseudoalteromonas genus, the Roseobacter clade, and the Photobacteriaceae and Vibrionaceae families produce both antibacterial and antivirulence compounds, and these organisms are interesting from an applied point of view as fish probiotics or as a source of pharmaceutical compounds. The application of either organisms or compounds requires that they do not cause any side effects, such as toxicity in eukaryotic organisms. The purpose of this study was to determine whether these bacteria or their compounds have any toxic side effects in the eukaryotic organisms Artemia sp. and Caenorhabditis elegans. Arthrobacter davidianii WX-11, Pseudoalteromonas luteoviolacea S4060, P. piscicida S2049, P. rubra S2471, Photobacterium halotolerans S2753, and Vibrio corallilyticus S2052 were lethal to either or both model eukaryotes. The toxicity of P. luteoviolacea S4060 could be related to the production of the antibacterial compound pentabromopseudilin, while the adverse effect observed in the presence of P. halotolerans S2753 and V. corallilyticus S2052 could not be explained by the production of holomycin nor andrimid, the respective antibiotic compounds in these organisms. In contrast, the tropodithietic acid (TDA)-producing bacteria Phaeobacter inhibens DSM17395 and Ruegeria mobilis F1926 and TDA itself had no adverse effect on the target organisms. These results reaffirm TDA-producing Roseobacter bacteria as a promising group to be used as probiotics in aquaculture, whereas Actinobacteria, Pseudoalteromonas, Photobacteriaceae, and Vibrionaceae should be used with caution.
Triclosan-Induced Aminoglycoside-Tolerant Listeria monocytogenes Isolates Can Appear as Small-Colony Variants

Exposure of the human food-borne pathogen Listeria monocytogenes to sublethal concentrations of triclosan can cause resistance to several aminoglycosides. Aminoglycoside-resistant isolates exhibit two colony morphologies: normal-size...
and pinpoint colonies. The purposes of the present study were to characterize the small colonies of L. monocytogenes and to determine if specific genetic changes could explain the triclosan-induced aminoglycoside resistance in both pinpoint and normal-size isolates. Isolates from the pinpoint colonies grew poorly under aerated conditions, but growth was restored by addition of antibiotics. Pinpoint isolates had decreased hemolytic activity under stagnant conditions and a changed spectrum of carbohydrate utilization compared to the wild type and isolates from normal-size colonies. Genome sequence comparison revealed that all seven pinpoint isolates had a mutation in a heme gene, and addition of heme caused the pinpoint isolates to revert to normal colony size. Triclosan-induced gentamicin-resistant isolates had mutations in several different genes, and it cannot be directly concluded how the different mutations caused gentamicin resistance. However, since many of the mutations affected proteins involved in respiration, it seems likely that the mutations affected the active transport of the antibiotic and thereby caused resistance by decreasing the amount of aminoglycoside that enters the bacterial cell. Our study emphasizes that triclosan likely has more targets than just fabI and that exposure to triclosan can cause resistance to antibiotics that enters the cell via active transport. Further studies are needed to elucidate if L. monocytogenes pinpoint isolates could have any clinical impact, e.g., in persistent infections.
Vibriophages and Their Interactions with the Fish Pathogen Vibrio anguillarum.

Vibrio anguillarum is an important pathogen in aquaculture, responsible for the disease vibriosis in many fish and invertebrate species. Disease control by antibiotics is a concern due to potential development and spread of antibiotic resistance. The use of bacteriophages to control the pathogen may offer a non-antibiotic-based approach to reduce vibriosis. A detailed understanding of the phage-host interaction is needed to evaluate the potential of phages to control the pathogen. In this study, we examined the diversity and interactions of 11 vibriophages, 24 V. anguillarum strains, and 13 Vibrio species strains. Together, the host ranges of the 11 phages covered all of the tested 37 Vibrio sp. host strains, which represented considerable temporal (20 years) and geographical (9 countries) differences in their origins of isolation. Thus, despite the occurrence of unique susceptibility patterns of the individual host isolates, key phenotypic properties related to phage susceptibility are distributed worldwide and maintained in the global Vibrio community for decades. The phage susceptibility pattern of the isolates did not show any relation to the physiological relationships obtained from Biolog GN2 profiles, demonstrating that similar phage susceptibility patterns occur across broad phylogenetic and physiological differences in Vibrio strains. Subsequent culture experiments with two phages and two V. anguillarum hosts demonstrated an initial strong lytic potential of the phages. However, rapid regrowth of both phage-resistant and phage-sensitive cells following the initial lysis suggested that several mechanisms of protection against phage infection had developed in the host populations.

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Organisations: Department of Systems Biology, University of Copenhagen
Authors: Tan, D. (Forskerdatabase), Gram, L. (Intern), Middelboe, M. (Ekstem)
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Adaptive Evolution of Escherichia coli to an α-Peptide/β-Peptoid Peptidomimetic Induces Stable Resistance.

Antimicrobial peptides (AMPs) and synthetic analogues thereof target conserved structures of bacterial cell envelopes and hence, development of resistance has been considered an unlikely event. However, recently bacterial resistance to AMPs has been observed, and the aim of the present study was to determine whether bacterial resistance may also evolve against synthetic AMP analogues, e.g. α-peptide/β-peptoid peptidomimetics. E. coli ATCC 25922 was exposed to increasing concentrations of a peptidomimetic (10 lineages), polymyxin B (10 lineages), or MilliQ water (4 lineages) in a re-inoculation culturing setup covering approx. 500 generations. All 10 lineages exposed to the peptidomimetic adapted to 32×MIC while this occurred for 8 out of 10 of the polymyxin B-exposed lineages. All lineages exposed to 32×MIC of either the peptidomimetic or polymyxin B had a significantly increased MIC (16-32×) to the selection agent. Five transfers (~35 generations) in unsupplemented media did not abolish resistance indicating that resistance was heritable. Single isolates from peptidomimetic-exposed lineage populations displayed MICs against the peptidomimetic from wild-type MIC to 32×MIC revealing heterogeneous populations. Resistant isolates showed no cross-resistance against a panel of membrane-active AMPs. These isolates were highly susceptible to blood plasma antibacterial activity and were killed when plasma concentrations exceeded ~30%. Notably, MIC of the peptidomimetic against resistant isolates returned to wild-type level upon addition of 25% plasma. Whole-genome sequencing of twenty isolates from four resistant lineages revealed mutations, in murein transglycosylase D (mltD) and outer-membrane proteins, which were conserved within and between lineages. However, no common resistance-conferring mutation was identified. We hypothesise that alterations in cell envelope structure result in peptidomimetic resistance, and that this may occur via several distinct mechanisms. Interestingly, this type of resistance result in a concomitant high susceptibility towards plasma, and therefore the present study does not infer additional concern for peptidomimetics as future therapeutics.

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Organisations: National Food Institute, Division of Industrial Food Research, Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, University of Copenhagen
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Scopus rating (2016): CiteScore 3.11 SJR 1.236 SNIP 1.101
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.427 SNIP 1.136 CiteScore 3.32
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.559 SNIP 1.148 CiteScore 3.54
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
**Aquaculture application and ecophysiology of marine bacteria from the Roseobacter clade**

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Organisations: National Food Institute, Division of Industrial Food Research  
Authors: D'Alvise, P. (Intern), Gram, L. (Intern)  
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**Disruption of Cell-to-Cell Signaling Does Not Abolish the Antagonism of Phaeobacter gallaeciensis toward the Fish Pathogen Vibrio anguillarum in Algal Systems**
Quorum sensing (QS) regulates Phaeobacter gallaeciensis antagonism in broth systems; however, we demonstrate here that QS is not important for antagonism in algal cultures. QS mutants reduced Vibrio anguillarum to the same extent as the wild type. Consequently, a combination of probiotic Phaeobacter and QS inhibitors is a feasible strategy for aquaculture disease control.
**Pseudoalteromonas spp. Serve as Initial Bacterial Attractants in Mesocosms of Coastal Waters but Have Subsequent Antifouling Capacity in Mesocosms and when Embedded in Paint**

The purpose of the present study was to determine if the monoculture antifouling effect of several pigmented pseudoalteromonads was retained in in vitro mesocosm systems using natural coastal seawater and when the bacteria were embedded in paint used on surfaces submerged in coastal waters. Pseudoalteromonas piscicida survived on a steel surface and retained antifouling activity for at least 53 days in sterile seawater, whereas P. tunicata survived and had antifouling activity for only 1 week. However, during the first week, all Pseudoalteromonas strains facilitated rather than prevented bacterial attachment when used to coat stainless steel surfaces and submerged in mesocosms with natural seawater. The bacterial density on surfaces coated with sterile growth medium was 105 cells/cm² after 7 days, whereas counts on surfaces precoated with Pseudoalteromonas were significantly higher, at 106 to 108 cells/cm². However, after 53 days, seven of eight Pseudoalteromonas strains had reduced total bacterial adhesion compared to the control. P. piscicida, P. antarctica, and P. ulvae remained on the surface, at levels similar to those in the initial coating, whereas P. tunicata could not be detected. Larger fouling organisms were observed on all plates precoated with Pseudoalteromonas; however, plates coated only with sterile growth medium were dominated by a bacterial biofilm. Suspensions of a P. piscicida strain and a P. tunicata strain were incorporated into ship paints (Hempasil x3 87500 and Hempasil 77500) used on plates that were placed at the Hempel A/S test site in Jyllinge Harbor. For the first 4 months, no differences were observed between control plates and treated plates, but after 5 to 6 months, the control plates were more fouled than the plates with pseudoalteromonad-based paint. Our study demonstrates that no single laboratory assay can predict antifouling effects and that a combination of laboratory and real-life methods must be used to determine the potential antifouling capability of new agents or organisms.

**General information**

State: Published
Organisations: National Food Institute, Division of Industrial Food Research, Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, Hempel A/S
Authors: Bernbom, N. (Intern), Ng, Y. (Intern), Møller, S. (Ekstern), Gram, L. (Intern)
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BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Genome Sequencing Identifies Two Nearly Unchanged Strains of Persistent Listeria monocytogenes Isolated at Two Different Fish Processing Plants Sampled 6 Years Apart
Listeria monocytogenes is a food-borne human-pathogenic bacterium that can cause infections with a high mortality rate. It has a remarkable ability to persist in food processing facilities. Here we report the genome sequences for two L. monocytogenes strains (N53-1 and La111) that were isolated 6 years apart from two different Danish fish processors. Both strains are of serotype 1/2a and belong to a highly persistent DNA subtype (random amplified polymorphic DNA [RAPD] type 9). We demonstrate using in silico analyses that both strains belong to the multilocus sequence typing (MLST) type ST121 that has been isolated as a persistent subtype in several European countries. The purpose of this study was to use genome analyses to identify genes or proteins that could contribute to persistence. In a genome comparison, the two persistent strains were extremely similar and collectively differed from the reference lineage II strain, EGD-e. Also, they differed markedly from a lineage I strain (F2365). On the proteome level, the two strains were almost identical, with a predicted protein homology of 99.94%, differing at only 2 proteins. No single-nucleotide polymorphism (SNP) differences were seen between the two strains; in contrast, N53-1 and La111 differed from the EGD-e reference strain by 3,942 and 3,471 SNPs, respectively. We included a persistent L. monocytogenes strain from the United States (F6854) in our comparisons. Compared to nonpersistent strains, all three persistent strains were distinguished by two genome deletions: one, of 2,472 bp, typically contains the gene for inlF, and the other, of 3,017 bp, includes three genes potentially related to bacteriocin production and transport (lmo2774, lmo2775, and the 3′-terminal part of lmo2776). Further studies of highly persistent strains are required to determine if the absence of these genes promotes persistence. While the genome comparison did not point to a clear physiological explanation of the persistent phenotype, the remarkable similarity between the two strains indicates that subtypes with specific traits are selected for in the food processing environment and that particular genetic and physiological factors are responsible for the persistent phenotype.

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State: Published
Organisations: National Food Institute, Department of Systems Biology, Center for Biological Sequence Analysis, Allegheny College, U.S. Department of Agriculture
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BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014):Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
During our search for new natural products from the marine environment, we discovered a wide range of cyclic peptides from a marine Photobacterium, closely related to P. halotolerans. The chemical fingerprint of the bacterium showed primarily non-ribosomal peptide synthetase (NRPS)-like compounds, including the known pyrrothine antibiotic holomycin and a wide range of peptides, from diketopiperazines to cyclodepsipeptides of 500–900 Da. Purification of components from the pellet fraction led to the isolation and structure elucidation of four new cyclodepsipeptides, ngercheumicin F, G, H, and I. The ngercheumicins interfered with expression of virulence genes known to be controlled by the agr quorum sensing system of Staphylococcus aureus, although to a lesser extent than the previously described solonamides from the same strain of Photobacterium.
Influence of natural substrates and co-occurring marine bacteria on the production of secondary metabolites by Photobacterium halotolerans

Genome sequences reveal that our current standard laboratory conditions only support a fraction of the potential secondary metabolism in bacteria. Thus, we must rethink cultivation, detection, and isolation strategies for bacterial secondary metabolites in order to explore the huge, so far uncharacterized chemical potential of these organisms. We are currently investigating the use of natural substrates and co-cultures with commensal bacteria to elicit or alter production of antibacterial compounds in marine bacteria.

General information
State: Published
Organisations: Department of Systems Biology, Natural Product Chemistry, Bacterial Ecophysiology and Biotechnology, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, University of California
Listeria monocytogenes strains encoding premature stop codons in inlA invade mice and guinea pig fetuses in orally dosed dams

Listeria monocytogenes is an important food-borne bacterial pathogen and listeriosis can result in abortions in pregnant women. The bacterium can colonize food-processing environments, where specific molecular subtypes can persist for years. The purpose of this study was to determine the virulence potential of a group of food-processing persistent L. monocytogenes strains encoding a premature stop codon in inlA (encoding internalin A) by using two orally dosed models, pregnant mice and pregnant guinea pigs. A food-processing persistent strain of L. monocytogenes invaded placentas (n = 58; 10 % positive) and fetuses (3 % positive) of pregnant mice (n = 9 animals per strain), similar to a genetically manipulated murinized strain, EGD-e InlAm* (n = 61; 3 and 2 %, respectively). In pregnant guinea pigs (n = 9 animals per bacterial strain), a maternofetal strain (from a human fetal clinical fatal case) was isolated from 34 % of placenta samples (n = 50), whereas both food-processing persistent strains were found in 5 % of placenta samples (n = 36 or 37). One of the food-processing persistent strains, N53-1, was found in up to 8 % of guinea pig fetal liver and brain samples, whereas the maternofetal control was found in 6 % of fetal tissue samples. As the food-processing persistent strains carry a premature stop codon in InlA but are invasive in orally dosed pregnant mice and guinea pigs, we hypothesize that listerial crossing of the placental barrier can occur by a mechanism that is independent of an interaction between E-cadherin and InlA.
Physicochemical characterization of fish protein adlayers with bacteria repelling properties

Materials coated with aqueous fish protein extracts can reduce bacterial adhesion, but the mechanism behind the observed effect is not fully understood. In this study we explore the physicochemical properties of fish muscle protein adlayers on four substrates: gold, stainless steel, polystyrene and silicon dioxide. The aims were (i) to determine if the anti-adhesive effect is independent of the underlying substrate chemistry, (ii) to link the physicochemical properties of the adlayer to its ability to repel bacteria, and (iii) to elucidate the mechanism behind this effect. The main proteins on all surfaces were the muscle proteins troponin, tropomyosin, and myosin, and the lipid binding protein apolipoprotein. The quantity, viscoelasticity, and hydration of the protein adlayers varied greatly on the different substrates, but this variation did not affect the bacterial repelling properties. Our results imply that these proteins adsorb to all substrates and provide a steric barrier towards bacterial adhesion, potentially providing a universal antifouling solution.

General information
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Organisations: National Food Institute, Division of Industrial Food Research, Aarhus University
Authors: Meyer, R. L. (Ekstern), Arpanaei, A. (Ekstern), Pillai, S. (Ekstern), Bernbom, N. (Intern), Enghild, J. J. (Ekstern), Ng, Y. (Intern), Gram, L. (Intern), Besenbacher, F. (Ekstern), Kingshott, P. (Ekstern)
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Bacterial adhesion, Antifouling, Protein adsorption, MALDI, XPS, QCM-D

DOI: 10.1016/j.colsurfb.2012.08.044
Source: dtu
Source-ID: u::5745
Publication: Research - peer-review › Journal article – Annual report year: 2013
Protection of cod larvae from vibriosis by Phaeobacter spp.: A comparison of strains and introduction times

Infections with Vibrio anguillarum and other pathogenic Vibrio spp. are a major problem for marine larviculture, and improved control of the microbiota in marine larvae cultures could ensure a more reliable and cost-effective production of juvenile fish. Phaeobacter gallaeciensis is capable of reducing V. anguillarum in live feed cultures and can, in challenge trials, protect fish larvae from vibriosis. The purpose of the present study was to estimate the probiotic potential of Phaeobacter isolates that produce different levels of the antagonistic compound tropodithietic acid (TDA). We compared the capability of three wild type Phaeobacter strains to reduce cod larvae mortalities in challenge trials with single cod (Gadus morhua) embryo/larvae cultures, and assessed the importance of the time point at which the probiotic bacteria were introduced relative to the pathogen. All three Phaeobacter strains reduced larvae mortalities, however to different degrees. The capability of the strains to prevent disease was correlated with their in vitro TDA production, as measured by in vitro inhibition of V. anguillarum. The most effective time to apply the probiotics was in advance of the pathogen, while simultaneous introduction was only effective for the two strains with the highest TDA production. This suggests that prophylactic use of Phaeobacter spp., where the probiotic bacterium is introduced early into the system, is most efficient in disease prevention.

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, National Food Institute, Division of Industrial Food Research, Institute of Marine Research, University of Bergen
Authors: D’Alvise, P. (Intern), Lillebø, S. (Ekstern), Wergeland, H. I. (Ekstern), Gram, L. (Intern), Bergh, Ø. (Ekstern)
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Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 2.75 SJR 1.122 SNIP 1.51
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.107 SNIP 1.256 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.01 SNIP 1.33 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.151 SNIP 1.293 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.222 SNIP 1.485 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.281 SNIP 1.536 CiteScore 2.39
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.161 SNIP 1.39
Pseudoalteromonas strains are potent immunomodulators owing to low-stimulatory LPS

Many species of marine bacteria elicit a weak immune response. In this study, the aim was to assess the immunomodulatory properties of Gram-negative Pseudoalteromonas strains compared with other marine Gram-negative bacteria and to identify the molecular cause of the immunomodulation. Using murine bone-marrow derived dendritic cells (DCs), it was found that Pseudoalteromonas strains induced low cytokine production and modest up-regulation of surface markers CD40 and CD86 compared with other marine bacteria and Escherichia coli LPS. Two strains, Ps. luteoviolacea and Ps. ruthenica, were further investigated with respect to their immunomodulatory properties in DCs. Both inhibited IL-12 and increased IL-10 production induced by E. coli LPS. LPS isolated from the two Pseudoalteromonas strains had characteristic lipid A bands in SDS-PAGE. Stimulation of HEK293 TLR4/MD2 cells with the isolated LPS confirmed the involvement of LPS and TLR4 and established Pseudoalteromonas LPS as TLR4 antagonists. The isolated LPS was active in the endotoxin limulus amoebocyte lysate assay and capable of inducing increased endocytosis in DCs. This study highlights that antagonistic LPS from Pseudoalteromonas strains has potential as a new candidate of therapeutic agent capable of modulating immune responses.
Selectivity in the potentiation of antibacterial activity of α-peptide/β-peptoid peptidomimetics and antimicrobial peptides by human blood plasma

Antimicrobial peptides (AMPs) are promising leads for novel antibiotics; however, their activity is often compromised under physiological conditions. The purpose of this study was to determine the activity of alpha-peptide/beta-peptoid peptidomimetics and AMPs against Escherichia coli and Staphylococcus aureus in the presence of human blood-derived matrices and immune effectors. The minimum inhibitory concentration (MIC) of two peptidomimetics against E. coli decreased by up to one order of magnitude when determined in 50% blood plasma as compared to MHB media. The MIC of a membrane-active AMP, LL-I/3, also decreased, whereas two intracellularly acting AMPs were not potentiated by...
plasma. Blood serum had no effect on activity against E. coli and neither matrix had an effect on activity against S. aureus. Unexpectedly, physiological concentrations of human serum albumin did not influence activity. Plasma potentiation was not mediated by an LL-37 analogue, lysozyme or hydrogen peroxide; however, plasma potentiation of activity was abolished when the complement system was heat-inactivated. Time-course experiments indicated that potentiation was due to plasma-mediated effects on bacterial cells prior to activities of peptidomimetics. The unexpected enhancement of antibacterial activity of peptidomimetics and AMPs under physiological conditions significantly increases the therapeutic potential of these compounds. (C) 2013 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.

**General information**

State: Published
Organisations: National Food Institute, Division of Industrial Food Research, Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, University of Copenhagen
Authors: Hein-Kristensen, L. (Intern), Knapp, K. M. (Ekstern), Franzyk, H. (Ekstern), Gram, L. (Intern)
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- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 1
- Scopus rating (2017): SNIP 0.848 SJR 0.82 CiteScore 2.11
- Web of Science (2017): Indexed Yes
- BFI (2016): BFI-level 1
- Scopus rating (2016): CiteScore 2.27 SJR 1.01 SNIP 0.855
- Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 1
- Scopus rating (2015): SJR 1.089 SNIP 0.747 CiteScore 2.08
- BFI (2014): BFI-level 1
- Scopus rating (2014): SJR 1.508 SNIP 1.136 CiteScore 2.78
- Web of Science (2014): Indexed yes
- BFI (2013): BFI-level 1
- Scopus rating (2013): SJR 1.256 SNIP 1.104 CiteScore 2.88
- ISI indexed (2013): ISI indexed yes
- Web of Science (2013): Indexed yes
- BFI (2012): BFI-level 1
- Scopus rating (2012): SJR 1.373 SNIP 1.133 CiteScore 2.95
- ISI indexed (2012): ISI indexed yes
- Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 1
- Scopus rating (2011): SJR 1.428 SNIP 1.011 CiteScore 2.78
- ISI indexed (2011): ISI indexed yes
- BFI (2010): BFI-level 1
- Scopus rating (2010): SJR 1.333 SNIP 0.841
- BFI (2009): BFI-level 1
- Scopus rating (2009): SJR 1.169 SNIP 1.048
- BFI (2008): BFI-level 1
- Scopus rating (2008): SJR 1.018 SNIP 0.92
- Web of Science (2008): Indexed yes
- Scopus rating (2007): SJR 1.146 SNIP 0.885
- Scopus rating (2006): SJR 1.418 SNIP 0.967
- Scopus rating (2005): SJR 1.183 SNIP 0.821
- Scopus rating (2004): SJR 1.319 SNIP 0.991
Staphylococcus aureus but not Listeria monocytogenes adapt to triclosan and adaptation correlates with increased fabI expression and agr deficiency.

Background. The ability of pathogens to adapt to the widely used biocide, triclosan, varies substantially. The purpose of the study was to examine bacterial adaptation over an extended period of time to low increments of triclosan concentrations. Focus was two human pathogens, S. aureus and L. monocytogenes that previously have displayed inherent high and low adaptability, respectively. Results. Three strains of L. monocytogenes and two strains of S. aureus including the community-acquired USA300 were exposed to increasing, sub-lethal concentrations of triclosan in triclosan-containing agar gradients. Following 25 days of exposure on agar plates to sub-lethal concentrations of triclosan with a twofold concentration increase every second day, minimum inhibitory concentration (MIC) for S. aureus increased from 0.125 (8325-4) and 0.0625 (USA 300) mg/L to 4 mg/L. The MIC of all three L. monocytogenes strains was initially 4 mg/L and remained unaltered by the exposure. The adapted S. aureus isolates retained normal colony size but displayed increased expression of fabI encoding an essential enzyme in bacterial fatty acid synthesis. Also, they displayed decreased or no expression of the virulence associated agrC of the agr quorum sensing system. While most adapted strains of USA300 carried mutations in fabI, none of the adapted strains of 8325-4 did. Conclusions. Adaptability to triclosan varies substantially between Gram positive human pathogens. S. aureus displayed an intrinsically lower MIC for triclosan compared to L. monocytogenes but was easily adapted leading to the same MIC as L. monocytogenes. Even though all adapted S. aureus strains over-expressed fabI and eliminated expression of the agr quorum sensing system, adaptation in USA300 involved fabI mutations whereas this was not the case for 8325-4. Thus, adaptation to triclosan by S. aureus appears to involve multiple genetic pathways.

Staphylococcus aureus but not Listeria monocytogenes adapt to triclosan and adaptation correlates with increased fabI expression and agr deficiency.

Background. The ability of pathogens to adapt to the widely used biocide, triclosan, varies substantially. The purpose of the study was to examine bacterial adaptation over an extended period of time to low increments of triclosan concentrations. Focus was two human pathogens, S. aureus and L. monocytogenes that previously have displayed inherent high and low adaptability, respectively. Results. Three strains of L. monocytogenes and two strains of S. aureus including the community-acquired USA300 were exposed to increasing, sub-lethal concentrations of triclosan in triclosan-containing agar gradients. Following 25 days of exposure on agar plates to sub-lethal concentrations of triclosan with a twofold concentration increase every second day, minimum inhibitory concentration (MIC) for S. aureus increased from 0.125 (8325-4) and 0.0625 (USA 300) mg/L to 4 mg/L. The MIC of all three L. monocytogenes strains was initially 4 mg/L and remained unaltered by the exposure. The adapted S. aureus isolates retained normal colony size but displayed increased expression of fabI encoding an essential enzyme in bacterial fatty acid synthesis. Also, they displayed decreased or no expression of the virulence associated agrC of the agr quorum sensing system. While most adapted strains of USA300 carried mutations in fabI, none of the adapted strains of 8325-4 did. Conclusions. Adaptability to triclosan varies substantially between Gram positive human pathogens. S. aureus displayed an intrinsically lower MIC for triclosan compared to L. monocytogenes but was easily adapted leading to the same MIC as L. monocytogenes. Even though all adapted S. aureus strains over-expressed fabI and eliminated expression of the agr quorum sensing system, adaptation in USA300 involved fabI mutations whereas this was not the case for 8325-4. Thus, adaptation to triclosan by S. aureus appears to involve multiple genetic pathways.
Survival of Bactericidal Antibiotic Treatment by a Persister Subpopulation of Listeria monocytogenes

Listeria monocytogenes can cause the serious infection listeriosis, which despite antibiotic treatment has a high mortality. Understanding the response of L. monocytogenes to antibiotic exposure is therefore important to ensure treatment success. Some bacteria survive antibiotic treatment by formation of persisters, which are a dormant antibiotic-tolerant subpopulation. The purpose of this study was to determine whether L. monocytogenes can form persisters and how bacterial physiology affects the number of persisters in the population. A stationary-phase culture of L. monocytogenes was adjusted to 108 CFU ml\(^{-1}\), and 103 to 104 CFU ml\(^{-1}\) survived 72-h treatment with 100 μg of norfloxacin ml\(^{-1}\), indicating a persister subpopulation. This survival was not caused by antibiotic resistance as regrown persisters were as sensitive to norfloxacin as the parental strain. Higher numbers of persisters (105 to 106) were surviving when older stationary phase or surface-associated cells were treated with 100 μg of norfloxacin ml\(^{-1}\). The number of persisters was similar when a ΔsigB mutant and the wild type were treated with norfloxacin, but the killing rate was higher in the ΔsigB
mutant. Dormant norfloxacin persisters could be activated by the addition of fermentable carbohydrates and subsequently killed by gentamicin; however, a stable surviving subpopulation of 103 CFU ml−1 remained. Nitrofurantoin that has a growth-independent mode of action was effective against both growing and dormant cells, suggesting that eradication of persisters is possible. Our study adds L. monocytogenes to the list of bacterial species capable of surviving bactericidal antibiotics in a dormant stage, and this persister phenomenon should be borne in mind when developing treatment regimens.
**ABSTRACT:** BACKGROUND: The increase in antibiotic resistant bacteria has led to renewed interest in development of alternative antimicrobial compounds such as antimicrobial peptides (AMPs), either naturally-occurring or synthetically-derived. Knowledge of the mode of action (MOA) of synthetic compounds mimicking the function of AMPs is highly valuable both when developing new types of antimicrobials and when predicting resistance development. Despite many functional studies of AMPs, only a few of the synthetic peptides have been studied in detail. RESULTS: We investigated the MOA of the lysine-peptoid hybrid, LP5, which previously has been shown to display antimicrobial activity against Staphylococcus aureus. At concentrations of LP5 above the minimal inhibitory concentration (MIC), the peptoid caused ATP leakage from bacterial cells. However, at concentrations close to the MIC, LP5 inhibited the growth of S. aureus without ATP leakage. Instead, LP5 bound DNA and inhibited macromolecular synthesis. The binding to DNA also led to inhibition of DNA gyrase and topoisoisomerase IV and caused induction of the SOS response. CONCLUSIONS: Our data demonstrate that LP5 may have a dual mode of action against S. aureus. At MIC concentrations, LP5 binds DNA and inhibits macromolecular synthesis and growth, whereas at concentrations above the MIC, LP5 targets the bacterial membrane leading to disruption of the membrane. These results add new information about the MOA of a new synthetic AMP and aid in the future design of synthetic peptides with increased therapeutic potential.

**General information**
State: Published
Organisations: National Food Institute, Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, University of Copenhagen, University of Minnesota
Authors: Gottschalk, S. (Ekstern), Ifrah, D. (Ekstern), Lerche, S. (Ekstern), Gottlieb, C. T. (Intern), Cohn, M. T. (Ekstern), Hiasa, H. (Ekstern), Hansen, P. R. (Ekstern), Gram, L. (Intern), Ingmer, H. (Ekstern), Thomsen, L. E. (Ekstern)
Publication date: 2013
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BFI (2017): BFI-level 1
Oxidative stress can be an important contributor to the lethal effect of bactericidal antibiotics in some bacteria, such as Escherichia coli and Staphylococcus aureus. Thus, despite the different target-specific actions of bactericidal antibiotics, they have a common mechanism leading to bacterial self-destruction by internal production of hydroxyl radicals. The purpose of the present study was to determine if a similar mechanism is involved in antibiotic killing of the infectious human pathogen, Listeria monocytogenes. We treated wild-type L. monocytogenes and oxidative stress mutants (Δsod
and Δfrt) with three different bactericidal antibiotics and found no difference in killing kinetics. In contrast, wild-type E. coli and an oxidative stress mutant (ΔsodA ΔsodB) differed significantly in their sensitivity to bactericidal antibiotics. We conclude that bactericidal antibiotics did not appear to cause oxidative stress in L. monocytogenes and propose that this is caused by its noncyclic tricarboxylic acid (TCA) pathway. Hence, in this noncyclic metabolism, there is a decoupling between the antibiotic-mediated cellular requirement for NADH and the induction of TCA enzyme activity, which is believed to mediate the oxidative stress reaction.

General information
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Organisations: Cognitive Systems, National Food Institute, Division of Industrial Food Research
Authors: Feld, L. (Intern), Knudsen, G. M. (Intern), Gram, L. (Intern)
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BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
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Chitin elicitation of natural product production in marine bacteria

Genome sequences reveal that our current standard laboratory conditions only support a fraction of the potential secondary metabolism in bacteria. Thus, we must rethink cultivation, detection, and isolation strategies for bacterial secondary metabolites in order to explore the huge, so far uncharacterized chemical potential of these organisms. As part of a new project on ecology-driven drug discovery at the Technical University of Denmark, we investigate the use of chitin to elicit or alter production of antibacterial compounds in marine bacteria. Within our large collection of Gram-negative bacteria (mainly Pseudoalteromonas and Vibrio), we found that some strains were capable of producing antibacterial compounds when grown on chitin, an N-acetyl-D-glucosamine polymer found in the exoskeleton of zooplankton. A strain of Vibrio coralliilyticus solely produced the antibiotic andrimid, indicating that andrimid serves a function while growing on chitin-containing surfaces. In contrast, a Photobacterium halotolerans sustained production of all metabolites including the antibiotic holomycin. Furthermore, chitin stimulated the production of two potentially novel metabolites not observed on glucose-based medium. The different phenotypic responses to a natural growth substrate may reflect different niche-adaptations or ecological functions of the compounds produced and it represents a fruitful approach for elicitation of natural product production in marine bacteria.

Gene Sequence Based Clustering Assists in Dereplication of Pseudoalteromonas luteoviolacea Strains with Identical Inhibitory Activity and Antibiotic Production

Some microbial species are chemically homogenous, and the same secondary metabolites are found in all strains. In contrast, we previously found that five strains of P. luteoviolacea were closely related by 16S rRNA gene sequence but produced two different antibiotic profiles. The purpose of the present study was to determine whether such bioactivity differences could be linked to genotypes allowing methods from phylogenetic analysis to aid in selection of strains for biodiscovery. Thirteen P. luteoviolacea strains divided into three chemotypes based on production of known antibiotics and four antibacterial profiles based on inhibition assays against Vibrio anguillarum and Staphylococcus aureus. To determine whether chemotype and inhibition profile are reflected by phylogenetic clustering we sequenced 16S rRNA,
gyrB and recA genes. Clustering based on 16S rRNA gene sequences alone showed little correlation to chemotypes and inhibition profiles, while clustering based on concatenated 16S rRNA, gyrB, and recA gene sequences resulted in three clusters, two of which uniformly consisted of strains of identical chemotype and inhibition profile. A major time sink in natural products discovery is the effort spent rediscovering known compounds, and this study indicates that phylogeny clustering of bioactive species has the potential to be a useful dereplication tool in biodiscovery efforts.

