Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries

Antimicrobial resistance (AMR) in bacteria and associated human morbidity and mortality is increasing. The use of antimicrobials in livestock selects for AMR that can subsequently be transferred to humans. This flow of AMR between reservoirs demands surveillance in livestock and in humans. We quantified and characterized the acquired resistance gene pools (resistomes) of 181 pig and 178 poultry farms from nine European countries, sequencing more than 5,000 Gb of DNA using shotgun metagenomics. We quantified acquired AMR using the ResFinder database and a second database constructed for this study, consisting of AMR genes identified through screening environmental DNA. The pig and poultry resistomes were very different in abundance and composition. There was a significant country effect on the resistomes, more so in pigs than in poultry. We found higher AMR loads in pigs, whereas poultry resistomes were more diverse. We detected several recently described, critical AMR genes, including mcr-1 and optrA, the abundance of which differed both between host species and between countries. We found that the total acquired AMR level was associated with the overall country-specific antimicrobial usage in livestock and that countries with comparable usage patterns had similar resistomes. However, functionally determined AMR genes were not associated with total drug use.
Genomics-Based Identification of Microorganisms in Human Ocular Body Fluid

Advances in genomics have the potential to revolutionize clinical diagnostics. Here, we examine the microbiome of vitreous (intraocular body fluid) from patients who developed endophthalmitis following cataract surgery or intravitreal injection. Endophthalmitis is an inflammation of the intraocular cavity and can lead to a permanent loss of vision. As controls, we included vitreous from endophthalmitis-negative patients, balanced salt solution used during vitrectomy and DNA extraction blanks. We compared two DNA isolation procedures and found that an ultraclean production of reagents appeared to reduce background DNA in these low microbial biomass samples. We created a curated microbial genome database (>5700 genomes) and designed a metagenomics workflow with filtering steps to reduce DNA sequences originating from: (i) human hosts, (ii) ambiguous/contaminants in public microbial reference genomes and (iii) the environment. Our metagenomic read classification revealed in nearly all cases the same microorganism that was determined in cultivation- and mass spectrometry-based analyses. For some patients, we identified the sequence type of the microorganism and antibiotic resistance genes through analyses of whole genome sequence (WGS) assemblies of isolates and metagenomic assemblies. Together, we conclude that genomics-based analyses of human ocular body fluid specimens can provide actionable information relevant to infectious disease management.

General information
State: Published
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Web of Science (2017): Impact factor 4.122
Web of Science (2017): Indexed yes
Draft Genome Sequence of Acinetobacter johnsonii C6, an Environmental Isolate Engaging in Interspecific Metabolic Interactions

Acinetobacter johnsonii C6 originates from creosote-polluted groundwater and performs ecological and evolutionary interactions with Pseudomonas putida in biofilms. The draft genome of A. johnsonii C6 is 3.7 Mbp and was shaped by mobile genetic elements. It reveals genes facilitating the biodegradation of aromatic hydrocarbons and resistance to antimicrobials and metals.

General information
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Organisations: National Food Institute, Research Group for Genomic Epidemiology, Novo Nordisk Foundation Center for Biosustainability, Infection Microbiology, Infection Microbiology
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A sampling and metagenomic sequencing-based methodology for monitoring antimicrobial resistance in swine herds

Objectives
Reliable methods for monitoring antimicrobial resistance (AMR) in livestock and other reservoirs are essential to understand the trends, transmission and importance of agricultural resistance. Quantification of AMR is mostly done using culture-based techniques, but metagenomic read mapping shows promise for quantitative resistance monitoring.

Methods
We evaluated the ability of: (i) MIC determination for Escherichia coli; (ii) cfu counting of E. coli; (iii) cfu counting of aerobic bacteria; and (iv) metagenomic shotgun sequencing to predict expected tetracycline resistance based on known antimicrobial consumption in 10 Danish integrated slaughter pig herds. In addition, we evaluated whether fresh or manure floor samples constitute suitable proxies for intestinal sampling, using cfu counting, qPCR and metagenomic shotgun sequencing.

Results
Metagenomic read-mapping outperformed cultivation-based techniques in terms of predicting expected tetracycline resistance based on antimicrobial consumption. Our metagenomic approach had sufficient resolution to detect antimicrobial-induced changes to individual resistance gene abundances. Pen floor manure samples were found to represent rectal samples well when analysed using metagenomics, as they contain the same DNA with the exception of a few contaminating taxa that proliferate in the extraintestinal environment.

