Basics of antibody phage display technology
Antibody discovery has become increasingly important in almost all areas of modern medicine. Different antibody discovery approaches exist, but one that has gained increasing interest in the field of toxinology and antivenom research is phage display technology. In this review, the lifecycle of the M13 phage and the basics of phage display technology are presented together with important factors influencing the success rates of phage display experiments. Moreover, the pros and cons of different antigen display methods and the use of naïve versus immunized phage display antibody libraries is discussed, and selected examples from the field of antivenom research are highlighted. This review thus provides in-depth knowledge on the principles and use of phage display technology with a special focus on discovery of antibodies that target animal toxins.

General information
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Organisations: Department of Biotechnology and Biomedicine, Section for Microbial and Chemical Ecology, Metabolic Signaling and Regulation, Tropical Pharmacology and Biotherapeutics, Network Engineering of Eukaryotic Cell factories, IONTAS Ltd, Technical University of Denmark
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Isolation and characterization of bacteriophages with therapeutic potential

The concerning spread of antibiotic resistant bacteria has directed the spotlight upon bacteriophages, in short phages, as potential candidates for therapeutic purposes. Far for being a novelty, phage therapy has been widely used in the 20s and 30s in western countries until the discovery of antibiotics, which, coupled with a lack of knowledge of phage biology at that time, let to the replacement of phage therapy by antibiotics. On the other side of the planet, the Georgian Eliava Institute has been using phages for treating bacterial diseases since short after phage discovery a century ago. Georgian pharmacies commonly sell phage cocktails from the Institute without the need of a doctors prescription. A thorough characterisation of the cocktail is though required for it to be accepted as pharmaceutical in the European Union. The potential to investigate the genetic material of microbial communities directly from the environment through metagenomics, allows for genomic characterisation of these cocktail. Furthermore, metagenomics analyses may lead to the discovery of novel phages with therapeutic potential, opening up a promising new horizon for phage therapy.

This thesis is divided into five parts, each assigned a chapter. Chapter 1 provides the reader with an introduction to phage biology, history and metagenomics. Here, the main bioinformatics methods used throughout the studies of the following chapters are also presented and briefly described. Chapter 2 presents the paper "HostPhinder: A Phage Host Prediction Tool" published in May 2016. The tool predicts the bacterial host of a given phage based on co-occurrent k-mers between a query sequence and reference phage genomes with known host. HostPhinder’s accuracy in predicting the host species and genus of an evaluation set was higher than 74% and 81%, respectively. The tool can be applied to identify the host of phage sequences found for instance in metagenomes allowing for a first step characterisation. Chapter 3 presents the paper "Metagenomic analysis of therapeutic PYO phage cocktails from 1997 to 2014" submitted in October 2017 and currently under peer-revision. In this study, the compositions of 3 batches of a Georgian cocktail from 1997 to 2014 was compared by means of Next Generation Sequencing (NGS) and metagenomic analysis. Thirty and 29 phage draft genomes were found in the cocktails from 1997 and 2014, respectively. One of them was present in both sample and did not resemble any known phage genomes, strongly suggesting its novelty. Phage representatives of all bacterial targets supposedly targeted by the cocktail's were found, as predicted using HostPhinder. A comparison between cocktails from
1997, 2000, and 2014 showed a closer composition between the first two cocktails. Chapter 4 presents the characterisation of historical S. aureus phages, once used for phage typing. Finally, the conclusive Chapter 5, recapitulates the main findings of this thesis and frame them into the perspective of potential future investigations.

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Organisations: Department of Bio and Health Informatics, Immunoinformatics and Machine Learning, Center for Biological Sequence Analysis, Department of Biotechnology and Biomedicine, Metabolic Signaling and Regulation

Contributors: Villarroel, J., Nielsen, M., Larsen, M. V., Kilstrup, M.

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**Linear peptidomimetics as potent antagonists of Staphylococcus aureus agr quorum sensing**

Staphylococcus aureus is an important pathogen causing infections in humans and animals. Increasing problems with antimicrobial resistance has prompted the development of alternative treatment strategies, including antivirulence approaches targeting virulence regulation such as the agr quorum sensing system. agr is naturally induced by cyclic auto-inducing peptides (AIPs) binding to the AgrC receptor and cyclic peptide inhibitors have been identified competing with AIP binding to AgrC. Here, we disclose that small, linear peptidomimetics can act as specific and potent inhibitors of the S. aureus agr system via intercepting AIP-AgrC signal interaction at low micromolar concentrations. The corresponding linear peptide did not have this ability. This is the first report of a linear peptide-like molecule that interferes with agr activation by competitive binding to AgrC. Prospectively, these peptidomimetics may be valuable starting scaffolds for the development of new inhibitors of staphylococcal quorum sensing and virulence gene expression.

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Organisations: Department of Biotechnology and Biomedicine, Metabolic Signaling and Regulation, University of Copenhagen

Contributors: Karathanasi, G., Bojer, M. S., Baldry, M., Johannessen, B. A., Wolff, S., Greco, I., Kilstrup, M., Hansen, P. R., Ingmer, H.

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Web of Science (2016): Impact factor 4.259

Web of Science (2016): Indexed yes

BFI (2015): BFI-level 1

Scopus rating (2015): CiteScore 5.3 SJR 2.034 SNIP 1.597

Web of Science (2015): Impact factor 5.228
Butanol is cytotoxic to Lactococcus lactis while ethanol and hexanol are cytostatic

Lactic acid bacteria currently used extensively by the dairy industry have a superior tolerance towards small chain alcohols, which makes them interesting targets for use in future bio-refineries. The mechanism underlying the alcohol tolerance of lactic acid bacteria has so far received little attention. In the present study the physiological alcohol stress response of Lactococcus lactis subsp. cremoris MG1363 towards the primary, even-chain alcohols; ethanol, butanol, and hexanol was characterized. The alcohol tolerance of L. lactis was found comparable to those reported for highly alcohol resistant lactic acid bacteria. Combined results from alcohol survival rate, live/dead staining, and a novel usage of the beta-galactosidase assay, revealed that while high concentrations of ethanol and hexanol were cytostatic to L. lactis, high concentrations of butanol were cytotoxic, causing irreparable damages to the cell membrane.
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
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Scopus rating (2017): CiteScore 1.78 SJR 0.924 SNIP 0.6
Web of Science (2017): Impact factor 1.866
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Scopus rating (2016): CiteScore 1.56 SJR 1.035 SNIP 0.663
Web of Science (2016): Impact factor 2.151
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.05 SJR 1.352 SNIP 0.859
Web of Science (2015): Impact factor 2.268
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.69 SJR 1.461 SNIP 0.97
Web of Science (2014): Impact factor 2.557
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 3.34 SJR 1.674 SNIP 1.028
Web of Science (2013): Impact factor 2.835
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Scopus rating (2012): CiteScore 3.12 SJR 1.6 SNIP 0.969
Web of Science (2012): Impact factor 2.852
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 3.18 SJR 1.659 SNIP 1.036
Web of Science (2011): Impact factor 3.061
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.804 SNIP 0.988
Web of Science (2010): Impact factor 2.957
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.71 SNIP 0.995
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BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.743 SNIP 1.011
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.739 SNIP 1.062
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.794 SNIP 1.063
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.76 SNIP 1.024
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.705 SNIP 1.065
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.682 SNIP 1.04
Metagenomic Analysis of Therapeutic PYO Phage Cocktails from 1997 to 2014

Phage therapy has regained interest in recent years due to the alarming spread of antibiotic resistance. Whilst phage cocktails are commonly sold in pharmacies in countries such as Georgia and Russia, this is not the case in western countries due to western regulatory agencies requiring a thorough characterization of the drug. Here, DNA sequencing of constituent biological entities constitutes a first step. The pyophage (PYO) cocktail is one of the main commercial products of the Georgian Eliava Institute of Bacteriophage, Microbiology and Virology and is used to cure skin infections. Since its first production in the 1930s, the composition of the cocktail has been periodically modified to add phages effective against emerging pathogenic strains. In this paper, we compared the composition of three PYO cocktails from 1997 (PYO97), 2000 (PYO2000) and 2014 (PYO2014). Based on next generation sequencing, de novo assembly and binning of contigs into draft genomes based on tetranucleotide distance, thirty and twenty-nine phage draft genomes were predicted in PYO97 and PYO2014, respectively. Of these, thirteen and fifteen shared high similarity to known phages. Eleven draft genomes were found to be common in the two cocktails. One of these showed no similarity to publicly available phage genomes. Representatives of phages targeting E. faecalis, E. faecium, E. coli, Proteus, P. aeruginosa and S. aureus were found in both cocktails. Finally, we estimated larger overlap of the PYO2000 cocktail to PYO97 compared to PYO2014. Using next generation sequencing and metagenomics analysis, we were able to characterize and compare the content of PYO cocktails separated by 17 years in time. Even though the cocktail composition is upgraded every six months, we found it to remain relatively stable over the years.