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Authors: Vynne, N. G. (Intern), Månsson, M. (Intern), Gram, L. (Intern)
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Scopus rating (2010): SJR 0.751 SNIP 1.294
Web of Science (2010): Indexed yes
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Scopus rating (2008): SJR 0.438 SNIP 0.337
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Industrial disinfectants do not select for resistance in *Listeria monocytogenes* following long term exposure

*Listeria monocytogenes* is a food-borne pathogen that can persist for years in food processing plants. It has been hypothesized that this could be due to the development of tolerance or resistance to the disinfectants used. The purpose of the present study was to determine whether biocide resistance or tolerance would evolve in *L. monocytogenes* under continued selection in three industrial disinfectants. *L. monocytogenes* EGD was exposed to Desinfect CL (hypochlorite) and Incimaxx DES (peracetic acid and hydrogen peroxide) for several hundred generations. This caused no increase in the minimal inhibitory concentration (MIC) to the disinfectants, whereas exposure to Triquart SUPER (quaternary ammonium compounds) caused a two- to four-fold increase in MIC. Exposure to gentamicin, which was used as a positive control, caused an 8 to 256-fold increase in MIC for several aminoglycosides. Despite the low level of tolerance, the populations adapted to Triquart SUPER were still sensitive to killing with this disinfectant at 0.0125%, which is much lower than in-use concentrations (1–5%). Our data are in agreement with the fact that finding strains with high acquired resistance to disinfectants is rare, and that the disinfectants are still efficient for controlling microorganisms such as *L. monocytogenes*.

**General information**

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Organisations: National Food Institute, Division of Industrial Food Research  
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Scopus rating (2016): CiteScore 3.97 SJR 1.481 SNIP 1.553  
Web of Science (2016): Indexed yes  
BFI (2015): BFI-level 2  
Scopus rating (2015): SJR 1.614 SNIP 1.683 CiteScore 4.02  
Web of Science (2015): Indexed yes  
BFI (2014): BFI-level 2  
Scopus rating (2014): SJR 1.493 SNIP 1.695 CiteScore 3.62  
Web of Science (2014): Indexed yes  
BFI (2013): BFI-level 2  
Scopus rating (2013): SJR 1.612 SNIP 1.841 CiteScore 3.8  
ISI indexed (2013): ISI indexed yes  
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BFI (2012): BFI-level 2  
Scopus rating (2012): SJR 1.603 SNIP 1.705 CiteScore 3.7  
ISI indexed (2012): ISI indexed yes  
Web of Science (2012): Indexed yes  
BFI (2011): BFI-level 2  
Scopus rating (2011): SJR 1.607 SNIP 1.713 CiteScore 3.63  
ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 2  
Scopus rating (2010): SJR 1.61 SNIP 1.666  
Web of Science (2010): Indexed yes
Nigribactin, a Novel Siderophore from Vibrio nigripulchritudo, Modulates Staphylococcus aureus Virulence Gene Expression

Staphylococcus aureus is a serious human pathogen that employs a number of virulence factors as part of its pathogenesis. The purpose of the present study was to explore marine bacteria as a source of compounds that modulate virulence gene expression in S. aureus. During the global marine Galathea 3 expedition, a strain collection was established comprising bacteria that express antimicrobial activity against Vibrio anguillarum and/or Staphylococcus aureus. Within this collection we searched colony material, culture supernatants, and cell extracts for virulence modulating activity showing that 68 out of 83 marine bacteria (affiliated with the Vibrionaceae and Pseudoalteromonas sp.) influenced expression of S. aureus hla encoding α-hemolysin toxin and/or spa encoding Protein A. The isolate that upon initial screening showed the highest degree of interference (crude ethyl acetate extract) was a Vibrio nigripulchritudo. Extraction, purification and structural elucidation revealed a novel siderophore, designated nigribactin, which induces spa transcription. The effect of nigribactin on spa expression is likely to be independent from its siderophore activity, as another potent siderophore, enterobactin, failed to influence S. aureus virulence gene expression. This study shows that marine microorganisms produce compounds with potential use in therapeutic strategies targeting virulence rather than viability of human pathogens.

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**Phaeobacter gallaeciensis** cell-to-cell signalling does not influence antagonism in algae cultures

**General information**

*State:* Published  
*Organisations:* National Food Institute, Division of Industrial Food Research  
*Authors:* Prol García, M. J. (Intern), D’Alvise, P. (Intern), Gram, L. (Intern)  
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Phaeobacter gallaeciensis Reduces Vibrio anguillarum in Cultures of Microalgae and Rotifers, and Prevents Vibriosis in Cod Larvae

Phaeobacter gallaeciensis can antagonize fish-pathogenic bacteria in vitro, and the purpose of this study was to evaluate the organism as a probiont for marine fish larvae and their feed cultures. An in vivo mechanism of action of the antagonistic probiotic bacterium is suggested using a non-antagonistic mutant. P. gallaeciensis was readily established in axenic cultures of the two microalgae Tetraselmis suecica and Nannochloropsis oculata, and of the rotifer Brachionus plicatilis. P. gallaeciensis reached densities of 10^7 cfu/ml and did not adversely affect growth of algae or rotifers. Vibrio anguillarum was significantly reduced by wild-type P. gallaeciensis, when introduced into these cultures. A P. gallaeciensis mutant that did not produce the antibacterial compound tropodithietic acid (TDA) did not reduce V. anguillarum numbers, suggesting that production of the antibacterial compound is important for the antagonistic properties of P. gallaeciensis. The ability of P. gallaeciensis to protect fish larvae from vibriosis was determined in a bath challenge experiment using a multidish system with 1 larva per well. Unchallenged larvae reached 40% accumulated mortality which increased to 100% when infected with V. anguillarum. P. gallaeciensis reduced the mortality of challenged cod larvae (Gadus morhua) to 10%, significantly below the levels of both the challenged and the unchallenged larvae. The TDA mutant reduced mortality of the cod larvae in some of the replicates, although to a much lesser extent than the wild type. It is concluded that P. gallaeciensis is a promising probiont in marine larviculture and that TDA production likely contributes to its probiotic effect.

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Organisations: National Food Institute, Division of Industrial Food Research, Department of Systems Biology, Center for Microbial Biotechnology, Institute of Marine Research, University of Bergen
Authors: D’Alvise, P. (Intern), Lillebø, S. (Ekstern), Prol García, M. J. (Intern), Wergeland, H. I. (Ekstern), Nielsen, K. F. (Intern), Bergh, O. (Ekstern), Gram, L. (Intern)
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Scopus rating (2015): SJR 1.427 SNIP 1.136 CiteScore 3.32
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.559 SNIP 1.148 CiteScore 3.54
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Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
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Scopus rating (2011): SJR 2.425 SNIP 1.233 CiteScore 4.58
Antibiotics have been an effective weapon against bacterial infections for over 50 years. However, bacterial resistance towards conventional antibiotics has increased considerably within the last decades and the number of antibacterial agents available for treating complicated bacterial infections is becoming increasingly limited. In the search for alternatives therapies, antimicrobial peptides (AMPs) have received considerable attention since they target the bacterial Achilles’ heel i.e. their distinct membrane structure. These host defence molecules are ubiquitous in nature by forming part of the immune system among all classes of life. Several of these compounds have therefore been characterised and developed into future antibacterials. Furthermore, in an attempt to improve the antibacterial activity, synthetic analogues i.e. peptidomimetics have been designed based on the structural properties of natural AMPs.

The purpose of this PhD study was to establish the potential correlation between structure and antibacterial activity for a series of α-peptides/β-peptoid peptidomimetics and additionally to determine if mechanistic differences could explain observed variations in activity. We determined the activity of the peptidomimetics against a range food borne and nosocomial pathogenic bacteria. These structure-activity studies demonstrated that peptide length was important for high antibacterial activity since analogues with a length shorter than 12 residues were virtually inactive. In the present design, with a 1:1 ratio between cationic α-amino acids and hydrophobic β-peptoids, amino acid composition and chirality in the β-peptoid unit only had a minor influence on antibacterial activity.

By using an ATP leakage assay we determined that the mechanism of action of the chimeras was permeabilization or disruption of the bacterial cell membrane. The resulting changes to the cell surface were visualised with Scanning Electron Microscopy (SEM). Importantly, our leakage studies were performed with viable bacterial cells and using a concentration that was close to the Minimum Inhibitory Concentration (MIC). The findings show that all of the chimeras included in the study have a similar mechanism of action that was independent on bacterial species. However, the study showed that the detailed interaction with the cell membrane may be different, since there were large variations in the amount of leaked ATP and subsequent loss of viability. A series of three peptides differing only in length all caused ATP leakage but only the longest of the three caused complete depletion of intracellular ATP, which correlated with a substantial loss in the number of viable cells.

In a continuous selection protocol encompassing 500 generations, 10 out of 10 lineages of Escherichia coli developed resistance towards the chimera they had been exposed to. This was the first time resistance was successfully developed towards peptidomimetics, though several studies have reported resistance towards AMPs. Resistance was specific to compounds within the peptidomimetics library, since we were unable to demonstrate cross-resistance to other AMPs. We sequenced the entire genome of six highly resistant isolates from two separate lineages, and identified a single-nucleotide-polymorphism (SNP) in the gene encoding the MltD protein. This protein functions in the reorganization of the peptidoglycan layer, and we consider it likely that a change in this protein is the cause of resistance, since the SNP was found exclusively in isolates with high levels of resistance.
Conversely, these resistant isolates displayed increased sensitivity towards human blood plasma possibly due to immune effector compounds present in this. The addition of 50% blood plasma also increased the activity of the chimeras against wild type bacteria by up to 32 times. This effect was abolished by heat-treatment, which is a method known to inactivate the complement system.

The findings in this thesis have elucidated how central structural determinants influence antibacterial activity. Peptidomimetics can be regarded as promising future antibiotics since the possibility to optimize their properties through structural modification allows for continuous variation. This thesis concludes that antibacterial activity can be improved further and that in the future resistance may be circumvented by optimizing the existing scaffold.

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Subinhibitory concentrations of antibiotics affect biofilm formation in Listeria monocytogenes

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Authors: Knudsen, G. M. (Intern), Gram, L. (Intern)
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Subinhibitory concentrations of antibiotics affect stress and virulence gene expression in Listeria monocytogenes and cause enhanced stress sensitivity but do not affect Caco-2 cell invasion

Antibiotics can act as signal molecules and affect bacterial gene expression, physiology and virulence. The purpose of this study was to determine whether subinhibitory antibiotic concentrations alter gene expression and physiology of Listeria monocytogenes. Using an agar-based screening assay with promoter fusions, 14 of 16 antibiotics induced or repressed expression of one or more stress and/or virulence genes. Despite ampicillin-induced up-regulation of PmlA-lacZ expression, Caco-2 cell invasion was not affected. Subinhibitory concentrations of ampicillin and tetracycline caused up- and down-regulation of stress response genes, respectively, but both antibiotics caused increased sensitivity to acid stress. Six combinations of gene-antibiotic were quantified in broth cultures and five of the six resulted in the same expression pattern as the agar-based assay. Antibiotics affect virulence and/or stress gene expression; however, altered expression could not predict changes in phenotypic behaviour. Subinhibitory concentrations of antibiotics led to increased acid sensitivity, and we speculate that this is attributed to changes in cell envelope or reduced σB-dependent gene expression. Although subinhibitory concentrations of antibiotics affect gene expression in L. monocytogenes, the changes did not increase virulence but did enhance the acid sensitivity.

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Authors: Knudsen, G. M. (Intern), Holch, A. (Intern), Gram, L. (Intern)
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Tropolodithietic acid producing bacteria – A novel tool for improving food safety of molluscan shellfish?
Wide Distribution of Closely Related, Antibiotic-Producing Arthrobacter Strains throughout the Arctic Ocean

We isolated 16 antibiotic-producing bacterial strains throughout the central Arctic Ocean, including seven Arthrobacter spp. with almost identical 16S rRNA gene sequences. These strains were numerically rare, as revealed using 454 pyrosequencing libraries. Arthrobacter spp. produced arthrobacilins A to C under different culture conditions, but other, unidentified compounds likely contributed to their antibiotic activity.
Global patterns of marine bacterioplankton diversity and characterisation of bioactive Vibrionaceae isolates

The purpose of the present study was to analyze the composition of marine bacterial communities around the world, and to investigate bacterial isolates regarding the production of antibiotics. This included molecular analyses of marine bacterioplankton, as well as culture-based studies of marine bacterial isolates with antagonistic activity. The work was based on samples collected during the Galathea 3 and LOMROG-II marine research expeditions that have explored many different oceanic regions worldwide. A molecular survey of marine bacterioplankton at 24 worldwide stations investigated the abundance of major bacterial groups, potential biogeographical patterns, and their relation to environmental parameters. The original aim was to determine whether the composition of the total microbiota correlates with the occurrence of culturable bioactive bacteria. No such correlation was found. Quantitative community analyses showed latitudinal patterns in bacterial distribution, revealing significantly different relative abundances of Bacteroidetes, unclassified Bacteria and Vibrio between warmer and colder oceans. Absolute cell numbers of most bacterial groups were positively correlated with nutrient concentrations in warmer oceans, and negatively with oxygen saturation in colder oceans. The finding of differing communities in warmer and colder oceans underlined the presence of biogeographical patterns among marine bacteria and the influence of environmental parameters on bacterial distribution. Studies of antagonistic isolates focused on six bioactive Vibrionaceae isolated during Galathea 3. The six strains were identified as Vibrio coralliilyticus (two strains), V. neptunius (two strains), V. nigripulchritudo (one strain), and Photobacterium halotolerans (one strain) by sequencing of housekeeping genes. Chemical metabolite profiling underlined genetic relationships by showing highly similar production of secondary metabolites for each species. Two known antibiotics were purified; andrimid from V. coralliilyticus and holomycin from P. halotolerans. In addition, two novel cyclic peptides from P. halotolerans and a novel siderophore-like compound from V. nigripulchritudo were isolated. All three compounds interfere with quorum sensing in S. aureus. During LOMROG-II further seventeen strains with antagonistic activity were isolated, affiliating with the Actinobacteria (8 strains), Pseudoalteromonas (4 strains), the Vibrionaceae (3 strains), and
Psychrobacter (2 strains). Seven of the eight bioactive Actinobacteria, being isolated from different sources throughout the Arctic Ocean, were related to Arthrobacter davidanieli. Its broad antibiotic spectrum was likely based on production of the known arthrobacilin antibiotics. The eighth actinomycete, tentatively identified as Brevibacterium sp., produces a potentially novel antimicrobial compound. Most studies of antagonistic marine bacteria have been conducted with the aim of isolating novel antimicrobials with potential clinical applications. However, little is known about production and role of these compounds in the natural environment. This thesis took one step in this direction and demonstrated that V. coralliilyticus S2052 produced its antibiotic andrimid when grown with chitin as the sole carbon source. Whilst the strain produced an array of secondary metabolites in laboratory media, it focused on andrimid production with chitin. This indicates that the antibiotic is likely produced in the natural habitat and may serve an ecophysiological function. The finding that two related strains from public culture collections do not produce andrimid and have different biosynthetic temperature optima suggested that V. coralliilyticus may comprise different subspecies with different niches. In summary, the present study shows biogeographical patterns of marine bacterioplankton on a global scale. In addition, the thesis work has demonstrated that marine Vibrionaceae and polar Actinobacteria are a resource of antibacterial compounds and may have potential for future natural product discovery.

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Bacterial membrane activity of a-peptide/b-peptoid chimeras: Influence of amino acid composition and chain length on the activity against different bacterial strains
BACKGROUND: Characterization and use of antimicrobial peptides (AMPs) requires that their mode of action is determined. The interaction of membrane-active peptides with their target is often established using model membranes, however, the actual permeabilization of live bacterial cells and subsequent killing is usually not tested. In this report, six α-peptide/β-peptoid chimeras were examined for the effect of amino acid/peptoid substitutions and chain length on the membrane perturbation and subsequent killing of food-borne and clinical bacterial isolates. RESULTS: All six AMP analogues inhibited growth of twelve food-borne and clinical bacterial strains including Extended Spectrum Beta-Lactamase-producing Escherichia coli. In general, the Minimum Inhibitory Concentrations (MIC) against Gram-positive and -negative bacteria were similar, ranging from 1 to 5 μM. The type of cationic amino acid only had a minor effect on MIC values, whereas chain length had a profound influence on activity. All chimeras were less active against Serratia marcescens (MICs above 46 μM). The chimeras were bactericidal and induced leakage of ATP from Staphylococcus aureus and S. marcescens with similar time of onset and reduction in the number of viable cells. EDTA pre-treatment of S. marcescens and E. coli followed by treatment with chimeras resulted in pronounced killing indicating that disintegration of the Gram-negative outer membrane eliminated innate differences in susceptibility. Chimera chain length did not influence the degree of ATP leakage, but the amount of intracellular ATP remaining in the cell after treatment was influenced by chimera length with the longest analogue causing complete depletion of intracellular ATP. Hence some chimeras caused a complete disruption of the membrane, and this was parallel by the largest reduction in number of viable bacteria. CONCLUSION: We found that chain length but not type of cationic amino acid influenced the antibacterial activity of a series of synthetic α-peptide/β-peptoid chimeras. The synthetic chimeras exert their killing effect by permeabilization of the bacterial cell envelope, and the outer membrane may act as a barrier in Gram-negative bacteria. The tolerance of S. marcescens to chimeras may be due to differences in the composition of the lipopolysaccharide layer also responsible for its resistance to polymyxin B.

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Bioactivity and phylogeny of the marine bacterial genus Pseudoalteromonas

The purpose of this Ph.D. project was to evaluate a global collection of marine Pseudoalteromonas bacteria as a source of novel bioactive compounds, and to investigate the distribution and production of such compounds among different species within the Pseudoalteromonas genus. The strain collection was obtained during the research cruise “Galathea 3”, which circumnavigated the Earth while screening marine bacteria for the ability to inhibit Vibrio anguillarum 90-11-287. Pseudoalteromonas strains were one of the most frequently isolated genera. The Pseudoalteromonas strains were evaluated for their ability to repeatedly inhibit the fish pathogen Vibrio anguillarum 90-11-287 or Staphylococcus aureus 8325. Based on previous work, a hypothesis that antagonistic Pseudoalteromonas strains primarily were pigmented and hence surface associated was investigated.

This Ph.D. work confirmed that surface-associated strains were significantly more likely to possess stable antibacterial activity and be pigmented. Pseudoalteromonas strains are known as prolific producers of bioactive secondary metabolites; hence screening the global strain collection for production of novel antibiotics was initiated. Novel quinolone-related compounds were described, but were not antibacterial. Several antibacterial compounds known from other sources were identified, for instance indolmycin which was hitherto only known from terrestrial Streptomycetes. Genome sequencing of P. luteoviolacea S4054 revealed up to 11 biosynthetic pathways with unknown products, confirming the potential for discovery of new secondary metabolites from Pseudoalteromonas strains.

The elaborate secondary metabolite production led me to speculate whether it was possible to use secondary metabolites to assist in species identification within this genus. This would also provide information on the use of 16S rRNA gene sequences to dereplicate strain collections in biodiscovery efforts. A phylogenetic study of 16S rRNA gene sequences of the Pseudoalteromonas strains confirmed the division into two clades; one consisted of bioactive pigmented strains and one predominantly of inactive nonpigmented strains. Correlating this to a dendrogram based on the secondary metabolites in each strain showed that some strains clustered together in a species-specific way, whereas other strains did not cluster near strains of the same species. Hence, secondary metabolite production was not unequivocally reflected in the secondary metabolite profile, possibly due to the limited resolving power of the 16S rRNA gene. The species P. luteoviolacea showed an interesting pattern indicative of phylotype specific antibiotic production strains. Detailed phylogenetic analysis of an expanded collection of P. luteoviolacea strains showed confirmed that production of antibiotics was related to phylogeny within this species, which indicates that the underlying biosynthetic pathways are maintained under selective pressure and hence are important traits for the organism.

One strain stood out during work with the strain collection, in part because of its production of an intense black pigment in contrast to its phylogenetic placement within the non-pigmented clade. This strain was subsequently shown to represent a new bacterial species named Pseudoalteromonas galatheae.

Initial studies revealed the potential production of regulatory compounds involved in cell to cell signaling within some strains of the species P. luteoviolacea. Since such mechanisms are known to govern antibiotic production in some bacteria, this was investigated. A quorum sensing system controlling a putative novel biosynthetic pathway with high homology to the lux system of Vibrio fischeri was identified in P. luteoviolacea S4054. The signal molecule was potentially a new acylated homoserine lactone (AHL) like compound, and the AHL synthetase was phylogenetically distinct from related synthetases. This expands our knowledge of bacterial signaling and lux homologue system, and further work will resolve if this system has implications for antibiotic production.

In summary, this Ph.D. work explored the phylogeny and chemical diversity of the genus Pseudoalteromonas. Novel compounds were discovered but they possessed no antibiotic activity. However, analysis of the genome sequence of P. luteoviolacea S4054 revealed genetic potential for discovery of secondary metabolites not known within this species. Secondary metabolites were not unequivocally representative of species assignments, but on an intra-species level the use of detailed phylogenetic analysis showed phylotype specific production of antibiotics within the species P. luteoviolacea. These findings validate the genus Pseudoalteromonas as a potential source of novel secondary metabolites and may be useful when designing future biodiscovery strategies. The novel species P. galatheae was described which contributed to resolving the taxonomy of the genus. This thesis also provides evidence of a quorum sensing system related to the lux system of Vibrio fischeri but relying on putatively novel signaling molecule encoded by a distinct synthetase, which might be involved in the regulation of antibiotics production.

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Main Research Area: Technical/natural sciences
Bioactivity, Chemical Profiling, and 16S rRNA-Based Phylogeny of Pseudoalteromonas Strains Collected on a Global Research Cruise

One hundred one antibacterial Pseudoalteromonas strains that inhibited growth of a Vibrio anguillarum test strain were collected on a global research cruise (Galathea 3), and 51 of the strains repeatedly demonstrated antibacterial activity. Here, we profile secondary metabolites of these strains to determine if particular compounds serve as strain or species markers and to determine if the secondary metabolite profile of one strain represents the bioactivity of the entire species. 16S rRNA gene similarity divided the strains into two primary groups: One group (51 strains) consisted of bacteria which retained antibacterial activity, 48 of which were pigmented, and another group (50 strains) of bacteria which lost antibacterial activity upon sub-culturing, two of which were pigmented. The group that retained antibacterial activity consisted of six clusters in which strains were identified as Pseudoalteromonas aurantia, Pseudoalteromonas phenolica, Pseudoalteromonas ruthenica, Pseudoalteromonas rubra, and Pseudoalteromonas piscicida. HPLC-UV/VIS analyses identified key peaks, such as violacein in P. luteoviolacea. Some compounds, such as a novel bromoalterochromide, were detected in several species. HPLC-UV/VIS detected systematic intra-species differences for some groups, and testing several strains of a species was required to determine these differences. The majority of non-antibacterial, non-pigmented strains were identified as Pseudoalteromonas agarivorans, and HPLC-UV/VIS did not further differentiate this group. Pseudoalteromonas retaining antibacterial were more likely to originate from biotic or abiotic surfaces in contrast to planktonic strains. Hence, the pigmented, antibacterial Pseudoalteromonas have a niche specificity, and sampling from marine biofilm environments is a strategy for isolating novel marine bacteria that produce antibacterial compounds.
Chitin stimulates production of the antibiotic andrimid in a *Vibrio coralliilyticus* strain

*Vibrio coralliilyticus* is a putative coral pathogen in tropical oceans, but also possesses antagonistic traits. We previously reported antibacterial activity in *Vibrio coralliilyticus* strain S2052 based upon the antibiotic andrimid. The purpose of the present study was to determine whether *V. coralliilyticus* S2052 produces the antibiotic under conditions mimicking natural habitats of vibrios. S2052 synthesized andrimid with both chitin and macroalgal extracts as sole nutrient source. With chitin, the biosynthesis of metabolites other than andrimid was largely abolished, and the yield of the antibiotic per cell was twofold higher. In cultures with Artemia as live chitin model system, S2052 reached up to 10^8 cells ml^-1, produced andrimid and showed attachment to the exoskeleton and chitinous exuviae. The metabolic focus on andrimid production with chitin indicates that the antibiotic could serve an ecophysiological function. S2052 was compared with two related *V. coralliilyticus* strains (LMG20984^T^ and LMG10953). Despite overall similar secondary metabolomes, LMG20984^T^ and LMG10953 did not produce andrimid, and their optimum biosynthetic temperature was 30 as compared with 25°C for S2052. In addition, S2052 appeared less pathogenic towards Artemia than reported for the type strain. Different physiologies of S2052 and closely related strains indicated that *V. coralliilyticus* subspecies may be adapted to different niches.

**General information**

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Organisations: National Food Institute, Center for Microbial Biotechnology, Department of Systems Biology, Division of Industrial Food Research
Authors: Wietz, M. (Intern), Månsson, M. (Intern), Gram, L. (Intern)
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Main Research Area: Technical/natural sciences

**Publication information**

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Exploring a Global Collection of Marine Bacteria for new Antibacterial Compounds: Oral presentation

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Authors: Månsson, M. (Intern), Nielsen, K. F. (Intern), Gram, L. (Intern), Larsen, T. O. (Intern)
Publication date: 2011
Main Research Area: Technical/natural sciences
Electronic versions:
EXPLORING A GLOBAL.pdf
Source: orbit
Source-ID: 316722
Publication: Research › Conference abstract for conference – Annual report year: 2011

Global patterns of marine bacterioplankton diversity and characterization of bioactive vibrionaceae isolates

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Organisations: National Food Institute, Bacterial Ecophysiology and Biotechnology
Authors: Wietz, M. (Intern), Gram, L. (Intern)
Number of pages: 91
Publication date: 2011

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Original language: English
Inhibition of Virulence Gene Expression in Staphylococcus aureus by Novel Depsipeptides from a Marine Photobacterium

During a global research expedition, more than five hundred marine bacterial strains capable of inhibiting the growth of pathogenic bacteria were collected. The purpose of the present study was to determine if these marine bacteria are also a source of compounds that interfere with the agr quorum sensing system that controls virulence gene expression in Staphylococcus aureus. Using a gene reporter fusion bioassay, we recorded agr interference as enhanced expression of spa, encoding Protein A, concomitantly with reduced expression of hla, encoding α-hemolysin, and rnaIII encoding RNAIII, the effector molecule of agr. A marine Photobacterium produced compounds interfering with agr in S. aureus strain 8325-4, and bioassay-guided fractionation of crude extracts led to the isolation of two novel cyclodepsipeptides, designated solonamide A and B. Northern blot analysis confirmed the agr interfering activity of pure solonamides in both S. aureus strain 8325-4 and the highly virulent, community-acquired strain USA300 (CA-MRSA). To our knowledge, this is the first report of inhibitors of the agr system by a marine bacterium.
Listeria monocytogenes strains encoding inlA with premature stop codons are able to infect pregnant mice

General information
State: Published
Organisations: National Food Institute, Division of Microbiology and Risk Assessment
Authors: Holch, A. (Intern), Licht, T. R. (Intern), Gram, L. (Intern)
Publication date: 2011
Event: Abstract from 9th Symposium on Food Microbiology, Helsingør, Denmark.
Main Research Area: Technical/natural sciences

Listeria monocytogenes survival of UV-C radiation is enhanced by presence of sodium chloride, organic food material and by bacterial biofilm formation

The bactericidal effect on food processing surfaces of ceiling-mounted UV-C light (wavelength 254nm) was determined in a fish smoke house after the routine cleaning and disinfection procedure. The total aerobic counts were reduced during UV-C light exposure (48h) and the number of Listeria monocytogenes positive samples went from 30 (of 68) before exposure to 8 (of 68). We therefore in a laboratory model determined the L. monocytogenes reduction kinetics by UV-C light with the purpose of evaluating the influence of food production environmental variables, such as presence of NaCl, organic material and the time L. monocytogenes was allowed to adhere to steel before exposure. L. monocytogenes grown and attached in tryptone soy broth (TSB) with glucose were rapidly killed (after 2min) by UV-C light. However, bacteria grown and adhered in TSB with glucose and 5% NaCl were more resistant and numbers declined with 4–5log units during exposure of 8–10min. Bacteria grown in juice prepared from cold-smoked salmon were protected and numbers were reduced with 2–3log when UV-C light was used immediately after attachment whereas numbers did not change at all if bacteria had been allowed to form a biofilm for 7days before exposure. It is not known if this enhanced survival is due to physiological changes in the attached bacterial cells, a physical protection of the cells in the food matrix or a combination. In conclusion, we demonstrate that UV-C light is a useful extra bacteriocidal step and that it, as all disinfecting procedures, is hampered by the presence of organic material.

General information
State: Published
Organisations: National Food Institute
Authors: Bernbom, N. (Intern), Vogel, B. F. (Intern), Gram, L. (Intern)
Pages: 69-73
Publication date: 2011
Main Research Area: Technical/natural sciences
Marine Bacteria from Danish Coastal Waters Show Antifouling Activity against the Marine Fouling Bacterium Pseudoalteromonas sp. Strain S91 and Zoospores of the Green Alga Ulva australis Independent of Bacteriocidal Activity

The aims of this study were to determine if marine bacteria from Danish coastal waters produce antifouling compounds and if antifouling bacteria could be ascribed to specific niches or seasons. We further assess if antibacterial effect is a good proxy for antifouling activity. We isolated 110 bacteria with anti-Vibrio activity from different sample types and locations during a 1-year sampling from Danish coastal waters. The strains were identified as Pseudoalteromonas, Phaeobacter, and Vibrionaceae based on phenotypic tests and partial 16S rRNA gene sequence similarity. The numbers of bioactive bacteria were significantly higher in warmer than in colder months. While some species were isolated at all sampling locations, others were niche specific. We repeatedly isolated Phaeobacter gallaeciensis at surfaces from one site and Pseudoalteromonas tunicata at two others. Twenty-two strains, representing the major taxonomic groups, different seasons, and isolation strategies, were tested for antiadhesive effect against the marine biofilm-forming bacterium Pseudoalteromonas sp. strain S91 and zoospores of the green alga Ulva australis. The antiadhesive effects were assessed by quantifying the number of strain S91 or Ulva spores attaching to a preformed biofilm of each of the 22 strains. The strongest antifouling activity was found in Pseudoalteromonas strains. Biofilms of Pseudoalteromonas piscicida, Pseudoalteromonas tunicata, and Pseudoalteromonas ulvae prevented Pseudoalteromonas S91 from attaching to steel surfaces. P. piscicida killed S91 bacteria in the suspension cultures, whereas P. tunicata and P. ulvae did not; however, they did prevent adhesion by nonbactericidal mechanism(s). Seven Pseudoalteromonas species, including P. piscicida and P. tunicata, reduced the number of settling Ulva zoospores to less than 10% of the number settling on control surfaces. The antifouling alpP gene was detected only in P. tunicata strains (with purple and yellow pigmentation), so other compounds/mechanisms must be present in the other Pseudoalteromonas strains with antifouling activity.

General information

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Organisations: Division of Industrial Food Research, National Food Institute, Technical University of Denmark, University of New South Wales
Authors: Bernbom, N. (Intern), Ng, Y. Y. (Ekstern), Kjelleberg, S. (Ekstern), Harder, T. (Ekstern), Gram, L. (Intern)
Pages: 8557-8567
Publication date: 2011
Main Research Area: Technical/natural sciences

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Scopus rating (2017): CiteScore 3.99
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Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
Vibrionaceae are Gram-negative bacteria found widespread in the marine environment where they are particularly abundant on the surface of marine macroorganisms. Production of antibacterial compounds appears to be common among vibrios, yet vibrios are largely underexplored for their proclivity to produce secondary metabolites. We have studied the production of antibacterial compounds in Vibrionaceae collected during a global marine expedition, Galathea 3. Apart from growth inhibitory compounds, we searched for compounds that interfere with virulence regulation in Staphylococcus aureus. We found that some strains were capable of producing antibacterial compounds when grown on natural substrates such as chitin or seaweed. One Vibrio corallilyticus strain was capable of producing the antibacterial compound when using chitin as the sole carbon source and in a live chitin model system, suggesting an ecological function. Using chemical profiling, vibrio strains were compared on a global scale, revealing that the production of certain compounds is a conserved feature independent of sample locations. Chemical screening techniques such as explorative solid-phase extraction led to the isolation of two novel depsipeptides, solonamide A and B, as potent inhibitors of the agr QS system involved in virulence expression in S. aureus. Of special interest was a pronounced effect against a highly virulent, CA-MRSA strain (USA300). In conclusion, we found that vibrios are competent producers of secondary metabolites, some of which possess biological activities attractive for alternative strategies in antibacterial therapy.