Conclusions
We present a workflow, from sampling to interpretation, showing how resistance monitoring can be carried out in swine herds using a metagenomic approach. We propose metagenomic sequencing should be part of routine livestock resistance monitoring programmes and potentially of integrated One Health monitoring in all reservoirs.
Comparative genomics of toxigenic and non-toxigenic Staphylococcus hyicus

The most common causative agent of exudative epidermitis (EE) in pigs is Staphylococcus hyicus. S. hyicus can be grouped into toxigenic and non-toxigenic strains based on their ability to cause EE in pigs and specific virulence genes have been identified. A genome wide comparison between non-toxigenic and toxigenic strains has never been performed. In this study, we sequenced eleven toxigenic and six non-toxigenic S. hyicus strains and performed comparative genomic and phylogenetic analysis. Our analyses revealed two genomic regions encoding genes that were predominantly found in toxigenic strains and are predicted to encode for virulence determinants for EE. All toxigenic strains encoded for one of the exfoliative toxins ExhA, ExhB, ExhC, or ExhD. In addition, one of these regions encoded for an ADP-ribosyltransferase (EDIN, epidermal cell differentiation inhibitor) and a novel putative RNase toxin (polymorphic toxin) and was associated with the gene encoding ExhA. A clear differentiation between toxigenic and non-toxigenic strains based on genomic and phylogenetic analyses was not apparent. The results of this study support the observation that exfoliative toxins of S. hyicus and S. aureus are located on genetic elements such as pathogenicity islands, phages, prophages and plasmids.

General information
State: Published
Organisations: National Food Institute, Research Group for Genomic Epidemiology, National Veterinary Institute
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Journal: Veterinary Microbiology
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Web of Science (2018): Indexed yes
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Scopus rating (2017): CiteScore 2.7 SJR 1.175 SNIP 1.241
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.65 SJR 1.363 SNIP 1.206
Web of Science (2016): Impact factor 2.628
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.413 SNIP 1.21 CiteScore 2.56
Web of Science (2015): Impact factor 2.564
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Impact of Sample Type and DNA Isolation Procedure on Genomic Inference of Microbiome Composition

Explorations of complex microbiomes using genomics greatly enhance our understanding about their diversity, biogeography, and function. The isolation of DNA from microbiome specimens is a key prerequisite for such examinations, but challenges remain in obtaining sufficient DNA quantities required for certain sequencing approaches, achieving accurate genomic inference of microbiome composition, and facilitating comparability of findings across specimen types and sequencing projects. These aspects are particularly relevant for the genomics-based global surveillance of infectious agents and antimicrobial resistance from different reservoirs. Here, we compare in a stepwise approach a total of eight commercially available DNA extraction kits and 16 procedures based on these for three specimen types (human feces, pig feces, and hospital sewage). We assess DNA extraction using spike-in controls and different types of beads for bead beating, facilitating cell lysis. We evaluate DNA concentration, purity, and stability and microbial community composition using 16S rRNA gene sequencing and for selected samples using shotgun metagenomic sequencing. Our results suggest that inferred community composition was dependent on inherent specimen properties as well as DNA extraction method. We further show that bead beating or enzymatic treatment can increase the extraction of DNA from Gram-positive bacteria. Final DNA quantities could be increased by isolating DNA from a larger volume of cell lysate than that in standard protocols. Based on this insight, we designed an improved DNA isolation procedure optimized for microbiome genomics that can be used for the three examined specimen types and potentially also for other biological specimens. A standard operating procedure is available from https://dx.doi.org/10.6084/m9.figshare.3475406.

General information
State: Published
Organisations: National Food Institute, Research Group for Genomic Epidemiology, University of Copenhagen
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Development of Spatial Distribution Patterns by Biofilm Cells

Confined spatial patterns of microbial distribution are prevalent in nature, such as in microbial mats, soil communities, and water stream biofilms. The symbiotic two-species consortium of Pseudomonas putida and Acinetobacter sp. C6, originally isolated from a creosote-polluted aquifer, has evolved a distinct spatial organization in the laboratory that is characterized by an increased fitness and productivity. In this consortium, P. putida is reliant on microcolonies formed by Acinetobacter sp. C6 — to which it attaches. Here we describe the processes that lead to the microcolony-pattern by Acinetobacter sp. C6. Ecological spatial pattern analyses revealed that the microcolonies were not entirely randomly distributed, and instead arranged in a uniform pattern. Detailed time-lapse confocal microscopy at the single cell level demonstrated that the spatial pattern was the result of an intriguing self-organization: Small multicellular clusters moved along the surface to fuse with one another to form microcolonies. This active distribution capability was dependent on environmental factors (carbon source, oxygen) and historical contingency (formation of phenotypic variants). The findings of this study are discussed in the context of species distribution patterns observed in macroecology, and we summarize observations about the
processes involved in co-adaptation between *P. putida* and *Acinetobacter* sp. C6. Our results contribute to an understanding of spatial species distribution patterns as they are observed in nature, as well as the ecology of engineered communities that have the potential for enhanced and sustainable bioprocessing capacity.