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Scopus rating (2017): CiteScore 3.88 SJR 1.805 SNIP 1.13
Web of Science (2017): Impact factor 3.761
Web of Science (2017): Indexed yes
Scopus rating (2016): CiteScore 3.6 SJR 1.747 SNIP 1.02
Web of Science (2016): Impact factor 3.465
Web of Science (2016): Indexed yes
Scopus rating (2015): CiteScore 3.74 SJR 1.832 SNIP 1.034
Phosphoribosyl Diphosphate (PRPP): Biosynthesis, Enzymology, Utilization, and Metabolic Significance

Phosphoribosyl diphosphate (PRPP) is an important intermediate in cellular metabolism. PRPP is synthesized by PRPP synthase, as follows: ribose 5-phosphate + ATP → PRPP + AMP. PRPP is ubiquitously found in living organisms and is used in substitution reactions with the formation of glycosidic bonds. PRPP is utilized in the biosynthesis of purine and pyrimidine nucleotides, the amino acids histidine and tryptophan, the cofactors NAD and tetrahydromethanopterin, arabinosyl monophosphodecaprenol, and certain aminoglycoside antibiotics. The participation of PRPP in each of these metabolic pathways is reviewed. Central to the metabolism of PRPP is PRPP synthase, which has been studied from all kingdoms of life by classical mechanistic procedures. The results of these analyses are unified with recent progress in molecular enzymology and the elucidation of the three-dimensional structures of PRPP synthases from eubacteria, archaea, and humans. The structures and mechanisms of catalysis of the five diphosphoryltransferases are compared, as are those of selected enzymes of diphosphoryl transfer, phosphoryl transfer, and nucleotidyl transfer reactions. PRPP is used as a substrate by a large number phosphoribosyltransferases. The protein structures and reaction mechanisms of these phosphoribosyltransferases vary and demonstrate the versatility of PRPP as an intermediate in cellular physiology. PRPP synthases appear to have originated from a phosphoribosyltransferase during evolution, as demonstrated by phylogenetic analysis. PRPP, furthermore, is an effector molecule of purine and pyrimidine nucleotide biosynthesis, either by binding to PurR or PyrR regulatory proteins or as an allosteric activator of carbamoylphosphate synthetase. Genetic analyses have disclosed a number of mutants altered in the PRPP synthase-specifying genes in humans as well as bacterial species.

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Clear Plaque Mutants of Lactococcal Phage TP901-1

We report a method for obtaining turbid plaques of the lactococcal bacteriophage TP901-1 and its derivative TP901-BC1034. We have further used the method to isolate clear plaque mutants of this phage. Analysis of 8 such mutants that were unable to lysogenize the host included whole genome resequencing. Four of the mutants had different mutations in structural genes with no relation to the genetic switch. However all 8 mutants had a mutation in the cI repressor gene region. Three of these were located in the promoter and Shine-Dalgarno sequences and five in the N-terminal part of the encoded CI protein involved in the DNA binding. The conclusion is that cI is the only gene involved in clear plaque formation i.e. the CI protein is the determining factor for the lysogenic pathway and its maintenance in the lactococcal...
phage TP901-1.

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Organisations: Department of Systems Biology, Metabolic Signaling and Regulation, Aarhus University, University of Copenhagen
Contributors: Kot, W., Kilstrup, M., Vogensen, F. K., Hammer, K.
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BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.01 SJR 1.164 SNIP 1.111
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
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): CiteScore 3.32 SJR 1.427 SNIP 1.136
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.54 SJR 1.559 SNIP 1.148
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.94 SJR 1.772 SNIP 1.153
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 4.15 SJR 1.982 SNIP 1.156
Web of Science (2012): Impact factor 3.73
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.233
Web of Science (2011): Impact factor 4.092
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Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.705 SNIP 1.178
Web of Science (2010): Impact factor 4.411
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
Design of Fab-based chimeric antibodies against Bothrops asper toxins
Snakebite is one of the world’s most neglected tropical diseases, with an estimated 5 million bites per year, resulting in about 125,000 deaths. The only current treatment for snakebite envenoming is antiserum derived from the blood of immunized mammals (typically horses). These antisera are expensive to produce and carry a high risk of causing hypersensitive reactions in human recipients due to their heterologous origin. Here we report the discovery of chimeric scFvs against Bothrops asper toxins.

Innovation from the Perspective of a Natural Scientist: The SAND Model
Engineers and natural scientists are required to suggest successful utilization of their discoveries and secure property rights to their universities whenever possible. Here I develop a novel model that optimizes the process of innovation by dividing it into three separate phases following the pre-innovative discovery; i.e., an application phase, a design phase, and an entrepreneurial phase. The phases are identified in the well-described innovation of the electron tube from Edison’s light bulb. Each phase consists of an abductive process, where a large selection of possible solutions is created, followed by selection of viable solutions among them according to their fitness in an entrepreneurial niche. An innovation is described in an evolutionary setting, starting with a novel discovery which becomes the Source (S) of an innovation. In the application phase, a viable application (A) of the Source is selected among a variety of possible applications. This again becomes the basis for a viable design (D) in the design phase. Finally, to become a viable innovation the particular discovery, application and design has to fit into an entrepreneurial niche (N) with a high fitness. To become a successful innovation all four elements (SAN D) need to be optimized by abduction. The present SAND model is different from all other innovative models in its focus on three separate creative abductive processes, yet current innovative theories can be described in the four dimensional innovation space by mapping along its four SAND axes. Analysis of fitness landscapes is in the present report used to visualize the events leading to incremental versus radical innovation, sustaining versus disruptive innovation, as well as the difference between technology and meaning-changes in design. Leading innovation models thus fit in as specialized scenarios under the general model. A low level of redundancy was found between the SAND model and the Stage-Gate model, but the differing theoretical foundations have the effect that the two models are complementary rather than overlapping.
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 violobiosynthetic 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 play a role in the antibacterial activity of P. luteoviolacea.

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Contributors: Schmidt Thøgersen, M., Delpin, M., Melchiorsen, J., Kilstrup, M., Månsson, M., Bunk, B., Spröer, C., Overmann, J., Nielsen, K. F., Gram, L.
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Ribosomal dimerization factor YfiA is the major protein synthesized after abrupt glucose depletion in *Lactococcus lactis*

We analysed the response of the model bacterium *Lactococcus lactis* to abrupt depletion of glucose after several generations of exponential growth. Glucose depletion resulted in a drastic drop in the energy charge accompanied by an extremely low GTP level and an almost total arrest of protein synthesis. Strikingly, the cell prioritized the continued synthesis of a few proteins, of which the ribosomal dimerization factor YfiA was the most highly expressed. Transcriptome analysis showed no immediate decrease in total mRNA levels despite the lowered nucleotide pools and only marginally increased levels of the yfiA transcript. Severe up-regulation of genes in the FruR, CcpA, ArgR and AhrC regulons were consistent with a downshift in carbon and energy source. Based upon the results, we suggest that transcription proceeded long enough to record the transcriptome changes from activation of the FruR, CcpA, ArgR and AhrC regulons, while protein synthesis stopped due to an extremely low GTP concentration emerging a few minutes after glucose depletion. The yfiA deletion mutant exhibited a longer lag phase upon replenishment of glucose and a faster death rate after prolonged starvation supporting that YfiA-mediated ribosomal dimerization is important for keeping long-term starved cells viable and competent for growth initiation.
Structural and dynamics studies of a truncated variant of CI repressor from bacteriophage TP901-1

The CI repressor from the temperate bacteriophage TP901-1 consists of two folded domains, an N-terminal helix-turn-helix DNA-binding domain (NTD) and a C-terminal oligomerization domain (CTD), which we here suggest to be further divided into CTD1 and CTD2. Full-length CI is a hexameric protein, whereas a truncated version, CIΔ58, forms dimers.

We identify the dimerization region of CIΔ58 as CTD1 and determine its secondary structure to be helical both within the context of CIΔ58 and in isolation. To our knowledge this is the first time that a helical dimerization domain has been found in a phage repressor. We also precisely determine the length of the flexible linker connecting the NTD to the CTD. Using electrophoretic mobility shift assays and native mass spectrometry, we show that CIΔ58 interacts with the O-L operator site as one dimer bound to both half-sites, and with much higher affinity than the isolated NTD domain thus demonstrating cooperativity between the two DNA binding domains. Finally, using small angle X-ray scattering data and state-of-the-art ensemble selection techniques, we delineate the conformational space sampled by CIΔ58 in solution, and we discuss the possible role that the dynamics play in CI-repressor function.