Marine Vibrionaceae as a source of bioactive natural products
Vibrionaceae are Gram-negative bacteria found widespread in the marine environment where they are particularly abundant on the surface of marine macroorganisms. Production of antibacterial compounds appears to be common among vibrios, yet vibrios are largely underexplored for their proclivity to produce secondary metabolites. We have studied the production of antibacterial compounds in Vibrionaceae collected during a global marine expedition, Galathea 3. Apart from growth inhibitory compounds, we searched for compounds that interfere with virulence regulation in Staphylococcus aureus. We found that some strains were capable of producing antibacterial compounds when grown on natural substrates such as chitin or seaweed. One Vibrio corallilyticus strain was capable of producing the antibacterial compound when using chitin as the sole carbon source and in a live chitin model system, suggesting an ecological function. Using chemical profiling, vibrio strains were compared on a global scale, revealing that the production of certain compounds is a conserved feature independent of sample locations. Chemical screening techniques such as explorative solid-phase extraction led to the isolation of two novel depsipeptides, solonamide A and B, as potent inhibitors of the agr QS system involved in virulence expression in S. aureus. Of special interest was a pronounced effect against a highly virulent, CA-MRSA strain (USA300). In conclusion, we found that vibrios are competent producers of secondary metabolites, some of which possess biological activities attractive for alternative strategies in antibacterial therapy.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, National Food Institute, Division of Industrial Food Research
Authors: Månsson, M. (Intern), Wietz, M. (Intern), Gram, L. (Intern), Larsen, T. O. (Intern)
Publication date: 2011
Event: Abstract from The 7th European Conference on Marine Natural Products, Strömstad, Sweden.
Production of bioactive secondary metabolites by marine Vibrionaceae

Bacteria belonging to the Vibrionaceae family are widespread in the marine environment. Today, 128 species of vibrios are known. Several of them are infamous for their pathogenicity or symbiotic relationships. Despite their ability to interact with eukaryotes, the vibrios are greatly underexplored for their ability to produce bioactive secondary metabolites and studies have been limited to only a few species. Most of the compounds isolated from vibrios so far are non-ribosomal peptides or hybrids thereof, with examples of N-containing compounds produced independent of nonribosomal peptide synthetases (NRPS). Though covering a limited chemical space, vibrios produce compounds with attractive biological activities, including antibacterial, anticancer, and antivirulence activities. This review highlights some of the most interesting structures from this group of bacteria. Many compounds found in vibrios have also been isolated from other distantly related bacteria. This cosmopolitan occurrence of metabolites indicates a high incidence of horizontal gene transfer, which raises interesting questions concerning the ecological function of some of these molecules. This account underlines the pending potential for exploring new bacterial sources of bioactive compounds and the challenges related to their investigation.
Quorum sensing in Aeromonas salmonicida subsp. achromogenes and the effect of the autoinducer synthase AsaI on bacterial virulence

The Gram-negative fish pathogenic bacterium Aeromonas salmonicida possesses the LuxIR-type quorum sensing (QS) system, termed AsaIR. In this study the role of QS in A. salmonicida subsp. achromogenes virulence and pigment production was investigated. Five wild-type Asa strains induced the N-acyl-homoserine lactone (AHL) monitor bacteria. HPLC–HR-MS analysis identified only one type of AHL, N-butanoyl-L-homoserine lactone (C4-HSL). A knock out mutant of Asal, constructed by allelic exchange, did not produce a detectable QS signal and its virulence in fish was significantly impaired, as LD50 of the AsaI deficient mutant was 20-fold higher than that of the isogenic wt strain and the mean day to death of the mutant was significantly prolonged. Furthermore, the expression of two virulence factors (a toxic protease, AsaP1, and a cytotoxic factor) and a brown pigment were reduced in the mutant. AsaP1 production was inhibited by synthetic QS inhibitors (N-(propylsulfanylacetyl)-L-homoserine lactone; N-(pentylsulfanylacetyl)-L-homoserine lactone; and N-(heptylsulfanylacetyl)-L-homoserine lactone) at concentrations that did not affect bacterial growth. It is a new finding that the AHL synthase of Aeromonas affects virulence in fish and QS has not previously been associated with A. salmonicida infections in fish. Furthermore, AsaP1 production has not previously been shown to be QS regulated. The simplicity of the A. salmonicida subsp. achromogenes LuxIR-type QS system and the observation that synthetic QSI can inhibit an important virulence factor, AsaP1, without affecting bacterial growth, makes A. salmonicida subsp. achromogenes an interesting target organism to study the effects of QS in disease development and QSI in disease control.
Virulence, AsaI, Quorum sensing, Aeromonas salmonicida subsp. achromogenes, Quorum sensing inhibitors, Arctic charr

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Source: orbit
Source-ID: 277294
Publication: Research - peer-review › Journal article – Annual report year: 2011
Resistance and Tolerance to Tropodithietic Acid, an Antimicrobial in Aquaculture, Is Hard To Select

The antibacterial compound tropodithietic acid (TDA) is produced by bacteria of the marine Roseobacter clade and is thought to explain the fish probiotic properties of some roseobacters. The aim of the present study was to determine the antibacterial spectrum of TDA and the likelihood of development of TDA resistance. A bacterial extract containing 95% TDA was effective against a range of human-pathogenic bacteria, including both Gram-negative and Gram-positive bacteria. TDA was bactericidal against Salmonella enterica serovar Typhimurium SL1344 and Staphylococcus aureus NCTC 12493 and killed both growing and nongrowing cells. Several experimental approaches were used to select mutants resistant to TDA or subpopulations of strains with enhanced tolerance to TDA. No approach (single exposures to TDA extract administered via different methods, screening of a transposon library for resistant mutants, or prolonged exposure to incremental concentrations of TDA) resulted in resistant or tolerant strains. After more than 300 generations exposed to sub-MIC and MIC concentrations of a TDA-containing extract, strains tolerant to 2x the MIC of TDA for wild-type strains were selected, but the tolerance disappeared after one passage in medium without TDA extract. S. Typhimurium mutants with nonfunctional efflux pump and porin genes had the same TDA susceptibility as wild-type strains, suggesting that efflux pumps and porins are not involved in innate tolerance to TDA. TDA is a promising broad-spectrum antimicrobial in part due to the fact that enhanced tolerance is difficult to gain and that the TDA-tolerant phenotype appears to confer only low-level resistance and is very unstable.

General information
State: Published
Organisations: National Food Institute, Center for Microbial Biotechnology, Department of Systems Biology, University of Birmingham
Authors: Porsby, C. H. (Intern), Webber, M. A. (Ekstern), Nielsen, K. F. (Intern), Piddock, L. J. V. (Ekstern), Gram, L. (Intern)
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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.343 SNIP 1.361 CiteScore 4.28
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.361 SNIP 1.428 CiteScore 4.45
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 2.423 SNIP 1.411 CiteScore 4.67
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 2.363 SNIP 1.5 CiteScore 4.88
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.523 SNIP 1.574 CiteScore 5.02
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Screening and dereplication of microbial natural products extracts

Cosmopolitan occurrence of a lot of antibiotics and other bioactives among microorganisms, stresses the need for efficient dynamic screening and dereplication methods to avoid redundancy in isolation of compounds. Exploring our large collection of marine bacteria collected during the Galathea 3 expedition, we use a combination of chemical profiling and explorative solid-phase extraction (E-SPE) to assess the bacteria's potential to produce new and interesting molecules. We found the use of chemical profiling by LC-UV/MS very useful for marine bacteria such as Vibrio and Pseudoalteromonas. It enabled the grouping of similar strains at species and subspecies level disregarding geographical sampling locations. However, intraspecies differences were still observed. In P. luteoviolacea and V. coralliilyticus some of the differences were related to the production of antibacterial compounds. The chemical profile could be linked to a bioactivity profile using E-SPE which through the use of three different ion-exchangers and a size-exclusion column gives information about the charge, size, and polarity of active components in an extract. This can be used to discriminate between possible candidates during dereplication and allows detailed mapping of bioactives.
Sublethal Triclosan Exposure Decreases Susceptibility to Gentamicin and Other Aminoglycosides in Listeria monocytogenes

The human food-borne pathogen Listeria monocytogenes is capable of persisting in food processing plants despite cleaning and sanitation and is likely exposed to sublethal biocide concentrations. This could potentially affect susceptibility of the bacterium to biocides and other antimicrobial agents. The purpose of the present study was to determine if sublethal biocide concentrations affected antibiotic susceptibility in L. monocytogenes. Exposure of L. monocytogenes strains EGD and N53-1 to sublethal concentrations of Incimaxx DES (containing peroxy acids and hydrogen peroxide) and Triquart Super (containing quaternary ammonium compound) in four consecutive cultures did not alter the frequency of antibiotic-tolerant isolates, as determined by plating on 2x the MIC for a range of antibiotics. Exposure of eight strains of L. monocytogenes to 1 and 4 µg/ml triclosan did not alter triclosan sensitivity. However, all eight strains became resistant to gentamicin (up to 16-fold increase in MIC) after exposure to sublethal triclosan concentrations. Gentamicin-resistant isolates of strains N53-1 and 4446 were also resistant to other aminoglycosides, such as kanamycin, streptomycin, and tobramycin. Gentamicin resistance remained at a high level also after five subcultures without triclosan or gentamicin. Aminoglycoside resistance can be caused by mutations in the target site, the 16S rRNA gene. However, such mutations were not detected in the N53-1-resistant isolates. A combination of gentamicin and ampicillin is commonly used in listeriosis treatment. The triclosan-induced resistance is, hence, of great concern. Further investigations are needed to determine the molecular mechanisms underlying the effect of triclosan.

General information
State: Published
Organisations: Division of Microbiology and Risk Assessment, National Food Institute
Authors: Christensen, E. G. (Intern), Gram, L. (Intern), Kastbjerg, V. G. (Intern)
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Main Research Area: Technical/natural sciences

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Web of Science (2017): Indexed Yes
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Scopus rating (2016): CiteScore 4.21 SJR 2.275 SNIP 1.328
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BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.343 SNIP 1.361 CiteScore 4.28
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.361 SNIP 1.428 CiteScore 4.45
Web of Science (2014): Indexed yes
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ISI indexed (2013): ISI indexed yes
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ISI indexed (2012): ISI indexed yes
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Response of Listeria monocytogenes and Staphylococcus aureus to host defense peptides and behavior in eukaryotic cells

General information
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Organisations: Division of Seafood Research, National Institute of Aquatic Resources, National Food Institute
Authors: Gottlieb, C. T. (Intern), Gram, L. (Intern)
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Electronic versions:
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Antibacterial activity of marine culturable bacteria collected from a global sampling of ocean surface waters and surface swabs of marine organisms

The purpose of the present study was to isolate marine culturable bacteria with antibacterial activity and hence a potential biotechnological use. Seawater samples (244) and 309 swab samples from biotic or abiotic surfaces were collected on a global Danish marine research expedition (Galathea 3). Total cell counts at the seawater surface were 5 × 105 to 106 cells/ml, of which 0.1–0.2% were culturable on dilute marine agar (20°C). Three percent of the colonies cultured from seawater inhibited Vibrio anguillarum, whereas a significantly higher proportion (13%) of colonies from inert or biotic surfaces was inhibitory. It was not possible to relate a specific kind of eukaryotic surface or a specific geographic location to a general high occurrence of antagonistic bacteria. Five hundred and nineteen strains representing all samples and geographic locations were identified on the basis of partial 16S rRNA gene sequence homology and belonged to three major groups: Vibrionaceae (309 strains), Pseudoalteromonas spp. (128 strains), and the Roseobacter clade (29 strains). Of the latter, 25 strains were identified as Ruegeria mobilis or pelagia. When re-testing against V. anguillarum, only 409 (79%) retained some level of inhibitory activity. Many strains, especially Pseudoalteromonas spp. and Ruegeria spp., also inhibited Staphylococcus aureus. The most pronounced antibacterial strains were pigmented Pseudoalteromonas strains and Ruegeria spp. The inhibitory, pigmented Pseudoalteromonas were predominantly isolated in warmer waters from swabs of live or inert surfaces. Ruegeria strains were isolated from all ocean areas except for Arctic and Antarctic waters and inhibitory activity caused by production of tropodithietic acid.

General information
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Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Melchiorsen, J. (Intern), Bruhn, J. B. (Intern)
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Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.06 SNIP 0.986 CiteScore 3.09
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.097 SNIP 0.957 CiteScore 2.89
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.157 SNIP 1.177 CiteScore 3.09
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
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ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.882 SNIP 1.22 CiteScore 2.87
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.951 SNIP 1.246 CiteScore 3.21
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.95 SNIP 1.107
Web of Science (2010): Indexed yes
Antibacterial compounds from marine Vibrionaceae isolated on a global expedition

On a global research expedition, over 500 bacterial strains inhibitory towards pathogenic bacteria were isolated. Three hundred of the antibacterial strains were assigned to the Vibrionaceae family. The purpose of the present study was to investigate the phylogeny and bioactivity of five Vibrionaceae strains with pronounced antibacterial activity. These were identified as Vibrio coralliilyticus (two strains), V. neptunius (two strains), and Photobacterium halotolerans (one strain) on the basis of housekeeping gene sequences. The two related V. coralliilyticus and V. neptunius strains were isolated from distant oceanic regions. Chemotyping by LC-UV/MS underlined genetic relationships by showing highly similar metabolite profiles for each of the two V. coralliilyticus and V. neptunius strains, respectively, but a unique profile for P. halotolerans. Bioassay-guided fractionation identified two known antibiotics as being responsible for the antibacterial activity; andrimid (from V. coralliilyticus) and holomycin (from P. halotolerans). Despite the isolation of already known antibiotics, our findings show that marine Vibrionaceae are a resource of antibacterial compounds and may have potential for future natural product discovery.

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Organisations: Division of Seafood Research, National Food Institute, Center for Microbial Biotechnology, Department of Systems Biology, Organic Chemistry, Department of Chemistry
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 1.537 SJR 0.978 CiteScore 4.58
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.83 SJR 0.883 SNIP 1.313
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Bioactive bacteria from Arctic marine environments

General information
State: Published
Organisations: Division of Seafood Research, National Food Institute, Center for Microbial Biotechnology, Department of Systems Biology
Authors: Wietz, M. (Intern), Månsson, M. (Intern), Bernbom, N. (Intern), Ng, Y. (Intern), Gram, L. (Intern)
Publication date: 2010
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 271828
Publication: Research - peer-review › Poster – Annual report year: 2010

Explorative Solid-Phase Extraction (E-SPE) for Accelerated Microbial Natural Product Discovery, Dereplication, and Purification

Microbial natural products (NP) cover a high chemical diversity, and in consequence extracts from microorganisms are often complex to analyze and purify. A distribution analysis of calculated pK(a) values from the 34390 records in Antibase2008 revealed that within pH 2-11, 44% of all included compounds had an acidic functionality, 17% a basic functionality, and 9% both. This showed a great potential for using ion-exchange chromatography as an integral part of the separation procedure, orthogonal to the classic reversed-phase strategy. Thus, we investigated the use of an "explorative solid-phase extraction" (E-SPE) protocol using SAX, Oasis MAX, SCX, and LH-20 columns for targeted exploitation of chemical functionalities. E-SPE provides a minimum of fractions (15) for chemical and biological analyses and implicates development into a preparative scale methodology. Overall, this allows fast extract prioritization, easier dereplication, mapping of biological activities, and formulation of a purification strategy.
Inactivation of Vibrio anguillarum by attached and planktonic Roseobacter cells

The purpose of the present study was to investigate inhibition of Vibrio by Roseobacter in a combined liquid-surface system. Exposure of Vibrio anguillarum to surface-attached roseobacters (10^7 cfu/cm²) resulted in significant reduction or complete killing of the pathogen inoculated at 10^2 – 10^4 cfu/ml. The effect was likely associated with production of tropodithietic acid (TDA), as a TDA-negative mutant did not affect survival or growth of V. anguillarum.

General information
State: Published
Organisations: National Food Institute, Center for Microbial Biotechnology, Department of Systems Biology
Authors: D’Alvise, P. (Intern), Melchiorsen, J. (Intern), Porsby, C. H. (Intern), Nielsen, K. F. (Intern), Gram, L. (Intern)
Pages: 2366-2370
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 76
Issue number: 7
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Influence of sublethal concentrations of common disinfectants on expression of virulence genes in Listeria monocytogenes

Listeria monocytogenes is a food-borne human pathogen that causes listeriosis, a relatively rare infection with a high fatality rate. The regulation of virulence gene expression is influenced by several environmental factors, and the aim of the present study was to determine how disinfectants used routinely in the food industry affect the expression of different virulence genes in L. monocytogenes when added at sublethal concentrations. An agar-based assay was developed to screen the effect of disinfectants on virulence gene promoter expression and was validated at the transcriptional level by Northern blot analysis. Eleven disinfectants representing four different groups of active components were evaluated in this study. Disinfectants with the same active ingredients had a similar effect on gene expression. Peroxy and chlorine compounds reduced the expression of the virulence genes, and quaternary ammonium compounds (QAC) induced the expression of the virulence genes. In general, a disinfectant had similar effects on the expression of all four virulence genes examined. Northern blot analyses confirmed the downregulation of prfA and inlA expression by Incimaxx DES (a peroxy compound) and their upregulation by Triquart Super (a QAC) in L. monocytogenes EGD. Hence, sublethal concentrations of disinfectants routinely used in the food industry affect virulence gene expression in the human pathogen L. monocytogenes, and the effect depends on the active components of the disinfectant. From a practical perspective, the study underlines that disinfectants should be used at the lethal concentrations recommended by the manufacturers.
Further studies are needed to elucidate whether the changes in virulence gene expression induced by the disinfectants have impact on virulence or other biological properties, such as antibiotic resistance.
Latitudinal patterns in the abundance of major marine bacterioplankton groups

This study describes the abundance of major marine bacterioplankton taxa and two bacterial genera (Pseudoalteromonas and Vibrio) in surface seawater at 24 stations around the world. Catalyzed Reporter Deposition-Fluorescence in situ Hybridization (CARD-FISH) showed that Alphaproteobacteria (average relative abundance 37%, average absolute abundance $3.7 \times 10^5$ cells mL$^{-1}$) including SAR11 (30%/3×10$^5$), Gammaproteobacteria (14%/1.2×10$^5$), and Bacteroidetes (12%/1.3×10$^5$) globally dominated the bacterioplankton. The SAR86 clade (4.6%/4.1×10$^4$) and Actinobacteria (4.5%/4×10$^4$) were detected ubiquitously, whereas Archaea were scarce (0.6%/4.2×10$^3$). The Roseobacter clade (averaging 3.8%/3.5×10$^4$), Pseudoalteromonas (2.6%/2.1×10$^4$), and Vibrio (1.5%/1.3×10$^4$) showed cosmopolitan occurrence. Principal Component Analysis revealed a latitudinal pattern in bacterial abundances by clustering samples according to lower and higher latitudes. This was related to significantly different relative abundances of Bacteroidetes (peaking at higher latitudes), unclassified Bacteria and Vibrio (both peaking at lower latitudes) between warmer and colder oceans. Relative abundances of Alphaproteobacteria (peaking at subtropical) and Gammaproteobacteria (polar stations) varied between major oceanic regions (biomes), as did absolute abundances of Roseobacter (peaking at temperate and polar stations). For almost all groups absolute abundances were positively correlated with nutrient concentrations in warmer oceans, and negatively with oxygen saturation in colder oceans. On a global scale, Roseobacter and SAR86 were correlated with chlorophyll a. Linkages of environmental parameters with relative abundances were more complex, with e.g. Bacteroidetes being associated with chlorophyll a. The finding of differing communities in warmer and colder oceans underlined the presence of biogeographical patterns among marine bacteria and the influence of environmental parameters on bacterial distribution.
Mutating the heme sensing response regulator HssR in Staphylococcus aureus but not in the Listeria monocytogenes homologue results in increased tolerance to the antimicrobial peptide Plectasin

Background Host defence peptides (HDPs), also known as antimicrobial peptides (AMPs), have emerged as potential new therapeutics and their antimicrobial spectrum covers a wide range of target organisms. However, the mode of action and the genetics behind the bacterial response to HDPs is incompletely understood and such knowledge is required to evaluate their potential as antimicrobial therapeutics. Plectasin is a recently discovered HDP active against Gram-positive bacteria with the human pathogen, Staphylococcus aureus (S. aureus) being highly susceptible and the food borne pathogen, Listeria monocytogenes (L. monocytogenes) being less sensitive. In the present study we aimed to use transposon mutagenesis to determine the genetic basis for S. aureus and L. monocytogenes susceptibility to plectasin.
Results In order to identify genes that provide susceptibility to plectasin we constructed bacterial transposon mutant libraries of S. aureus NCTC8325-4 and L. monocytogenes 4446 and screened for increased resistance to the peptide. No resistant mutants arose when L. monocytogenes was screened on plates containing 5 and 10 fold Minimal Inhibitory Concentration (MIC) of plectasin. However, in S. aureus, four mutants with insertion in the heme response regulator (hssR) were 2-4 fold more resistant to plectasin as compared to the wild type. The hssR mutation also enhanced resistance to the plectasin-like defensin eurocin, but not to other classes of HDPs or to other stressors tested. Addition of plectasin did not influence the expression of hssR or hrtA, a gene regulated by HssR. The genome of L. monocytogenes LO28 encodes a putative HssR homologue, RR23 (in L. monocytogenes EGD-e lmo2583) with 48% identity to the S. aureus HssR, but a mutation in the rr23 gene did not change the susceptibility of L. monocytogenes to plectasin. Conclusions S. aureus HssR, but not the homologue RR23 from L. monocytogenes, provides susceptibility to the defensins plectasin and eurocin. Our data suggest that a functional difference between response regulators HssR and RR23 is responsible for the difference in plectasin susceptibility observed between S. aureus and L. monocytogenes.

General information
State: Published
Organisations: Department of Microbiology, Division of Seafood Research, National Food Institute
Authors: Thomsen, L. E. (Ekstern), Gottlieb, C. T. (Intern), Gottschalk, S. (Ekstern), Wodskou, T. (Ekstern), Kristensen, H. (Ekstern), Gram, L. (Intern), Ingmer, H. (Ekstern)
Pages: 307
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: B M C Microbiology
Volume: 10
ISSN (Print): 1471-2180
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.953 SJR 1.242 CiteScore 2.95
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.82 SJR 1.282 SNIP 0.993
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.42 SNIP 0.994 CiteScore 2.93
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.519 SNIP 1.069 CiteScore 2.95
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.571 SNIP 1.179 CiteScore 3.32
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.507 SNIP 1.146 CiteScore 3.38
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.487 SNIP 1.125 CiteScore 3.4
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.433 SNIP 1.034
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.474 SNIP 0.964
Poor Invasion of Trophoblastic Cells but Normal Plaque Formation in Fibroblastic Cells despite actA Deletion in a Group of Listeria monocytogenes Strains Persisting in Some Food Processing Environments

We determined mammalian cell invasion and virulence gene (inlA, inlB, and actA) sequences of Listeria monocytogenes strains belonging to a molecular subtype (RAPD 9) that often persists in Danish fish-processing plants. These strains invaded human placental trophoblasts less efficiently than other L. monocytogenes strains, including clinical strains, and they carry a premature stop codon in inlA. Eight of 15 strains, including the RAPD 9 and maternofetal strains, had a 105-nucleotide deletion in actA that did not affect cell-to-cell spread in mouse fibroblasts. The RAPD 9 strains may still be regarded as of low virulence with respect to human listeriosis.

General information
State: Published
Organisations: Division of Seafood Research, National Food Institute, University of Copenhagen
Authors: Holch, A. (Intern), Gottlieb, C. T. (Intern), Larsen, M. H. (Ekstern), Ingmer, H. (Ekstern), Gram, L. (Intern)
Pages: 3391-3397
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 76
Issue number: 10
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Sub-lethal concentrations of common disinfectants do not influence survival and growth of Listeria monocytogenes in whole blood

General information
State: Published
The survival of Listeria monocytogenes during long term desiccation is facilitated by sodium chloride and organic material
One specific DNA-subtype, as determined by RAPD, of Listeria monocytogenes persisted in a fish slaughterhouse for years, even during months with no production where the plant was cleaned and kept dry. We hypothesised that tolerance to desiccation could be a factor in explaining the persistence of L monocytogenes in food processing environments and the purpose of the present study was to determine ability of L monocytogenes to survive desiccation on stainless steel under simulated food processing conditions. Viable counts of eight different L. monocytogenes strains exposed to different soils and relative humidities (RHs) during desiccation decreased significantly (p
Adhesion of food-borne bacteria to stainless steel is reduced by food conditioning films

Preconditioning of stainless steel with aqueous cod muscle extract significantly impedes subsequent bacterial adhesion most likely due to repelling effects of fish tropomyosin. The purpose of this study was to determine if other food conditioning films decrease or enhance bacterial adhesion to stainless steel. Attachment of Pseudomonas fluorescens AH2 to stainless steel coated with water-soluble coatings of animal origin was significantly reduced as compared with noncoated stainless steel or stainless steel coated with laboratory substrate or extracts of plant origin. Coating with animal extracts also decreases adhesion of other food-relevant bacteria. The manipulation of adhesion was not attributable to growth inhibitory effects. Chemical analysis revealed that the stainless steels were covered by homogenous layers of
adsorbed proteins. The presence of tropomyocin was indicated by appearance of proteins with similar molecular weight based in sodium dodecyl sulfate-polyacrylamide gel electrophoresis, in several extracts that reduced adhesion but also extracts not containing this protein reduced bacterial adhesion, indicating that several molecular species may be involved in the phenomenon. It is a common perception that food materials facilitate bacterial adhesion to surfaces; however, this study demonstrates that aqueous coatings of food origin may actually reduce bacterial adhesion. Compounds from food extracts may potentially be used as nontoxic coatings to reduce bacterial attachment to inert surfaces.
Antifouling capacity of Danish marine bacteria – a new project.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Bernbom, N. (Intern), Ng, Y. (Intern), Gram, L. (Intern)
Publication date: 2009
Event: Abstract from 7th Symposium of Food Microbiology, Helsingør, Denmark.
Main Research Area: Technical/natural sciences

Bakterier der kan begrænse brug af antibiotika

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Gram, L. (Intern)
Pages: 1
Publication date: 2009
Main Research Area: Technical/natural sciences

Bioprospecting a global collection of marine bioactive bacteria

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Publication date: 2009
Event: Abstract from The Marine Chemistry and Geochemistry Seminar, Woods Hole Oceanograhhic Institute, June 2nd, Woods Hole, MA, USA.
Main Research Area: Technical/natural sciences

Den evige kamp mod Listeria monocytogenes

General information
Desinficér uden kemikalier

**General information**
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Pages: 7-7
Publication date: 2009
Main Research Area: Technical/natural sciences

**Publication Information**
Journal: D T U Avisen
Issue number: 2
ISSN (Print): 1604-1232
Ratings:
ISI indexed (2013): ISI indexed no
ISI indexed (2012): ISI indexed no
ISI indexed (2011): ISI indexed no
Original language: Danish
Source: orbit
Source-ID: 238354
Publication: Communication › Journal article – Annual report year: 2009

DNA-based methods for tracing contamination patterns of especially Listeria monocytogenes

**General information**
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Publication date: 2009
E-SPE: Explorative Solid-Phase-Extraction for Accelerated Natural Product Discovery and Purification

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, Division of Seafood Research, National Food Institute, FoodDTU
Publication date: 2009
Event: Poster session presented at Nordic Natural Products Conference, Reykjavik, Island.
Main Research Area: Technical/natural sciences
Electronic versions:
MariaJohansen_NNPC_poster_2009_MAJ-4.pdf
Source: orbit
Source-ID: 259174
Publication: Research › Poster – Annual report year: 2009

Explorative Solid-Phase Extraction for Accelerated Natural Products Discovery and Purification: Abstract of poster presentation

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, National Institute of Aquatic Resources
Authors: Johansen, M. (Intern), Nielsen, K. F. (Intern), Gram, L. (Intern), Larsen, T. O. (Intern)
Publication date: 2009
Main Research Area: Technical/natural sciences
Electronic versions:
Explorative Solid-Phase Extraction.pdf
Source: orbit
Source-ID: 316719
Publication: Research › Conference abstract for conference – Annual report year: 2009

Hygiejniske fødevareprocesser på DTU

General information
State: Published
Organisations: Division of Food Production Engineering, National Food Institute, Division of Seafood Research
Authors: Jørgensen, S. B. (ed.) (Intern), Kastbjerg, V. G. (Intern), Gram, L. (Intern)
Publication date: 2009
Main Research Area: Technical/natural sciences
Publication information
Journal: FoodDTU Midt i Ugen
Issue number: 71
Original language: Danish
Source: orbit
Source-ID: 258169
Publication: Communication › Journal article – Annual report year: 2009

Kan fiskeproteiner forhindre bakterier i at klister til overflader?

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Bernbom, N. (Intern), Gram, L. (Intern)
Marine bacteria – a potential source of novel bioactive compounds

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Publication date: 2009
Event: Abstract from The SMBB XIII National Congress of Biotechnology and Bioengineering, Acapulco, Mexico, June, Acapulco, Mexico.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 253076
Publication: Research › Conference abstract for conference – Annual report year: 2009

Microbiological spoilage of seafood

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Number of pages: 367
Pages: 87-120
Publication date: 2009

Host publication information
Title of host publication: Compendium of the Microbiological Spoilage of Foods and Beverages
Publisher: Kluwer Academic Publishers
Editors: Sperber, W., Doyle, M.
ISBN (Print): 978-1-4419-0825-4
Series: Food Microbiology and Food Safety
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 231127
Publication: Research - peer-review › Book chapter – Annual report year: 2009

Microorganisms - the good, the bad and the indispensable

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Division of Microbiology and Risk Assessment, National Food Institute
Authors: Gram, L. (Intern), Aarestrup, F. M. (Intern)
Pages: 190-200
Model systems allowing quantification of sensitivity to disinfectants and comparison of disinfectant susceptibility of persistent and presumed nonpersistent Listeria monocytogenes

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Kastbjerg, V. G. (Intern), Gram, L. (Intern)
Pages: 1667-1681
Publication date: 2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 106
Issue number: 5
ISSN (Print): 1364-5072
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.41
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.41
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.57
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.56
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.69
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.51
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.55
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
Preventing protein adsorption from a range of surfaces using an aqueous fish protein extract

We utilize an aqueous extract of fish proteins (FPs) as a coating for minimizing the adsorption of fibrinogen (Fg) and human serum albumin (HSA). The surfaces include stainless steel (SS), gold (Au), silicon dioxide (SiO2), and poly(styrene) (PS). The adsorption processes (kinetics and adsorbed mass) are followed by quartz crystal microbalance with dissipation (QCM-D). Complementary surface information is provided by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). QCM-D shows no mass increases to any of the FP-coated surfaces upon treating with Fg or HSA. Also, when Fg- or HSA-coated surfaces are exposed to the FPs, a significant increase in adsorbed mass occurs because the FPs are highly surface-active displacing Fg. Additionally, fluorescence microscopy confirms that very little Fg adsorbs to the FP-coated surfaces. We propose that FP coatings prevent protein adsorption by steric stabilization and could be an alternative method for preventing unwanted bioadhesion on medical materials.
Real-time PCR detection and quantification of fish probiotic Phaeobacter strain 27-4 and fish pathogenic Vibrio in microalgae, rotifer, Artemia and first feeding turbot (Psetta maxima) larvae

To develop a SYBR Green quantitative real-time PCR protocol enabling detection and quantification of a fish probiotic and two turbot pathogenic Vibrio spp. in microcosms. Phaeobacter 27-4, Vibrio anguillarum 90-11-287 and Vibrio splendidus DMC-1 were quantified as pure and mixed cultures and in presence of microalgae (Isochrysis galbana), rotifers (Brachionus plicatilis), Artemia nauplii or turbot (Psetta maxima) larvae by real-time PCR based on primers directed at genetic loci coding for antagonistic and virulence-related functions respectively. The optimized protocol was used to study bioencapsulation and maintenance of the probiont and pathogens in rotifers and for the detection and quantification of Phaeobacter and V. anguillarum in turbot larvae fed rotifers loaded with the different bacteria in a challenge trial. Our real-time PCR protocol is reproducible and specific. The method requires separate standard curve for each host organism and can be used to detect and quantify probiotic Phaeobacter and pathogenic Vibrio bioencapsulated in rotifers and in turbot larvae. Our method allows monitoring and quantification of a turbot larvae probiotic bacteria and turbot pathogenic vibrios in in vivo trials and will be useful tools for detecting the bacteria in industrial rearing units.
Reduction of Listeria monocytogenes on stainless steel by SonoSteam® treatment

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Vogel, B. F. (Intern), Larsen, B. (Ekstern), Gram, L. (Intern)
Publication date: 2009
Event: Poster session presented at ASM General Meeting, June, Philadelphia, USA.
Main Research Area: Technical/natural sciences
Source: orbit
Publication: Research – Poster – Annual report year: 2009

Response of Listeria monocytogenes to disinfection stress at the single-cell and population levels as monitored by intracellular pH measurements and viable-cell counts
Listeria monocytogenes has a remarkable ability to survive and persist in food production environments. The purpose of the present study was to determine if cells in a population of L. monocytogenes differ in sensitivity to disinfection agents as this could be a factor explaining persistence of the bacterium. In situ analyses of Listeria monocytogenes single cells were performed during exposure to different concentrations of the disinfectant Incimaxx DES to study a possible population subdivision. Bacterial survival was quantified with plate counting and disinfection stress at the single-cell level by measuring intracellular pH (pHi) over time by fluorescence ratio imaging microscopy. pHi values were initially 7 to 7.5 and decreased in both attached and planktonic L. monocytogenes cells during exposure to sublethal and lethal concentrations of Incimaxx DES. The response of the bacterial population was homogenous; hence, subpopulations were not detected. However, pregrowth with NaCl protected the planktonic bacterial cells during disinfection with Incimaxx (0.0015%) since pHi was higher (6 to 6.5) for the bacterial population pregrown with NaCl than for cells grown without NaCl (pHi 5 to 5.5) (P <0.05). The protective effect of NaCl was reflected by viable-cell counts at a higher concentration of Incimaxx (0.0031%), where the salt-grown population survived better than the population grown without NaCl (P <0.05). NaCl protected attached cells through drying but not during disinfection. This study indicates that a population of L. monocytogenes cells, whether planktonic or attached, is homogenous with respect to sensitivity to an acidic disinfectant studied on the single-cell level. Hence a major subpopulation more tolerant to disinfectants, and hence more persistent, does not appear to be present.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Kastbjerg, V. G. (Intern), Nielsen, D. S. (Ekstern), Arneborg, N. (Ekstern), Gram, L. (Intern)
Pages: 4550-4556
Publication date: 2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 75
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BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Screening tentative probiotics in vivo in cod and turbot larvae