**General information**
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factories, National Food Institute, Research Group for Genomic Epidemiology, Department of Systems Biology, Infection Microbiology, University of Copenhagen
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Scopus rating (2017): CiteScore 3.99
Web of Science (2017): Impact factor 3.633
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Impact factor 3.807
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): Impact factor 3.668
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
Web of Science (2013): Impact factor 3.952
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
Web of Science (2012): Impact factor 3.678
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
Web of Science (2011): Impact factor 3.829
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
The indigenous microbiota of the nasal cavity plays important roles in human health and disease. Patterns of spatial variation in microbiota composition may help explain Staphylococcus aureus colonization and reveal interspecies and species-host interactions. To assess the biogeography of the nasal microbiota, we sampled healthy subjects, representing both S. aureus carriers and noncarriers at three nasal sites (anterior naris, middle meatus, and sphenoethmoidal recess). Phylogenetic compositional and sparse linear discriminant analyses revealed communities that differed according to site epithelium type and S. aureus culture-based carriage status. Corynebacterium accolens and C. pseudodiphtheriticum were identified as the most important microbial community determinants of S. aureus carriage, and competitive interactions were only evident at sites with ciliated pseudostratified columnar epithelium. In vitro cocultivation experiments provided supporting evidence of interactions among these species. These results highlight spatial variation in nasal microbial communities and differences in community composition between S. aureus carriers and noncarriers.
Gut Immune Maturation Depends on Colonization with a Host-Specific Microbiota

Germ-free mice that lack intestinal microbiota are immunodeficient, with failed maturation of gut immune cells. Inoculation of these mice with either human or rat microbiota does not support gut immunity either, suggesting that mammalian hosts have coevolved with a specific consortium of bacterial species that stimulates intestinal immune maturation.
Single-cell sequencing provides clues about the host interactions of segmented filamentous bacteria (SFB)

Segmented filamentous bacteria (SFB) are host-specific intestinal symbionts that comprise a distinct clade within the Clostridiaceae, designated Candidatus Arthromitus. SFB display a unique life cycle within the host, involving differentiation into multiple cell types. The latter include filaments that attach intimately to intestinal epithelial cells, and from which "holdfasts" and spores develop. SFB induce a multifaceted immune response, leading to host protection from intestinal pathogens. Cultivation resistance has hindered characterization of these enigmatic bacteria. In the present study, we isolated five SFB filaments from a mouse using a microfluidic device equipped with laser tweezers, generated genome sequences from each, and compared these sequences with each other, as well as to recently published SFB genome sequences. Based on the resulting analyses, SFB appear to be dependent on the host for a variety of essential nutrients. SFB have a relatively high abundance of predicted proteins devoted to cell cycle control and to envelope biogenesis, and have a group of SFB-specific autolysins and a dynamin-like protein. Among the five filament genomes, an average of 8.6% of predicted proteins were novel, including a family of secreted SFB-specific proteins. Four ADP-ribosyltransferase (ADPRT) sequence types, and a myosin-cross-reactive antigen (MCRA) protein were discovered; we hypothesize that they are involved in modulation of host responses. The presence of polymorphisms among mouse SFB genomes suggests the evolution of distinct SFB lineages. Overall, our results reveal several aspects of SFB adaptation to the mammalian intestinal tract.

General information
State: Published
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Main Research Area: Technical/natural sciences