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While the field of semiotics has been active since it was started by Peirce, it appears like the last decade has been especially productive with a number of important new concepts being developed within the biosemiotics community. The novel concept of the Semiotic scaffold by Hoffmeyer is an important addition that offers insight into the hardware requirements for bio-semiosis. As any type of semiosis must be dependent upon Semiotic scaffolds, I recently argued that the process of semiosis has to be divided into two separate processes of sign establishment and sign interpretation, and that misalignment between the two processes result in faulty sign interpretation and over-signification. Such faulty signs were forbidden in the sign classification system of Peirce, so I defined them as forbidden signs. Here I present an analysis of the forbidden sign categories with examples from Occult semiotics. I also show that biological semiosis offers examples of forbidden signs, where the faulty interpretation of signs may lead to decimation of whole evolutionary lines of organisms. A new concept of Evolutionary memory which is applicable to both human and biological semiosis is explained as the combination of two processes; one leading to diversity generation within semiotic scaffolds followed by a second process of decimation of faulty signs during selection in specific learning environments. The analysis suggests that forbidden signs are always used as early stages in the iterative sign establishment process during semiosis.
The in vitro redundant enzymes PurN and PurT are both essential for systemic infection of mice in *Salmonella enterica* serovar Typhimurium

Metabolic enzymes show a high degree of redundancy, and for that reason they are generally ignored when searching for novel targets for anti-infective substances. The enzymes PurN and PurT are redundant *in vitro* in *Salmonella enterica* serovar Typhimurium (S. Typhimurium), where they perform the third step in the purine synthesis. Surprisingly the results of the current study demonstrated that single gene deletions of each of the genes encoding these enzymes caused attenuation (competitive infection index <0.03) in mouse infections. While the ΔpurT mutant multiplied as fast as the wild
type strain in cultured J774A.1 macrophages, net multiplication of the ΔpurN mutant was reduced by approximately 50 %
in 20 hours. The attenuation of the ΔpurT mutant was abolished by simultaneous removal of the enzyme PurU, responsible for formation of formate, indicating that the attenuation was related to formate accumulation or wasteful consumption of formyl-tetrahydrofolate by PurU. In the process of further characterization, we disclosed that in vivo the enzyme-complex GCV was the most important for formation of C-1 units in vivo (CI: 0.03 ± 0.03). In contrast, GlyA was the only important enzyme for the formation of C-1 units in vitro. The results with the ΔgcvT mutant further revealed that formation of serine by SerA and further conversion of serine into C-1 units and glycine by GlyA was not sufficient to ensure C-1 formation in S. Typhimurium in vivo. The study calls for re-investigations of the concept of metabolic redundancy in S. Typhimurium in vivo.

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Scopus rating (2017): CiteScore 3.43 SJR 1.954 SNIP 0.953
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Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 3.34 SJR 2.04 SNIP 0.915
Web of Science (2016): Indexed yes
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Scopus rating (2015): CiteScore 3.72 SJR 2.361 SNIP 1.053
Web of Science (2015): Impact factor 3.603
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.74 SJR 2.344 SNIP 1.08
Web of Science (2014): Impact factor 3.731
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Scopus rating (2013): CiteScore 4.25 SJR 2.433 SNIP 1.168
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BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 4.32 SJR 2.386 SNIP 1.167
Web of Science (2012): Impact factor 4.074
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.25 SJR 2.298 SNIP 1.166
Web of Science (2011): Impact factor 4.165
Bistability in a Metabolic Network Underpins the De Novo Evolution of Colony Switching in Pseudomonas fluorescens

Phenotype switching is commonly observed in nature. This prevalence has allowed the elucidation of a number of underlying molecular mechanisms. However, little is known about how phenotypic switches arise and function in their early evolutionary stages. The first opportunity to provide empirical insight was delivered by an experiment in which populations of the bacterium Pseudomonas fluorescens SBW25 evolved, de novo, the ability to switch between two colony phenotypes. Here we unravel the molecular mechanism behind colony switching, revealing how a single nucleotide change in a gene enmeshed in central metabolism (carB) generates such a striking phenotype. We show that colony switching is underpinned by ON/OFF expression of capsules consisting of a colanic acid-like polymer. We use molecular genetics, biochemical analyses, and experimental evolution to establish that capsule switching results from perturbation of the pyrimidine biosynthetic pathway. Of central importance is a bifurcation point at which uracil triphosphate is partitioned towards either nucleotide metabolism or polymer production. This bifurcation marks a cell-fate decision point whereby cells with relatively high pyrimidine levels favour nucleotide metabolism (capsule OFF), while cells with lower pyrimidine levels divert resources towards polymer biosynthesis (capsule ON). This decision point is present and functional in the wild-type strain. Finally, we present a simple mathematical model demonstrating that the molecular components of the decision point are capable of producing switching. Despite its simple mutational cause, the connection between genotype and phenotype is complex and multidimensional, offering a rare glimpse of how noise in regulatory networks can provide opportunity for evolution.

General information
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Organisations: Department of Systems Biology, Center for Systems Microbiology, Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factories, Metabolic Signaling and Regulation, Massey University, Swiss Federal Institute of Technology Zurich, University Paris Diderot - Paris 7
Naturalizing semiotics: The triadic sign of Charles Sanders Peirce as a systems property

The father of pragmatism, Charles Sanders Peirce, gave in 1903 the following definition of a sign: "A Sign, or Representamen, is a First which stands in such a genuine triadic relation to a Second, called its Object, as to be capable of determining a Third, called its Interpretant, to assume the same triadic relation to its Object in which it stands itself to the same Object. The triadic relation is genuine, that is its three Objects are triadized together by it in a way that does not consist in any complexus of dyadic relations". Despite its cult status and its pragmatic foundation, the Peircean sign has never revealed its true potential by being integrated into a formal system. In the present report, a reconstruction of the sign model is presented, which may at first appear somewhat obvious and superficial. However by use of the reconstructed model, the above statement and the majority of Peirce's other statements about the nature of signs fall into place. Instead of defining three links between Object (O), Representamen (R), and Interpretant (I), the sign is described as having a single three-dimensional link, specifying its location in a three dimensional (O,R,I) linkage space. To understand and explain sign function, the process of sign utilization (semiosis) has to be divided into two temporally separated phases, a sign-establishment phase where a three-dimensional link (Ψ(O,R,I)) is formed between three sign elements, and a later sign-interpretation phase where the established linkage is used for inferring significance to a novel phenomenon, if this satisfies the criteria for being a Representamen for the sign. Numerous statements from Peirce indicate that he used a two-staged semiosis paradigm although he did not state that explicitly. The three-dimensional model was primarily constructed for use in biosemiotics, as an exploratory frame for mapping the evolutionary establishment of sign links, which logically must have preceded the fixation of any regulatory process in molecular biological systems. It became clear, however, that the model is able to clarify many of the difficult explanations offered by Peirce about his sign model. I make no claim that Peirce used a similar type of three-dimensional model, because he explicitly used the chemical atom as naturalization (natural scientific explanation) for his sign model, an interesting but problematic analogy. In order to discuss common versus specific semiotic scaffolds for molecular biosemiotics, biosemiotics and semiotics proper, I start with a generic definition of the three-dimensional sign system, using human semiosis as examples. After this, the major part of the paper, I define the specific biochemical and evolutionary scaffolds that is used for obtaining the evolutionary memory that is needed for sign establishment. To exemplify semiosis according to the present model I present a typical situation where a Representamen (R_{O}) and an object (O_{R}) in the establishment phase are frequently encountered together by a sign interpreter. The process that links specific Representamen to specific Objects will first involve the recognition of the specific traits that distinguish the two sign elements. Subsequently the establishment process leads to the creation of a specific systems-state, called the Interpretant, which links the two traits in a way that allows retrieval of the information (a memory function). During a later interpretation phase, a hypothetical Object will be inferred by the interpreter when a Representamen (R_{I}) harboring the required characteristics is encountered. This inference happens through a memory retrieval process, irrespective of the fact that relevant Objects of the sign may never be encountered after establishment. A simplified scheme for computer neural network algorithms is introduced as an example of such a system. Since the Peircean sign according to this definition is a systems property, there can be no sign without a sign interpreting systems or without some kind of memory function. A sign interpreter will thus harbor a semiotic scaffold that consists of at least an input sensor and an interpreting system coupled to a memory function. Further border conditions for semiotic scaffolds will be introduced. Peirce published a comprehensive sign definition system, but he allowed only ten sign classes, selected from the twenty-seven sign classes that result from his three main subdivisions, each containing three classes. His allowed sign classes are here identified as those which do not infer more significance during interpretation than was warranted during establishment. The excluded sign classes are either undefinable in his system or are of such a nature that the objects during interpretation are inferred to be much more significant than what was warranted during establishment. Occult signs are of these forbidden free-wheeling types, and it is postulated that they were omitted because Peirce defined his sign classes for use in a novel sign based logical system, where such over-signification would be detrimental.
Two Lactococcus lactis thioredoxin paralogues play different roles in responses to arsenate and oxidative stress

Thioredoxin (Trx) maintains intracellular thiol groups in a reduced state and is involved in a wide range of cellular processes, including ribonucleotide reduction, sulphur assimilation, oxidative stress responses and arsenate detoxification. The industrially important lactic acid bacterium Lactococcus lactis contains two Trxs. TrxA is similar to the well-characterized Trx homologue from Escherichia coli and contains the common WCGPC active site motif, while TrxD is atypical and contains an aspartate residue in the active site (WCGDC). To elucidate the physiological roles of the two Trx paralogues, deletion mutants ΔtrxA, ΔtrxD and ΔtrxAΔtrxD were constructed. In general, the ΔtrxAΔtrxD strain was significantly more sensitive than either of the ΔtrxA and ΔtrxD mutants. Upon exposure to oxidative stress, growth of the ΔtrxA strain was diminished while that of the ΔtrxD mutant was similar to the wild-type. The lack of TrxA also appears to impair methionine sulfoxide reduction. Both ΔtrxA and ΔtrxD strains displayed growth inhibition after treatment with sodium arsenate and tellurite as compared with the wild-type, suggesting partially overlapping functions of TrxA and TrxD. Overall the phenotype of the ΔtrxA mutant matches established functions of WCGPC-type Trx while TrxD appears to play a more restricted role in stress resistance of Lac. lactis.