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Berg, Ø. (Ekstern), Sandlund, N. (Ekstern), Porsby, C. H. (Intern), Gram, L. (Intern)
Survival and growth of Salmonella and Vibrio in som-fak, a Thai low-salt garlic containing fermented fish product

Fermentation of raw fish is a common process in Asia for improvement of shelf life and safety, however, little is known about the survival of pathogenic bacteria in these products. Raw fish may be contaminated with Salmonella and Vibrio species. The purpose of this study was to determine survival and potential growth of Salmonella enterica serovar Weltevreden, S. enterica serovar Enteritidis, Vibrio cholerae and V. parahaemolyticus as influenced by the preservation parameters (sodium chloride, garlic and lactic acid) present in the Thai fermented fish product som-fak. The inhibitory effects of sodium chloride (0–4%), garlic (0–10%) and lactic acid (pH levels as in som-fak) were measured in modified brain heart infusion (BHI) broth at 30 °C. All bacteria were inhibited by 8–10% sodium chloride. Salmonella grew in all concentrations of garlic whereas Vibrio spp. were inhibited by 1.0–1.5%. Lactic acid was inhibitory at levels above 1.5%. The combinations of sodium chloride, lactic acid and garlic showed a distinct hurdle effect in the broth system. Neither S. Enteritidis, V. cholerae nor V. parahaemolyticus grew in garlic (0.5–1%), regardless of the level of sodium chloride (0.5–4% (w/v)), when lactic acid (0.5–2%) was present. S. Weltevreden was the least inhibited of the four bacteria and grew in the combination of 0.5% garlic and 0.5% lactic acid regardless of the NaCl level (0.5–4% (w/v)). Som-fak with 0 to 10% garlic or 2% glucose was inoculated with either (i) 103 CFU/g Salmonella Weltevreden, (ii) 106 CFU/g garlic fermenting Lactobacillus plantarum strain 509 or (iii) a combination of the two strains and stored at 30 °C. The Salmonella count increased to N108 CFU/g (N106 CFU/g for 10% garlic) in all types of som-fak inoculated with S. Weltevreden within the first day. Only a combination of at least 6% garlic and L. plantarum 509 was enough to prevent growth of the inoculated Salmonella whereas adding the Lactobacillus strain alone or in combination with glucose was insufficient to prevent growth. Our results show that Salmonella Weltevreden can grow in som-fak independently of the inhibitory substances normally present in this type of product, emphasising the importance of preventing contamination. However, our results also suggest that the use of garlic fermenting starter cultures in combination with garlic could improve safety of fermented fish products. © 2009

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Survival of dessicated Listeria monocytogenes on stainless steel and transfer to salmon products

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Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Hansen, L. T. (Intern), Gram, L. (Intern), Vogel, B. F. (Intern)
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Vibrio vulnificus produces quorum sensing signals of the AHL-class

Vibrio vulnificus is an aquatic pathogenic bacterium that can cause vibriosis in humans and fish. The species is subdivided into three biotypes with the fish-virulent strains belonging to biotype 2. The quorum sensing (QS) phenomenon mediated by furanosyl borate diester or autoinducer 2 (AI-2) has been described in human strains of biotype 1, and here we show that the luxS gene which encodes AI-2 is present in all strains of V. vulnificus regardless of origin, biotype or serovar. In this study, we also demonstrate that V. vulnificus produces QS signals of the acylated homoserine lactone (AHL) class (AI-1). AHLs were detected in strains of biotype 1 and 2 from water, fish and human wound infections but not in strains isolated from human septicaemic cases. The AHL compound was identified as N-butanoyl-homoserine-lactone (C4-HL) by both reporter strains and by HPLC-high-resolution MS. C4-HL was detected when AHL-positive strains were grown in low-nutrient medium [modified sea water yeast extract (MSWYE)] but not in rich media (tryptic soy broth or brain–heart infusion) and its production was enhanced when blood factors were added to MSWYE. C4-HL was detected in vivo, in eels infected with AHL-positive biotype 2 strains. No known AHL-related gene was detected by PCR or Southern blot suggesting that AHL-related genes in V. vulnificus are different from those found in other Gram-negative bacteria.
Bacterial Adhesion & Blocking Bacterial Adhesion

Microorganisms are an integral part of all natural ecosystems, and as such are ubiquitous in nature. They often live adhered to or in association with surfaces of either organic or inorganic nature, and all surfaces will almost inevitably be colonized by microorganisms. This often results in the formation of highly complex sessile communities, referred to as biofilms. Such microbial communities are often highly dynamic and heterogeneous in nature. Microbial biofilms are of great importance in a wide range of natural processes and industrial settings, from the commensal flora of the gastrointestinal tract to the microbial flocs in waste water treatment facilities. Microbial biofilms may however also cause a wide range of industrial and medical problems, and have been implicated in a wide range of persistent infectious diseases, including implant-associated microbial infections. Bacterial adhesion is the first committing step in biofilm formation, and has therefore been intensely scrutinized. Much however, still remains elusive. Bacterial adhesion is a highly complex process, which is influenced by a variety of factors. In this thesis, a range of physico-chemical, molecular and environmental parameters, which influence the transition from a planktonic lifestyle to a sessile lifestyle, have been studied. Protein conditioning film formation was found to influence bacterial adhesion and subsequent biofilm formation considerably, and an aqueous extract of fish muscle tissue was shown to significantly reduce or delay bacterial biofilm formation of a range of urinary tract infectious E.coli and Klebsiella isolates. Several other proteinaceous coatings were also found to display anti-adhesive properties, possibly providing a measure for controlling the colonization of implant materials. Several other parameters controlling bacterial adhesion were also studied. Subinhibitory concentrations of certain antimicrobial compounds and several surfactants were found to significantly affect bacterial adhesion and biofilm formation, most likely by affecting the production of biofilm extracellular polymeric matrix components. These substances may both mediate and stabilize the bacterial biofilm. Finally, several adhesive structures were examined, and a novel physiological biofilm phenotype in E.coli biofilms was characterized, namely cell chain formation. The autotransporter protein, antigen 43, was implicated in this structural biofilm phenotype, at least in some bacterial strains. Understanding the fundamental requirements of bacterial adhesion and biofilm formation may aid in the development of effective preventive measures.
Adhesion of food-borne pathogenic and spoilage bacteria to stainless steel is reduced by some food substrate conditioning film

Anti-adhesive properties of fish tropomyosins

Aims: We have recently found that preconditioning of stainless steel surfaces with an aqueous fish muscle extract can significantly impede bacterial adhesion. The purpose of this study was to identify and characterize the primary components associated with this bacteria-repelling effect. Methods and Results: The anti-adhesive activity was assayed against Escherchia coli K-12, and bacterial adhesion was quantified by crystal violet staining and sonication methods. Proteolytic digestion, elution and fractionation experiments revealed that the anti-adhesive activity of the extract was linked to the formation of a proteinaceous conditioning film composed primarily of fish tropomyosins. These fibrous proteins formed a considerable anti-adhesive conditioning layer on and reduced bacterial adhesion to several different materials including polystyrene, vinyl plastic, stainless steel and glass. The protein adsorption profiles obtained from the various materials did not differ significantly, but elution was often incomplete making minor qualitative/quantitative differences indiscernible. Conclusions: The data highlights the significance of protein conditioning films on bacterial adhesion and emphasizes the importance of substratum's physiochemical properties and exposure time with regards to protein adsorption/elution efficiency and subsequent bacterial adhesion. Significance and Impact of the Study: Fish tropomyosin-coatings could potentially offer a nontoxic and relatively inexpensive measure of reducing bacterial colonization of inert surfaces.
Antimicrobial peptides effectively kill a broad spectrum of Listeria monocytogenes and Staphylococcus aureus strains independently of origin, subtype, or virulence factor expression

Background Host defense peptides (HDPs), or antimicrobial peptides (AMPs), are important components of the innate immune system that bacterial pathogens must overcome to establish an infection and HDPs have been suggested as novel antimicrobial therapeutics in treatment of infectious diseases. Hence it is important to determine the natural variation in susceptibility to HDPs to ensure a successful use in clinical treatment regimes. Results Strains of two human bacterial pathogens, Listeria monocytogenes and Staphylococcus aureus, were selected to cover a wide range of origin, subtype, and phenotypic behavior. Strains within each species were equally sensitive to HDPs and oxidative stress representing important components of the innate immune defense system. Four non-human peptides (protamine, plectasin, novicidin, and novispirin G10) were similar in activity profile (MIC value spectrum) to the human β-defensin 3 (HBD-3). All strains were inhibited by concentrations of hydrogen peroxide between 0.1% – 1.0%. Sub-selections of both species differed in expression of several virulence-related factors and in their ability to survive in human whole blood and kill the nematode virulence model Caenorhabditis elegans. For L. monocytogenes, proliferation in whole blood was paralleled by high invasion in Caco-2 cells and fast killing of C. elegans, however, no such pattern in phenotypic behavior was observed for S. aureus and none of the phenotypic differences were correlated to sensitivity to HDPs. Conclusion Strains of L. monocytogenes and S. aureus were within each species equally sensitive to a range of HDPs despite variations in subtype, origin, and phenotypic behavior. Our results suggest that therapeutic use of HDPs will not be hampered by occurrence of naturally tolerant strains of the two species investigated in the present study.
A processing plant persistent strain of Listeria monocytogenes crosses the fetoplacental barrier in a pregnant guinea pig model

The foodborne pathogen Listeria monocytogenes can cause infection in immunocompromised humans and in the fetuses of pregnant women. We have demonstrated that one group of genetically similar L. monocytogenes strains (random amplified polymorphic DNA [RAPD] type 9) dominate and persist in several independent fish processing plants. The purpose of the present study was to determine the virulence potential of one RAPD type 9 strain (La111), one human clinical strain (Scott A), and one monkey clinical strain (12443) in a pregnant guinea pig model. Animals were orally exposed to 10^8 CFU of L. monocytogenes in whipping cream on gestation day (GD) 36 and euthanized on GD 42, 45, or 56. Strains 12443 and Scott A were shed from treated animals for 20 days, whereas La111 was shed only in the first 10 days. Strains 12443 and Scott A were recovered from maternal liver, spleen, and gallbladder on all 3 days of euthanization, whereas La111 was recovered only at GD 45 and 56. Scott A was not isolated from any placenta or fetuses. For dams treated with 12443, 22% of the fetuses were positive for L. monocytogenes, and surprisingly, treatment of dams with La111 resulted in 56% infected fetuses. L. monocytogenes was isolated from 16 and 20% of placenta for 12443 and La111, respectively. The study demonstrates that a food processing plant persistent strain of L. monocytogenes is able to cross the fetoplacental barrier in pregnant guinea pigs. Furthermore, we demonstrate that although information can be gained from model virulence assays, assessment of the virulence potential of a strain may require more complex hosts.

General information
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Authors: Jensen, A. (Intern), Williams, D. (Ekstern), Irving, E. A. (Ekstern), Gram, L. (Intern), Smith, M. A. (Ekstern)
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Culturable, antagonistic Roseobacter clade strains isolated from turbot farms are genetically distinct from strains isolated from oceanic environments

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Authors: Porsby, C. H. (Intern), Stenvall, D. P. (Intern), Bruhn, J. B. (Intern), Gram, L. (Intern)
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Dereplication Strategies for Discovery of Marine Microbial Natural Products

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Organisations: Center for Microbial Biotechnology, Department of Systems Biology, Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
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Effect of ozone gas disinfection in a salmon smokehouse

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Authors: Vogel, B. F. (Intern), Mohr, M. (Ekstern), Gram, L. (Intern)
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Genetic Dissection of Tropodithietic Acid Biosynthesis by Marine Roseobacters

The symbiotic association between the roseobacter Silicibacter sp. strain TM1040 and the dinoflagellate Pfiesteria piscicida involves bacterial chemotaxis to dinoflagellate-produced dimethylsulfoniopropionate (DMSP), DMSP demethylation, and ultimately a biofilm on the surface of the host. Biofilm formation is coincident with the production of an antibiotic and a yellow-brown pigment. In this report, we demonstrate that the antibiotic is a sulfur-containing compound, tropodithietic acid (TDA). Using random transposon insertion mutagenesis, 12 genes were identified as critical for TDA biosynthesis by the bacteria, and mutation in any one of these results in a loss of antibiotic activity (Tda(-)) and pigment production. Unexpectedly, six of the genes, referred to as tdaA-F, could not be found on the annotated TM1040 genome and were instead located on a previously unidentified plasmid (ca. 130 kb; pSTM3) that exhibited a low frequency of spontaneous loss. Homologs of tdaA and tdaB from Silicibacter sp. strain TM1040 were identified by mutagenesis in another TDA-producing roseobacter, Phaeobacter sp. strain 27-4, which also possesses two large plasmids (ca. 60 and ca. 70 kb, respectively), and tda genes were found by DNA-DNA hybridization in 88% of a diverse collection of nine roseobacters with known antibiotic activity. These data suggest that roseobacters may use a common pathway for TDA biosynthesis that involves plasmid-encoded proteins. Using metagenomic library databases and a bioinformatics approach, differences in the biogeographical distribution between the critical TDA synthesis genes were observed. The implications of these results to roseobacter survival and the interaction between TM1040 and its dinoflagellate host are discussed.

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Organisations: National Institute of Aquatic Resources, Center for Microbial Biotechnology, Department of Systems Biology, Section for Aquatic Microbiology and Seafood Hygiene, University of Maryland
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Influence of processing steps in cold-smoked salmon production on survival and growth of persistent and presumed non-persistent Listeria monocytogenes

Cold-smoked salmon is a ready-to-eat product in which Listeria monocytogenes sometimes can grow to high numbers. The bacterium can colonize the processing environment and it is believed to survive or even grow during the processing steps. The purpose of the present study was to determine if the steps in the processing of cold-smoked salmon affect survival and subsequent growth of a persistent strain of L. monocytogenes to a lesser degree than presumed non-persistent strains. We used a sequence of experiments increasing in complexity: (i) small salmon blocks salted, smoked or dried under model conditions, (ii) fillets of salmon cold-smoked in a pilot plant and finally, (iii) assessment of the bacterial levels before and after processing during commercial scale production. L. monocytogenes proliferated on salmon blocks that were brined or dipped in liquid smoke and left at 25 degrees C in a humidity chamber for 24 h. However, combining brining and liquid smoke with a drying (25 degrees C) step reduced the bacterium 10-100 fold over a 24 h period. Non-salted, brine injected or dry salted salmon fillets were surface inoculated with L. monocytogenes and cold-smoked in a pilot plant. L. monocytogenes was reduced from 103 to 10-10(2) CFU/cm(2) immediately after cold-smoking. The greatest reductions were observed in dry salted and brine injected fillets as compared to cold-smoking of non-salted fresh fillets. Levels of L. monocytogenes decreased further when the cold-smoked fish was vacuum-packed and stored at 5 degrees C. A similar decline was seen when inoculating brine injected fillets after cold-smoking. High phenol concentrations are a likely cause of this marked growth inhibition. In a commercial production facility, the total viable count of salmon fillets was reduced 10-1000 fold by salting, cold-smoking and process-freezing (a freezing step after smoking and before slicing). The prevalence of L. monocytogenes in the commercial production facility was too low to determine any quantitative effects, however, one of nine samples was positive before processing and none after. Taken together, the processing steps involved in cold-smoking of salmon are bactericidal and reduce, but do not eliminate L. monocytogenes. A persistent strain was no less sensitive to the processing steps than a clinical strain or strain EGD. (C) 2008 Elsevier B.V. All rights reserved

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På fisketur efter nye bioaktive og bakteriehæmmende stoffer - en rapport fra verdenshavene

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Authors: Johansen, M. (Intern), Vynne, N. G. (Intern), Nielsen, K. F. (Intern), Larsen, T. O. (Intern), Gram, L. (Intern)
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Phaeobacter and Ruegeria Species of the Roseobacter Clade Colonize Separate Niches in a Danish Turbot (Scophthalmus maximus)-Rearing Farm and Antagonize Vibrio anguillarum under Different Growth Conditions

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Processing plant persistent strains of Listeria monocytogenes appear to have a lower virulence potential than clinical strains in selected virulence models

Listeria monocytogenes is an important foodborne bacterial pathogen that can colonize food processing equipment. One group of genetically similar L. monocytogenes strains (RAPID type 9) was recently shown to reside in several independent fish processing plants. Persistent strains are likely to contaminate food products, and it is important to determine their virulence potential to evaluate risk to consumers. We compared the behaviour of food processing persistent and clinical L. monocytogenes strains in four virulence models: Adhesion, invasion and intracellular growth was studied in an epithelial cell line, Caco-2; time to death in a nematode model, Caenorhabditis elegans and in a fruit fly model, Drosophila melanogaster and fecal shedding in a guinea pig model. All strains adhered to and grew in Caco-2 cells in similar levels. When exposed to 10^6 CFU/ml, two strains representing the persistent RAPD type 9 invaded Caco-2 cells in lower numbers (10^2-10^3 CFU/ml) as compared to the four other strains (10^4-10^6 CFU/ml), including food and human clinical strains. In the D. melanogaster model, the two RAPD type 9 strains were among the slowest to kill. Similarly, the time to reach 50% killed C elegans worms was longer (110 h) for the RAPD type 9 strains than for the other four strains (80 h). The Scott A strain and one RAPD type 9 strain were suspended in whipping cream before being fed to guinea pigs and the persistent RAPD type 9 strain was isolated from feces in a lower level (approximately 10^2 CFU/g) than the Scott A strain (approximately 10^5 CFU/g) (P

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Authors: Jensen, A. (Intern), Thomsen, L. (Ekstern), Jørgensen, R. (Ekstern), Larsen, M. (Ekstern), Roldgaard, B. (Intern), Christensen, B. B. (Intern), Vogel, B. F. (Intern), Gram, L. (Intern), Ingmer, H. (Ekstern)
Responses of Listeria monocytogenes to desinfection stress monitored by measurement of intracellular pH and viable counts

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Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Kastbjerg, V. G. (Intern), Nielsen, D. (Ekstern), Arneborg, N. (Ekstern), Gram, L. (Intern)
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Survival and growth of Salmonella enterica serovar Weltevreden in Som-fak, a Thai low-salt fermented product

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Factors of importance for virulence and persistence of Listeria monocytogenes

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Organisations: Division of Seafood Research, National Institute of Aquatic Resources
Authors: Holch, A. (Intern), Vogel, B. F. (Intern), Gram, L. (Intern)
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ATP leakage as a way to detect different mechanisms of action of antimicrobial peptides

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Authors: Gottlieb, C. T. (Intern), Mygind, P. (Ekstern), Kristensen, H. (Ekstern), Gram, L. (Intern)
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BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.329 SNIP 1.15 CiteScore 3.08
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.421 SNIP 1.238 CiteScore 3.7
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.417 SNIP 1.284 CiteScore 3.36
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.31 SNIP 1.189 CiteScore 3.04
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
Comparative study of methods for quantification of bacteria attached to stainless steel

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Ng, Y. (Intern), Bernbom, N. (Intern), Gram, L. (Intern)
Publication date: 2007
Event: Poster session presented at 4th ASM Conference on Biofilms, March, Quebec, Canada.
Main Research Area: Technical/natural sciences

Bibliographical note
Poster
Source: orbit
Source-ID: 226608
Publication: Research › peer-review › Journal article – Annual report year: 2007

Comparison of invasiveness and virulence potential of processing plant persistent and clinical strains of Listeria monocytogenes in different virulence models

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Jensen, A. (Ekstern), Thomsen, L. (Ekstern), Jensen, R. (Ekstern), Hansen, M. (Ekstern), Roldgaard, B. (Ekstern), Christensen, B. (Ekstern), Ingmer, H. (Ekstern), Vogel, B. F. (Intern), Gram, L. (Intern)
Publication date: 2007
Event: Poster session presented at International Association for Food Protection Annual Meeting, Lake Buena Vista, Florida, USA, July 8-11.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225969
Publication: Research › Poster – Annual report year: 2007
Description of Shewanella glacialipiscicola sp. nov. and Shewanella algidipiscicola sp. nov., isolated from marine fish of the Danish Baltic Sea, and proposal that Shewanella affinis is a later heterotypic synonym of Shewanella colwelliana

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Satomi, M. (Ekstern), Vogel, B. F. (Intern), Venkateswaran, K. (Ekstern), Gram, L. (Intern)
Pages: 347-352
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: International Journal of Systematic and Evolutionary Microbiology
Volume: 57
Issue number: 2
ISSN (Print): 1466-5026
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.29 SJR 0.943 SNIP 1.194
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.22 SJR 0.892 SNIP 1.164
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.098 SNIP 1.484 CiteScore 2.74
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.952 SNIP 1.174 CiteScore 2.42
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.996 SNIP 1.564 CiteScore 2.57
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.084 SNIP 1.203 CiteScore 1.96
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.105 SNIP 1.349 CiteScore 2.2
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.056 SNIP 1.195
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.955 SNIP 1.251
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.068 SNIP 1.344
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.103 SNIP 1.585
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.394 SNIP 1.554
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.565 SNIP 1.579
Web of Science (2005): Indexed yes
Influence of food soiling matrix on cleaning and disinfection efficiency on surface attached Listeria monocytogenes

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Bagge, D. (Intern), Ng, Y. (Intern), Gymoese, P. (Ekstern), Vogel, B. F. (Intern)
Pages: 1165-1171
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Food Control
Volume: 18
Issue number: 10
ISSN (Print): 0956-7135
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 4.06 SJR 1.502 SNIP 1.69
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.86 SJR 1.492 SNIP 1.709
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.498 SNIP 1.73 CiteScore 3.65
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.38 SNIP 1.717 CiteScore 3.27
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.278 SNIP 1.728 CiteScore 3.14
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.245 SNIP 1.931 CiteScore 3.1
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Possible bacterial interaction in development of winter ulcer disease in farmed fish

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Johansen, R. (Ekstern), Karlsen, C. (Ekstern), Nielsen, K. (Ekstern), Berg, K. (Ekstern), Gudmundsdottir, B. (Ekstern), Willadsen, N. (Ekstern), Gram, L. (Intern), Sørum, H. (Ekstern)
Publication date: 2007
Main Research Area: Technical/natural sciences

Bibliographical note
Poster
Source: orbit
Source-ID: 226050
Publication: Research › Poster – Annual report year: 2007

Production of antibacterial compound and biofilm formation by Roseobacter species are influenced by culture conditions

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Bruhn, J. B. (Intern), Gram, L. (Intern), Belas, R. (Ekstern)
Pages: 442-450
Publication date: 2007
Main Research Area: Technical/natural sciences
Profiling acylated homoserine lactones in Yersinia ruckeri and influence of exogenous acyl homoserine lactones and known quorum-sensing inhibitors on protease production

To profile the quorum-sensing (QS) signals in Yersinia ruckeri and to examine the possible regulatory link between QS signals and a typical QS-regulated virulence phenotype, a protease. Methods and Results: Liquid chromatography-high resolution mass spectrometry (HPLC-HRMS) showed that Y. ruckeri produced at least eight different acylated homoserine lactones (AHLs) with N-(3-oxooctanoyl)-l-homoserine lactone (3-oxo-C8-HSL) being the dominant molecule. Also, some uncommon AHL, N-(3-oxoheptanoyl)-l-homoserine lactone (3-oxo-C7-HSL) and N-(3-oxononanoyl)-l-homoserine lactone (3-oxo-C9-HSL), were produced. 3-oxo-C8-HSL was detected in organs from fish infected with Y. ruckeri. Protease production was significantly lower at temperatures above 23 degrees C than below although growth was faster at the higher temperatures. Neither addition of sterile filtered high-density Y. ruckeri culture supernatant nor the addition of pure exogenous AHLs induced protease production. Furthermore, three QS inhibitors (QSI s), sulfur-containing AHL analogues, did not inhibit protease production in Y. ruckeri. Conclusions: Exogenous AHL or sulfur-containing AHL analogues did not influence the protease production indicating that protease production may not be QS regulated in Y. ruckeri. Significance and Impact of the Study: The array of different AHLs produced indicates that the QS system of Y. ruckeri is complex and could involve several regulatory systems. In this case, neither AHLs nor QSI would be likely to directly affect a QS-regulated phenotype.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Center for Microbial Biotechnology, Department of Systems Biology, National Veterinary Institute, Center for Biomedical Microbiology
Authors: Kastbjerg, V. G. (Intern), Nielsen, K. F. (Intern), Dalsgaard, I. (Intern), Rasch, M. (Intern), Bruhn, J. B. (Intern), Givskov, M. C. (Intern), Gram, L. (Intern)
Pages: 363-374
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 102
Issue number: 2
ISSN (Print): 1364-5072
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.41
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.41
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.57
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.56
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.69
ISI indexed (2013): ISI indexed yes
Quorum sensing signals are produced by Aeromonas salmonicida and quorum sensing inhibitors can reduce production of a potential virulence factor

Many pathogens control production of virulence factors by self-produced signals in a process called quorum sensing (QS). We demonstrate that acyl homoserine lactone (AHL) signals, which enable bacteria to express certain phenotypes in relation to cell density, are produced by a wide spectrum of Aeromonas salmonicida strains. All 31 typical strains were AHL producers as were 21 of 26 atypical strains, but on a strain population basis, production of virulence factors such as protease, lipase, A-layer or pigment did not correlate with the production and accumulation of AHLs in the growth medium. Pigment production was only observed in broth under highly aerated conditions. Quorum sensing inhibitors (QSIs) are compounds that specifically block QS systems without affecting bacterial growth and 2 such compounds, sulphur-containing AHL-analogues, reduced production of protease in a typical strain of Aeromonas salmonicida. The most efficient compound N-(heptylsulfanylacetyl)-L-homoserine lactone (HepS-AHL), reduced protease production by a factor of 10. Five extracellular proteases were detected on gelatin-containing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) gels and 3 of these were completely down regulated by HepS-AHL. Hence, QSIs can curb virulence in some strains and could potentially be pursued as bacterial disease control measures in aquaculture.
Sodium chloride enhances adherence and aggregation and strain variation influences invasiveness of Listeria monocytogenes strains

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Jensen, A. (Intern), Larsen, M. (Ekstern), Ingmer, H. (Ekstern), Vogel, B. F. (Intern), Gram, L. (Intern)
Pages: 592-599
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Food Protection
Volume: 70
Issue number: 3
ISSN (Print): 0362-028X
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.63 SJR 0.761 SNIP 0.823
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.68 SJR 0.769 SNIP 0.811
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.954 SNIP 1.024 CiteScore 2.03
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.914 SNIP 0.953 CiteScore 1.94
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.101 SNIP 1.09 CiteScore 2.11
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.083 SNIP 0.981 CiteScore 2.03
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.994 SNIP 0.958 CiteScore 1.96
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.011 SNIP 0.949
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Survival of surface attached Listeria monocytogenes in drying up stress models

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Vogel, B. F. (Intern), Porsby, C. H. (Intern), Gram, L. (Intern)
Publication date: 2007
Event: Poster session presented at International Association for Food Protection Annual Meeting. July 8-11, Lake Buena Vista, Florida, USA.
Main Research Area: Technical/natural sciences

Bibliographical note
Poster presented at International Association for Food Protection Annual Meeting. July 8-11, Lake Buena Vista, Florida, USA
Source: orbit
Source-ID: 225972
Publication: Research - peer-review › Journal article – Annual report year: 2007

Well-known quorum sensing inhibitors do not affect bacterial quorum sensing-regulated bean sprout spoilage

General information
State: Published
Organisations: National Institute of Aquatic Resources, Department of Systems Biology, Division of Microbiology and Risk Assessment, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Rasch, M. (Intern), Rasmussen, T. B. (Intern), Andersen, J. B. (Intern), Persson, T. (Ekstern), Nielsen, J. (Ekstern), Givskov, M. C. (Intern), Gram, L. (Intern)
Pages: 826-837
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Attachment of Pseudomonas fluorescens AH2 to stainless steel surfaces is reduced by conditioning with fractions of fish extract

General information
State: Published
Bacterial Adhesion to Stainless Steel Is Reduced by Aqueous Fish Extract Coatings

Culture conditions of Roseobacter strain 27-4 affect its attachment and biofilm formation as quantified by real-time PCR

Culture conditions of Roseobacter strain 27-4 affect its attachment and biofilm formation as quantified by real-time PCR
Influence of processing steps in cold-smoked fish production on survival and growth of Listeria monocytogenes

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Porsby, C. H. (Intern), Mohr, M. (Ekstern), Wiekman, A. (Ekstern), Vogel, B. F. (Intern), Gram, L. (Intern)
Publication date: 2006
Event: Poster session presented at 93rd Annual Meeting International Association for Food Protein, Calgary Alberta, Canada.
Main Research Area: Technical/natural sciences

Bibliographical note
Poster at IAFP yearly meeting, August 2006, Canada
Source: orbit
Source-ID: 225602
Publication: Research › Poster – Annual report year: 2006

Listeria monocytogenes: kommer de ind udefra?