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BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 11.65 SJR 12.367 SNIP 2.35
Web of Science (2017): Impact factor 10.101
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 11.88 SJR 12.594 SNIP 2.373
Web of Science (2016): Impact factor 11.922
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 14.278 SNIP 2.943 CiteScore 14.3
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 14.817 SNIP 2.954 CiteScore 13.75
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 15.228 SNIP 2.897 CiteScore 14.86
Web of Science (2013): Impact factor 13.852
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 14.306 SNIP 3.072 CiteScore 15.03
Web of Science (2012): Impact factor 14.397
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 14.017 SNIP 2.968 CiteScore 13.83
Web of Science (2011): Impact factor 13.608
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 12.476 SNIP 2.567
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 10.404 SNIP 2.572
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 8.588 SNIP 2.101
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 6.922 SNIP 2.228
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 7.935 SNIP 2.388
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 7.78 SNIP 2.314
Scopus rating (2003): SJR 6.795 SNIP 2.301
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 6.762 SNIP 2.031
Scopus rating (2001): SJR 6.272 SNIP 2.134
Scopus rating (2000): SJR 5.823 SNIP 2.113
Scopus rating (1999): SJR 5.892 SNIP 2.315
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genome sequence, host interaction, Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Endospore-forming Gram-Positives [07810] Candidatus Arthromitus genus, Microorganisms (Bacteria, Eubacteria, Microorganisms) - Bacteria [05000] segmented filamentous bacteria common, Rodentia Mammalia Vertebrata Chordata Animalia (Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals, Rodents, Vertebrates) - Muridae [86375] mouse common, ADP-ribosyltransferase 58319-92-9, myosin-cross-reactive antigen, 10060, Biochemistry studies - General, 14004, Digestive system - Physiology and biochemistry, 31000, Physiology and biochemistry of bacteria, intestinal tract digestive system, microfluidic device laboratory equipment, single-cell sequencing laboratory techniques, genetic techniques, Biochemistry and Molecular Biophysics
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Source-ID: n::oai:DTIC-ART:biosis/366350369::38868
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The metabolically active subpopulation in Pseudomonas aeruginosa biofilms survives exposure to membrane-targeting antimicrobials via distinct molecular mechanisms

Biofilms are reported to be inherently refractory toward antimicrobial attack and, therefore, cause problems in industrial and medical settings. Pseudomonas aeruginosa biofilms contain subpopulations that exhibit high metabolic activity and subpopulations that exhibit low metabolic activity. We have found that membrane-targeting antimicrobials such as colistin, EDTA, SDS, and chlorhexidine specifically kill the inactive subpopulation in P. aeruginosa biofilms, whereas the active subpopulation survives exposure to these compounds. Because treatment of P. aeruginosa biofilms with the membrane-targeting compounds colistin, EDTA, SDS, and chlorhexidine resulted in the same spatial distribution of live and dead bacteria, we investigated whether tolerance to these compounds originated from the same molecular mechanisms. Development of colistin-tolerant subpopulations was found to depend on the pmr genes encoding lipopolysaccharide modification enzymes, as well as on the mexAB-oprM, mexCD-oprJ, and muxABC-opmB genes encoding antimicrobial efflux pumps, but does not depend on the mexPQ-opmE efflux pump genes. Development of chlorhexidine-tolerant subpopulations was found to depend on the mexCD-oprJ genes, but does not depend on the pmr, mexAB-oprM, mexPQ-opmE, or muxABC-opmB genes. Tolerance to SDS and EDTA in P. aeruginosa biofilms is linked to metabolically active cells, but does not depend on the pmr, mexAB, mexCD, mexPQ, or muxABC genes. Our data suggest that the active subpopulation in P. aeruginosa biofilms is able to adapt to exposure to membrane-targeting agents through the use of different genetic determinants, dependent on the specific membrane-targeting compound.
An update on Pseudomonas aeruginosa biofilm formation, tolerance, and dispersal

We review the recent advances in the understanding of the Pseudomonas aeruginosa biofilm lifestyle from studies using in vitro laboratory setups such as flow chambers and microtiter trays. Recent work sheds light on the role of nutrients, motility, and quorum sensing in structure formation in P. aeruginosa biofilms. The second messenger, c-di-GMP, is established as an important regulator of the synthesis of polysaccharide and protein components of the biofilm matrix. Extracellular DNA is shown to be an essential component of the biofilm matrix. It has become apparent that biofilm formation involves interactions between different subpopulations. The molecular mechanisms underlying the tolerance of biofilm bacteria to antimicrobial agents are beginning to be unraveled, and new knowledge has been obtained regarding the environmental cues and regulatory mechanisms involved in biofilm dispersal.

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
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Pages: 253-268
Publication date: 2010
Main Research Area: Technical/natural sciences
Inactivation of the rhlA gene in Pseudomonas aeruginosa prevents rhamnolipid production, disabling the protection against polymorphonuclear leukocytes

Many of the virulence factors produced by the opportunistic human pathogen Pseudomonas aeruginosa are quorum-sensing (QS) regulated. Among these are rhamnolipids, which have been shown to cause lysis of several cellular components of the human immune system, e.g. monocyte-derived macrophages and polymorphonuclear leukocytes (PMNs). We have previously shown that rhamnolipids produced by P. aeruginosa cause necrotic death of PMNs in vitro. This raises the possibility that rhamnolipids may function as a 'biofilm shield' in vivo, which contributes significantly to the increased tolerance of P. aeruginosa biofilms to PMNs. In the present study, we demonstrate the importance of the production of rhamnolipids in the establishment and persistence of P. aeruginosa infections, using an in vitro biofilm system, an intraperitoneal foreign-body model and a pulmonary model of P. aeruginosa infections in mice. Our experimental data showed that a P. aeruginosa strain, unable to produce any detectable rhamnolipids due to an inactivating mutation in the single QS-controlled rhlA gene, did not induce necrosis of PMNs in vitro and exhibited increased clearance compared with its wild-type counterpart in vivo. Conclusively, the results support our model that rhamnolipids are key protective agents of P. aeruginosa against PMNs.