General information

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Scopus rating (2015): CiteScore 2.05 SJR 1.352 SNIP 0.859
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Scopus rating (2014): CiteScore 2.69 SJR 1.461 SNIP 0.97
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Identification of Metabolic Pathways Essential for Fitness of *Salmonella Typhimurium* In Vivo

Bacterial infections remain a threat to human and animal health worldwide, and there is an urgent need to find novel targets for intervention. In the current study we used a computer model of the metabolic network of *Salmonella enterica* serovar Typhimurium and identified pairs of reactions (cut sets) predicted to be required for growth in vivo. We termed such cut sets synthetic auxotrophic pairs. We tested whether these would reveal possible combined targets for new antibiotics by analyzing the performance of selected single and double mutants in systemic mouse infections. One
hundred and two cut sets were identified. Sixty-three of these included only pathways encoded by fully annotated genes, and from this sub-set we selected five cut sets involved in amino acid or polyamine biosynthesis. One cut set (asnA/asnB) demonstrated redundancy in vitro and in vivo and showed that asparagine is essential for S. Typhimurium during infection. tvpB/trpA as well as single mutants were attenuated for growth in vitro, while only the double mutant was a cut set in vivo, underlining previous observations that tryptophan is essential for successful outcome of infection. speB/speF,speC was not affected in vitro but was attenuated during infection showing that polyamines are essential for virulence apparently in a growth independent manner. The serA/glyA cut-set was found to be growth attenuated as predicted by the model. However, not only the double mutant, but also the glyA mutant, were found to be attenuated for virulence. This adds glycine production or conversion of glycine to THF to the list of essential reactions during infection. One pair (thrC/kbl) showed true redundancy in vitro but not in vivo demonstrating that threonine is available to the bacterium during infection. These data add to the existing knowledge of available nutrients in the intra-host environment, and have identified possible new targets for antibiotics.

**General information**

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Organisations: Department of Systems Biology, Center for Systems Microbiology, Metabolic Signaling and Regulation, Systems Biotechnology, National Food Institute, Division of Industrial Food Research, Oxford Brookes University, University of Copenhagen
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Web of Science (2012): Impact factor 3.73
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.233
Web of Science (2011): Impact factor 4.092
Multi-stress resistance in *Lactococcus lactis* is actually escape from purine-induced stress sensitivity

Multi-stress resistance is a widely documented and fascinating phenotype of lactococci where single mutations, preferentially in genes involved in nucleotide metabolism and phosphate uptake, result in elevated tolerance to multiple stresses simultaneously. In this report, we have analysed the metabolic basis behind this multi-stress-resistance phenotype in *Lactococcus lactis* subsp. cremoris MG1363 using acid stress as a model of multi-stress resistance. Surprisingly, we found that *L. lactis* MG1363 is fully resistant to pH 3.0 in the chemically defined SA medium, contrary to its sensitivity in the rich and complex M17 medium. When salvage of purines and subsequent conversion to GTP was permitted in various genetic backgrounds of *L. lactis* MG1363, the cells became sensitive to acid stress, indicating that an excess of guanine nucleotides induces stress sensitivity. The addition of phosphate to the acid-stress medium increased the stress sensitivity of *L. lactis* MG1363. It is also shown that high intracellular guanine nucleotide pools confer increased sensitivity to high temperatures, thus showing that it is indeed a multi-stress phenotype. Our analysis suggests that an increased level of guanine nucleotides is formed as a result of an improved conversion of guanosine in the salvage pathway. Based upon our findings, we suggest that *L. lactis* MG1363 is naturally multi-stress resistant in habitats devoid of any purine source. However, any exogenous purine that results in increased guanine nucleotide pools renders the bacterium sensitive to environmental stresses.

**General information**

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Organisations: Department of Systems Biology, Metabolic Signaling and Regulation, University of Copenhagen, Technical University of Denmark
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Towards in vivo regulon kinetics: PurR activation by 5-phosphoribosyl-a-1-pyrophosphate during purine depletion in Lactococcus lactis.

Short-term adaptation to changing environments relies on regulatory elements translating shifting metabolite concentrations into a specifically optimized transcriptome. So far the focus of analyses has been divided between regulatory elements identified in vivo and kinetic studies of small molecules interacting with the regulatory elements in vitro. Here we describe how in vivo regulon kinetics can describe a regulon through the effects of the metabolite controlling it, exemplified by temporal purine exhaustion in Lactococcus lactis. We deduced a causal relation between the pathway precursor 5-phosphoribosyl-a-1-pyrophosphate (PRPP) and individual mRNA levels, whereby unambiguous and homogeneous relations could be obtained for PurR regulated genes, thus linking a specific regulon to a specific metabolite. As PurR activates gene expression upon binding of PRPP, the pur mRNA curves reflect the in vivo kinetics of PurR PRPP binding and activation. The method singled out the xpt-pbuX operon as kinetically distinct, which was found to be caused by a guanine riboswitch whose regulation was overlaying the PurR regulation. Importantly, genes could be clustered according to regulatory mechanism and long-term consequences could be distinguished from transient changes – many of which would not be seen in a long-term adaptation to a new environment. The strategy outlined here can be adapted to analyse the individual effects of members from larger metabolomes in virtually any organism, for elucidating regulatory networks in vivo.

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Scopus rating (2016): CiteScore 1.56 SJR 1.035 SNIP 0.663
Web of Science (2016): Impact factor 2.151
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.05 SJR 1.352 SNIP 0.859
The Transcriptional and Gene Regulatory Network of Lactococcus lactis MG1363 during Growth in Milk

In the present study we examine the changes in the expression of genes of Lactococcus lactis subspecies cremoris MG1363 during growth in milk. To reveal which specific classes of genes (pathways, operons, regulons, COGs) are important, we performed a transcriptome time series experiment. Global analysis of gene expression over time showed that L. lactis adapted quickly to the environmental changes. Using upstream sequences of genes with correlated gene expression profiles, we uncovered a substantial number of putative DNA binding motifs that may be relevant for L. lactis fermentative growth in milk. All available novel and literature-derived data were integrated into network reconstruction building blocks, which were used to reconstruct and visualize the L. lactis gene regulatory network. This network enables easy mining in the chrono-transcriptomics data. A freely available website at http://milkts.molgenrug.nl gives full access to all transcriptome data, to the reconstructed network and to the individual network building blocks.

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Organisations: Department of Systems Biology, Enzyme and Protein Chemistry, Center for Systems Microbiology, University of Groningen
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Scopus rating (2016): CiteScore 3.11 SJR 1.236 SNIP 1.101
Web of Science (2016): Indexed yes
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Scopus rating (2015): CiteScore 3.32 SJR 1.427 SNIP 1.136
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Scopus rating (2013): CiteScore 3.94 SJR 1.772 SNIP 1.153
ISI indexed (2013): ISI indexed yes
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BFI (2012): BFI-level 1
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Web of Science (2012): Impact factor 3.73
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.233
Web of Science (2011): Impact factor 4.092
ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
The PurR regulon in Lactococcus lactis – transcriptional regulation of the purine nucleotide metabolism and translational machinery

Purine nucleotides are either synthesized de novo from 5-phosphoribosyl-1-pyrophosphate (PRPP) or salvaged from the environment. In Lactococcus lactis, transcription of the de novo synthesis operons, purCSQLF and purDEK, has genetically been shown to be activated by the PurR protein when bound to a conserved PurBox motif present on the DNA at a fixed distance from the promoter -10 element. PurR contains a PRPP-binding site, and activation occurs when the intracellular PRPP pool is high as a consequence of low exogenous purine nucleotide pools. By an iterative approach of bioinformatics searches and motif optimization, 21 PurR-regulated genes were identified and used in a redefinition of the PurBox consensus sequence. In the process a new motif, the double-PurBox, which is present in a number of promoters and contains two partly overlapping PurBox motifs, was established. Transcriptional fusions were used to analyse wild-type promoters and promoters with inactivating PurBox mutations to confirm the relevance of the PurBox motifs as PurR-binding sites. The promoters of several operons were shown to be devoid of any -35 sequence, and found to be completely dependent on PurR-mediated activation. This suggests that binding of the PurR protein to the PurBox takes over the role of the -35 sequence. The study has expanded the PurR regulon to include promoters in nucleotide metabolism, C(1) compound metabolism, phosphonate transport, pyrophosphatase activity, (p)ppGpp metabolism, and translation-related functions. Of special interest is the presence of PurBox motifs in rrn promoters, suggesting a novel connection between nucleotide availability and the translational machinery.