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Porsby, C. H. (Intern), Vogel, B. F. (Intern), Gram, L. (Intern)
Pages: 20-29
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information
Journal: Fisk og Hav
Issue number: 61
ISSN (Print): 0105-9211
Ratings:
ISI indexed (2013): ISI indexed no
ISI indexed (2012): ISI indexed no
ISI indexed (2011): ISI indexed no
Original language: Danish
Links:
http://www.aqua.dtu.dk/Publikationer/Fisk-og-hav.aspx
Source: orbit
Source-ID: 225603
Publication: Research › Journal article – Annual report year: 2006

Microbial food spoilage

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Publication date: 2006

Host publication information
Title of host publication: Handbook of food science
Minimisation of biofilm formation by surfaces coated with fish proteins

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Kingshott, P. (Ekstern), Bernbom, N. (Intern), Meyer, R. (Ekstern), Xu, S. (Ekstern), Besenbacher, F. (Ekstern), Gram, L. (Intern)
Publication date: 2006
Main Research Area: Technical/natural sciences

Bibliographical note
Poster
Source: orbit
Source-ID: 225549
Publication: Research › Book chapter – Annual report year: 2006

One group of genetically similar Listeria monocytogenes strains frequently dominates and persists in several fish slaughter- and smokehouses

Contamination of foods with the human pathogen Listeria monocytogenes may occur during processing, and the purpose of this study was to determine whether genetically similar strains colonize different processing plants or whether specific persistent strains are unique to each processing plant. We hypothesized that specific L. monocytogenes strains may be better adapted to specific environmental niches in the processing environment. L. monocytogenes contamination patterns were identified by the collection of 686 and 267 samples from the processing environments: raw fish and products of four fish smokehouses and four fish slaughterhouses, respectively. Samples were collected both during production and after cleaning and disinfection. Typically, these samplings were separated by 1 to 3 months. Sampling sites were targeted toward areas likely to harbor the bacterium. L. monocytogenes was isolated from 213 samples, and one strain from each positive sample was typed by RAPD (random amplified polymorphic DNA) analysis with four different primers. The 213 strains were divided into 37 RAPD types. One RAPD type was predominant; 86 of 213 strains belonged to this type. This type was found in three smokehouses and two slaughterhouses and was predominant in three of these plants. A subset of 35 strains was also analyzed by amplified fragment length polymorphism typing, which confirmed the genetic similarity of the groups. Moreover, strains of the dominant RAPD type were indistinguishable from strains isolated frequently from smoked fish products 10 years ago. One smokehouse was surveyed for a year and a half, and the dominant RAPD type persisted throughout the survey period and accounted for 94 of 118 isolates. Our study indicates that strains of L. monocytogenes that are genetically very closely related may be especially adapted to colonizing the processing equipment or especially resistant to cleaning and disinfection.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, National Veterinary Institute, Technical University of Denmark
Authors: Wulff, G. (Ekstern), Gram, L. (Intern), Ahrens, P. (Intern), Vogel, B. F. (Intern)
Pages: 4313-4322
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 72
Issue number: 6
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.043 SNIP 1.647
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.054 SNIP 1.602
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.074 SNIP 1.653
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 2.108 SNIP 1.648
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 2.097 SNIP 1.821
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 2.046 SNIP 1.754
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.989 SNIP 1.736
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.957 SNIP 1.758
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 2.3 SNIP 1.732
Original language: English
DOIs:
10.1128/AEM.02288-05
Source: orbit
Source-ID: 227819
Potassium lactate combined with sodium diacetate can inhibit growth of Listeria monocytogenes in vacuum-packed cold-smoked salmon and has no adverse sensory effects

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Section for Aquatic Process and Product Technology
Authors: Vogel, B. F. (Intern), Ng, Y. (Intern), Hyldig, G. (Intern), Mohr, M. (Ekstern), Gram, L. (Intern)
Pages: 2134-2142
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Food Protection
Volume: 69
Issue number: 9
ISSN (Print): 0362-028X
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.63 SJR 0.761 SNIP 0.823
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.68 SJR 0.769 SNIP 0.811
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.954 SNIP 1.024 CiteScore 2.03
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.914 SNIP 0.953 CiteScore 1.94
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.101 SNIP 1.09 CiteScore 2.11
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.083 SNIP 0.981 CiteScore 2.03
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.994 SNIP 0.958 CiteScore 1.96
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.011 SNIP 0.949
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.119 SNIP 1.147
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.064 SNIP 0.996
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.043 SNIP 1.143
Prevalence and survival of Listeria monocytogenes in danish aquatic and fish-processing environments

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Porsby, C. H. (Intern), Vogel, B. F. (Intern), Gram, L. (Intern)
Pages: 2113-2122
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Food Protection
Volume: 69
Issue number: 9
ISSN (Print): 0362-028X
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.63 SJR 0.761 SNIP 0.823
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.68 SJR 0.769 SNIP 0.811
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.954 SNIP 1.024 CiteScore 2.03
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.914 SNIP 0.953 CiteScore 1.94
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.101 SNIP 1.09 CiteScore 2.11
ISI indexed (2013): ISI indexed yes
Probiotic effect in vivo of Roseobacter strain 27-4 against Vibrio anguillarum infections in turbot (Scophthalmus maximus L.) larvae

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Planas, M. (Ekstern), Pérez-Lorenzo, M. (Ekstern), Hjelm, M. (Intern), Gram, L. (Intern), Uгляnes Fiksdal, I. (Ekstern), Bergh, Ø. (Ekstern), Pintado, J. (Ekstern)
Pages: 323-333
Publication date: 2006
Main Research Area: Technical/natural sciences
Production of antibacterial compound and attachment confer a selective advantage to Roseobacter species when colonizing algae

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Bruhn, J. B. (Intern), Gram, L. (Intern), Belas, R. (Ekstern)
Publication date: 2006
Event: Poster session presented at Poster at Gordon Conference on Marine Microorganisms, 26-29 July, Maine, USA.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 227138
Publication: Research - peer-review › Journal article – Annual report year: 2006

Profiling of acylated homoserine lactones of Vibrio anguillarum in vitro and in vivo: influence of growth conditions and serotype

Vibrio anguillarum produces several interlinked acylated homoserine lactone (AHL) signal molecules which may influence expression of its virulence factors such as exoprotease production and biofilm formation. Using both thin layer chromatography and HPLC-high resolution mass spectrometry (HPLC-HRMS), we demonstrate in this study that the same types of AHLs are produced by many serotypes of V. anguillarum and that altering in vitro growth conditions (salinity, temperature and iron concentration) has little influence on the AHL-profile. Most strains produced N-(3-oxodecanoyl)-l-homoserine lactone (3-oxo-C10-HSL) and N-(3-hydroxy-hexanoyl)-l-homoserine lactone (3-hydroxy-C6-HSL) as the dominant molecules. Also, two spots with AHL activity appeared on TLC plates, which could not be identified as AHL structures. Trace amounts of N-(3-hydroxy-octanoyl)-l-homoserine lactone, N-(3-hydroxy-decanoyl)-l-homoserine lactone and N-(3-hydroxy-dodecanoyl)-l-homoserine lactone (3-hydroxy-C8-HSL, 3-hydroxy-C10-HSL and 3-oxo-C12-HSL, respectively) were also detected by HPLC-HRMS analysis from in vitro cultures. Most studies of quorum sensing (QS) systems have been conducted in vitro, the purpose of our study was to determine if the same acylated homoserine lactones were produced in vivo during infection. Extracts from infected fish were purified using several solid phase extraction strategies to allow chromatographic detection and separation by both TLC and HPLC-HRMS. 3-oxo-C10-HSL and 3-hydroxy-C6-HSL were detected in organs from fish dying from vibriosis, however, compared to in vitro culturing where 3-oxo-C10-HSL is the dominant molecule, 3-hydroxy-C6-HSL was prominent in the infected fish tissues. Hence, the balance between the QS systems may be different during infection compared to in vitro cultures. For future studies of QS systems and the possible specific interference with expression of virulence factors, in vitro cultures should be optimised to reflect the in vivo situation.
Shewanella hafniensis sp. nov. and Shewanella morhuae sp. nov., isolated from marine fish of the Baltic Sea

Two novel species belonging to the genus Shewanella are described on the basis of their phenotypic characteristics, phylogenetic analyses of 16S rRNA and gyrB gene sequences and levels of DNA-DNA hybridization. A total of 47 strains belonging to two novel Gram-negative, psychrotolerant, H2S-producing bacterial species were isolated from marine fish (cod and flounder) caught from the Baltic Sea off Denmark. The phenotypic characteristics of strains belonging to group 1 (14 strains) indicated that these represented a non-sucrose-assimilating variant of Shewanella baltica with a DNA G+C content of 47.0 mol%. Strains of group 2 (33 isolates) did not utilize the carbon substrates assimilated by S. baltica except gluconate, N-acetylglucosamine and malate. Their DNA G+C content was 44.0 mol%. Phylogenetic analysis of the 16S rRNA gene sequence data placed the two novel species within the genus Shewanella. Group 1 strains showed greatest sequence similarity to Shewanella putrefaciens ATCC 8071T (99.0 mol%) and with S. baltica NCTC 10375T (98.0 mol%). However, gyrB gene sequence analysis showed these isolates to share only 90.0 mol% sequence similarity with S. putrefaciens ATCC 8071T and 93.0 mol% with S. baltica NCTC 10375T. Similarly, DNA-DNA hybridization experiments revealed DNA relatedness levels of 38% between the group 1 isolates and S. putrefaciens ATCC 8071T and 43% with S. baltica NCTC 10375T. The group 2 strains shared less than 97% 16S rRNA gene sequence similarities with recognized Shewanella species. Comparisons between the two novel species indicated 16S rRNA gene sequence similarity of [~]98%, gyrB gene sequence similarity of [~]89% and DNA-DNA reassociation values of 20-34%. Based on the evidence presented, two novel species, Shewanella hafniensis sp. nov. (type strain P010T=ATCC BAA-1207T=NBRC 100975T) and Shewanella morhuae sp. nov. (type strain U1417T=ATCC...
BAA-1205T=NBRC 100978T), are described.

**General information**
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Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Satomi, M. (Ekstern), Vogel, B. F. (Intern), Gram, L. (Intern), Venkateswaran, K. (Ekstern)
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BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.098 SNIP 1.484 CiteScore 2.74
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Scopus rating (2014): SJR 0.952 SNIP 1.174 CiteScore 2.42
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.996 SNIP 1.564 CiteScore 2.57
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.084 SNIP 1.203 CiteScore 1.96
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.105 SNIP 1.349 CiteScore 2.2
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Scopus rating (2010): SJR 1.056 SNIP 1.195
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.955 SNIP 1.251
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.068 SNIP 1.344
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.103 SNIP 1.585
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Scopus rating (2006): SJR 1.394 SNIP 1.554
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.565 SNIP 1.579
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.932 SNIP 1.858
Scopus rating (2003): SJR 1.809 SNIP 1.829
Achieving continuous improvement in reductions in foodborne listeriosis: A risk-based approach

Listeria monocytogenes is a foodborne pathogen that can cause listeriosis, a severe disease that can lead to septicemia, meningitis, and spontaneous abortion. Ongoing efforts are needed to further reduce the incidence of listeriosis, due to its high mortality rate. The focus of this report is the use of a risk-based approach to identify strategies that will have the greatest impact on reducing foodborne listeriosis. A continuum of risk for listeriosis is observed in the human population, ranging from exquisitely sensitive groups, who are highly immunocompromised and at very high risk of listeriosis, through the normal healthy population younger than 65 years of age, who appear to have a minimal risk for listeriosis. In addition, unique subpopulations may exist; for example, pregnant Latina women appear to have a higher risk of listeriosis than pregnant women of other ethnic groups, most likely due to consumption of contaminated soft cheeses such as queso fresco and queso blanco. The International Life Sciences Institute Risk Science Institute Expert Panel concluded that certain foods pose a high risk for causing listeriosis. High-risk foods have all of the following properties: (1) have the potential for contamination with L. monocytogenes; (2) support the growth of L. monocytogenes to high numbers; (3) are ready to eat; (4) require refrigeration; and (5) are stored for an extended period of time. Control strategies are needed in the food chain from preharvest through consumption to minimize the likelihood that food will become contaminated by L. monocytogenes and to prevent the growth of the organism to high numbers. The Expert Panel identified three main strategies for ensuring continuous improvement in reducing foodborne listeriosis: (1) preventing contamination of foods with L. monocytogenes; (2) preventing growth of L. monocytogenes to high numbers in foods; and (3) science-based education messages targeted to susceptible populations and their caregivers. Of these strategies, the Expert Panel concluded that preventing growth of L. monocytogenes to high numbers would have the greatest impact in reducing cases of listeriosis. Dose-response models predict that the risk of listeriosis increases as the number of organisms in a food increases and can be used as a scientific basis for a target level below which the organism should be reduced to minimize the likelihood of listeriosis in high-risk populations. This requires implementation of effective food safety control measures and ensuring that these control strategies are consistently met. Most effective strategies to control L. monocytogenes in high-risk foods include (1) good manufacturing practices, sanitation standard operating procedures, and hazard analysis critical control point programs to minimize environmental L. monocytogenes contamination and to prevent cross-contamination in processing plants and at retail; (2) an intensive environmental sampling program in plants processing high-risk foods and an effective corrective action plan to reduce the likelihood of contamination of high-risk foods; (3) time and temperature controls throughout the entire distribution and storage period, including establishing acceptable storage times of foods that support growth of L. monocytogenes to high numbers; (4) reformulating foods to prevent or retard the growth of L. monocytogenes; and (5) using postpackaging treatments to destroy L. monocytogenes on products. Science-based education and risk communication strategies aimed at susceptible populations and focused on high-risk foods should be delivered through health care providers or other credible sources of information. Exquisitely sensitive consumers may become ill when exposed to low numbers of L. monocytogenes or other opportunistic pathogens, so reducing the risk to this population could be achieved by maintaining them on restricted low-microbe diets during those periods when they are most severely immunocompromised. High-risk individuals (i.e., the elderly, pregnant women, and most immunocompromised individuals) should be provided with guidance on healthy eating, including specific information on high-risk foods that they should avoid, and strategies to reduce their risk, such as thorough cooking, avoidance of cross-contamination, and short-term refrigerated storage of cooked perishable foods. Those at low risk for listeriosis should receive information on safe food handling practices, preferably starting at a preschool age.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Pages: 1932-1994
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Antilisterial activity of a Carnobacterium piscicola isolated from Brazilian smoked fish (Surubim [Pseudoplatystoma sp.]) and its activity against a persistent strain of Listeria monocytogenes isolated from Surubim
Bias in the Listeria monocytogenes enrichment procedure: Lineage 2 strains outcompete lineage 1 strains in University of Vermont selective enrichments

Listeria monocytogenes can be isolated from a range of food products and may cause food-borne outbreaks or sporadic cases of listeriosis. L. monocytogenes is divided into three genetic lineages and 13 serotypes. Strains of three serotypes (1/2a, 1/2b, and 4b) are associated with most human cases of listeriosis. Of these, strains of serotypes 1/2b and 4b belong to lineage 1, whereas strains of serotype 1/2a and many other strains isolated from foods belong to lineage 2. L. monocytogenes is isolated from foods by selective enrichment procedures and from patients by nonselective methods. The aim of the present study was to investigate if the selective enrichment procedure results in a true representation of the subtypes of L. monocytogenes present in a sample. Eight L. monocytogenes strains (four lineage 1 strains and four lineage 2 strains) and one Listeria innocua strain grew with identical growth rates in the nonselective medium brain heart infusion (BHI), but differed in their growth rate in the selective medium University of Vermont medium I (UVM I). When coinoculated in UVM I, some strains completely outgrew other strains. This outcome was dependent on the lineage of L. monocytogenes rather than the individual growth rate of the strains. When inoculated at identical cell densities in UVM I, L. innocua outcompeted L. monocytogenes lineage 1 strains but not lineage 2 strains. In addition, lineage 2 L. monocytogenes strains outcompeted lineage 1 L. monocytogenes strains in all combinations tested, indicating a bias in strains selected by the enrichment procedures. Bias also occurred when coinoculating two lineage 2 or lineage 1 strains; however, it did not appear to correlate with origin (clinical versus food). Identical coinoculation experiments in BHI suggested that the selective compounds in UVM I and II influenced this bias. The results of the present study demonstrate that the selective procedures used for isolation of L. monocytogenes may not allow a true representation of the types present in foods. Our results could have a significant impact on epidemiological studies, as lineage 1 strains, which are often isolated from clinical cases of listeriosis, may be suppressed during enrichment by other L. monocytogenes lineages present in a food sample.

General information

State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Bruhn, J. B. (Intern), Vogel, B. F. (Intern), Gram, L. (Intern)
Cell-to-cell communication signals are produced by non-bioluminescent strains of Photobacterium phosphoreum

General information
State: Published
Organisations: Department of Systems Biology, Center for Microbial Biotechnology, Center for Biomedical Microbiology
Authors: Flodgaard, L. (Intern), Dalsgaard, P. (Intern), Jensen, P. (Intern), Andersen, J. B. (Intern), Nielsen, K. F. (Intern), Givskov, M. C. (Intern), Gram, L. (Intern)
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Ecology, Inhibitory Activity, and Morphogenesis of a Marine Antagonistic Bacterium Belonging to the Roseobacter Clade

Roseobacter strain 27-4 has been isolated from a turbot larval rearing unit and is capable of reducing mortality in turbot egg yolk sac larvae. Here, we demonstrate that the supernatant of Roseobacter 27-4 is lethal to the larval pathogens Vibrio anguillarum and Vibrio splendidus in a buffer system and inhibited their growth in marine broth. Liquid chromatography (LC) with both UV spectral detection and high-resolution mass spectrometry (HR-MS) identified the known antibacterial compound thiotropocin or its closely related precursor tropodithietic acid in the bioactive fractions. Antibacterial activity correlated with the appearance of a brownish pigment and was only formed in marine broth under static growth conditions. A thick biofilm of multicellular star-shaped aggregated cells formed at the air-liquid interface under static growth conditions. Here, the bioactive compound was the base peak in the LC-UV chromatograms of the extracts where it constituted 15% of the total peak area. Aerated conditions results in 10-fold-higher cell yield, however, cultures were nonpigmented, did not produce antibacterial activity, and grew as single cells. Production of antibacterial compounds may be quorum regulated, and we identified the acylated homoserine lactone (3-hydroxy-decanoyl homoserine lactone) from cultures of Roseobacter 27-4 using LC-HR-MS. The signal molecule was primarily detected in stagnant cultures. Roseobacter 27-4 grew between 10 and 30{degrees}C but died rapidly at 37{degrees}C. Also, the antibacterial compounds was sensitive to heat and was inactivated at 37{degrees}C in less than 2 days and at 25{degrees}C in 8 days. Using Roseobacter 27-4 as a probiotic culture will require that is be established in stagnant or adhered conditions and, due to the temperature sensitivity of the active compound, constant production must be ensured.

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Effect of environmental and physiological factors on the antibacterial activity of Curvularia haloperoxidase system against Escherichia coli.

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Organisations: Center for Systems Microbiology, Department of Systems Biology, Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Hansen, E. H. (Ekstern), Schafer, T. (Ekstern), Molin, S. (Intern), Gram, L. (Intern)
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Growth inhibition of Listeria monocytogenes by a nonbacteriocinogenic Carnobacterium piscicola

Aims: This study elucidates the mechanisms by which a nonbacteriocinogenic Carnobacterium piscicola inhibits growth of Listeria monocytogenes. Methods and Results: Listeria monocytogenes was exposed to live cultures of a bacteriocin-negative variant of C. piscicola A9b in co-culture, in a diffusion chamber system, and to a cell-free supernatant. Suppression of maximum cell density (0-3.5 log units) of L. monocytogenes was proportional to initial levels of C. piscicola (10^3-10^7 CFU ml^(-1)). Cell-to-cell contact was not required to cause inhibition. The cell-free C. piscicola supernatant caused a decrease in L. monocytogenes maximum cell density, which was abolished by glucose addition but not by amino acid, vitamin or mineral addition. The fermentate also gave rise to a longer lag phase and a reduction in growth rate. These effects were independent of glucose and may have been caused by acetate production by C. piscicola. 2D gel-electrophoretic patterns of L. monocytogenes exposed to C. piscicola or to L. monocytogenes fermentate did not differ. Treatment with C. piscicola fermentate resulted in down-regulation (twofold) of genes involved in purine- or pyrimidine metabolism, and up-regulation (twofold) of genes from the regulon for vitamin B-12 biosynthesis and propanediol and ethanolamine utilization. Conclusions: A nonbacteriocinogenic C. piscicola reduced growth of L. monocytogenes partly by glucose depletion. Significance and Impact of the Study: Understanding the mechanism of microbial interaction enhances prediction of growth in mixed communities as well as use of bioprotective principles for food preservation.
Identification of Shewanella baltica as the most important H2S-producing species during iced storage of danish marine fish

Shewanella putrefaciens has been considered the main spoilage bacteria of low-temperature stored marine seafood. However, psychrotrophic Shewanella have been reclassified during recent years, and the purpose of the present study was to determine whether any of the new Shewanella species are important in fish spoilage. More than 500 H2S-producing strains were isolated from iced stored marine fish (cod, plaice, and flounder) caught in the Baltic Sea during winter or summer time. All strains were identified as Shewanella species by phenotypic tests. Different Shewanella species were present on newly caught fish. During the warm summer months the mesophilic human pathogenic S. algae dominated the H2S-producing bacterial population. After iced storage, a shift in the Shewanella species was found, and most of the H2S-producing strains were identified as S. baltica. The 16S rRNA gene sequence analysis confirmed the identification of these two major groups. Several isolates could only be identified to the genus Shewanella level and were separated into two subgroups with low (44%) and high (47%) G+C mol%. The low G+C% group was isolated during winter months, whereas the high G+C% group was isolated on fish caught during summer and only during the first few days of iced storage. Phenotypically, these strains were different from the type strains of S. putrefaciens, S. oneidensis, S. colwelliana, and S. affinis, but the high G+C% group clustered close to S. colwelliana by 16S rRNA gene sequence comparison. The low G+C% group may constitute a new species. S. baltica, and the low G+C% group of Shewanella spp. strains grew well in cod juice at 0\(^\circ\)C, but three high G+C Shewanella spp. were unable to grow at 0\(^\circ\)C. In conclusion, the spoilage reactions of iced Danish marine fish remain unchanged (i.e., trimethylamine-N-oxide reduction and H2S production); however, the main H2S-producing organism was identified as S. baltica.
Involvement of bacterial quorum-sensing signals in spoilage of bean sprouts

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Authors: Rasch, M. (Intern), Andersen, J. B. (Intern), Nielsen, K. F. (Intern), Flodgaard, L. (Intern), Christensen, H. (Ekstern), Givskov, M. C. (Intern), Gram, L. (Intern)
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Web of Science (2012): Indexed yes
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Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
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Nonbioluminescent strains of Photobacterium phosphoreum produce the cell-to-cell communication signal N-(3-Hydroxyoctanoyl)homoserine lactone.

Bioluminescence is a common phenotype in marine bacteria, such as Vibrio and Photobacterium species, and can be quorum regulated by N-acylated homoserine lactones (AHLs). We extracted a molecule that induced a bacterial AHL monitor (Agrobacterium tumefaciens NT1 [pZLR4]) from packed cod fillets, which spoil due to growth of Photobacterium phosphoreum. Interestingly, AHLs were produced by 13 nonbioluminescent strains of P. phosphoreum isolated from the product. Of 177 strains of P. phosphoreum (including 18 isolates from this study), none of 74 bioluminescent strains elicited a reaction in the AHL monitor, whereas 48 of 103 nonbioluminescent strains did produce AHLs. AHLs were also detected in Aeromonas spp., but not in Shewanella strains. Thin-layer chromatographic profiles of cod extracts and P. phosphoreum culture supernatants identified a molecule similar in relative mobility (R-f value) and shape to N-(3-hydroxyoctanoyl)homoserine lactone, and the presence of this molecule in culture supernatants from a nonbioluminescent strain of P. phosphoreum was confirmed by high-performance liquid chromatography-positive electrospray high-resolution mass spectrometry. Bioluminescence (in a non-AHL-producing strain of P. phosphoreum) was strongly up-regulated during growth, whereas AHL production in a nonbioluminescent strain of P. phosphoreum appeared constitutive. AHLs apparently did not influence bioluminescence, as the addition of neither synthetic AHLs nor supernatants delayed or reduced this phenotype in luminescent strains of P. phosphoreum. The phenotypes of nonbioluminescent P. phosphoreum strains regulated by AHLs remains to be elucidated.

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Authors: Flodgaard, L. (Intern), Dalgaard, P. (Intern), Andersen, J. B. (Intern), Nielsen, K. F. (Intern), Givskov, M. C. (Intern), Gram, L. (Intern)

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Production of quorum sensing signal molecules, acylated homoserine lactones, is widespread among fish pathogenic bacteria

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Authors: Bruhn, J. B. (Ekstern), Dalsgaard, I. (Ekstern), Nielsen, K. F. (Intern), Buch, C. (Ekstern), Larsen, J. L. (Ekstern), Gram, L. (Intern)
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Prospects of fish probiotics

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Authors: Gram, L. (Intern), Ringø, E. (Ekstern)
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Quorum sensing signal molecules (acylated homoserine lactones) in Gram-negative fish pathogenic bacteria

The aim of the present study was to investigate the production of quorum sensing signals (specifically acylated homoserine lactones, AHLs) among a selection of strains of Gram-negative fish bacterial pathogens. These signals are involved in the regulation of virulence factors in some human and plant-pathogenic bacteria. A total of 59 strains, representing 9 different fish pathogenic species, were tested against 2 AHL monitor bacteria (Agrobacterium tumefaciens NT1 [pZLR4] and Chromobacterium violaceum CV026) in a well diffusion assay and by thin-layer chromatography (TLC).

Representative samples were further characterized by high performance liquid chromatography-high resolution mass spectrometry (HPLC-HR-MS). AHLs were produced by all strains of Aeromonas salmonicida, Aeromonas hydrophila, Yersinia ruckeri, Vibrio salmonicida, and Vibrio vulnificus. Some strains of atypical Aeromonas salmonicida and Vibrio splendidus were also positive. Aeromonas species produced N-butanoyl homoserine lactone (BHL) and N-hexanoyl
homoserine lactone (HHL) and 1 additional product, whereas N-3-oxo-hexanoyl homoserine lactone (OHHL) and HHL were detected in Vibrio salmonicida. N-3-oxo-octanoyl homoserine lactone (OOHL) and N-3-octanoyl homoserine lactone (OHL) were detected in Y. ruckeii. AHLs were not detected from strains of Photobacterium damselae, Flavobacterium psychrophilum or Moritella viscosa. AHLs were extracted from fish infected with Y. ruckeri but not from fish infected with A. salmonicida. In conclusion, the production of quorum sensing signals, AHLs, is common among the strains that we examined. If the AHL molecules regulate the expression of the virulence phenotype in these bacteria, as shown to occur in some bacterial pathogens, novel disease control measures may be developed by blocking AHL-mediated communication and suppressing virulence.

**General information**

State: Published
Organisations: National Institute of Aquatic Resources, Section for Fish Diseases, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Bruhn, J. B. (Intern), Dalsgaard, I. (Intern), Nielsen, K. (Ekstern), Buchholtz, C. (Ekstern), Larsen, J. (Ekstern), Gram, L. (Intern)
Pages: 43-52
Publication date: 2005
Main Research Area: Technical/natural sciences

**Publication information**

Journal: Diseases of Aquatic Organisms
Volume: 65
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Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.7 SJR 0.675 SNIP 0.95
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.95 SJR 0.893 SNIP 0.92
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.973 SNIP 0.943 CiteScore 1.96
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.895 SNIP 0.889 CiteScore 1.86
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.831 SNIP 0.928 CiteScore 1.77
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.919 SNIP 1.092 CiteScore 2.04
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.12 SNIP 1.164 CiteScore 2.29
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.918 SNIP 0.948
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.897 SNIP 0.985
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.865 SNIP 0.995
Scopus rating (2007): SJR 0.951 SNIP 1.05
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.875 SNIP 0.966
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.909 SNIP 1.033
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.992 SNIP 1.097
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 0.942 SNIP 1.188
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.199 SNIP 1.217
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.35 SNIP 1.193
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.16 SNIP 1.215
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.193 SNIP 1.139
Original language: English
AHL, acylated homoserine lactones, quorum sensing, fish pathogenic bacteria, signal molecules
Source: orbit
Source-ID: 196251
Publication: Research - peer-review › Journal article – Annual report year: 2005

Roseobacter: En stjerne blandt bakterier

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Bruhn, J. B. (Intern), Gram, L. (Intern)
Pages: 46-53
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: Fisk og Hav
Issue number: 59
ISSN (Print): 0105-9211
Ratings:
ISI indexed (2013): ISI indexed no
ISI indexed (2012): ISI indexed no
ISI indexed (2011): ISI indexed no
Original language: Danish
Links:
http://www.aqua.dtu.dk/Publikationer/Fisk-og-hav.aspx
Source: orbit
Source-ID: 225014
Publication: Research › Journal article – Annual report year: 2005

Salmonella isolates with different behavior in adhesion to surfaces studied by DNA micro array assisted analysis

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Pages: 7
Publication date: 2005
Main Research Area: Technical/natural sciences
An inhibitor of bacterial quorum sensing reduces mortalities caused by vibriosis in rainbow trout (Oncorhynchus mykiss, Walbaum)

The fish pathogen Vibrio anguillarum produces quorum sensing signal molecules, N-acyl homoserine lactones (AHLs), which in several Gram-negative human and plant pathogenic bacteria regulate virulence factors. Expression of these factors can be blocked using specific quorum-sensing inhibitors (QSIs). The purpose of this study was to investigate the effect of a QSI, furanone C-30, on mortality of rainbow trout during challenge with V. anguillarum. Addition of 0.01 or 0.1 μM furanone C-30 to rainbow trout infected by cohabitation caused a significant reduction in accumulated mortality from 80-100% in challenge controls to 4-40%, in treated groups. Furanone C-30 had no effect in an immersion challenge system, probably due to a very high water exchange and a rapid dilution of furanone C-30. Growth and Survival of V. anguillarum were not affected by the concentrations of furanone C-30 used in the challenge experiments, thus avoiding selection for resistance. To elucidate the mechanism of disease control by furanone C-30, we determined its effect on the bacterial proteome, motility, and respiration. No effects were seen of furanone C-30 in any of these experiments. Although no cytotoxic effect on HeLa cells were observed, exposure to 1 μM (or higher) concentrations of furanone C-30 had detrimental effects on the rainbow trout. Our results indicate that QSIs can be used in non-antibiotic based control of fish diseases. However, they also underline the need for development of novel, less toxic QSI compounds and the need for understanding the exact mechanism(s) of action.
Assessment and management of seafood safety

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Huss, H. H. (Intern), Ababouch, L. (Ekstern), Gram, L. (Intern)
Number of pages: 230
Publication date: 2004

Elucidation of the antibacterial mechanism of the Curvularia haloperoxidase system by DNA microarray profiling
A novel antimicrobial enzyme system, the Curvularia haloperoxidase system, was examined with the aim of elucidating its mechanism of antibacterial action. Escherichia coli strain MG1655 was stressed with sublethal concentrations of the enzyme system, causing a temporary arrest of growth. The expression of genes altered upon exposure to the Curvularia haloperoxidase system was analyzed by using DNA microarrays. Only a limited number of genes were involved in the response to the Curvularia haloperoxidase system. Among the induced genes were the ibpA and ibpB genes encoding small heat shock proteins, a gene cluster of six genes (b0301-b0306) of unknown function, and finally, cpxP, a member of the Cpx pathway. Knockout mutants were constructed with deletions in b0301-b0306, cpxP, and cpxARP, respectively. Only the mutant lacking cpxARP was significantly more sensitive to the enzyme system than was the wild type. Our results demonstrate that DNA microarray technology cannot be used as the only technique to investigate the mechanisms of action of new antimicrobial compounds. However, by combining DNA microarray analysis with the subsequent creation of knockout mutants, we were able to pinpoint one of the specific responses of E. coli—namely, the Cpx pathway, which is important for managing the stress response from the Curvularia haloperoxidase system.

General information
State: Published
Organisations: Center for Biomedical Microbiology, Department of Systems Biology, Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Hansen, E. (Ekstern), Schembri, M. (Intern), Klemm, P. (Intern), Schafer, T. (Ekstern), Molin, S. (Intern), Gram, L. (Intern)
Pages: 1749-1757
Publication date: 2004
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 70
Issue number: 3
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
High-resolution genotyping of Listeria monocytogenes by fluorescent amplified fragment length polymorphism analysis compared to pulsed-field gel electrophoresis, random amplified polymorphic DNA analysis, ribotyping, and PCR-restriction fragment length polymorphism analysis

The purpose of this study was to evaluate fluorescent amplified fragment length polymorphism (AFLP) analysis for the inter- and intraspecies differentiation of a collection of 96 strains of Listeria monocytogenes and 10 non- L. monocytogenes strains representing six other Listeria species of different origin. The AFLP technique was compared with three other molecular typing methods - ribotyping, random amplified polymorphic DNA analysis (RAPD), and pulsed-field gel electrophoresis (PFGE) - in terms of discriminatory ability. PCR-restriction fragment length polymorphism was included for virulence gene allele characterization. The 96 L. monocytogenes strains were divided into two major clusters by AFLP fingerprinting at a similarity level of 82% in concordance with the results of PFGE, RAPD, and ribotyping. One main cluster consisted of all of the 24 L. monocytogenes hly allele 1 strains, while another main cluster consisted of all of the 72 L. monocytogenes hly allele 2 strains. This indicates the existence of two distinct phylogenetic divisions. Isolates of the remaining Listeria species were not included in the clusters. AFLP, PFGE, and RAPD typing were highly discriminatory methods, with discrimination (D) indices of 0.974, 0.969, and 0.954, respectively, whereas ribotyping had a lower D index of 0.874. AFLP, PFGE, and RAPD typing showed some level of agreement in terms of strain grouping and differentiation. However, all three methods subdivided types of strains grouped by the other methods. Isolates with identical DNA profiles were distributed across the spectrum of origin. It was not possible to associate certain types with specific food sectors or clinical cases, which is indicative of the spread of L. monocytogenes clones across species. Overall, AFLP fingerprinting was suitable for the high-resolution genotyping of L. monocytogenes and had an equally high or higher differentiation power compared to PFGE or RAPD typing.
How to meet an FSO - Control of Listeria monocytogenes in the smoked fish industry

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Pages: 59-67
Publication date: 2004
Main Research Area: Technical/natural sciences

Publication information
Journal: Mitteilungen aus Lebensmitteluntersuchung und Hygiene
Volume: 95
ISSN (Print): 1424-1307
Ratings:
ISI indexed (2013): ISI indexed no
ISI indexed (2012): ISI indexed no
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Original language: English

Bibliographical note
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Source: orbit
Source-ID: 225541
Publication: Research › Journal article – Annual report year: 2004

Overfladeruhed: Giver lave Ra-værdier bedre hygiejne?