General information
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Organisations: Department of Systems Biology, Center for Microbial Biotechnology, Center for Systems Microbiology
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Journal: Acta Pathologica Microbiologica et Immunologica Scandinavica
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Scopus rating (2017): CiteScore 1.95
Web of Science (2017): Impact factor 2.026
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.87
Web of Science (2016): Impact factor 1.795
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 1.92
Web of Science (2015): Impact factor 1.933
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 1.95
Web of Science (2014): Impact factor 2.042
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.07
Web of Science (2013): Impact factor 1.922
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.06
Web of Science (2012): Impact factor 2.068
Insight into the Microbial Multicellular Lifestyle via Flow-Cell Technology and Confocal Microscopy

Biofilms are agglomerates of microorganisms surrounded by a self-produced extracellular matrix. During the last 10 years, there has been an increasing recognition of biofilms as a highly significant topic in microbiology with relevance for a variety of areas in our society including the environment, industry, and human health. Accordingly a number of biofilm model systems, molecular tools, microscopic techniques, and image analysis programs have been employed for the study of biofilms under controlled and reproducible conditions. Studies using confocal laser scanning microscopy (CLSM) of biofilms formed in flow-chamber experimental systems by genetically color-coded bacteria have provided detailed knowledge about biofilm developmental processes, cell differentiations, spatial organization, and function of laboratory-grown biofilms, in some cases down to the single cell level. In addition, the molecular mechanisms underlying the increased tolerance that biofilm cells often display towards antibiotic treatment are beginning to be unravelled.
Roles of type IV pili, flagellum-mediated motility and extracellular DNA in the formation of mature multicellular structures in Pseudomonas aeruginosa biofilms.

General information
State: Published
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Pages: 2331-2343
Publication date: 2008
Main Research Area: Technical/natural sciences

Publication information
Tolerance to the antimicrobial peptide colistin in Pseudomonas aeruginosa biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes

Bacteria living as biofilm are frequently reported to exhibit inherent tolerance to antimicrobial compounds, and might therefore contribute to the persistence of infections. Antimicrobial peptides are attracting increasing interest as new potential antimicrobial therapeutics; however, little is known about potential mechanisms, which might contribute to resistance or tolerance development towards these compounds in biofilms. Here we provide evidence that a spatially distinct subpopulation of metabolically active cells in Pseudomonas aeruginosa biofilms is able to develop tolerance to the antimicrobial peptide colistin. On the contrary, biofilm cells exhibiting low metabolic activity were killed by colistin. We demonstrate that the subpopulation of metabolically active cells is able to adapt to colistin by inducing a specific adaptation mechanism mediated by the pmr operon, as well as an unspecific adaptation mechanism mediated by the mexAB-oprM genes. Mutants defective in either pmr-mediated lipopolysaccharide modification or in mexAB-oprM-mediated antimicrobial efflux were not able to develop a tolerant subpopulation in biofilms. In contrast to the observed pattern of colistin-mediated killing in biofilms, conventional antimicrobial compounds such as ciprofloxacin and tetracycline were found to specifically kill the subpopulation of metabolically active biofilm cells, whereas the subpopulation exhibiting low metabolic activity survived the treatment. Consequently, targeting the two physiologically distinct subpopulations by combined antimicrobial treatment with either ciprofloxacin and colistin or tetracycline and colistin almost completely eradicated all biofilm cells.
Differentiation in Pseudomonas Aeruginosa Biofilms

General information
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Organisations: Center for Biomedical Microbiology, Department of Systems Biology
Multiple roles of biosurfactants in structural biofilm development by Pseudomonas aeruginosa