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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.56 SJR 1.035 SNIP 0.663
Web of Science (2016): Impact factor 2.151
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.05 SJR 1.352 SNIP 0.859
Web of Science (2015): Impact factor 2.268
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.69 SJR 1.461 SNIP 0.97
Web of Science (2014): Impact factor 2.557
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 3.34 SJR 1.674 SNIP 1.028
Web of Science (2013): Impact factor 2.835
ISI indexed (2013): ISI indexed yes
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BFI (2012): BFI-level 2
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Web of Science (2012): Impact factor 2.852
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 3.18 SJR 1.659 SNIP 1.036
Web of Science (2011): Impact factor 3.061
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.804 SNIP 0.988
Web of Science (2010): Impact factor 2.957
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.71 SNIP 0.995
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.743 SNIP 1.011
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.739 SNIP 1.062
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.794 SNIP 1.063
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.76 SNIP 1.024
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.705 SNIP 1.065
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.682 SNIP 1.04
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.45 SNIP 1.01
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.54 SNIP 1.049
A simplified method for rapid quantification of intracellular nucleoside triphosphates by one-dimensional thin-layer chromatography

Quantification of nucleotides is an important part of metabolomics but has been hampered by the lack of fast, sensitive, and reliable methods. We present a less time-consuming, more sensitive, and more precise method for the quantitative determination of nucleoside triphosphates (NTPs), d-riboyl-1-pyrophosphate (PRPP), and inorganic pyrophosphate (Pi) in cell extracts. The method uses one-dimensional thin-layer chromatography (TLC) and radiolabeled biological samples. Nucleotides are resolved at the level of ionic charge in an optimized acidic ammonium formate and chloroform solvent, permitting quantification of NTPs. The method is significantly simpler and faster than both current two-dimensional methods and high-performance liquid chromatography (HPLC)-based procedures, allowing a higher throughput while common sources of inaccuracies and technical problems are avoided. For determination of Pi, treatment with inorganic pyrophosphatase (PiPase) of the radiolabeled phosphate is employed for removal of contaminating pyrophosphate. Biological examples performed in triplicates showed standard deviations of approximately 10% of the mean for the determined concentrations of NTPs.
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Web of Science (2019): Indexed yes
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Scopus rating (2017): CiteScore 2.16 SJR 0.633 SNIP 0.649
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Scopus rating (2016): CiteScore 2.34 SJR 0.719 SNIP 0.743
Web of Science (2016): Impact factor 2.334
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.28 SJR 0.729 SNIP 0.777
Web of Science (2015): Impact factor 2.243
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BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.37 SJR 0.825 SNIP 0.836
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BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 2.46 SJR 0.857 SNIP 0.932
Web of Science (2013): Impact factor 2.305
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
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BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.046 SNIP 0.97
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.097 SNIP 0.922
Scopus rating (2007): SJR 1.149 SNIP 0.977
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.097 SNIP 0.952
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.105 SNIP 0.944
Scopus rating (2004): SJR 1.007 SNIP 0.973
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.057 SNIP 0.977
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.017 SNIP 0.993
“Good teaching practice” at DTU Systems Biology - sustaining quality in teaching and learning

Success in developing teaching and learning in engineering education in general, as well as in a CDIO context, depends on continuous development of teaching competences among faculty members. Thus, it is essential to develop systems that promote understanding of how teaching and assessment can support student learning within disciplinary knowledge as well as development of professional skills. Development and maintenance of high quality teaching and learning furthermore requires that teachers have the ability to reflect critically on their teaching activities and understand its impact on the students’ learning process. To succeed in reaching these goals, development of teaching competences and knowledge in the fields of teaching and learning must be combined with continuous possibilities to reflect on teaching practice in a structured way. Development of successful teaching also requires that faculty members are inspired and encouraged to try new ways and methods in teaching, and gaining an extended understanding in how students learning can be efficiently supported. In this paper we describe a novel initiative, a concept of Good Teaching Practice, that has been developed through a process involving faculty at the department of Systems Biology at the Technical University of Denmark. The GTP initiative addresses important factors for effective teaching and enhancement of student learning. On the surface GTP is structured as an online tool, which makes six statements about important factors that support student learning from teaching practice among faculty. By formulating a teaching and learning profile at the department level the importance of teaching for the department are emphasized and at the same time, the wiki-based resource for sharing teaching experience shows that teaching is a shared responsibility among the entire faculty. On the website, the theoretical framework underlying GTP provides a shorthand introduction to the important prerequisites for students learning and provides definitions that provide the faculty members with a common language to use in discussions of teaching and learning. The GTP concept addresses standard 10 in the CDIO context which focuses on the enhancement of the development of teaching and learning at department level and provides the teachers with tools to conduct teaching proficiently.

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Research output: Research - peer-review › Conference abstract in proceedings – Annual report year: 2011

Nucleotide Metabolism
Metabolic pathways are connected through their utilization of nucleotides as supplier of energy, allosteric effectors, and their role in activation of intermediates. Therefore, any attempt to exploit a given living organism in a biotechnological process will have an impact on nucleotide metabolism and vice versa. Here, we provide an overview of the pyrimidine and purine biosynthesis, the interconversion pathways, the formation of deoxyribonucleotides, and the salvage reactions used in the utilization of exogenous precursors. The enzymatic and genetic regulation of these pathways are addressed. The aim of this article is to provide knowledge of nucleotide metabolism and its regulation to facilitate interpretation of data arising from genetics, proteomics, and transcriptomics in connection with biotechnological processes and beyond.
Two nucleoside transporters in Lactococcus lactis with different substrate specificities

In an alternative to biosynthesis of nucleotides, most organisms are capable of exploiting exogenous nucleotide sources. In order to do so, the nucleotide precursors must pass the membrane, which requires the presence of transporters. Normally, phosphorylated compounds are not subject to transport, and the utilization of nucleotides is dependent on exogenous phosphatases. The composition of transporters with specificity for purine and pyrimidine nucleosides and nucleobases is subject to variation. The ability of Lactococcus lactis to transport different nucleosides across the cell membrane was characterized at both genetic and physiological level, using mutagenesis and by measuring the growth and uptake of nucleosides in the different mutants supplemented with different nucleosides. Two high affinity transporters were identified: BmpA-NupABC was shown to be an ABC transporter with the ability to actively transport all common nucleosides, whereas UriP was shown to be responsible for the uptake of only uridine and deoxyuridine. Interestingly, the four genes encoding the ABC transporter were found at different positions on the chromosome. The bmpA gene was separated from the nupABC operon by 60 kb. Moreover, bmpA was subject to regulation by purine availability, whereas the nupABC operon was constitutively expressed.
An optimized method for measurements of nucleotide and PRPP pools applied to the evaluation of a model of PurR-mediated activation in Lactococcus lactis

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Jendresen, C. B., Kilstrup, M., Martinussen, J.
Publication date: 2009
Peer-reviewed: No
Event: Poster session presented at 7th Symposium of Food Microbiology, Helsingør, Denmark.
Source: orbit
Source-ID: 251399
Research output: Research › Poster – Annual report year: 2009

An optimized method for measurements of nucleotide and PRPP pools applied to the evaluation of a model of PurR-mediated activation in Lactococcus lactis

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Jendresen, C. B., Kilstrup, M., Martinussen, J.
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Peer-reviewed: No
Event: Poster session presented at 3rd Congress of European Microbiologists, Goteborg, Sweden.
Source: orbit
Source-ID: 251400
Research output: Research › Poster – Annual report year: 2009

Control analysis of the purine biosynthesis in Lactococcus lactis

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Jessing, S. G., Haaber, J. B. B., Jendresen, C. B., Jensen, P. R., Kilstrup, M.
Publication date: 2009
Peer-reviewed: No
Event: Poster session presented at 7th Symposium of Food Microbiology, Helsingør, Denmark.
Source: orbit
Source-ID: 251398
Research output: Research › Poster – Annual report year: 2009

Control analysis of the purine biosynthesis in Lactococcus lactis

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Jessing, S. G., Haaber, J. B. B., Jendresen, C. B., Jensen, P. R., Kilstrup, M.
Publication date: 2009
Peer-reviewed: No
Event: Abstract from 3rd Congress of European Microbiologists, Goteborg, Sweden.
Source: orbit
Source-ID: 251402
Research output: Research › Conference abstract for conference – Annual report year: 2009