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Bagge-Ravn, D. (Ekstern), Kold, J. (Ekstern), Hilbert, L. (Ekstern)
Pages: 16-18
Publication date: 2004
Main Research Area: Technical/natural sciences

Publication information
Journal: Plus proces
Volume: 18
Issue number: 6
ISSN (Print): 0902-5057
Ratings:
BFI (2018): BFI-level 1
BFI (2017): BFI-level 1
BFI (2016): BFI-level 1
BFI (2015): BFI-level 1
BFI (2014): BFI-level 1
BFI (2013): BFI-level 1
Phylogenetic analysis and in situ identification of the intestinal microbial community of rainbow trout (Oncorhynchus mykiss, Walbaum)

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Huber, I. (Ekstern), Spanggaard, B. (Intern), Appel, K. (Ekstern), Rossen, L. (Ekstern), Nielsen, T. (Ekstern), Gram, L. (Intern)
Pages: 117-132
Publication date: 2004
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 96
Issue number: 1
ISSN (Print): 1364-5072
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.41
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.41
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.57
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.56
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.69
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.51
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.55
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Presence of acylated homoserine lactones (AHLs) and AHL-producing bacteria in meat and potential role of AHL in spoilage of meat

Quorum-sensing (QS) signals (N-acyl homoserine lactones [AHLs]) were extracted and detected from five commercially produced vacuum-packed meat samples. Ninety-six AHL-producing bacteria were isolated, and 92 were identified as Enterobacteriaceae. Hafnia alvei was the most commonly identified AHL-producing bacterium. Thin-layer chromatographic profiles of supernatants from six H. alvei isolates and of extracts from spoiling meat revealed that the major AHL species had an R-f value and shape similar to N-3-oxo-hexanoyl homoserine lactone (OHHL). Liquid chromatography-mass spectrometry (MS) (high-resolution MS) analysis confirmed the presence of OHHL in pure cultures of H. alvei. Vacuum-packed meat spoiled at the same rate when inoculated with the H. alvei wild type compared to a corresponding AHL-lacking mutant. Addition of specific QS inhibitors to the AHL-producing H. alvei inoculated in meat or to naturally contaminated meat did not influence the spoilage of vacuum-packed meat. An extracellular protein of approximately 20 kDa produced by the H. alvei wild-type was not produced by the AHL-negative mutant but was restored in the mutant when complemented by OHHL, thus indicating that AHLs do have a regulatory role in H. alvei. Coinoculation of H. alvei wild-type with an AHL-deficient Serratia proteamaculans B5a, in which protease secretion is QS regulated, caused spoilage of liquid milk. By contrast, coinoculation of AHL-negative strains of H. alvei and S. proteamaculans B5a did not cause spoilage. In conclusion, AHL and AHL-producing bacteria are present in vacuum-packed meat during storage and spoilage, but AHL does not appear to influence the spoilage of this particular type of conserved meat. Our data indicate that AHL-producing H. alvei may induce food quality-relevant phenotypes in other bacterial species in the same environment. H. alvei may thus influence spoilage of food products in which Enterobacteriaceae participate in the spoilage process.
Probe - Improved procedures for flatfish larval rearing through the use of probiotic bacteria - Annual progress report year three

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Birkbech, T. (Ekstern), Bergh, Ø. (Ekstern), Gram, L. (Intern), Planas, M. (Ekstern), Skjermo, J. (Ekstern), Riaza, A. (Ekstern)
Number of pages: 68
Publication date: 2004

Publication information
Place of publication: Glasgow
Publisher: University of Glasgow, Institute of Biochemical and Life Science, Division of Infection and Immunity
Main Research Area: Technical/natural sciences

Bibliographical note
Quality of life management of living resources
Source: orbit
Source-ID: 224960
Publication: Research › Report – Annual report year: 2004

Seasonal incidence of autochthonous antagonistic Roseobacter spp. and Vibrionaceae strains in a turbot larva (Scophthalmus maximus) rearing system

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Hjelm, M. (Ekstern), Riaza, A. (Ekstern), Formoso, F. (Ekstern), Melchiorsen, J. (Intern), Gram, L. (Intern)
Pages: 7288-7294
Publication date: 2004
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 70
Issue number: 12
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Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
Selection and identification of autochthonous potential probiotic bacteria from turbot larvae (*Scophthalmus maximus*) rearing units

**General information**

State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Technical University of Denmark
Authors: Hjelm, M. (Ekstern), Bergh, Ø. (Ekstern), Riaza, A. (Ekstern), Nielsen, J. (Ekstern), Melchior森, J. (Intern), Jensen, S. (Ekstern), Duncan, H. (Ekstern), Ahrens, P. (Ekstern), Birkbeck, H. (Ekstern), Gram, L. (Intern)
Pages: 360-372
Publication date: 2004
Main Research Area: Technical/natural sciences

**Publication information**

Journal: Systematic and Applied Microbiology
Volume: 27
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ISSN (Print): 0723-2020
Ratings:
BFI (2018): BFI-level 1
The contribution of bacteriocin to inhibition of Listeria monocytogenes by Carnobacterium piscicola strains in cold-smoked salmon systems

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Nilsson, L. (Intern), Ng, Y. (Intern), Christiansen, J. (Ekstern), Jørgensen, B. (Ekstern), Grótinum, D. (Ekstern), Gram, L. (Intern)
Covalent Attachment of Poly(ethylene glycol) to Surfaces, Critical for Reducing Bacterial Adhesion

The effects of different poly(ethylene glycol) (PEG) attachment strategies upon the adhesion of a Gram-negative bacteria (Pseudomonas sp.) was tested. PEG was covalently immobilized, at the lower critical solution temperature of PEG, to a layer of branched poly(ethyleneimine) (PEI). PEI was both physically adsorbed to a stainless-steel (SS) substrate and covalently immobilized to a carboxylated poly(ethylene terephthalate) (PET-COOH) surface. On both substrates, the PEI and PEG grafting conditions were optimized so that the levels of surface coverage after each step were maximized and were the same on both substrates, as judged by X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry (ToF-SIMS). Also, ToF-SIMS imaging showed that both substrates were chemically uniform after each surface modification step. Thus, the two surfaces differ only in the mode of attachment of PEI to the substrate. In bacterial adhesion experiments, the optimal SS-PEG surface was not capable of reducing the number of adherent Pseudomonas sp. when compared to the controls. However, the PET-PEG surface reduced the level of adhesion by between 2 and 4 orders of magnitude for up to 5 h. ToF-SIMS analysis showed that both PEG surfaces adsorbed low but comparable levels of proteinaceous growth medium components (tryptic soy broth), as indicated by the addition of unique amino acid fragment ions in the spectra, most likely small peptides. Thus, bacterial adhesion was strongly dependent on the PEG immobilization strategy and not on the extent of peptide/protein adsorption. However, for the best PEG surfaces the residual bacterial adhesion is most likely from recognition of the small amount of adsorbed peptides. This highlights the necessity for preventing the adsorption of small biological species that can even penetrate PEG layers of high graft density, in the quest for the ultimate "nonfouling" surface.

General information
State: Published
Organisations: Solar Energy Programme, Risø National Laboratory for Sustainable Energy, National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Kingshott, P. (Intern), Wei, J. (Intern), Bagge, D. (Intern), Gadegaard, N. H. (Intern), Gram, L. (Intern)
Pages: 6912-6921
Publication date: 19 Aug 2003
Main Research Area: Technical/natural sciences

Publication information
Journal: Langmuir
Volume: 19
Issue number: 17
ISSN (Print): 0743-7463
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 4 SJR 1.479 SNIP 1.148
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 3.99 SJR 1.559 SNIP 1.178
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.65 SNIP 1.281 CiteScore 4.33
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.81 SNIP 1.371 CiteScore 4.59
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.896 SNIP 1.343 CiteScore 4.55
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 2.179 SNIP 1.369 CiteScore 4.37
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 2.051 SNIP 1.349 CiteScore 4.42
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
Microbial adhesion and biofilm formation in the food industry

General information
State: Published
Organisations: National Institute of Aquatic Resources
Authors: Bagge, D. (Intern), Gram, L. (Intern), Johansen, C. (Intern)
Number of pages: 118
Publication date: Mar 2003

Publication Information
Publisher: Danish Institute for Fisheries Research, Department of Seafood Research and Technical University of Denmark, BioCentrum
Original language: English
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 305792
Publication: Research - peer-review › Journal article – Annual report year: 2003

Comparison of sodium hypochlorite-based foam and peroxyacetic acid-based fog sanitizing procedures in a salmon smokehouse: Survival of the general microflora and Listeria monocytogenes

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Bagge, D. (Intern), Gardshodn, K. (Ekstern), Gram, L. (Intern), Vogel, B. F. (Intern)
COMPOSITION AND METHOD FOR CONTROLLING MICROBIAL ADHESION AND BIOFILM FORMATION OF SURFACES

The present invention describes how coating of surfaces with an extract, particularly a fish extract, can significantly reduce microbial adhesion, attachment, colonization and biofilm formation on surfaces. Such reduction of microbial adherence, attachment and colonization will be applicable in a large range of areas. The reduced numbers of adhered, attached or colonized microbial organisms is not due to a general growth inhibitory effect and therefore the anti-adhesive effect may not be caused by the presence of antimicrobials (antibiotics or non-antibiotics) in the fish extract.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Vogel, B. F. (Intern), Bagge, D. (Intern)
Publication date: 2003

Publication information
Patent number: WO2003092382
Date: 13/11/2003
Original language: English

Bibliographical note
International application published under the World Intellectual Property Organization (WIPO)
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 228625
Publication: Research › Patent – Annual report year: 2003

Curvularia haloperoxidase: Antimicrobial activity and potential application as a surface disinfectant

A presumed antimicrobial enzyme system, the Curvularia haloperoxidase system, was examined with the aim of evaluating its potential as a sanitizing agent. In the presence of hydrogen peroxide, Curvularia haloperoxidase facilitates the oxidation of halides, such as chloride, bromide, and iodide, to antimicrobial compounds. The Curvularia haloperoxidase system caused several-log-unit reductions in counts of bacteria (Pseudomonas spp., Escherichia coli, Serratia marcescens, Aeromonas salmonicida, Shewanella putrefaciens, Staphylococcus epidermidis, and Listeria monocytogenes), yeasts (Candida sp. and Rhodotorula sp.), and filamentous fungi (Aspergillus niger, Aspergillus tubigensis, Aspergillus versicolor, Fusarium oxysporum, Penicillium chrysogenum, and Penicillium paullii) cultured in suspension. Also, bacteria adhering to the surfaces of contact lenses were killed. The numbers of S. marcescens and S. epidermidis cells adhering to contact lenses were reduced from 4.0 and 4.9 log CFU to 1.2 and 2.7 log CFU, respectively, after treatment with the Curvularia haloperoxidase system. The killing effect of the Curvularia haloperoxidase system was rapid, and 10⁶ CFU of E. coli cells/ml were eliminated within 10 min of treatment. Furthermore, the antimicrobial effect was short lived, causing no antibacterial effect against E. coli 10 min after the system was mixed. Bovine serum albumin (1%) and alginate (1%) inhibited the antimicrobial activity of the Curvularia haloperoxidase system, whereas glucose and Tween 20 did not affect its activity. In conclusion, the Curvularia haloperoxidase system is an effective sanitizing system and has the potential for a vast range of applications, for instance, for disinfection of contact lenses or medical devices.

General information
State: Published
Organisations: Department of Systems Biology, National Institute of Aquatic Resources, Center for Microbial Biotechnology, Center for Systems Microbiology, Section for Aquatic Microbiology and Seafood Hygiene, Novozymes A/S
Pages: 4611-4617
Publication date: 2003
Elucidation of the *Vibrio anguillarum* genetic response to the potential fish probiont *Pseudomonas fluorescens* AH2, using RNA-arbitrarily primed PCR

The antagonistic interaction between a potential fish probiont, *Pseudomonas fluorescens* strain AH2, and its target organism, *Vibrio anguillarum*, was investigated by studying the genetic response of the target organism when it was exposed to the antagonist. We compared the differential display of arbitrarily PCR-amplified gene transcripts in *V. anguillarum* serotype O1 when it was exposed to AH2 supernatant with the display of transcripts in nonexposed control cultures. Growth of *V. anguillarum* was immediately arrested when the organism was exposed to 50% (vol/vol) AH2 supernatant. A total of 10 potentially differentially expressed transcripts were identified. Among these we identified a gene homologous to *rpoS* that was induced in a dose-dependent manner when *V. anguillarum* was cultured in media supplemented with sterile filtered supernatant from AH2. *rpoS* was also induced when growth was arrested with the iron chelator 2,2-dipyridyl. A chromosomal transcript homologous to *vibE* that participates in vibriobactin synthesis in *Vibrio cholerae* was also upregulated during AH2 exposure. This transcript could represent a functionally active gene in *V. anguillarum* involved in biosynthesis of anguibactin or another *V. anguillarum* siderophore. On the pJM1 plasmid of *V. anguillarum* serotype O1, a pseudogene designated open reading frame E (ORF E) that contains a frameshift mutation was previously identified. The gene homologous to *vibE* identified in this study, interestingly, also has significant homology to ORF E on the amino acid level and does not possess the frameshift mutation. Thus, the chromosomally encoded *vibE* homologue could fulfill the role of the inactive plasmid-encoded ORF E pseudogene. Addition of Fe3+ to the system eliminated the growth arrest, and the genes homologous to *rpoS* and *vibE* were not induced. To our knowledge, this is the first study linking *rpoS* induction to iron starvation. Taken together, the results of this study suggest that a major part of the antagonistic property exhibited by strain AH2 is caused by the ability of siderophores in the supernatant to efficiently chelate iron, which results in instant iron deprivation of the pathogen *V. anguillarum* and complete growth arrest.

**General information**

- **State:** Published
- **Organisations:** Biotechnological Institute, Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
- **Authors:** Holmstrøm, K. (Intern), Gram, L. (Intern)
- **Pages:** 831-842
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- **Main Research Area:** Technical/natural sciences

**Publication information**

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  - BFI (2018): BFI-level 1
  - Web of Science (2018): Indexed yes
  - BFI (2017): BFI-level 1
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  - Web of Science (2017): Indexed Yes
  - BFI (2016): BFI-level 1
  - Scopus rating (2016): CiteScore 3.08 SJR 1.943 SNIP 0.877
  - BFI (2015): BFI-level 1
  - Scopus rating (2015): SJR 2.154 SNIP 0.95 CiteScore 2.84
  - Web of Science (2015): Indexed yes
  - BFI (2014): BFI-level 1
  - Scopus rating (2014): SJR 2.084 SNIP 0.931 CiteScore 2.72
  - Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 2.151 SNIP 1.013 CiteScore 3
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 2.125 SNIP 1.085 CiteScore 3.42
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.471 SNIP 1.154 CiteScore 3.83
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.64 SNIP 1.144
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.71 SNIP 1.181
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.639 SNIP 1.088
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.653 SNIP 1.148
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.665 SNIP 1.137
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.66 SNIP 1.164
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 2.497 SNIP 1.188
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 2.71 SNIP 1.148
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 2.412 SNIP 1.111
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 2.661 SNIP 1.182
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 2.728 SNIP 1.157
Web of Science (2000): Indexed yes
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Genetic variability among isolates of Listeria monocytogenes from food products, clinical samples and processing environments, estimated by RAPD-typing

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Martinez, I. (Ekstern), Rørvik, L. (Ekstern), Seppola, M. (Ekstern), Brox, V. (Ekstern), Lassen, J. (Ekstern), Gram, L. (Intern), Vogel, B. F. (Intern)
Pages: 285-297
Publication date: 2003
Main Research Area: Technical/natural sciences

Publication information
Journal: International Journal of Food Microbiology
Influence of food preservation parameters and associated microbiota on production rate, profile and stability of acylated homoserine lactones from food-derived Enterobacteriaceae

Quorum-dependent regulation is mediated by N-acyl-L-homoserine lactones (AHLs) in several Gram-negative bacteria. The production of AHLs has typically been studied using pure bacteria cultures grown in nutrient-rich media at optimal temperature. AHLs are produced in several chill-stored foods by Gram-negative bacteria participating in spoilage. As part of our investigation of the role of AHLs in food quality, we studied the AHL production in two Enterobacteriaceae isolated from cold-smoked salmon under growth conditions typical of those found in cold-smoked salmon. We tested the influence of carbon source (glucose, sucrose, xylose, arabinose, mannose, mannitol and sorbitol), temperature (5 and 25 degreesC), salt concentration (0-7%), pH (6, 7 and 8) and co-existing lactic acid bacteria microflora on the AHL profile and production rate from Serratia proteamaculans strain B5a and Enterobacter agglomerans strain B6a. The two strains produced the same types of AHLs under all conditions tested. The specific AHL concentrations (moles/liter/OD450) changed slightly for both strains at the various conditions. S. proteamaculans strain B5a produced approximately 150 nM/OD450 N-3-oxo-hexanoyl homoserine lactone (OHHL) and E. agglomerans strain B6a produced two major signals, OHHL and N-3-oxo-octanoyl homoserine lactone (OOHL) in a 1:9 ratio with a total concentration of approximately 3000 nM/OD450. The AHL signal molecules became unstable with increasing pH (>7.5). In cold-smoked salmon, pH is approximately 6 and therefore only a low degree of pH-induced turnover is expected to occur in this product. Overall, our study demonstrates that food-derived Enterobacteriaceae produce AHLs of the same type and in the same magnitude when grown under food-relevant conditions as when grown in laboratory media at high temperature. Also, the AHLs produced in foods will be relatively stable and their regulatory impact lasting during storage.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Center for Biomedical Microbiology, Department of Systems Biology, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Flodgaard, L. (Intern), Christensen, A. B. (Intern), Molin, S. (Intern), Givskov, M. C. (Intern), Gram, L. (Intern)
Pages: 145-156
Publication date: 2003
Main Research Area: Technical/natural sciences

Publication information
Journal: International Journal of Food Microbiology
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Ratings:
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.76 SJR 1.366 SNIP 1.436
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BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 3.97 SJR 1.481 SNIP 1.553
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.614 SNIP 1.683 CiteScore 4.02
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.493 SNIP 1.695 CiteScore 3.62
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.612 SNIP 1.841 CiteScore 3.8
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Influence of surface roughness of stainless steel on microbial adhesion and corrosion resistance

Abstract The aim of this study was to evaluate if hygienic characteristics of stainless steel used in the food industry could be improved by smoothing surface roughness from an Ra of 0.9 to 0.01 μm. The adherence of Pseudomonas sp., Listeria monocytogenes and Candida lipolytica to stainless steel was not affected by surface roughness (Ra) ranging from grit 4000 polished stainless steel (Ra <0.01) to ground stainless steel (Ra 0.9). Neither adhesion of Ps. aeruginosa nor its removal by an alkaline commercial cleaner in a flow system was affected by surface roughness. Pitting corrosion resistance was evaluated in a commercial disinfectant and in 1 M NaCl. Electropolished and grit 4000 polished steel proved more corrosion resistant as opposed to grit 80 and 120 polished surfaces. In conclusion, the surface finish did not influence bacterial attachment, colonisation, or removal, but is an important parameter for the corrosion resistance of the surface.

General information
State: Published
Organisations: Department of Management Engineering, Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Hilbert, L. R. (Intern), Bagge-Ravn, D. (Ekstern), Kold, J. (Ekstern), Gram, L. (Intern)
Pages: 175-185
microbial adhesion, bacterial adhesion, stainless steel, roughness Ra, corrosion resistance

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Source: orbit
Source-ID: 63544
Publication: Research - peer-review › Journal article – Annual report year: 2003

Listeria monocytogenes i fiskeindustrien - hvad er problemet?

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Vogel, B. F. (Intern), Gram, L. (Intern)
Pages: 38-47
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Journal: Fisk og Hav
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ISI indexed (2013): ISI indexed no
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http://www.difres.dk/dk/publication/files/22122003$FH56.PDF
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Publication: Research › Journal article – Annual report year: 2003

Probiotika i akvakultur - kan gode bakterier bekæmpe dårlige

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Pages: 9-11
Publication date: 2003
Main Research Area: Technical/natural sciences

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Journal: Dansk kemi
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ISI indexed (2013): ISI indexed no
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Web of Science (2007): Indexed yes
Web of Science (2004): Indexed yes
Original language: Danish
Source: orbit
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Processing of seminal plasma hCAP-18 to ALL-38 by gastricsin - a novel mechanism of generating antimicrobial peptide in vagina

General information
State: Published
Production of acylated homoserine lactones by different serotypes of Vibrio anguillarum both in culture and during infection of rainbow trout

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Buch, C. (Ekstern), Sigh, J. (Ekstern), Nielsen, J. (Ekstern), Larsen, J. (Ekstern), Gram, L. (Intern)
Pages: 338-349
Publication date: 2003
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Journal: Systematic and Applied Microbiology
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 1.309 SJR 1.367 CiteScore 3.66
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.589 SNIP 1.518 CiteScore 3.56
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.397 SNIP 1.307 CiteScore 3.42
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.49 SNIP 1.172 CiteScore 3.37
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.42 SNIP 1.315 CiteScore 3.64
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.823 SNIP 1.185 CiteScore 3.42
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.906 SNIP 1.438 CiteScore 3.75
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Quorum-sensing-directed protein expression in Serratia proteamaculans B5a

N-Acyl-L-homoserine-lactone-producing Serratia species are frequently encountered in spoiling foods of vegetable and protein origin. The role of quorum sensing in the food spoiling properties of these bacteria is currently being investigated. A set of luxR luxI homologous genes encoding a putative quorum sensor was identified in the N-(3-oxo-hexanoyl)-L-homoserine lactone (3-oxo-C6-HSL)-producing Serratia proteamaculans strain B5a. The 3-oxo-C6-HSL synthase SprI showed 79% similarity with Esal from Pantoea stewartii and the putative regulatory protein SprR was 86% similar to the SpnR of Serratia marcescens. Proteome analysis suggested that the presence of at least 39 intracellular proteins was affected by the 3-oxo-C6-HSL-based quorum sensing system. The lipB-encoded secretion system was identified as one target gene of the quorum sensing system. LipB was required for the production of extracellular lipolytic and proteolytic activities, thus rendering the production of food-deterioration-relevant exoenzymes indirectly under the control of quorum sensing. Strain B5a caused quorum-sensing-controlled spoilage of milk. Furthermore, chitinolytic activity was controlled by quorum sensing. This control appeared to be direct and not mediated via LipB. The data presented here demonstrate that quorum-sensing-controlled exoenzymic activities affect food quality.
Stainless steel modified with poly(ethylene glycol) can prevent protein adsorption but not bacterial adhesion

The surface of AISI 316 grade stainless steel (SS) was modified with a layer of poly(ethylene glycol) (PEG) (molecular weight 5000) with the aim of preventing protein adsorption and bacterial adhesion. Model SS substrates were first modified to introduce a very high density of reactive amine groups by the adsorption of branched poly(ethylenimine) (PEI) from water. Methoxy-terminated aldehyde-poly(ethylene glycol) (M-PEG-CHO) was then grafted onto the PEI layers using reductive amination at the lower critical solution temperature (LCST) of the PEG in order to optimize the graft density of the linear PEG chains. The chemical composition and uniformity of the surfaces were determined using X-ray photoelectron spectroscopy (XPS) and time-of-flight static secondary ion mass spectrometry (ToF-SSIMS) in the imaging mode. The effects of PEI concentration and different substrate pre-cleaning methods on the structure and stability of the final PEG layer was examined. Piranha solution proved to be the most effective method for removing adventitious hydrocarbon contamination, compared to cleaning with ultrasonication in organic solvents, and was the SS substrate that produced the most stable and thickest PEI layer. The surface density of PEI was shown to increase with increasing PEI concentration (up to 30 mg/ml), as determined from XPS measurements, and subsequently produced the PEG layer with the highest density of attached chains. In model experiments using beta-lactoglobulin no protein adsorption was detected on the optimized PEG surface as determined by XPS and ToF-SSIMS analysis. However, neither the adhesion of a Gram-negative (Pseudomonas sp.) nor a Gram-positive (Listeria monocytogenes) bacterium was affected by the coating as equal numbers adhered to all surfaces tested. Our results show that preventing protein adsorption is not a prerequisite stopping bacterial adhesion, and that other mechanisms most likely play a role. (C) 2003 Elsevier B.V. All rights reserved.
The microbial ecology of processing equipment in different fish industries - analysis of the microflora during processing and following cleaning and disinfection

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Bagge, D. (Intern), Ng, Y. (Intern), Hjelm, M. (Ekstern), Christiansen, J. (Ekstern), Johansen, C. (Ekstern), Gram, L. (Intern)
Pages: 239-250
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Main Research Area: Technical/natural sciences

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Scopus rating (2017): CiteScore 3.76 SJR 1.366 SNIP 1.436
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 3.97 SJR 1.481 SNIP 1.553
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.614 SNIP 1.683 CiteScore 4.02
Comparison of adhesion of the food spoilage bacterium Shewanella putrefaciens to stainless steel and silver surfaces

The aim of this study is to compare the number of attached bacteria, Shewanella putrefaciens, on stainless steel with different silver surfaces. Thus evaluating if silver surfaces could contribute to a higher hygienic status in the food industry. Bacterial adhesion to three types of silver surfaces (new silver, tarnished silver and sulphide treated silver) was compared to adhesion to stainless steel (AISI 316). Numbers of attached bacteria (cfu cm-2) were estimated using the Malthus indirect conductance method. A lower number of attached bacteria were measured on new silver surfaces compared to stainless steel for samples taken after 24 hours. However this was not significant (P > 0.05). The numbers of attached bacteria were consistently lower when tarnished silver surfaces were compared to stainless steel and some, but not all, experiments showed statistically significant. A difference of more than one log unit in bacterial numbers on the two types
of materials was observed, but for most samples the difference was within one log unit. Treating new silver with sulphide
to try to reproduce a tarnished silver surface did not result in a similar lowering of adhering cells when compared to steel
\((P > 0.05)\). To conclude new or tarnished silver surfaces caused a slight reduction in numbers of attached bacteria,
however, the difference was only sometimes statistically different.

**General information**

**State:** Published

**Organisations:** Department of Management Engineering, National Food Institute, Division of Industrial Food Research,
National Institute of Aquatic Resources

**Authors:** Hjelm, M. (Intern), Hilbert, L. R. (Intern), Møller, P. (Intern), Gram, L. (Intern)

**Pages:** 903-911

**Publication date:** 2002

**Main Research Area:** Technical/natural sciences

**Publication information**

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**Volume:** 92

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- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 1
- Scopus rating (2017): CiteScore 2.41
- Web of Science (2017): Indexed Yes
- BFI (2016): BFI-level 1
- Scopus rating (2016): CiteScore 2.41
- BFI (2015): BFI-level 1
- Scopus rating (2015): CiteScore 2.57
- Web of Science (2015): Indexed yes
- BFI (2014): BFI-level 1
- Scopus rating (2014): CiteScore 2.56
- Web of Science (2014): Indexed yes
- BFI (2013): BFI-level 1
- Scopus rating (2013): CiteScore 2.69
- ISI indexed (2013): ISI indexed yes
- Web of Science (2013): Indexed yes
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- Scopus rating (2012): CiteScore 2.51
- ISI indexed (2012): ISI indexed yes
- Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 1
- Scopus rating (2011): CiteScore 2.55
- ISI indexed (2011): ISI indexed yes
- Web of Science (2011): Indexed yes
- BFI (2010): BFI-level 1
- Web of Science (2010): Indexed yes
- BFI (2009): BFI-level 1
- Web of Science (2009): Indexed yes
- BFI (2008): BFI-level 1
- Web of Science (2008): Indexed yes
- Web of Science (2007): Indexed yes
- Web of Science (2006): Indexed yes
- Web of Science (2005): Indexed yes
- Web of Science (2004): Indexed yes
- Web of Science (2003): Indexed yes
- Web of Science (2002): Indexed yes
Fermentation and microflora of plaa-som, a Thai fermented fish product prepared with different salt concentrations

Plaa-som is a Thai fermented fish product prepared from snakehead fish, salt, palm syrup and sometimes roasted rice. We studied the effects of different salt concentrations on decrease in pH and on microflora composition during fermentation. Two low-salt batches were prepared, containing 6% and 7% salt (w/w) as well as two high-salt batches, containing 9% and 11% salt. pH decreased rapidly from 6 to 4.5 in low-salt batches, whereas in high-salt batches, a slow or no decrease in pH was found. Lactic acid bacteria (LAB) and yeasts were isolated as the dominant microorganisms during fermentation. LAB counts increased to 108-109 cfu g⁻¹ and yeast counts to 107-5 x 10⁷ cfu g⁻¹ in all batches, except in the 11% salt batch, where counts were 1-2 log lower. Phenotypic tests, ITS-PCR, carbohydrate fermentations and 16S rRNA gene sequencing identified LAB isolates as Pediococcus pentosaceus, Lactobacillus alimentarius/farciiminis, Weisella confusa, L. plantarum and Lactococcus garviae. The latter species was only isolated from high-salt batches. Phenotypic characteristics, ITS-PCR and carbohydrate assimilation identified 95% of the yeasts as Zygosaccharomyces rouxii. It is concluded that the fermentation of plaa-som is delayed by a salt-level of 9% due to an inhibition of LAB growth. The growth of Z. rouxii has no influence on the fermentation rate, but may contribute positively to the flavour development of the product.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Paludan-Müller, C. (Intern), Madsen, M. (Ekstern), Sophanodora, P. (Ekstern), Gram, L. (Intern), Møller, P. (Ekstern)
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Main Research Area: Technical/natural sciences

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BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.76 SJR 1.366 SNIP 1.436
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 3.97 SJR 1.481 SNIP 1.553
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.614 SNIP 1.683 CiteScore 4.02
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.493 SNIP 1.695 CiteScore 3.62
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.612 SNIP 1.841 CiteScore 3.8
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.603 SNIP 1.705 CiteScore 3.7
Fish spoilage bacteria - problems and solutions

Microorganisms are the major cause of spoilage of most seafood products. However, only a few members of the microbial community, the specific spoilage organisms (SSOs), give rise to the offensive off-flavours associated with seafood spoilage. Combining microbial ecology, molecular techniques, analytical chemistry, sensory analysis and mathematical modelling allows us to characterise the SSOs and to develop methods to determine, predict and extend the shelf life of products.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Dalgaard, P. (Intern)
Pages: 262-266
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
Journal: Current Opinion in Biotechnology
Volume: 13
Issue number: 3
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Food spoilage - interactions between food spoilage bacteria

Food spoilage is a complex process and excessive amounts of foods are lost due to microbial spoilage even with modern day preservation techniques. Despite the heterogeneity in raw materials and processing conditions, the microflora that develops during storage and in spoiling foods can be predicted based on knowledge of the origin of the food, the substrate base and a few central preservation parameters such as temperature, atmosphere, $a(w)$ and pH. Based on such knowledge, more detailed sensory, chemical and microbiological analysis can be carried out on the individual products to determine the actual specific spoilage organism. Whilst the chemical and physical parameters are the main determining factors for selection of spoilage microorganisms, a level of refinement may be found in some products in which the interactive behavior of microorganisms may contribute to their growth and/or spoilage activity. This review gives three such examples. We describe the competitive advantage of Pseudomonas spp. due to the production of iron-chelating siderophores, the generation of substrates for spoilage reactions by one organism from another microorganism (so-called metabiosis) and the up-regulation of phenotypes potentially involved in spoilage through cell-to-cell communication. In particular, we report for the first time the widespread occurrence of N-acyl homoserine lactones (AHL) in stored and spoiling fresh foods and we discuss the potential implications for spoilage and food preservation.
Scopus rating (2010): SJR 1.61 SNIP 1.666
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.475 SNIP 1.539
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 1.442 SNIP 1.509
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.349 SNIP 1.692
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.541 SNIP 1.788
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.511 SNIP 1.834
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.502 SNIP 1.638
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.233 SNIP 1.612
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.226 SNIP 1.289
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.031 SNIP 1.506
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.043 SNIP 1.306
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.071 SNIP 1.2

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Publication: Research - peer-review › Conference article – Annual report year: 2002

Genotypic and phenotypic characterization of garlic-fermenting lactic acid bacteria isolated from som-fak, a Thai low-salt fermented fish product

AIMS: To evaluate the importance of garlic for fermentation of a Thai fish product, and to differentiate among garlic-/inulin-fermenting lactic acid bacteria (LAB) at strain level. METHODS AND RESULTS: Som-fak was prepared by fermentation of a mixture of fish, salt, rice, sucrose and garlic. pH decreased to 4.5 in 2 days, but omitting garlic resulted in a lack of acidification. LAB were predominant and approximately one third of 234 isolated strains fermented garlic and inulin (the carbohydrate reserve in garlic). These strains were identified as Lactobacillus pentosus and Lact. plantarum. Randomly Amplified Polymorphic DNA (RAPD) analysis revealed one major RAPD type (29 strains) isolated from all stages of fermentation. CONCLUSION: Garlic was essential for acidification of som-fak and garlic-fermenting strains constituted a significant, homogeneous part of the LAB flora. SIGNIFICANCE AND IMPACT OF THE STUDY: The present study indicates the role of fructans (garlic/inulin) as carbohydrate sources for LAB. Fructan fermenters may have several biotechnological applications, for example, as probiotics.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Paludan-Müller, C. (Intern), Valyasevi, R. (Ekstern), Huss, H. H. (Intern), Gram, L. (Intern)
Pages: 307-314
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 92
Issue number: 2
Improving the control of pathogens in fish products

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Nilsson, L. (Intern), Gram, L. (Intern)
Pages: 54-84
Publication date: 2002

Host publication information
Microbiological testing in food safety management

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Tompkin, R. (Ekstern), Gram, L. (Intern), Roberts, T. (Ekstern), Buchanan, R. (Ekstern), van Schotjorst, M. (Ekstern), Dahms, S. (Ekstern), Cole, M. (Ekstern)
Number of pages: 362
Pages: 1-362
Publication date: 2002

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Title of host publication: Microorganisms in foods
Place of publication: New York
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Editors: Tompkin, R., Roberts, T., von Schothorst, M., Cole, M., Gram, L., Buchanan, R., Dahms, S.
ISBN (Print): 0306472627
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225876
Publication: Research - peer-review › Book chapter – Annual report year: 2002

Possible quorum sensing in marine snow bacteria: Production of acylated homoserine lactones by Roseobacter strains isolated from marine snow

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Section for Ocean Ecology and Climate
Authors: Gram, L. (Intern), Grossart, H. (Ekstern), Schlingloff, A. (Ekstern), Kiørboe, T. (Intern)
Pages: 4111-4116
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
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BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Purification and characterisation of an extracellular fructan beta-fructosidase from a Lactobacillus pentosus strain isolated from fermented fish

Lactobacillus pentosus B235, which was isolated as part of the dominant microflora from a garlic containing fermented fish product, was grown in a chemically defined medium with inulin as the sole carbohydrate source. An extracellular fructan beta-fructosidase was purified to homogeneity from the bacterial supernatant by ultrafiltration, anion exchange chromatography and hydrophobic interaction chromatography. The molecular weight of the enzyme was estimated to be approximately 126 kDa by gel filtration and by SDS-PAGE. The purified enzyme had the highest activity for levan (a beta(2--6)-linked fructan), but also hydrolysed garlic extract, (a beta(2--1)-linked fructan with beta(2--6)-linked fructosyl sidechains), 1,1,1-kestose, 1,1-kestose, 1-kestose, inulin (beta(2--1)-linked fructans) and Sucrose at 60, 45, 39, 12, 9 and 3%, respectively, of the activity observed for levan. Melezitose, raffinose and srachyose were not hydrolysed by the enzyme. The fructan P-fructosidase was inhibited by p-chloromercuribenzoate, EDTA, Fe2+, Cu2+, Zn2+ and Co2+, whereas Mn2+ and Cu2+ had no effect. The sequence of the first 20 N-terminal amino acids was: Ala-Thr-Ser-Ala-Ser-
Ser-Ser-Gln-Ile-Ser-Gln-Ast-Asn-Thr-Gln-Thr-Ser-Asp-Val-Val. The enzyme had temperature and pH optima at 25 degreesC and 5.5, respectively. At concentrations of up to 12%, NaCl no adverse effect on the enzyme activity was observed.
Role of acetate in production of an autoinducible Class IIa Bacteriocin in Carnobacterium piscicola A9b

Carnobacterium piscicola strain A9b isolated from cold smoked salmon inhibits growth of the food-borne pathogen Listeria monocytogenes partly due to the production of a proteinaceous compound (L. Nilsson, L. Gram, and H. H. Huss. J. Food Prot. 62:336-342, 1999). The purpose of the present study was to purify the compound and describe factors affecting its production, with particular emphasis on food-relevant factors. Amino acid sequencing showed that the compound is a class IIa bacteriocin with an N-terminal amino acid sequence identical to that of carnobacteriocin B2. The production of the bacteriocin was autoinducible, and the threshold level for induction was 9.6 x 10(-10) M. We also report, for the first time, that acetate acts as an induction factor, with a threshold concentration of 0.3 to 12 mM. Acetate could not act as an inducer during the late exponential phase of C. piscicola A9b. The induction of bacteriocin production showed a dose-dependent relationship at acetate concentrations of up to 10 to 20 mM (depending on the growth medium) and at a concentration of 1.9 x 10(-8) M for the bacteriocin itself; a saturation level of bacteriocin specific activity was reached at these concentrations of induction factors. The combined use of both inducers did not enhance the saturation level of bacteriocin production compared to that seen with the use of each inducer alone. Increasing NaCl and glucose concentrations negatively influenced the efficiency of acetate as an induction factor. Based on the results, carnobacteriocin B2 was used as an induction factor to manipulate the production of bacteriocin in cold smoked salmon juice and thus improve the ability to inhibit L. monocytogenes.