Recent studies have indicated that biosurfactants produced by Pseudomonas aeruginosa play a role both in maintaining channels between multicellular structures in biofilms and in dispersal of cells from biofilms. Through the use of flow cell technology and enhanced confocal laser scanning microscopy, we have obtained results which suggest that the biosurfactants produced by P. aeruginosa play additional roles in structural biofilm development. We present genetic evidence that during biofilm development by P. aeruginosa, biosurfactants promote microcolony formation in the initial phase and facilitate migration-dependent structural development in the later phase. P. aeruginosa rhl4 mutants, deficient in synthesis of biosurfactants, were not capable of forming microcolonies in the initial phase of biofilm formation. Experiments involving two-color-coded mixed-strain biofilms showed that P. aeruginosa rhl4 mutants were defective in migration-dependent development of mushroom-shaped multicellular structures in the later phase of biofilm formation. Experiments involving three-color-coded mixed-strain P. aeruginosa biofilms demonstrated that the wild-type and rhl4 and pilI4 mutant strains formed distinct subpopulations on top of each other dependent on their ability to migrate and produce biosurfactants.
The Biofilm Matrix – A Sticky Framework

General information
State: Published
Organisations: Bioscience and Technology, Department of Systems Biology
Authors: Pamp, S. J. (Intern), Gjermansen, M. (Intern), Tolker-Nielsen, T. (Intern)
Pages: 37-69
Publication date: 2007

Host publication information
Title of host publication: Bacterial Biofilm Formation and Adaptation
Spx is a global effector impacting stress tolerance and biofilm formation in Staphylococcus aureus

In Bacillus subtilis, Spx was recently characterized as a novel type of global regulator whose activity is regulated by the redox status of the cells. In the present study, we demonstrate that inactivation of Spx in the important pathogen Staphylococcus aureus renders the cells hypersensitive to a wide range of stress conditions including high and low temperature, high osmolarity, and hydrogen peroxide. Moreover, growth was restricted under nonstress conditions. Two-dimensional gel electrophoresis revealed that the proteome of the spx mutant differs substantially from the proteome of wild-type cells, supporting the finding that Spx is also a global regulator in S. aureus. More specifically, we demonstrated that Spx is required for transcription of trxB, encoding thioredoxin reductase, under all growth conditions examined. As trxB is essential in S. aureus, we speculate that the severely reduced trxB transcription could account for some of the growth defects of the spx mutant. Inactivation of spx also enhanced biofilm formation. S. aureus biofilm formation is associated with the production of the polysaccharide intercellular adhesin encoded by the ica operon. Interestingly, our data indicate that the augmented capacity of the spx mutant to form biofilms is due to Spx modulating the expression of icaR, encoding a repressor of the structural ica genes (icaABCD). In summary, we conclude that Spx fulfills an important role for growth, general stress protection, and biofilm formation in S. aureus.
Scopus rating (2012): SJR 2.125 SNIP 1.085 CiteScore 3.42
Web of Science (2012): Impact factor 3.177
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BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.471 SNIP 1.154 CiteScore 3.83
Web of Science (2011): Impact factor 3.825
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Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.64 SNIP 1.144
Web of Science (2010): Impact factor 3.726
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
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Scopus rating (1999): SJR 2.688 SNIP 1.205

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Projects:

Generic open science data platform for surveillance, exposure assessment and risk analysis
National Food Institute
Period: 01/12/2016 → 10/02/2020
Number of participants: 5
Phd Student: 
Backhaus, Liv Louise Victoria (Intern)
Supervisor: 
Lund, Ole (Intern)
Pamp, Sünje Johanna (Intern)
Vigre, Håkan (Intern)
Main Supervisor: 
Aarestrup, Frank Møller (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Metagenomic Approaches for Determining the Structure and Function of Complex Microbiomes
National Food Institute
Period: 01/01/2016 → 06/05/2019
Number of participants: 4
Phd Student: 
Kirstahler, Philipp (Intern)
Supervisor: 
Lund, Ole (Intern)
Pamp, Sünje Johanna (Intern)
Main Supervisor: 
Aarestrup, Frank Møller (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Samfinansieret - Andet
Project: PhD

Detection of Pathogens and Antimicrobial Resistance in Microbiomes
National Food Institute
Period: 01/10/2015 → 31/03/2019
Number of participants: 4
Phd Student: 
Poulsen, Casper Sahl (Intern)
Supervisor: 
Kaas, Rolf Sommer (Intern)
Pamp, Sünje Johanna (Intern)
Main Supervisor: 
Aarestrup, Frank Møller (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Samfinansieret - Andet
Project: PhD

Interactions Between the Microbiome and Host Immune System

Diagnostic use of Microbial Whole Genome Sequencing (WGS)