Activation of purine biosynthetic gene expression by PurR in Lactococcus lactis

General information
State: Published
Identification of DNA-binding sites for the activator involved in late transcription of the temperate lactococcal phage TP901-1

Alt, encoded by the lactococcal phage TP901-1, is needed for late transcription. We identify Alt as a DNA-binding protein, and footprint analysis shows that Alt binds to a region containing four imperfect direct repeats (ALT boxes) located -76 to -32 relative to the P-late transcriptional start site. The importance of the ALT boxes was confirmed by deletion of one or two ALT boxes and by introducing mutations in ALT boxes 1 and 4. Alt is proposed to act as a tetramer or higher multimer activating transcription of TP901-1 late genes by binding to the four ALT boxes, and bending of the DNA may be important for transcriptional activation of P-late. Furthermore, our results suggest that DNA replication may be required for late transcription in TP901-1. Additionally, we identify gp28 of the related lactococcal phage Tuc2009 as an activator and show that the activators required for late transcription in TP901-1 and Tuc2009 are interchangeable. (c) 2005 Elsevier Inc. All rights reserved.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, Center for Systems Microbiology
Contributors: Pedersen, M., Kilstrup, M., Hammer, K.
Pages: 446-456
Publication date: 2006
Peer-reviewed: Yes
Proteomics of Lactococcus lactis: phenotypes for a domestic bacterium.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Contributors: Kilstrup, M.
Pages: 149-178
Publication date: 2006
Peer-reviewed: Yes

Publication information
Journal: Methods of biochemical analysis
Volume: 49
ISSN (Print): 0076-6941
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Nucleoside triphosphate synthesis catalysed by adenylate kinase is ADP dependent

Adenylate kinase (Adk) that catalyses the synthesis of ADP from ATP and AMP has also been shown to perform an ATP-dependent phosphorylation of ribo- and deoxynucleoside diphosphates to their corresponding nucleoside triphosphate; ATP+(d)NDPADP+(d)NTP. This reaction, suggested to occur by the transfer of the gamma-phosphoryl from ATP to the nucleoside diphosphate, is overall similar to that normally carried out by nucleoside diphosphate kinase (Ndk).

Accordingly, Adk was proposed to be responsible for residual Ndk-like activity measured in a mutant strain of Escherichia coli, where the ndk gene was disrupted. We present data supporting a mechanism for the synthesis of nucleoside triphosphates by Adk that unlike the previously suggested mechanism mentioned above are in complete agreement with the current knowledge about the Adk enzyme and its various catalytic properties. We propose that nucleoside triphosphate synthesis occurs by beta-phosphoryl transfer from ADP to any bound nucleoside diphosphate. Our results point to the fact that the proposed Ndk-like mechanism of Adk originated from an erroneous interpretation of data, in that contamination of ATP preparations with AMP and ADP was not taken into account. Our results also address the proposed role of Adk in restoring a normal growth rate of mutant strains of E. coli lacking Ndk. These mutant strains apparently, in spite of a mutator phenotype, are able to synthesise nucleoside triphosphates by alternative pathways to maintain the same growth rate as the wildtype.

General information

State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Contributors: Willemoës, M., Kilstrup, M.
Pages: 195-199
Nucleotide Metabolism and Its Control in Lactic Acid Bacteria

Most metabolic reactions are connected through either their utilization of nucleotides or their utilization of nucleotides or their regulation by these metabolites. In this review the biosynthetic pathways for pyrimidine and purine metabolism in lactic acid bacteria are described including the interconversion pathways, the formation of deoxyribonucleotides and the salvage pathways for use of exogenous precursors. The data for the enzymatic and the genetic regulation of these pathways are reviewed, as well as the gene organizations in different lactic acid bacteria. Mutant phenotypes and methods for manipulation of nucleotide pools are also discussed. Our aim is to provide an overview of the physiology and genetics of nucleotide metabolism and its regulation that will facilitate the interpretation of data arising from genetics, metabolomics, proteomics, and transcriptomics in lactic acid bacteria.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Contributors: Kilstrup, M., Hammer, K., Jensen, P. R., Martinussen, J.
Pages: 555-590
Publication date: 2005
Peer-reviewed: Yes

Publication information
Journal: F E M S Microbiology Reviews
Volume: 29
ISSN (Print): 0168-6445
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 2
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 2
Scopus rating (2017): CiteScore 12.68 SJR 7.063 SNIP 3.444
Web of Science (2017): Impact factor 11.392
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 13.54 SJR 7.779 SNIP 3.916
Web of Science (2016): Impact factor 12.198
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 13.38 SJR 7.689 SNIP 4.216
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 12.9 SJR 7.192 SNIP 4.116
Web of Science (2014): Impact factor 13.244
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 13.78 SJR 7.687 SNIP 4.274
Web of Science (2013): Impact factor 13.806
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Proteome and transcriptome analysis of Lactococcus lactis stress responses

**General information**
State: Published
Organisations: Enzyme and Protein Chemistry, Department of Systems Biology, Center for Biomedical Microbiology, Center for Microbial Biotechnology
Pages: S138-S138
Publication date: 2005
Peer-reviewed: Yes

**Publication information**
Journal: Journal of Biotechnology
Volume: 118
ISSN (Print): 0168-1656
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.64 SJR 0.929 SNIP 0.86
Web of Science (2017): Impact factor 2.533
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.88 SJR 1.004 SNIP 0.929
Web of Science (2016): Impact factor 2.599
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.87 SJR 1.068 SNIP 0.988
Web of Science (2015): Impact factor 2.667
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.95 SJR 1.116 SNIP 1.13
Web of Science (2014): Impact factor 2.871
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.22 SJR 1.183 SNIP 1.175
Web of Science (2013): Impact factor 2.884
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.4 SJR 1.238 SNIP 1.312
Web of Science (2012): Impact factor 3.183
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 2.87 SJR 1.165 SNIP 1.043
Web of Science (2011): Impact factor 3.045
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.135 SNIP 1.175
Web of Science (2010): Impact factor 2.97
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.224 SNIP 1.231
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.147 SNIP 1.265
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.133 SNIP 1.27
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.109 SNIP 1.394
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.193 SNIP 1.358
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.028 SNIP 1.442
Web of Science (2004): Indexed yes
Identification of bacterial cultures from archaeological wood using molecular biological techniques

Anaerobic bacteria were isolated from a 1700-year-old wooden spear shaft, excavated from an archaeological site that dates from the iron age, in the southern part of Jutland, Denmark. The bacteria were cultivated in glucose- and xylose-supplemented media at 14°C and 20°C. A gene library with 21 clones was constructed by extracting and amplifying 16S rDNA sequences from the individual cultures. One clone was phylogenetically affiliated to the Spirochaeta. Eleven clones affiliated to an unidentified member of the alpha-Proteobacteria were present in all culture samples. Three clones were affiliated to the beta-Proteobacteria. Four clones were clustered among the Geobacteriaceae, in the delta-Proteobacteria. A single clone was clustered with gram-positives. All the identified bacterial families are commonly found in soil or bog environments and many are able to utilize cellulose as their carbon or energy source.

General information
State: Published
Organisations: Center for Biomedical Microbiology, Department of Systems Biology, Environmental Microbiology and Biotechnology, Center for Microbial Biotechnology
Contributors: Helms, A., Martiny, A. C., Hofman-Bang, H. J. P., Ahring, B. K., Kilstrup, M.
Pages: 79-88
Publication date: 2004
Peer-reviewed: Yes

Publication information
Journal: International Biodeterioration & Biodegradation
Volume: 53
ISSN (Print): 0964-8305
Ratings:
BFI (2019): BFI-level 1
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BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.75 SJR 1.086 SNIP 1.485
Web of Science (2017): Impact factor 3.562
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.38 SJR 1.032 SNIP 1.567
Web of Science (2016): Impact factor 2.962
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.71 SJR 0.904 SNIP 1.313
Web of Science (2015): Impact factor 2.429
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.53 SJR 0.879 SNIP 1.381
Web of Science (2014): Impact factor 2.131
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Proteome analysis of the purine stimulon from Lactococcus lactis