General information
State: Published
Organisations: Department of Biotechnology, National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Nilsson, L. (Intern), Nielsen, M. K. (Intern), Ng, Y. (Intern), Gram, L. (Intern)
Pages: 2251-2260
Publication date: 2002
Main Research Area: Technical/natural sciences

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Journal: Applied and Environmental Microbiology
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Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Sensory, chemical and bacteriological changes during storage of iced squid (Todaropsis eblanae)

Aims: To relate sensory shelf-life of iced whole and gutted squid to bacterial growth and chemical changes. Methods and Results: Cooked mantles from whole and gutted individuals were rejected after 10 and 12 days of storage, respectively, due to ammonia off-odours. Rate of production of both ammonia and trimethylamine was highest in the whole lot. Agmatine, which was only present in trace amounts in freshly-caught squid, increased rapidly in both lots. The main microflora at the time of sensory rejection of iced whole squid included Gram-negative, motile and non-fermentative rods, which were psychrophilic and had a requirement for NaCl. 16S rDNA sequence analyses identified the strains as belonging to the genus Pseudoalteromonas. Shewanella putrefaciens, Pseudoalteromonas sp. and Pseudomonas sp. dominated in spoiled gutted squid. Identification of strains from the stomach and digestive gland of recently-captured squid showed that the main flora consisted of Photobacterium phosphoreum. Conclusions: Spoilage of iced squid is likely to result from a combination of autolytic and bacterial changes. Agmatine seems to be an excellent freshness indicator. Photobacterium phosphoreum may contribute to spoilage through activity in the digestive gland, followed by diffusion of volatile compounds and amines to the mantle. Significance and Impact of the Study: Due to the psychrophilic nature of P. phosphoreum and Pseudoalteromonas sp., spread-plating and low temperature incubation are recommended for bacteriological evaluation of iced squid.
Diversity of Listeria monocytogenes isolates from cold-smoked salmon produced in different smokehouses as assessed by Random Amplified Polymorphic DNA analyses

One hundred and forty-eight Listeria monocytogenes isolates originating from vacuum packed cold-smoked salmon produced in 10 different Danish smokehouses were compared by Random Amplified Polymorphic DNA (RAPD) profiling. A total of 16 different reproducible RAPD profiles were obtained using a standardised RAPD analysis by four primers separately. The grouping of the 148 strains was exactly the same for the four primers used. For a sub-set of 20 strains typed by Pulsed Field Gel Electrophoresis (PFGE), only one strain was allocated into a different group as compared to the grouping by RAPD typing. Different RAPD types dominated in products from different smokehouses. Some identical RAPD types were isolated in several smokehouses. In each of four smokehouses, one particular RAPD type could he repeatedly isolated from products. Each smokehouse/product carried its own specific RAPD type and this may indicate a possible persistence of closely related strains of L. monocytogenes in smokehouses. (C) 2001 Elsevier Science B.V. All rights reserved
Elucidation of Listeria monocytogenes contamination routes in cold-smoked salmon processing plants detected by DNA-based typing methods

The contamination routes of Listeria monocytogenes in cold-smoked salmon processing plants were investigated by analyzing 3,585 samples from products (produced in 1995, 1996, 1998, and 1999) and processing environments (samples obtained in 1998 and 1999) of two Danish smokehouses. The level of product contamination in plant I varied from 31 to 85%, and no L. monocytogenes was found on raw fish (30 fish were sampled). In plant II, the levels of both raw fish and product contamination varied from 0 to 25% (16 of 185 raw fish samples and 59 of 1,000 product samples were positive for L. monocytogenes). A total of 429 strains of L. monocytogenes were subsequently compared by random amplified polymorphic DNA (RAPD) profiling, and 55 different RAPD types were found. The RAPD types detected on the products were identical to types found on the processing equipment and in the processing environment, suggesting that contamination of the final product (cold-smoked salmon) in both plants (but primarily in plant I) was due to contamination during processing rather than to contamination from raw fish. However, the possibility that raw fish was an important source of contamination of the processing equipment and environment could not be excluded. Contamination of the product occurred in specific areas (the brining and slicing areas). In plant I, the same RAPD type (RAPD type 12) was
found over a 4-year period, indicating that an established in-house flora persisted and was not eliminated by routine hygienic procedures. In plant II, where the prevalence of L. monocytogenes was much lower, no RAPD type persisted over long periods of time, and several different L. monocytogenes RAPD types were isolated. This indicates that persistent strains may be avoided by rigorous cleaning and sanitation; however, due to the ubiquitous nature of the organism, sporadic contamination occurred. A subset of strains was also typed by using pulsed-field gel electrophoresis and amplified fragment length polymorphism profiling, and these methods confirmed the type division obtained by RAPD profiling.

**General information**

State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, National Veterinary Institute
Authors: Vogel, B. F. (Intern), Huss, H. H. (Intern), Ojeniyi, B. (Ekstern), Ahrens, P. (Intern), Gram, L. (Intern)
Pages: 2586-2595
Publication date: 2001
Main Research Area: Technical/natural sciences

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BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Factors affecting production of an antilisterial bacteriocin by Carnobacterium piscicola strain A9b in laboratory media and model fish systems

Aims: To investigate factors influencing bacteriocin production and bacteriocin stability of the bioprotective culture Carnobacterium piscicola strain A9b. Methods and Results: Maximum activity was obtained in MRS7 broth (MRS adjusted to pH 7.2), with or without glucose. No bacteriocin was produced in APT broth when a low inoculum level (0.001%) was used. In contrast, inoculum level did not influence bacteriocin production in BHI and MRS7 without glucose. Bacteriocin production in APT was induced by the presence of an extracellular compound present in the sterile, filtered, cell-free supernatant fluid of a stationary-phase culture. Increasing concentrations of NaCl (2-7%) reduced bacteriocin production and maximum cell density of C. piscicola A9b when grown in cooked fish juice at VC. Conclusions: Media composition, inoculum level and sodium chloride concentration affected production. Significance and Impact of the Study: The influence of NaCl on bacteriocin production may negate the inhibitory effect of C. piscicola A9b against Listeria monocytogenes in salty foods.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Himelbloom, B. (Ekstern), Nilsson, L. (Intern), Gram, L. (Intern)
Pages: 506-513
Publication date: 2001
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 91
Issue number: 3
ISSN (Print): 1364-5072
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.41
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.41
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.57
Web of Science (2015): Indexed yes
In vitro antagonism of the probiont Pseudomonas fluorescens strain AH2 against Aeromonas salmonicida does not confer protection of salmon against furunculosis

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Løvold, T. (Ekstern), Nielsen, J. (Ekstern), MelchiorSEN, J. (Intern), Spanggaard, B. (Intern)
Pages: 1-11
Publication date: 2001
Main Research Area: Technical/natural sciences

Publication information
Journal: Aquaculture
Volume: 199
Issue number: 1-2
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Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.05 SJR 1.152 SNIP 1.58
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.75 SJR 1.122 SNIP 1.51
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.107 SNIP 1.256 CiteScore 2.12
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.01 SNIP 1.33 CiteScore 2.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.151 SNIP 1.293 CiteScore 2.18
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.222 SNIP 1.485 CiteScore 2.32
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.281 SNIP 1.536 CiteScore 2.39
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.161 SNIP 1.39
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.949 SNIP 1.27
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 0.917 SNIP 1.165
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.033 SNIP 1.315
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.021 SNIP 1.695
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.937 SNIP 1.238
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.072 SNIP 1.626
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.151 SNIP 1.909
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.969 SNIP 1.458
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.062 SNIP 1.319
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 0.981 SNIP 1.114
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.073 SNIP 1.24
Original language: English
Source: orbit
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Publication: Research - peer-review › Journal article – Annual report year: 2001

Kommunikation hos bakterier - i mad og mennesker

General information
Methods for detecting acylated homoserine lactones produced by Gram-negative bacteria and their application in studies of AHL-production kinetics

In the process of evaluating the role of acylated homoserine lactones (AHLs) in food-spoiling Gram-negative bacteria, we have combined a range of bacterial AHL monitor systems to determine the AHL-profile and the kinetics of AHL-production. AHL production from 148 strains of Enterobacteriaceae isolated from foods was tested using Escherichia coli pSB403 (LuxR), Agrobacterium tumefaciens A136 (TraR) and both induction and inhibition of Chromobacterium violaceum CV026 (CviR). All strains except one was found to produce AHL(s). In no case could a single monitor system identify more than 64% of the Enterobacteriaceae as AHL-producers, showing that the simultaneous use of monitor strains is required in the process of screening bacterial populations for AHL-production. AHLs from 20 selected strains were profiled by thin layer chromatography. Most strains produced more than one AHL with 3-N-oxo-hexanoyl homoserine lactone being the most prominent. It was found that the simultaneous use of monitor strains in the top-layer was necessary for the detection of (presumably) all the AHLs. An agar well-diffusion assay based on A. tumefaciens pDZLR4 was used for quantifying AHLs from bacterial supernatants and enabled an assessment of the kinetics of AHL-production of 3 strains (Serratia proteamaculans strain B5a, Erwinia carotovora ATCC 39048 and V. fischeri strain MJ-1). As expected, the production of AHL (OHHL) and luminescence in Vibrio fischeri strain MJ-1 increased faster than growth indicating up-regulation of the AHL regulated phenotype and auto-induction of AHL production. In contrast, production kinetics of AHL (OHHL) in the two Enterobacteriaceae indicated lack of auto-induction.

General information

State: Published
Organisations: Center for Biomedical Microbiology, Department of Systems Biology, Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Ravn, L. (Ekstern), Christensen, A. B. (Intern), Molin, S. (Intern), Givskov, M. C. (Intern), Gram, L. (Intern)
Pages: 239-251
Publication date: 2001
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Microbiological Methods
Volume: 44
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.95 SJR 0.696 SNIP 0.781
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.05 SJR 0.742 SNIP 0.817
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.819 SNIP 0.86 CiteScore 2.04
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Potential hazards in cold-smoked fish: Clostridium botulinum type E

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Pages: 1082-1087
Publication date: 2001
Main Research Area: Technical/natural sciences

Publication Information
Journal: Journal of Food Science
Potential hazards in cold-smoked fish: Listeria monocytogenes

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Pages: 1072-1081
Publication date: 2001
Main Research Area: Technical/natural sciences

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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.978 SJR 0.827 CiteScore 2.06
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.796 SNIP 0.992 CiteScore 1.92
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.829 SNIP 0.982 CiteScore 1.97
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.93 SNIP 1.112 CiteScore 2.07
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.019 SNIP 1.077 CiteScore 2.24
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.961 SNIP 1.08 CiteScore 1.98
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.936 SNIP 1.051 CiteScore 1.9
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.055 SNIP 1.114
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.982 SNIP 1.012
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.884 SNIP 0.922
Scopus rating (2007): SJR 0.703 SNIP 0.968
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.734 SNIP 0.897
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.685 SNIP 1.016
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.761 SNIP 1.021
Web of Science (2004): Indexed yes
Probiotika i akvakultur - en strategi til forebyggelse af fiskesygdom

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Spanggaard, B. (Intern), Gram, L. (Intern)
Pages: 44-48
Publication date: 2001
Main Research Area: Technical/natural sciences

Shewanella putrefaciens adhesion and biofilm formation on food processing surfaces

Laboratory model systems were developed for studying Shewanella putrefaciens adhesion and biofilm formation under batch and flow conditions. S. putrefaciens plays a major role in food spoilage and may cause microbially induced corrosion on steel surfaces. S. putrefaciens bacteria suspended in buffer adhered readily to stainless steel surfaces. Maximum numbers of adherent bacteria per square centimeter were reached in 8 h at 25 degreesC and reflected the cell density in suspension. Numbers of adhering bacteria from a suspension containing 10(8) CFU/ml were much lower in a laminar flow system (modified Robbins device) (reaching 10(2) CFU/cm(2)) than in a batch system (reaching 10(7) CFU/cm(2)), and maximum numbers were reached after 24 h. When nutrients were supplied, S. putrefaciens grew in biofilms with layers of bacteria. The rate of biofilm formation and the thickness of the film were not dependent on the availability of carbohydrate (lactate or glucose) or on iron starvation. The number of S. putrefaciens bacteria on the surface was partly influenced by the presence of other bacteria (Pseudomonas fluorescens) which reduced the numbers of S. putrefaciens bacteria in the biofilm. Numbers of bacteria on the surface must be quantified to evaluate the influence of environmental factors on adhesion and biofilm formation. We used a combination of fluorescence microscopy (4',6'-diamidino-2-phenylindole staining and in situ hybridization, for mixed-culture studies), ultrasonic removal of bacteria from surfaces, and indirect conductometry and found this combination sufficient to quantify bacteria on surfaces

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Bagge, D. (Intern), Hjelm, M. (Ekstern), Johansen, C. (Ekstern), Huber, I. (Ekstern), Gram, L. (Intern)
Pages: 2319-2325
Publication date: 2001
Main Research Area: Technical/natural sciences
The probiotic potential against vibriosis of the indigenous microflora of rainbow trout

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Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Spanggaard, B. (Intern), Huber, I. (Ekstern), Nielsen, J. (Ekstern), Sick, E. (Ekstern), Bressen Pipper, C. (Ekstern), Martinussen, T. (Ekstern), Slierendrecht, W. (Ekstern), Gram, L. (Intern)
Pages: 755-765
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Main Research Area: Technical/natural sciences

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Web of Science (2016): Indexed yes
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Scopus rating (2015): SJR 3.02 SNIP 1.571 CiteScore 5.61
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.862 SNIP 1.599 CiteScore 5.6
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.273 SNIP 1.823 CiteScore 6.37
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.165 SNIP 1.639 CiteScore 5.94
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 3.368 SNIP 1.7 CiteScore 6.1
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 2.775 SNIP 1.551
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 2.502 SNIP 1.378
Biokonservering af koldrøget laks - hvordan man forhinder vækst af Listeria i fiskeprodukter

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Nilsson, L. (Intern), Gram, L. (Intern)
Pages: 2-6
Publication date: 2000
Main Research Area: Technical/natural sciences

Publication Information
Journal: Fisk og Hav
Issue number: 51
ISSN (Print): 0105-9211
Ratings:
ISI indexed (2013): ISI indexed no
ISI indexed (2012): ISI indexed no
ISI indexed (2011): ISI indexed no
Original language: Danish
Links:
Source: orbit
Source-ID: 226941
Publication: Research - peer-review › Journal article – Annual report year: 2001

Carbon dioxide and nisin act synergistically on Listeria monocytogenes
This paper examines the synergistic action of carbon dioxide and nisin on Listeria monocytogenes Scott A wild-type and nisin-resistant (Nis(r)) cells grown in broth at 4 degrees C. Carbon dioxide extended the lag phase and decreased the specific growth rate of both strains, but to a greater degree in the Nis(r) cells. Wild-type cells grown in 100% CO2 were two to five times longer than cells grown in air. Nisin (2.5 mu g/ml) did not decrease the viability of Nis(r) cells but for wild-type cells caused an immediate 2-log reduction of viability when they were grown in air and a 4-log reduction when they were grown in 100% CO2. There was a quantifiable synergistic action between nisin and CO2 in the wild-type strain. The MIC of nisin for the wild-type strain grown in the presence of 2.5 mu g of nisin per ml increased from 3.1 to 12.5 mu g/ml over 35 days, but this increase was markedly delayed for cultures in CO2. This synergism between nisin and CO2 was examined mechanistically by following the leakage of carboxyfluorescein (CF) from listerial liposomes. Carbon dioxide enhanced nisin-induced CF leakage, indicating that the synergistic action of CO2 and nisin occurs at the cytoplasmic membrane. Liposomes made from cells grown in a CO2 atmosphere were even more sensitive to nisin action. Liposomes
made from cells grown at 4 degrees C were dramatically more nisin sensitive than were liposomes derived from cells
grown at 30 degrees C. Cells grown in the presence of 100% CO2 and those grown at 4 degrees C had a greater
proportion of short-chain fatty acids. The synergistic action of nisin and CO2 is consistent with a model where membrane
fluidity plays a role in the efficiency of nisin action.

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Nilsson, L. (Intern), Chen, Y. (Ekstern), Chikindas, M. (Ekstern), Huss, H. H. (Intern), Gram, L. (Intern), Montville,
T. (Ekstern)
Pages: 769-774
Publication date: 2000
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 66
Issue number: 2
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.043 SNIP 1.647
Web of Science (2007): Indexed yes
Fresh and processed fish and shellfish

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Huss, H. H. (Intern)
Pages: 472-506
Publication date: 2000

Host publication information
Title of host publication: The Microbiological Safety and Quality of Food
Place of publication: Gaithersburg, Md
Publisher: Aspen Publishers
Editors: Lund, B., Baird-Parker, T., Gould, G.
ISBN (Print): 0-8342-1323-0
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225539
Publication: Research - peer-review › Book chapter – Annual report year: 2000

Homogeneity of Danish environmental and clinical isolates of Shewanella algae
Danish isolates of Shewanella algae constituted by whole-cell protein profiling a very homogeneous group, and no clear distinction was seen between strains from the marine environment and strains of clinical origin. Although variation between all strains was observed by rbotyping and random amplified polymorphic DNA analysis, no clonal relationship between infective strains was found. From several patients, clonally identical strains of S. algae were reisolated up to 8 months after the primary isolation, indicating that the same strain may be able to maintain the infection.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Vogel, B. F. (Intern), Holt, H. (Ekstern), Gerner-Smidt, P. (Ekstern), Bundvad, A. (Ekstern), Søgaard, P. (Ekstern), Gram, L. (Intern)
Pages: 443-448
Publication date: 2000
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 66
Issue number: 1
ISSN (Print): 0099-2240
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.043 SNIP 1.647
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.054 SNIP 1.602
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.074 SNIP 1.653
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 2.108 SNIP 1.648
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 2.097 SNIP 1.821
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 2.046 SNIP 1.754
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.989 SNIP 1.736
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.957 SNIP 1.758
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 2.3 SNIP 1.732
A taxonomically unique bacterium is described on the basis of a physiological and biochemical characterization, fatty acid profiling and sequence analyses of 16S rRNA and gyrase B (gyrB) genes. This non-motile, non-fermentative bacterium was isolated from a human foetus in Uppsala, Sweden, and originally misidentified as Shewanella putrefaciens by conventional biochemical testing. The bacterium grew well at mesophilic temperatures with optimum growth at 37 degrees C. It was facultatively anaerobic and utilized various electron accepters (trimethylamine oxide, nitrate, nitrite and thiosulphate). The dominant fatty acids were 17:1B, 16:1 cis9 17.0 and 16:0. Fatty acids 13:0 iso and 15:0 iso, which have been found to be typical of Shewanella species were not detected. The G+C content of the DNA was 50.6 mol%.

Phylogenetic analysis of the 16S rRNA gene sequence revealed a clear affiliation with members of the gamma subclass of the Proteobacteria. No relationship was seen with any of the established genera in the gamma subclass of the Proteobacteria, although a distinct relationship with Vibrionaceae was observed. That the bacterium represents a novel bacterial genus distinct from Vibrionaceae was also supported by gyrB sequence analysis. Considering the source and close proximity to the genus Shewanella, the name Alishewanella fetalis gen. nov., sp. nov. is proposed, for which the type strain is strain CCUG 30811(T).
Proliferation and location of Vibrio anguillarum during infection of rainbow trout, Onchorhynchus mykiss (Walbaum)

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene, Technical University of Denmark
Authors: Spanggaard, B. (Intern), Huber, I. (Ekstern), Nielsen, J. (Ekstern), Nielsen, T. (Ekstern), Gram, L. (Intern)
Pages: 423-427
Publication date: 2000
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Fish Diseases
Volume: 23
Issue number: 6
ISSN (Print): 0140-7775
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.82
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.12
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.71
Shewanella

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Vogel, B. F. (Intern)
Pages: 2008-2015
Publication date: 2000

Host publication information
Title of host publication: Encyclopedia of Food Microbiology
Place of publication: San Diego
Publisher: Academic Press
Editors: Robinson, R., Batt, C., Patels, P.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 225559
Publication: Research - peer-review › Book chapter – Annual report year: 2000

Spoilage of fish
The microflora of rainbow trout intestine: a comparison of traditional and molecular identification

The culturability of the intestinal microflora of 48 rainbow trout was detected by comparing direct microscopic counts (4',6-diamidino-2-phenylindole, DAPI) with plate counts (tryptone soya agar, TSA). In general, a high percentage (average 50%) of the microflora could be cultured. The counts of the intestinal microflora varied 3-5 log units between fish within the same sampling point. A total of 504 bacteria were identified by physiologic criteria and 153 strains also by partial sequencing of the 16S rRNA gene. High agreement was found between classical and molecular identification. The dominant intestinal microflora was identified as bacteria belonging to the gamma subclass of Proteobacteria (of the genera Citrobacter, Aeromonas and Pseudomonas), to the Gram+ positive bacteria with low G + C-content (of the genus Carnobacterium) and as bacteria belonging to the beta subclass of Proteobacteria. However, the composition of the intestinal microflora showed high variation among three investigated fish farms and also at different time points within one fish farm. (C) 2000 Elsevier Science B.V. All rights reserved.
Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation

Lactic acid bacteria (LAB) isolated from raw materials (fish, rice, garlic and banana leaves) and processed som-fak (a Thai low-salt fermented fish product) were characterized by API 50-CH and other phenotypic criteria. Lactococcus lactis subsp. lactis and Leuconostoc citreum were specifically associated with fish fillet and minced fish, Lactobacillus paracasei subsp. paracasei with boiled rice and Weisella confusa with garlic mix and banana leaves. In addition, Lactobacillus plantarum, Lactobacillus pentosus and Pediococcus pentosaceus were isolated from raw materials. A succession of aciduric, homofermentative lactobacillus species, dominated by Lb. plantarum/pentosus, was found during fermentation. In total, 9% of the strains fermented starch and 19% fermented garlic, the two main carbohydrate components in som-fak. The ability to ferment garlic was paralleled by a capacity to ferment inulin. An increased percentage of garlic fermenting strains was found during fermentation of som-fak, from 8% at day 1 to 40% at day 5. No starch fermenting strains were isolated during fermentation. Three mixed LAB cultures, composed of either starch fermenting Lc. lactis subsp. lactis and Lb. paracasei subsp. paracasei, or garlic fermenting Lb. plantarum and Pd. pentosaceus, or a combination of these strains were inoculated into laboratory prepared som-fak with or without garlic. In som-fak without garlic, pH was above 4.8 after three days, irrespective of addition of mixed LAB cultures. The starch fermenting LAB were unable to ferment som-fak and sensory spoilage occurred after three days. Fermentation with the combined mix of starch and garlic fermenting strains led to production of 2.5% acid and a decrease in pH to 4.5 in two days. The fermentation was slightly slower with the garlic
fermenting strains alone. This is the first report describing the role of garlic as carbohydrate source for LAB in fermented fish products. (C) 1999 Elsevier Science B.V. All rights reserved

**General information**

*State:* Published  
*Organisations:* National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene  
*Authors:* Paludan-Müller, C. (Intern), Huss, H. H. (Intern), Gram, L. (Intern)  
*Pages:* 219-229  
*Publication date:* 1999  
*Main Research Area:* Technical/natural sciences

**Publication information**

*Journal:* International Journal of Food Microbiology  
*Volume:* 46  
*Issue number:* 3  
*ISSN (Print):* 0168-1605  
*Ratings:*  
BFI (2018): BFI-level 2  
Web of Science (2018): Indexed yes  
BFI (2017): BFI-level 2  
Scopus rating (2017): CiteScore 3.76 SJR 1.366 SNIP 1.436  
Web of Science (2017): Indexed yes  
BFI (2016): BFI-level 2  
Scopus rating (2016): CiteScore 3.97 SJR 1.481 SNIP 1.553  
Web of Science (2016): Indexed yes  
BFI (2015): BFI-level 2  
Scopus rating (2015): SJR 1.614 SNIP 1.683 CiteScore 4.02  
Web of Science (2015): Indexed yes  
BFI (2014): BFI-level 2  
Scopus rating (2014): SJR 1.493 SNIP 1.695 CiteScore 3.62  
Web of Science (2014): Indexed yes  
BFI (2013): BFI-level 2  
Scopus rating (2013): SJR 1.612 SNIP 1.841 CiteScore 3.8  
ISI indexed (2013): ISI indexed yes  
Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 2  
Scopus rating (2012): SJR 1.603 SNIP 1.705 CiteScore 3.7  
ISI indexed (2012): ISI indexed yes  
Web of Science (2012): Indexed yes  
BFI (2011): BFI-level 2  
Scopus rating (2011): SJR 1.607 SNIP 1.713 CiteScore 3.63  
ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 2  
Scopus rating (2010): SJR 1.61 SNIP 1.666  
Web of Science (2010): Indexed yes  
BFI (2009): BFI-level 2  
Scopus rating (2009): SJR 1.475 SNIP 1.539  
Web of Science (2009): Indexed yes  
BFI (2008): BFI-level 2  
Scopus rating (2008): SJR 1.442 SNIP 1.509  
Web of Science (2008): Indexed yes  
Scopus rating (2007): SJR 1.349 SNIP 1.692  
Web of Science (2007): Indexed yes  
Scopus rating (2006): SJR 1.541 SNIP 1.788  
Web of Science (2006): Indexed yes
Growth control of *Listeria monocytogenes* on cold-smoked salmon using a competitive lactic acid bacteria flora

A *Lactobacillus* sake strain LKE5 and four strains of *Carnobacterium piscicola* were evaluated as biopreservation cultures to control the growth of *Listeria monocytogenes* on vacuum-packed, cold-smoked salmon stored at 5 degrees C. All five strains were antilisterial as live cultures in an agar diffusion assay. Cell-free supernatants of two strains of *C. piscicola* and *L. sake* LKE5 were also antilisterial because of the production of bacteriocins. The presence of high cell numbers of strains of *C. piscicola* had no influence on the sensory quality of cold-smoked salmon stored at 5 degrees C, but *L. sake* LKE5 caused strong sulfurous off-flavors and was rejected as a culture for biopreservation of cold-smoked salmon. A bacteriocin-producing strain of *C. piscicola* (A9b) initially caused a 7-day lag phase of *L. monocytogenes*, followed by a reduction in numbers of *L. monocytogenes* from 10(3) CFU/ml to below 10 CFU/ml after 32 days of incubation, coinciding with the detection of antilisterial compounds. The presence of a nonbacteriocin-producing strain of *C. piscicola* (A10a) prevented the growth of *L. monocytogenes* during the 32- day incubation. The growth of *L. monocytogenes* was strongly repressed on cold-smoked salmon in the presence of *C. piscicola* A9b and A10a, respectively. The initial cell numbers of *L. monocytogenes* that were found on Oxford plates incubated at 25 degrees C reached low maximum cell counts of 10(4) and 2 x 10(3) after 14 and 20 days of storage in mixed culture with *C. piscicola* A9b and A10a.

**General information**

State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Nilsson, L. (Intern), Gram, L. (Intern), Huss, H. H. (Intern)
Pages: 336-342
Publication date: 1999
Main Research Area: Technical/natural sciences

**Publication information**

Journal: Journal of Food Protection
Volume: 62
Issue number: 4
ISSN (Print): 0362-028X
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.63 SJR 0.761 SNIP 0.823
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.68 SJR 0.769 SNIP 0.811
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.954 SNIP 1.024 CiteScore 2.03
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Inhibition of Vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish

To study the possible use of probiotics in fish farming, we evaluated the in vitro and in vivo antagonism of antibacterial strain Pseudomonas fluorescens strain AH2 against the fish-pathogenic bacterium Vibrio anguillarum. As iron is important in virulence and bacterial interactions, the effect of P. fluorescens AH2 was studied under iron-rich and iron-limited conditions. Sterile-filtered culture supernatants from iron-limited P. fluorescens AH2 inhibited the growth of V. anguillarum, whereas sterile-filtered supernatants from iron-replete cultures of P. fluorescens AH2 did not. P. fluorescens AH2 inhibited the growth of V. anguillarum during coculture, independently of the iron concentration, when the initial count of the antagonist was 100 to 1,000 times greater than that of the fish pathogen. These in vitro results were successfully repeated in vivo. A probiotic effect in vivo was tested by exposing rainbow trout (Oncorhynchus mykiss Walbaum) to P. fluorescens AH2 at a density of 10^5 CFU/ml for 5 days before a challenge with V. anguillarum at 10^4 to 10^5 CFU/ml for 1 h. Some fish were also exposed to P. fluorescens AH2 at 10^7 CFU/ml during the 1-h infection. The combined probiotic treatment resulted in a 46% reduction of calculated accumulated mortality; accumulated mortality was 25% after 7 days at 12 degrees C in the probiotic-treated fish, whereas mortality was 47% in fish not treated with the probiont.
Occurrence of Shewanella algae in Danish coastal water and effects of water temperature and culture conditions on its survival

The marine bacterium Shewanella algae, which was identified as the cause of human cases of bacteremia and ear infections in Denmark in the summers of 1994 and 1995, was detected in seawater only during the months (July, August, September, and October) when the water temperature was above 13 degrees C. The bacterium is a typical mesophilic organism, and model experiments were conducted to elucidate the fate of the organism under cold and nutrient-limited conditions. The culturable count of S. algae decreased rapidly from 10(7) CFU/ml to 10(1) CFU/ml in approximately 1 month when cells grown at 20 to 37 degrees C were exposed to cold (2 degrees C) seawater. In contrast, the culturable count of cells exposed to warmer seawater (10 to 25 degrees C) remained constant. Allowing the bacterium a transition period in seawater at 20 degrees C before exposure to the 2 degrees C seawater resulted in 100% survival over a period of 1 to 2 months. The cold protection offered by this transition (starvation) probably explains the ability of the organism to persist in Danish seawater despite very low (0 to 1 degrees C) winter water temperatures. The culturable counts of samples kept at 2 degrees C increased to 10(5) to 10(7) CFU/ml at room temperature. Most probable number analysis showed this result to be due to regrowth rather than resuscitation. It was hypothesized that S. algae would survive cold exposure better if in the biofilm state; however, culturable counts from S. algae biofilms decreased as rapidly as did counts of planktonic cells.
Production of acylated homoserine lactones by psychrotrophic members of the Enterobacteriaceae isolated from foods

Bacteria are able to communicate and gene regulation can be mediated through the production of acylated homoserine lactone (AHL) signal molecules. These signals play important roles in several pathogenic and symbiotic bacteria. The following study was undertaken to investigate whether AHLs are produced by bacteria found in food at temperatures and NaCl conditions commercially used for food preservation and storage. A minimum of 116 of 154 psychrotrophic Enterobacteriaceae strains isolated from cold-smoked salmon or vacuum-packed chilled meat produced AHLs. Analysis by thin-layer chromatography indicated that N-3-oxohexanoyl homoserine lactone was the major AHL of several of the strains isolated from cold-smoked salmon and meat. AHL-positive strains cultured at 5 degrees C in medium supplemented with 4% NaCl produced detectable amounts of AHL(s) at cell densities of 10^6 CFU/ml. AHLs were detected in cold-smoked salmon inoculated with strains of Enterobacteriaceae stored at 5 degrees C under an N-2 atmosphere when mean cell densities increased to 10^6 CFU/g and above. Similarly, AHLs were detected in uninoculated samples of commercially produced cold-smoked salmon when the level of indigenous Enterobacteriaceae reached 106 CFU/g. This level of Enterobacteriaceae is often found in lightly preserved foods, and AHL-mediated gene regulation may
play a role in bacteria associated with food spoilage or food toxicity

**General information**

**State:** Published

**Organisations:** Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Center for Systems Microbiology, Department of Systems Biology

**Authors:** Gram, L. (Intern), Christensen, A. (Ekstern), Flodgaard, L. (Intern), Molin, S. (Intern), Givskov, M. C. (Intern)

**Pages:** 3458-3463

**Publication date:** 1999

**Main Research Area:** Technical/natural sciences

**Publication information**

**Journal:** Applied and Environmental Microbiology

**Volume:** 65

**Issue number:** 8

**ISSN (Print):** 0099-2240

**Ratings:**

- BFI (2018): BFI-level 2
- Web of Science (2018): Indexed yes
- BFI (2017): BFI-level 2
- Scopus rating (2017): CiteScore 3.99
- Web of Science (2017): Indexed yes
- BFI (2016): BFI-level 2
- Scopus rating (2016): CiteScore 4.08
- Web of Science (2016): Indexed yes
- BFI (2015): BFI-level 2
- Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
- Web of Science (2015): Indexed yes
- BFI (2014): BFI-level 2
- Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
- Web of Science (2014): Indexed yes
- BFI (2013): BFI-level 2
- Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
- ISI indexed (2013): ISI indexed yes
- Web of Science (2013): Indexed yes
- BFI (2012): BFI-level 2
- Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
- ISI indexed (2012): ISI indexed yes
- Web of Science (2012): Indexed yes
- BFI (2011): BFI-level 2
- Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
- ISI indexed (2011): ISI indexed yes
- Web of Science (2011): Indexed yes
- BFI (2010): BFI-level 2
- Scopus rating (2010): SJR 1.887 SNIP 1.436
- Web of Science (2010): Indexed yes
- BFI (2009): BFI-level 2
- Scopus rating (2009): SJR 1.972 SNIP 1.528
- Web of Science (2009): Indexed yes
- BFI (2008): BFI-level 2
- Scopus rating (2008): SJR 2.156 SNIP 1.572
- Web of Science (2008): Indexed yes
- Scopus rating (2007): SJR 2.043 SNIP 1.647
- Web of Science (2007): Indexed yes
- Scopus rating (2006): SJR 2.054 SNIP 1.602
- Web of Science (2006): Indexed yes
Antibacterial activity of marine-derived fungi

A total of 227 marine isolates of ubiquitous fungi were cultivated on different media and the secondary metabolite content of the extracts (ethyl acetate/chloroform/methanol 3:2:1) characterized by HPLC. The fungi were secured from animals, plants and sediments of Venezuelan waters (0-10 m) including mangroves and lagoonal areas. The extracts were tested for antibacterial activity. A total of 7 were active towards Vibrio parahaemolyticus and 55 towards Staphylococcus aureus, representing 18 different fungal species from 8 ascomycetous genera. For 61 strains of Penicillium citrinum antibacterial activity correlated well with content of secondary metabolites as measured by HPLC. Thirteen isolates of Penicillium steckii produced very similar profiles of secondary metabolites and 6 of these had activity against either V. parahaemolyticus or S. aureus or both.