Department of Bio and Health Informatics
Period: 15/12/2014 → 05/07/2019
Number of participants: 4
Phd Student:
Tetzschner, Anna Maria Malberg (Intern)
Supervisor:
Aarestrup, Frank Møller (Intern)
Pamp, Sünje Johanna (Intern)
Main Supervisor:
Lund, Ole (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Samfinansieret - Andet
Project: PhD

Biogeography of Pathogens: Unveiling their Diversity, Distribution, and Function in Space and Time

National Food Institute
Division of Epidemiology and Microbial Genomics
Period: 01/04/2014 → 31/03/2015
Number of participants: 2
Microbial Biogeography, Microbial Ecology, Metagenomics, Staphylococcus aureus
Project participant:
Aarestrup, Frank Møller (Intern)
Project applicant:
Pamp, Sünje Johanna (Intern)

Financing sources
Source: Private funding (private)
Name of research programme: Carlsbergfondet
Project

Microbiota and Metabolic Diseases - Dietary intervention studies in animal models

National Food Institute
Period: 01/11/2012 → 02/06/2016
Number of participants: 7
Phd Student:
Zhang, Li (Intern)
Risk based control in pig slaughter

National Food Institute
Period: 01/09/2012 → 30/09/2017
Number of participants: 8
Phd Student:
Bollerslev, Anne Mette (Intern)
Supervisor:
Hald, Tine (Intern)
Hansen, Tina Beck (Intern)
Nauta, Maarten (Intern)
Main Supervisor:
Aabo, Søren (Intern)
Examiner:
Pamp, Sünje Johanna (Intern)
Ahrné, Siv (Ekstern)
Wichmann, Anita E. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU) Samf.
Project: PhD

Revealing Polymorphisms in Individual Microbiotas that Impact on the Host Immune System

Department of Systems Biology
Period: 01/06/2011 → 30/09/2012
Number of participants: 2
Single-Cell Genomics, Microbiome, Segmented Filamentous Bacteria, Host-Microbe Interactions
Project participant:
Relman, David (Ekstern)
Project applicant:
Pamp, Sünje Johanna (Intern)

Financing sources
Source: Private funding (private)
Name of research programme: Lundbeckfonden
Project

Symbiotic Microbe-Host Interactions

Department of Systems Biology
Period: 01/06/2009 → 31/05/2011
Number of participants: 2
Human Microbiome, Genomics, Microbial Ecology, Host-Microbe Interactions, Symbiosis
Project participant:
**Differentiation in Microbial Biofilms**

Department of Systems Biology  
Period: 01/08/2004 → 14/12/2007  
Number of participants: 5  
PhD Student:  
Pamp, Sünje Johanna (Intern)  
Main Supervisor:  
Tolker-Nielsen, Tim (Intern)  
Examiner:  
Gram, Lone (Intern)  
Kühl, Michael (Intern)  
Parsek, Matthew R. (Ekstern)

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: 1/3 DTU-stip, 2/3 FUR/andet  
Project: PhD

**Activities:**

**Tar-Eating Bacterial Duo may Transform Toxic Compounds into New Usable Materials**  
Period: 16 May 2017  
Sünje Johanna Pamp (Participant)  
Department of Biotechnology and Biomedicine  
Department of Bio and Health Informatics  
National Food Institute  
Research Group for Genomic Epidemiology

**Description**  
Danish researchers have sequenced and analyzed the genome of a bacterium that can feed off coal tar. It lives in symbiosis with another bacterium that can recycle its partner's waste. Researchers hope that this sustainable bacterial duo can transform toxic substances into useful materials. Nevertheless, mapping the genome also led to an unpleasant surprise.

**Interview person.**  
Degree of recognition: International  
Documents:  
Tar-eating bacterial duo may transform toxic compounds into new usable materials | Sciencenews.dk  
Links:  
Activity: Other

**Cover Illustration (APMIS) December 2014**  
Period: Dec 2014  
Sünje Johanna Pamp (Participant)  
National Food Institute  
Division of Epidemiology and Microbial Genomics
Description

Documents:
Pamp.CytA.2009-Fig_APMIS.December.2014
Activity: Other

Prizes:

Cover Illustration (Cytometry Part A): Insight into the Microbial Multicellular Lifestyle
Sünje Johanna Pamp (Recipient)
Department of Systems Biology

Details
Awarded date: Feb 2009
Prize: Prizes, scholarships, distinctions

Cover Illustration (Genome Research): SFB Single-Cell Genomics
Sünje Johanna Pamp (Recipient)
National Food Institute, Division of Epidemiology and Microbial Genomics

Details
Awarded date: Jun 2012
Prize: Prizes, scholarships, distinctions

Cover Illustration (Journal of Bacteriology): Microbial Interactions, 3-Colour-Coded Biofilm
Sünje Johanna Pamp (Recipient)
Department of Systems Biology