A comparative expression proteome analysis was carried out by analyzing differential expression patterns of pulse-labelled proteins on two-dimensional gels under standard conditions and during purine nucleotide starvation, followed by mass spectrometric identification of regulated proteins. Based upon the expression patterns, three stimulons could be identified in Lactococcus lactis subsp. cremoris. The Psu proteins (purine starvation up-regulated) had increased synthesis during purine depletion in a purine auxotroph. Among these proteins were enzymes of the purine biosynthesis pathways (PurE, PurS, PurM, PurL), and enzymes involved in the generation of C1 units (GlyA, Fhs). C1 units are primarily required for purine biosynthesis. Upon analysis of the nucleotide sequence preceding the structural genes for these proteins in the L. lactis IL1403 genome sequence showed that all contained PurBox-Pribnow box structures resembling the PurR activated promoters for the purDEK and purCSQLF operons. Most, and possibly all members of the Psu stimulon are thus members of the PurR regulon. Five Psu proteins could not be identified. The second stimulon, the Psd stimulon (purine starvation decreased), whose members are down-regulated during purine depletion, contained proteins related to protein synthesis (PpsB, EF-TS, trigger factor), or to GTPases (FtsZ, EF-TS); or are involved in energy metabolism (GapB, CcpA). No common regulatory elements could be found for members of this stimulon. Two Psd proteins escaped identification. The last, Dcu (decoynine up-regulated), stimulon contained proteins whose synthesis escaped the severe general depression during inhibition of the GMP synthetase by decoynine. This regulon was comprised of mostly glycolytic enzymes (fructose bisphosphate aldolase, enolase, pyruvate kinase) and translation elongation factors (GTPases: EF-TU, EF-G). Two Dcu proteins could not be identified. Out of 28 proteins subjected to mass spectrometry, 19 could be readily identified despite the fact that only the genome sequence of a strain of L. lactis subsp. lactis was available. The two
subspecies share about 85% sequence identity, comparable to the genetic distance between Escherichia coli and Salmonella typhimurium. A success rate of 68% indicates that it may be feasible to perform proteomics based upon genomic sequences of relatives outside the genus.
Proteome analysis of a Lactococcus lactis strain overexpressing gapA suggests that the gene product is an auxiliary glyceraldehyde 3-phosphate dehydrogenase.

The sequence of the genome from the Lactococcus lactis subspecies lactis strain IL1403 shows the presence of two reading frames, gapA and gapB, putatively encoding glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Previous
proteomic analysis of the L. lactis subspecies cremoris strain MG1363 has revealed two neighbouring protein spots, GapBI and GapBII, with amino terminal sequences identical to the product of gapA from the L. lactis subspecies cremoris strain LM0230 and that of the two IL1403 sequences. In order to assign the two protein spots to their respective genes we constructed an L. lactis strain that overexpressed the gapA gene derived from MG1363 upon nisin induction. Compared to the wild-type, the overexpressing strain had a 3.4-fold elevated level of specific GAPDH activity when grown in the presence of nisin. In both MG 1363 and the gapA overexpressing strain the GAPDH activity was specific for NAD. No NADP dependent activity was detected. Proteome analysis of the gapA overexpressing strain revealed two new protein spots, GapAI and GapAII, not previously detected in proteome analysis of MG1363. Results from mass spectrometry analysis of GapA and GapB and comparison with the deduced protein sequences for the GAPDH isozymes from the genome sequence of strain IL1403 allowed us to assign GapA and GapB to their apparent IL1403 homologues encoded by gapA and gapB, respectively. Furthermore, we suggest that a homologue of a gapB product, represented by GapB, is the main source of GAPDH activity in L. lactis during normal growth.
Changes in rRNA levels during stress invalidates results from mRNA blotting: Fluorescence in situ rRNA hybridization permits renormalization for estimation of cellular mRNA levels

Regulation of gene expression can be analyzed by a number of different techniques. Some techniques monitor the level of specific mRNA directly, and others monitor indirectly by determining the level of enzymes encoded by the mRNA. Each method has its own inherent way of normalization. When results obtained by these techniques are compared between experiments in which differences in growth rates, strains, or stress treatments occur, the normalization procedure may have a significant impact on the results. In this report we present a solution to the normalization problem in RNA slot blotting experiments, in which mRNA levels routinely are normalized to a fixed amount of extracted total RNA. The cellular levels of specific mRNA species were estimated using a renormalization with the total RNA content per cell. By a combination of fluorescence in situ rRNA hybridization, which estimates the relative level of rRNA per cell, and slot blotting to rRNA probes, which estimates the level of rRNA per extracted total RNA, the amount of RNA per cell was calculated in a series of heat shock experiments with the gram-positive bacterium Lactococcus lactis. It was found that the level of rRNA per cell decreased to 30% in the course of the heat shock. This lowered ribosome level led to a decrease in the total RNA content, resulting in a gradually increasing overestimation of the mRNA levels throughout the experiment. Using renormalized cellular mRNA levels, the HrcA-mediated regulation of the genes in the hrcA-grpE-dnaK operon was analyzed. The hybridization data suggested a complex heat shock regulation indicating that the mRNA levels continued to rise after 30 min, but after renormalization the calculated average cellular levels exhibited a much simpler induction pattern, eventually attaining a moderately increased value.

General information
State: Published
Organisations: Center for Biomedical Microbiology, Department of Systems Biology, Bacterial Physiology and Genetics Group, Center for Microbial Biotechnology
Contributors: Hansen, M., Nielsen, A., Molin, S., Hammer, K., Kilstrup, M.
Pages: 4747-4751
Publication date: 2001
Peer-reviewed: Yes

Publication information
Journal: Journal of Bacteriology
Volume: 183
Inactivation of gltB abolishes expression of the assimilatory nitrate reductase gene (nasB) in Pseudomonas putida KT2442

**General information**
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Eberl, L., Ammendola, A., Rothballer, M. H., Givskov, M. C., Sternberg, C., Kilstrup, M., Schleifer, K. H., Molin, S.
Pages: 3368-3376
Publication date: 2000
Peer-reviewed: Yes

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Journal: Journal of Bacteriology
Volume: 182
Issue number: 12
ISSN (Print): 0021-9193
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 2.94 SJR 1.885 SNIP 0.903
Web of Science (2017): Impact factor 3.219
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.08 SJR 1.943 SNIP 0.877
Web of Science (2016): Impact factor 3.143
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.84 SJR 2.154 SNIP 0.95
Web of Science (2015): Impact factor 3.198
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.72 SJR 2.084 SNIP 0.931
Web of Science (2014): Impact factor 2.808
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3 SJR 2.151 SNIP 1.013
Web of Science (2013): Impact factor 2.688
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.42 SJR 2.125 SNIP 1.085
Web of Science (2012): Impact factor 3.177
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 3.83 SJR 2.471 SNIP 1.154
Web of Science (2011): Impact factor 3.825
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): 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
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 2.688 SNIP 1.205
Original language: English
Source: orbit
Source-ID: 177513
Research output: Research - peer-review › Journal article – Annual report year: 2000

Short communication: Salt extends the upper temperature limit for growth of Lactococcus lactis ssp. cremoris on solid M17 medium

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Kilstrup, M., Hammer, K.
Pages: 1448-1450
Publication date: 2000
Peer-reviewed: Yes

Publication information
Journal: Journal of Dairy Science
Volume: 83
Issue number: 7
Ratings:
Disruption and analysis of the clpB, clpC, and clpE genes in Lactococcus lactis: ClpE, a new Clp family in gram-positive bacteria

In the genome of the gram-positive bacterium Lactococcus lactis MG1363, we have identified three genes (clpC, clpE, and clpB) which encode Clp proteins containing two conserved ATP binding domains. The proteins encoded by two of the genes belong to the previously described ClpB and ClpC families. The clpE gene, however, encodes a member of a new Clp protein family that is characterized by a short N-terminal domain including a putative zinc binding domain (-CX2CX22CX2C-). Expression of the 83-kDa ClpE protein as well as of the two proteins encoded by clpB was strongly induced by heat shock and, while clpC mRNA synthesis was moderately induced by heat, we were unable to identify the ClpC protein. When we analyzed mutants with disruptions in clpB, clpC, or clpE, we found that although the genes are part of the L. lactis heat shock stimulon, the mutants responded like wild-type cells to heat and salt treatments. However, when exposed to puromycin, a tRNA analogue that results in the synthesis of truncated, randomly folded proteins, clpE mutant cells formed smaller colonies than wild-type cells and clpB and clpC mutant cells. Thus, our data suggest that ClpE, along with ClpP, which recently was shown to participate in the degradation of randomly folded proteins in L. lactis, could be necessary for degrading proteins generated by certain types of stress.
Activation Control of purDEK and purC Gene Expression in Lactococcus lactis

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Technical University of Denmark, CHL
Contributors: Kilstrup, M., Jessing, S., Wichmand-Jørgensen, S. B., Madsen, M., Nilsson, D.
Pages: 3900-3906
Activation of pur Gene Expression by a Homologue of the Bacillus subtilis PurR repressor: Isolation of a purR::iss Mutant and cloning of the wild type purR gene from Lactococcus lactis.

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Kilstrup, M., Martinussen, J.
Pages: 3907-3916
Publication date: 1998
Peer-reviewed: Yes

A Transcriptional Activator, Homologous to the Bacillus subtilis PurR Repressor, is Required for Expression of Purine Biosynthetic Genes in Lactococcus lactis

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Kilstrup, M., Martinussen, J.
Pages: 3907-3916
Publication date: 1998
Peer-reviewed: Yes
Cloning and Expression of the Lactococcus lactis purDEK Genes, Required for Growth in Milk.