General information

State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Universidad De Oriente, University of Copenhagen
Authors: Christophersen, C. (Ekstern), Crescente, O. (Ekstern), Frisvad, J. C. (Intern), Gram, L. (Intern), Nielsen, J. (Ekstern), Nielsen, P. H. (Ekstern), Rahbæk, L. (Ekstern)
Pages: 135-138
Publication date: 1998
Main Research Area: Technical/natural sciences

Publication Information

Journal: Mycopathologia
Volume: 143
Issue number: 3
ISSN (Print): 0301-486X
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.776 SJR 0.597 CiteScore 1.75
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.755 SNIP 0.889 CiteScore 1.77
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.739 SNIP 0.846 CiteScore 1.8
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.611 SNIP 0.853 CiteScore 1.64
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.672 SNIP 1.06 CiteScore 1.79
ISI indexed (2013): ISI indexed yes
Characterization of anti-listerial lactic acid bacteria isolated from Thai fermented fish products

Thai fermented fish products were screened for lactic acid bacteria capable of inhibiting Listeria sp. (Listeria innocua). Of 4150 assumed lactic acid bacteria colonies from MRS agar plates that were screened by an agar-overlay method 58 (1.4%) were positive. Forty four of these strains were further characterized and 43 strains were inhibitory against Listeria monocytogenes. The strains were inhibitory to other Gram-positive (lactic acid) bacteria probably because of production of bacteriocins. All 44 strains inhibited both Vibrio cholerae and Vibrio parahaemolyticus and 37 were inhibitory to a mesophilic fish spoilage bacterium, Aeromonas sp.). Inhibition of Gram-negative bacteria was attributed to production of lactic acid. Most strains were identified as Lactobacillus spp., and all grew well at ambient temperatures (25-37 degrees C) and tolerated up to 6.5% NaCl. Glucose was fermented rapidly in laboratory media whereas pH decreased only very slowly in fish juice supplemented with 4% glucose and 3.5% NaCl or in a rice-fish mixture. Only four of 44 isolates could degrade and ferment complex carbohydrates such as rice, potatoes and maize starch. This indicates that other types of bacteria may be responsible for the rapid spontaneous fermentation of the products or that other yet-unknown factors ensure rapid fermentation. Overall anti-listerial lactic acid bacteria do occur in fermented fish products and the antibacterial activity against pathogenic bacteria indicates that they may be important in product safety. (C) 1998 Academic Press Limited
Chemical mediation of bacterial surface colonisation by secondary metabolites from the red alga Delisea pulchra
We investigated the effects of halogenated furanones from the red alga Delisea pulchra on colonisation of surfaces by marine bacteria. Bacterial abundance on the surface of D. pulchra, assessed using scanning electron microscopy (SEM),
was significantly lower than on the surfaces of 3 co-occurring algal species, all of which lack furanones. There was also a
strong inverse correlation between bacterial abundance and furanone content (previously determined) for different
sections of the thallus of D. pulchra, consistent with inhibition of bacteria by furanones. Based on these observations we
experimentally investigated inhibition of marine bacteria by furanones, initially testing the effects of crude extract of D.
pulchra (about 50 % of which is furanones) on the growth of 144 strains of bacteria isolated from the surfaces of D.
pulchra, nearby rocks, or a co-occurring alga (Sasgassum vestitum). This crude extract did not strongly inhibit growth of
these bacteria; 79% of the strains grew at 50 pg ml\(^{-1}\) of crude extract, and 63 % grew at 500 \(\mu\)g ml\(^{-1}\). Inhibition of
growth that did occur was strongly source dependent, with bacteria isolated from rocks the least affected, and strains from
D. pulchra the most. As inhibition of growth did not provide an adequate explanation for the inverse relationship between
levels of furanones and bacteria abundance on D. pulchra, we proceeded to investigate the effects of these metabolites on
other bacterial characteristics relevant to colonisation - attachment, swarming, and swimming. Individual furanones or
crude extract at natural concentrations strongly inhibited bacterial attachment in the laboratory and in the field. In
laboratory assays, attachment of 3 strains isolated from rocks was much more strongly affected than that of 3 isolates
from D. pulchra, in contrast to the pattern for growth inhibition. We also tested individual furanones against swimming and
swarming of the same 6 bacterial isolates (3 from rocks, 3 from D. pulchra) used in the attachment assays. At least some
furanones inhibited swimming or swarming at non-growth-inhibitory concentrations for all isolates, again indicating specific
effects against bacterial characteristics. As for attachment, there were significant differences in the responses of different
isolates to furanones. We also found that the ability to swarm was widespread among these surface associated marine
bacteria, suggesting that swimming may be ecologically important in these systems. Overall, we found that the effects of
furanones on bacteria varied among (1) furanones, (2) bacterial phenotypes, (3) different isolates and (4) different sources
of isolation (e.g. rocks or algae). This differential inhibition of different bacterial isolates or phenotypes by furanones, as
well as affecting overall bacterial abundance on the alga, should have strong effects on the species composition of the
bacterial community on the alga's surface. The effects of furanones on specific bacterial colonisation traits are discussed
in the light of recent evidence demonstrating that furanones interfere with bacterial acylated homoserine lactone regulatory
systems.

**General information**

**State:** Published  
**Organisations:** Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Department of Microbiology, University of New South Wales  
**Authors:** Maximilien, R. (Ekstern), de Nys, R. (Ekstern), Holmström, C. (Ekstern), Gram, L. (Intern), Givskov, M. C. (Intern), Crass, K. (Ekstern), Kjelleberg, S. (Ekstern), Steinberg, P. (Ekstern)  
**Pages:** 233-246  
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**Publication information**

**Journal:** Aquatic Microbial Ecology  
**Volume:** 15  
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**Ratings:**  
**BFI (2018):** BFI-level 1  
**Web of Science (2018):** Indexed yes  
**BFI (2017):** BFI-level 1  
**Scopus rating (2017):** SNIP 0.707 SJR 0.962 CiteScore 2.13  
**Web of Science (2017):** Indexed yes  
**BFI (2016):** BFI-level 1  
**Scopus rating (2016):** SJR 0.852 SNIP 0.587 CiteScore 1.77  
**BFI (2015):** BFI-level 1  
**Scopus rating (2015):** SJR 1.148 SNIP 0.781 CiteScore 2.21  
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**BFI (2014):** BFI-level 1  
**Scopus rating (2014):** SJR 0.907 SNIP 0.706 CiteScore 1.88  
**BFI (2013):** BFI-level 1  
**Scopus rating (2013):** SJR 1.029 SNIP 0.84 CiteScore 2.16  
**ISI indexed (2013):** ISI indexed yes  
**BFI (2012):** BFI-level 1  
**Scopus rating (2012):** SJR 1.403 SNIP 0.947 CiteScore 2.49  
**ISI indexed (2012):** ISI indexed yes  
**BFI (2011):** BFI-level 1
Evaluation of the role of Carnobacterium piscicola in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5 degrees C

The microflora on spoiled cold-smoked salmon often consists of a mixture of lactic acid bacteria (LAB) and Gram-negative bacteria. To elucidate the role of the different groups, a storage trial was carried out in which nisin and CO2 were used for the selective inhibition of the two bacterial groups. The shelf-life of vacuum-packed cold-smoked salmon, recorded by sensory evaluation, was four weeks at 5 degrees C and the microflora was composed of LAB (10^6-10^7 cfu/g) with an associate Gram-negative flora in varying levels (10^5-10^7 cfu/g). The addition of nisin and/or a CO2-atmosphere increased the shelf-life to five or six weeks and limited the level of LAB to about 10^4-10^6, 10^3-10^6 and 10^2-10^4 cfu/g, respectively. CO2-atmosphere +/- nisin inhibited the growth of Gram-negative bacteria, whereas nisin had no effect on these in vacuum packages. The Gram-negative flora on vacuum-packed salmon was dominated by a Vibrio sp., resembling V. marinus, Enterobacteriaceae (Enterobacter agglomerans, Serratia liquefaciens and Rahnella aquatilis) and occasionally Aeromonas hydrophila. Irrespective of the addition of nisin and/or CO2-atmosphere, the LAB microflora was dominated by Carnobacterium piscicola, which was found to account for 87% of the 255 LAB isolates characterized. Whole-cell-protein patterns analysed by SDS-PAGE confirmed the Carnobacterium species identification. The spoilage potential of C. piscicola isolates was further studied by inoculation of approx. 10^6 cfu/g in cold-smoked salmon stored at 5 degrees C. The salmon did not spoil within 4 weeks of storage in vacuum-or CO2-atmosphere, and it is concluded that despite high levels (> 10^7 cfu/g) of C. piscicola, sensory rejection was caused by autolytic changes. This was supported by the development of soft texture and sour, rancid and bitter off-flavours at the point of spoilage, irrespective of the length of shelf-life and low or high total counts of LAB and Gram-negative bacteria. (C) 1998 Elsevier Science B.V

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Paludan-Müller, C. (Intern), Dalgaard, P. (Intern), Huss, H. H. (Intern), Gram, L. (Intern)
Pages: 155-166
Publication date: 1998
Main Research Area: Technical/natural sciences
Fermentation and spoilage of som fak, a Thai low-salt fish product

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Østergaard, A. (Intern), Embarek, P. K. B. (Intern), Yamprayoon, J. (Ekstern), Wedell-Neergaard, C. (Ekstern), Huss, H. H. (Intern), Gram, L. (Intern)
Pages: 105-112
Publication date: 1998
Main Research Area: Technical/natural sciences

A convenient synthesis of pseudoceratidine and three analogs for biological evaluation
The recently isolated marine natural product pseudoceratidine (1) has been synthesized from 2-trichloroacetylpyrrole. Bromination in the 4- and 5-position followed by nucleophilic displacement of the trichloromethyl group with spermidine gave 1 in 79% yield. The procedure is general and can easily be adopted to the preparation of other derivatives. This was demonstrated by the synthesis of a 5,5'-didebromo derivative (2) and two analogs (3-4). The compounds 1-4 have been tested for antibacterial activity and the results compared to a previous study. Also activity against the marine brine shrimp Artemia salina is reported.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Behrens, C. (Ekstern), Christoffersen, M. (Ekstern), Gram, L. (Intern), Nielsen, P. (Ekstern)
Pages: 321-326
Publication date: 1997
Main Research Area: Technical/natural sciences

Publication information
Journal: Bioorganic & Medicinal Chemistry Letters
Volume: 7
Differentiation of Shewanella putrefaciens and Shewanella alga on the basis of whole-cell protein profiles, ribotyping, phenotypic characterization, and 16S rRNA gene sequence analysis

Seventy-six presumed Shewanella putrefaciens isolates from fish, oil drillings, and clinical specimens, the type strain of Shewanella putrefaciens (ATCC 8071), the type strain of Shewanella alga (IAM 14159), and the type strain of Shewanella hanedai (ATCC 33224) were compared by several typing methods. Numerical analysis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell protein and ribotyping patterns showed that the strains were separated into two distinct clusters with 56% +/- 10% and 40% +/- 14% similarity for whole-cell protein profiling and ribotyping, respectively. One cluster consisted of 26 isolates with 52 to 55 mol% G + C and included 15 human isolates, mostly clinical specimens, 8 isolates from marine waters, and the type strain of S. alga. This homogeneous cluster of mesophilic,
halotolerant strains was by all analyses identical to the recently defined species S. alga (U. Simidu et al., Int. J. Syst. Bacteriol. 40:331-336, 1990). Fifty-two typically psychrotolerant strains formed the other, more heterogeneous major cluster, with 43 to 47 mol% G + C. The type strain of S. putrefaciens was included in this group. The two groups were confirmed by 16S rRNA gene sequence analysis. It is concluded that the isolates must be considered two different species, S. alga and S. putrefaciens, and that most mesophilic isolates formerly identified as S. putrefaciens belong to S. alga. The ecological role and potential pathogenicity of S. alga can be evaluated only if the organism is correctly identified.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Vogel, B. F. (Intern), Jørgensen, K. (Ekstern), Christensen, H. (Ekstern), Olsen, J. (Ekstern), Gram, L. (Intern)
Pages: 2189-2199
Publication date: 1997
Main Research Area: Technical/natural sciences

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Journal: Applied and Environmental Microbiology
Volume: 63
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BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.043 SNIP 1.647
Do marine natural products interfere with prokaryotic AHL regulatory systems?
Recent studies indicate that a taxonomically diverse range of marine eukaryotes produce metabolites which inhibit phenotypic traits in bacteria, with no or minimal effects on growth. In this review, we present evidence for the existence of such eukaryotic interference with a conserved prokaryotic signalling system. We demonstrate that halogenated furanones, a class of secondary metabolites produced by the Australian subtidal red alga DeLisea pulchra, interfere with the acylated homoserine lactone (AHL) regulatory system in several Gram-negative bacteria. Furanones were found to interfere with the AHL mediated expression of bioluminescence, swarming (surface) motility, and exoenzyme synthesis in different bacterial species. Furthermore, adhesion and swarming in a range of marine bacteria, for which the identity of the signalling molecules is not yet determined, were inhibited by furanones at concentrations that did not affect growth. Evidence for these effects were obtained in both field and laboratory experiments. Competition experiments in the presence of different concentrations of AHLs and furanones showed that the expression of swarming and bioluminescence in laboratory strains is competitively inhibited in a fashion that suggests that both classes of compounds have affinity for the same receptor site in the AHL regulatory system. Finally, by performing structure- function experiments on the inhibition of AHL systems by a range of different furanones, we identified the structural prerequisites responsible for interference.

General information
State: Published
Organisations: Department of Systems Biology, Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Kjelleberg, S. (Ekstern), Steinberg, P. (Ekstern), Givskov, M. C. (Intern), Gram, L. (Intern), Manefield, M. (Ekstern), de Nys, R. (Ekstern)
Pages: 85-93
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Main Research Area: Technical/natural sciences

Publication information
Journal: Aquatic Microbial Ecology
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Issue number: 1
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Web of Science (2018): Indexed yes
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Scopus rating (2017): SNIP 0.707 SJR 0.962 CiteScore 2.13
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.852 SNIP 0.587 CiteScore 1.77
Enzymatic removal and disinfection of bacterial biofilms

Model biofilms of Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas fluorescens, and Pseudomonas aeruginosa were made on steel and polypropylene substrata. Plaque-resembling biofilms of Streptococcus mutans, Actinomyces, viscosus, and Fusobacterium nucleatum were made on saliva-coated hydroxyapatite. The activity of enzymes against bacterial cells in biofilm was measured by fluorescence microscopy and an indirect conductance test in which evolution of carbon dioxide was measured. Glucose oxidase combined with lactoperoxidase was bactericidal.
against biofilm bacteria but did not remove the biofilm from the substrata. A complex mixture of polysaccharide-
hydrolyzing enzymes was able to remove bacterial biofilm from steel and polypropylene substrata but did not have a
significant bactericidal activity. Combining oxidoreductases with polysaccharide-hydrolyzing enzymes resulted in
bactericidal activity as well as removal of the biofilm.

**General information**

State: Published
Organisations: National Institute of Aquatic Resources, Department of Systems Biology, Section for Aquatic Microbiology
and Seafood Hygiene
Authors: Johansen, C. (Intern), Falholt, P. (Intern), Gram, L. (Intern)
Pages: 3724-3728
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Main Research Area: Technical/natural sciences

**Publication information**

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Web of Science (2018): Indexed yes
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Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.043 SNIP 1.647
Web of Science (2007): Indexed yes
Inhibition of Listeria monocytogenes on cold-smoked salmon by nisin and carbon dioxide atmosphere

The bacteriostatic and bacteriocidal effect of nisin in combination with carbon dioxide, NaCl and low temperature on the survival of Listeria monocytogenes was investigated in in vitro model studies and in trials with cold-smoked salmon. Addition of nisin caused various degrees of inhibition and sometimes death of L. monocytogenes in model experiments performed at 10 degrees C. The anti-listerial effect of nisin was improved in the presence of 100% CO2 and increasing NaCl concentrations (0.5 to 5.0% w/v). Minimal bactericidal concentrations (MBC) of nisin varied from 30 to more than 500 IU/ml. The most pronounced effect of nisin was found when 10(2) cfu/ml was grown in media with 5.0% NaCl and incubated in CO2 atmosphere (MBC = 30 IU/ml). The bactericidal effect of nisin was reduced in air and vacuum, and did not increase systematically with increasing NaCl concentrations. In general, nisin concentration less than or equal to 50 IU/ml resulted in the survival and growth of L. monocytogenes in all combinations with other preservatives (NaCl, CO2). Addition of nisin (500 or 1000 IU/g) to cold-smoked salmon inoculated with L. monocytogenes and stored at 5 degrees C delayed, but did not prevent growth of L. monocytogenes in vacuum-packs. Numbers of L. monocytogenes increased to 10(8) cfu/g in vacuum packed cold-smoked salmon in 8 days, whereas CO2 packing of cold- smoked salmon resulted in an 8-day lag phase of L. monocytogenes, with numbers eventually reaching 10(6) cfu/g in 27 days. Addition of nisin to CO2 packed cold-smoked salmon resulted in a 1 to 2 log reduction of L. monocytogenes followed by a lag phase of 8 and 20 days in salmon with 500 and 1000 IU nisin/g, respectively. The levels of L. monocytogenes remained below 10(3) cfu/g during 27 days of storage at both concentrations of nisin. (C) 1997 Elsevier Science B.V.
Lone Gram, Danish Institute of Fisheries Research, Department of Seafood Research, Lyngby, Denmark - Biography

General information

State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern)
Microbiology of fish and fish products

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Huss, H. H. (Intern), Dalgaard, P. (Intern), Gram, L. (Intern)
Pages: 413-430
Publication date: 1997

Host publication information
Place of publication: Amsterdam
Publisher: Elsevier
Editors: Luten, J., Børresen, T., Oehlenschläger, J.
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Source: orbit
Source-ID: 225829
Publication: Research - peer-review › Book chapter – Annual report year: 1997

Pathogenicity of Vibrio anguillarum serogroup O1 strains compared to plasmids, outer membrane protein profiles and siderophore production

The virulence of 18 strains of Vibrio anguillarum serogroup O1 was compared to plasmid content, expression of siderophores and outer membrane proteins. All strains, irrespective of plasmid content, produced siderophores and inducible outer membrane proteins under iron-limited conditions. Only strains that carried the 67 kbp virulence plasmid or derivatives of it produced the outer membrane protein, OM2. All virulent strains harboured the 67 kbp plasmid or derivatives of it, indicating its importance for virulence. However, some strains carrying the virulence plasmid or a derivative of it, produced siderophores as well as OM2 but were non-pathogenic to fish. Likewise, among the virulent strains, considerable variation in LD_{50} values was recorded. Plasmid profiling and restriction analysis showed that the virulence plasmid existed in various molecular weights from 26 to 80 kbp, with 65-67 kbp being the most common, and that this plasmid displayed various restriction profiles. The presence of other plasmids did not seem to affect the pathogenic properties

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Pedersen, K. (Ekstern), Gram, L. (Intern), Austin, D. (Ekstern), Austin, B. (Ekstern)
Pages: 365-371
Publication date: 1997
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Microbiology
Volume: 82
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.41
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Protamine-induced permeabilization of cell envelopes of gram-positive and gram-negative bacteria

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Johansen, C. (Intern), Verheul, A. (Ekstern), Gram, L. (Intern), Gill, T. (Ekstern), Abee, T. (Ekstern)
Pages: 1155-1159
Publication date: 1997
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
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BFI (2018): BFI-level 2
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General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Nilsson, L. (Intern), Huss, H. H. (Intern), Johansen, C. (Ekstern)
Pages: 12-15
Publication date: 1996
Main Research Area: Technical/natural sciences

Publication information
Journal: Alimenta
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Ratings:
BFI (2015): BFI-level 1
BFI (2014): BFI-level 1
BFI (2013): BFI-level 1
ISI indexed (2013): ISI indexed no
BFI (2012): BFI-level 1
ISI indexed (2012): ISI indexed no
BFI (2011): BFI-level 1
ISI indexed (2011): ISI indexed no
BFI (2010): BFI-level 1
BFI (2009): BFI-level 1
BFI (2008): BFI-level 1
Original language: Danish
Source: orbit
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Publication: Research › Journal article – Annual report year: 1996

Changes in cell morphology of Listeria monocytogenesnes and Shewanella putrefaciens resulting from the action of protamine

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Johansen, C. (Intern), Gill, T. (Ekstern), Gram, L. (Intern)
Pages: 1058-1064
Publication date: 1996
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
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Ratings:
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Eukaryotic interference with homoserine lactone mediated procaryotic signalling.

Acylated homoserine lactones (AHLs) plays a widespread role in intercellular communication among bacteria. The Australian macroalga Delisea pulchra produces secondary metabolites which have structural similarities to AHL molecules. We report here that these metabolites inhibited AHL-controlled processes in prokaryotes. Our results suggest that the interaction between higher organisms and their surface-associated bacteria may be mediated by interference with bacterial regulatory systems.

General information
Inhibitory effects of secondary metabolites from the red alga Delisea pulchra on swarming motility of Proteus mirabilis

Abnormal, uncoordinated swarming motility of the opportunistic human pathogen Proteus mirabilis was seen when a crude extract of the Australian red alga Delisea pulchra was added to the medium. This occurred at concentrations at which growth rate, swimming motility, cell elongation, polynucleation, and hyperflagellation were not affected. One halogenated furanone from D. pulchra inhibited swarming motility at concentrations that did not affect growth rate and swimming motility. Other structurally similar D. pulchra furanones had no effect on swarming, suggesting considerable specificity in the effects of furanones on swarming motility by P. mirabilis.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources, Department of Systems Biology
Authors: Gram, L. (Intern), de Nys, R. (Ekstern), Maximilien, R. (Ekstern), Givskov, M. C. (Intern), Steinberg, P. (Ekstern), Kjelleberg, S. (Ekstern)
Pages: 4284-4287
Publication date: 1996
Main Research Area: Technical/natural sciences

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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
Interaction between fish spoilage bacteria Pseudomonas sp and Shewanella putrefaciens in fish extracts and on fish tissue

The interaction between fish spoilage bacteria, Pseudomonas sp. and Shewanella putrefaciens, was investigated using fish extract and fish tissue as model systems. Isolates of Pseudomonas that produced iron chelators, siderophores, inhibited growth of S. putrefaciens in a fish-extract-agar diffusion assay but no, or only weak, antagonistic activity was seen when the medium was supplemented with iron. Sterile-filtered supernatant fluid from a siderophore-producing Pseudomonas grown in fish extract was inhibitory to S. putrefaciens if the number of Pseudomonas was above 10(8) cfu ml(-1). In contrast, supernatant fluids from siderophore-negative Pseudomonas isolates did not inhibit growth of S. putrefaciens. The inhibitory effect was, except for one strain of Pseudomonas, not seen in supernatant fluids from iron-enriched cultures of Pseudomonas sp. Finally, siderophore-producing Pseudomonas sp. lowered the maximum cell level of S. putrefaciens 1-2 log units from 10(9) to 10(10) cfu g(-1) when the strains were grown on fish muscle blocks at 0 degrees C but the growth rate of S. putrefaciens was not affected.

General information

State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Melchiorsen, J. (Intern)
Pages: 589-595
Publication date: 1996
Main Research Area: Technical/natural sciences
Microbiological spoilage of fish and fish products

Spoilage of fresh and lightly preserved fish products is caused by microbial action. This paper reviews the current knowledge in terms of the microbiology of fish and fish products with particular emphasis on identification of specific spoilage bacteria and the qualitative and quantitative biochemical indicators of spoilage. Shewanella putrefaciens and Pseudomonas spp. are the specific spoilage bacteria of iced fresh fish regardless of the origin of the fish. Modified atmosphere stored marine fish from temperate waters are spoiled by the CO2 resistant Photobacterium phosphoreum whereas Gram-positive bacteria are likely spoilers of CO2 packed fish from fresh or tropical waters. Fish products with
High salt contents may spoil due to growth of halophilic bacteria (salted fish) or growth of anaerobic bacteria and yeasts (barrel salted fish). Whilst the spoilage of fresh and highly salted fish is well understood, much less is known about spoilage of lightly preserved fish products. It is concluded that the spoilage is probably caused by lactic acid bacteria, certain psychrotrophic Enterobacteriaceae and/or Photobacterium phosphoreum. However, more work is needed in this area.
Shewanella alga bacteremia in two patients with lower leg ulcers
The first Danish cases of Shewanella alga bacteremia in two patients with chronic lower leg ulcers are reported. Both patients were admitted to the hospital during the same month of a very warm summer and had been exposed to the same marine environment, thereby suggesting the same source of infection. Both patients survived; however, one of them had extensive myonecrosis, while the other patient had an uncomplicated course. The strains were initially believed to be Shewanella putrefaciens on the basis of key characteristics and results of the API 20NE identification system (bioMerieux, Marcy l'Etoile, France), but further genetic and physiological analyses identified them as Shewanella alga.

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Domínguez, H. (Ekstern), Vogel, B. F. (Intern), Gram, L. (Intern), Hoffmann, S. (Ekstern), Schaebel, S. (Ekstern)
Pages: 1036-1039
Publication date: 1996
Main Research Area: Technical/natural sciences

Publication information
Journal: Clinical Infectious Diseases
Volume: 22
Issue number: 6
ISSN (Print): 1058-4838
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 5.42 SJR 5.051 SNIP 2.795
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.88 SJR 4.614 SNIP 2.56
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 4.827 SNIP 3.001 CiteScore 5.47
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 5.258 SNIP 3.415 CiteScore 6.11
BFI (2013): BFI-level 2
Shewanella alga - en ny sygdomsfremkaldende bakterie af marin oprindelse

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Gram, L. (Intern), Vogel, B. F. (Intern)
Pages: 14-17
Publication date: 1996
Main Research Area: Technical/natural sciences

Publication information
Journal: Fisk & hav
Volume: 47
ISSN (Print): 0105-9211
Ratings:
ISI indexed (2013): ISI indexed no
ISI indexed (2012): ISI indexed no
ISI indexed (2011): ISI indexed no
Original language: Danish
Source: orbit
Source-ID: 167373
Siderophore production of fish spoilage bacteria (5 isolates of Shewanella putrefaciens and 5 of Pseudomonas sp.) was determined in fish extract, Tris-succinate medium, a liquid medium of the Chrome-Azurol-S (CAS) agar and in M9 medium supplemented with glucose and casamino acids (M9GC). One Pseudomonas isolate produced siderophores in all four media whereas the other isolates varied. One isolate was negative in all media except for the M9GC that supported siderophore production for all five Pseudomonas isolates. Neither the hydroxamate nor catechol reaction was found for any of the Pseudomonas isolates. S. putrefaciens produced siderophores of the hydroxamate type in fish extract and to a lesser extend in the M9GC medium. Growth was supported by the other media. S. putrefaciens grew weakly on the Chrome-azurol-S (CAS agar as this medium did not support siderophore-production. However, the isolates formed orange halos on a fish extract agar to which CAS was added. Outer membrane proteins (OMPs) were analyzed by SDS-PAGE and, for each siderophore positive isolate, the same iron repressed OMPs were expressed in the different media supporting siderophore production. One S. putrefaciens isolate expressed an assumed receptor protein (69- 72 kDa) in Tris-succinate medium in which a positive siderophore reaction as measured by the GAS-assay was not detected.
Antibacterial effect of protamine assayed by impedimetry

Impedimetric measurements were used to assay the antibacterial effect of protamine. A good linear correlation between the impedance detection time and the initial cell counts was obtained \((r = 0.99, n = 2)\). As basic peptides may cause clumping of cells, this correlation curve was used when estimating the cell number after protamine treatment, rather than colony counts. Protamine from salmon killed growing Gram-positive bacteria and significantly inhibited growth of Gram-negative bacteria in Tryptone Soy Broth (TSB) at 25 degrees C. In general Gram-positive bacteria were more sensitive to protamine than Gram-negative bacteria; the minimum inhibitory concentrations (MIC) determined for Gram-positive strains varied from 20 to 1000 \(\mu\)g ml\(^{-1}\) and for Gram-negative strains from 500 \(\mu\)g ml\(^{-1}\) to more than 4000 \(\mu\)g ml\(^{-1}\). The effect of protamine on non-growing Listeria monocytogenes Scott A suspended in buffer was not lethal as was the effect on growing cells; however, protamine (50-500 \(\mu\)g ml\(^{-1}\)) killed the Gram-negative fish spoilage bacteria Shewanella putrefaciens when the live cells were suspended in buffer.

General information

State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Johansen, C. (Intern); Gill, T. (Ekstern); Gram, L. (Intern)
Pages: 297-303
Publication date: 1995
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Applied Bacteriology
Volume: 78
Issue number: 3
ISSN (Print): 0021-8847
Ratings:
BFI (2008): BFI-level 1
Original language: English
Source: orbit
Source-ID: 226045
Biopreservation of fish products - A review of recent approaches and results

General information
State: Published
Organisations: National Institute of Aquatic Resources, Section for Aquatic Microbiology and Seafood Hygiene
Authors: Huss, H. H. (Intern), Jeppesen, V. (Ekstern), Johansen, C. (Intern), Gram, L. (Intern)
Pages: 5-26
Publication date: 1995
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Aquatic Food Product Technology
Volume: 4
Issue number: 2
ISSN (Print): 1049-8850
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.386 SJR 0.309 CiteScore 0.64
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.59 SJR 0.266 SNIP 0.611
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.293 SNIP 0.621 CiteScore 0.65
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.273 SNIP 0.638 CiteScore 0.62
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.279 SNIP 0.548 CiteScore 0.59
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.419 SNIP 0.538 CiteScore 0.64
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.384 SNIP 0.62 CiteScore 0.68
ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.294 SNIP 0.485
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.207 SNIP 0.209
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.199 SNIP 0
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 0.259 SNIP 0.467
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 0.22 SNIP 0.764
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.203 SNIP 0
Web of Science (2005): Indexed yes
Pseudomine, an isoxazolidone with siderophoric activity from Pseudomonas fluorescens AH2 isolated from Lake Victorian Nile Perch

A siderophore, pseudomonine, and sodium salicylate were isolated from the culture broth of iron-deficient cultures of Pseudomonas fluorescens AH2 isolated from the surface of spoiled Nile Perch from Lake Victoria

General information
State: Published
Organisations: Section for Aquatic Microbiology and Seafood Hygiene, National Institute of Aquatic Resources
Authors: Anthoni, U. (Ekstern), Christophersen, C. (Ekstern), Nielsen, P. (Ekstern), Gram, L. (Intern), Petersen, B. (Ekstern)
Pages: 1786-1789
Publication date: 1995
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Natural Products
Volume: 58
Issue number: 11
ISSN (Print): 0163-3864
Ratings:
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 3.81 SJR 1.368 SNIP 1.487
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 3.41 SJR 1.202 SNIP 1.438
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.382 SNIP 1.748 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.326 SNIP 1.821 CiteScore 3.68
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.512 SNIP 1.696 CiteScore 3.75
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.428 SNIP 1.531 CiteScore 3.23
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
The Combined Inhibitory Effect of Lysozyme and Low pH on Growth of Listeria Monocytogenes

General information
State: Published
Organisations: Department of Biotechnology, Department of Systems Biology, Department of Chemical and Biochemical Engineering
Authors: Johansen, C. (Intern), Gram, L. (Intern), Meyer, A. B. S. (Intern)
Pages: 561-566
Publication date: 1994
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Food Protection
Volume: 57
ISSN (Print): 0362-028X
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 1.63 SJR 0.761 SNIP 0.823
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.68 SJR 0.769 SNIP 0.811
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1