Details
Awarded date: Jan 2007
Prize: Prizes, scholarships, distinctions

F1000 - Exceptional: Development of Spatial Distribution Patterns by Biofilm Cells (AEM Vol. 81(18)).
Sünje Johanna Pamp (Recipient)
National Food Institute, Research Group for Genomic Epidemiology

Description
Article: Development of Spatial Distribution Patterns by Biofilm Cells., Applied and Environmental Microbiology, 2015 (DOI: 10.3410/f.725596154.793509444), has been recommended in F1000Prime as being of special significance in its field by F1000 Faculty Member Robert Palmer.

Details
Awarded date: 8 Sep 2015
Granting Organisations: Faculty of 1000 Ltd
Prize: Prizes, scholarships, distinctions

F1000Prime - Tolerance to the antimicrobial peptide colistin in Pseudomonas aeruginosa biofilms is linked to metabolically active cells (Mol.Microbiol. Vol. 68(1)).
Sünje Johanna Pamp (Recipient)
National Food Institute, Research Group for Genomic Epidemiology

Description
This study demonstrates that difficulties in treating infections caused by biofilm-forming bacteria may be due to differential sensitivities of metabolically distinct subpopulations of bacterial cells in the biofilm. The authors show that combination therapy, with antibiotics targeting each distinct subpopulation, may be a successful treatment strategy for infections of biofilm-forming bacteria [...].

Synergistic effects of antibiotics are well known, and this paper presents one interesting explanation: distinct subpopulations of cells in a biofilm that are susceptible to different classes of drugs [...].

This paper highlights the importance of studying distinct and well-defined sub-populations of cells in a physiologically...
Selected by Editors: Microbial Community Assembly and Spatial Ecology (AEM Vol. 81(18)): Articles of Significant Interest
Selected by the Editors from AEM
Sünje Johanna Pamp (Recipient)
National Food Institute, Research Group for Genomic Epidemiology

Description
The principles and mechanisms that govern multicellular community assembly are incompletely understood. Haagensen et al. (p. 6120 – 6128 [doi: 10.1128/AEM.01614-15]) integrated high-resolution time-lapse microscopy with ecological spatial pattern analysis to characterize microbial community assembly and spatial organization. Their work revealed that small multicellular clusters can move, interact with each other, and fuse to form symmetric patterns of larger multicellular assemblages. Knowledge about microbial spatial ecology is central to our understanding of the structure and function of environmental, host-associated, and synthetic microbial communities. Moreover, the observed formation of primordial cell groups and their aggregation to higher-level structures may be a model for studying the emergence of multicellular life.

Press clippings:

Staph can lurk deep within nose, study finds
Sünje Johanna Pamp
11/12/2013

Description
Scientists at the Stanford University School of Medicine have revealed that formerly overlooked sites deep inside the nose may be reservoirs for Staphylococcus aureus, a major bacterial cause of disease. Moreover, the relative abundance of S. aureus was inversely related to that of another bacterial species, C. pseudodiphtheriticum. When one was present at high levels, the other was present at low levels or absent. The researchers suspect that something C. pseudodiphtheriticum produces and secretes — perhaps a protein, or possibly a small molecule — is responsible for S. aureus' failure to thrive. If such a substance could be identified, Pamp said, it could provide clues to the development of new compounds to prevent or treat S. aureus infections.

National Food Institute, Division of Epidemiology and Microbial Genomics

Media contribution (1)

Staph can lurk deep within nose, study finds
11/12/2013
Stanford News, Web
http://med.stanford.edu/ism/2013/december/nose.html
Sünje Johanna Pamp
National Food Institute, Division of Epidemiology and Microbial Genomics

Relations
Research outputs:
Nasal Microenvironments and Interspecific Interactions Influence Nasal Microbiota Complexity and S. aureus Carriage
Press / Media

Staph Germs Hide Out In The Hidden Recesses Of Your Nose
Sünje Johanna Pamp
11/12/2013

Description
Researchers at Stanford University School of Medicine voyaged up the nose to meet the natives — the bacteria that live in these warm, dark places. They found that staph germs just love the middle and upper nose cavity, much more so than the relatively arid nostrils.
Staph Germs Hide Out In The Hidden Recesses Of Your Nose
11/12/2013
Shots - Health News from NPR, Web
Sünje Johanna Pamp
National Food Institute, Division of Epidemiology and Microbial Genomics

Nasal Microenvironments and Interspecific Interactions Influence Nasal Microbiota Complexity and S. aureus Carriage