An operon containing the genes purD and purE and part of the purK gene was cloned from the facultative anaerobic gram positive bacterium Lactococcus lactis by complementation of the purD mutation in Escherichia coli SO609. The genes encode enzymes in the de novo pathway of purine nucleotides. The expression of the genes was regulated approximately...
35-fold at the transcription level by the availability of purines in the growth medium. Deletion analysis of the nucleotide region upstream of purD indicated that a region of 145 bp is enough to give regulated expression of the reporter lacLM genes, which encode beta-galactosidase. Deletion of a region 79 bp upstream of the transcription start point reduced the promoter activity 33-fold when incubated in a purine-free medium and to values below the detection limit when incubated in a purine-containing medium. No secondary transcription start points were mapped in or close to this region, indicating that a putative activator site and not a promoter was deleted or partly destroyed.

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, CHL
Contributors: Nilsson, D., Kilstrup, M.
Pages: 4321-4327
Publication date: 1998
Peer-reviewed: Yes
Induced Levels of Heat Shock Proteins in dnaK mutants of Lactococcus lactis

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, University of Copenhagen
Contributors: Koch, B., Hammer, K., Vogensen, F. K., Kilstrup, M.
Pages: 3873-3881
Publication date: 1998
Peer-reviewed: Yes

Publication information
Volume: 180
Original language: English
Source: orbit
Source-ID: 174730
Research output: Research - peer-review › Journal article – Annual report year: 1998

Induction of heat shock proteins DnaK, GroEL, and GroES by salt stress in Lactococcus lactis

The bacterium Lactococcus lactis has become a model organism in studies of growth physiology and membrane transport, as a result of its simple fermentative metabolism. It is also used as a model for studying the importance of specific genes and functions during lie in excess nutrients, by comparison of prototrophic wild-type strains and auxotrophic domesticated (daily) strains. In a study of the capacity of domesticated strains to perform directed responses toward various stress conditions, we have analyzed the heat and salt stress response in the established L. lactis subsp. cremoris laboratory strain MG1363, which was originally derived from a dairy strain. After two-dimensional separation of proteins, the DnaK, GroEL, and GroES heat shock proteins, the HrcA (Orf1) heat shock repressor, and the glycolytic enzymes pyruvate kinase, glyceraldehyde-3-phosphate dehydrogenase, and phosphoglycerate kinase were identified by a combination of Western blotting and direct N-terminal amino acid sequencing of proteins from the gels. Of 400 to 500 visible proteins, 17
were induced more than twofold during heat stress. Two classes of heat stress proteins were identified from their temporal induction pattern. The fast-induced proteins (including DnaK) showed an abruptly increased rate of synthesis during the first 10 min, declining to intermediate levels after 15 min, GroEL and GroES, which also belong to this group, maintained a high rate of synthesis after 15 min. The class of slowly induced proteins exhibited a gradual increase in the rate of synthesis after the onset of stress. Unlike other organisms, all salt stress-induced proteins in L. lactis were also subjected to heat stress induction. DnaK, GroEL, and GroES showed similar temporal patterns of induction during salt stress, resembling the timing during heat stress although at a lower induction level. These data indicate an overlap between the heat shock and salt stress responses in L. lactis.

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Contributors: Kilstrup, M., Jacobsen, S., Hammer, K., Vogensen, F. K.
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Development of electrochemical metabolite sensors to monitor stress responses in-situ in large bioreactors
Hasanzadeh, A., PhD Student, Department of Chemical and Biochemical Engineering
Gernaey, K. V., Main Supervisor, Department of Chemical and Biochemical Engineering
Kilstrup, M., Supervisor, Department of Biotechnology and Biomedicine
Krühne, U., Supervisor, Department of Chemical and Biochemical Engineering
01/01/2019 → 31/12/2021
Project: PhD

Biosensor development and next-generation sequencing approaches for studying molecular evolution in bacteria
Frendorf, P. O., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Nørholm, M., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Kilstrup, M., Supervisor, Department of Biotechnology and Biomedicine
01/09/2018 → 31/08/2021
Project: PhD

Bacillus subtilis biofilm formation and evolution on Arabidopsis thaliana roots
Nordgaard Christensen, M., PhD Student, Department of Biotechnology and Biomedicine
Kovács, Á. T., Main Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Supervisor, Department of Biotechnology and Biomedicine
Institut stipendie (DTU)
01/08/2018 → 31/07/2021
Award relations: Bacillus subtilis biofilm formation and evolution on Arabidopsis thaliana roots
Molecular interactions of probiotics with Extracts from Medicinal Plants, Oligosaccharides and Adhesion to Mucin: Cell Wall and Membrane proteome Analysis, Protein Structure and Function

Celebioglu, H. U., PhD Student, Department of Biotechnology and Biomedicine
Svensson, B., Main Supervisor, Department of Biotechnology and Biomedicine
Abou Hachem, M., Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Emanuelsson, C., Examiner
Juge, N., Examiner
Emanuelsson, C., Examiner
Juge, N., Examiner

Stipendie fra udlandet
01/02/2013 → 15/03/2017

Award relations: Molecular interactions of probiotics with Extracts from Medicinal Plants, Oligosaccharides and Adhesion to Mucin: Cell Wall and Membrane proteome Analysis, Protein Structure and Function
Project: PhD

Udvikling af mikroorganismer til biobrændselsproduktion

Hansen, A. C. H., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
Solem, C., Supervisor
Workman, M., Supervisor, Department of Systems Biology
Kilstrup, M., Examiner
Købmann, B., Examiner, Department of Systems Biology
Mijakovic, I., Examiner

Anden EU-finansiering
01/12/2008 → 01/03/2013

Award relations: Udvikling af mikroorganismer til biobrændselsproduktion
Project: PhD

Totalt regulerbare promotorer til skræddersyede starterkulturer

Johansen, A. H., PhD Student, Department of Microbiology
Hammer, K., Main Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Haggård, E., Examiner
Israelsen, H., Examiner, Department of Microbiology
Program Stipendium-SU, Eksp

01/03/1997 → 30/03/2001

Award relations: Totalt regulerbare promotorer til skræddersyede starterkulturer
Project: PhD

Characterization of a high-temperature adaptive Lactococcus lactis mutant and it’s applivation in milk fermentation

Chen, J., PhD Student, Department of Biotechnology and Biomedicine
Jensen, P. R., Main Supervisor, Department of Biotechnology and Biomedicine
Solem, C., Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Poolman, B., Examiner
Serensen, K., Examiner, Department of Microbiology
Poolman, B., Examiner
Institut stipendie (DTU)

01/11/2011 → 28/03/2014

Award relations: Characterization of a high-temperature adaptive Lactococcus lactis mutant and it’s applivation in milk fermentation
Project: PhD

Protein misfolding and degradation at the Bacillus membrane

Quesada Ganuza, A., PhD Student, Department of Biotechnology and Biomedicine
Kilstrup, M., Main Supervisor, Department of Biotechnology and Biomedicine
Nielsen, A. K., Supervisor, Department of Microbiology
Eksternt EU-finansieret

01/09/2015 → 31/01/2019
Award relations: Protein misfolding and degradation at the Bacillus membrane
Project: PhD

Platform for cytochrome P450 reductases for optimized sustainable production of high value compounds in Escherichia coli
Hobel, T., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Nørholm, M., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Siedler, S., Supervisor, Novo Nordisk Foundation Center for Biosustainability
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Lindorff-Larsen, K., Examiner
Taxis, C., Examiner

Samfinansieret - Andet
01/01/2014 → 30/09/2018
Award relations: Platform for cytochrome P450 reductases for optimized sustainable production of high value compounds in Escherichia coli
Project: PhD

Regulering af "late" promoter hos den temperate laktokokfag TP901-1 og konstruktion af et fag aktiveret selvmordssystem
Pedersen, M., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Hammer, K., Main Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Martinussen, J., Examiner, Department of Biotechnology and Biomedicine
Haggård, E., Examiner
Ingmer, H., Examiner

Ansat eksternt CAMP
15/05/2000 → 19/11/2003
Award relations: Regulering af "late" promoter hos den temperate laktokokfag TP901-1 og konstruktion af et fag aktiveret selvmordssystem
Project: PhD

In situ studies of stress factor expression in Lactococcus lactis
Udsen, C., PhD Student, Department of Systems Biology
Molin, S., Main Supervisor, Novo Nordisk Foundation Center for Biosustainability
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Hill, P. J., Examiner
Vogensen, F. K., Examiner

DTU-lønnet stipendie
01/08/1998 → 07/06/2002
Award relations: In situ studies of stress factor expression in Lactococcus lactis
Project: PhD

Discovery and molecular biology of the abortive infection phage resistance system AbiV from Lactococcus lactis
Haaber, J. K., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Hammer, K., Main Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Vogensen, F. K., Examiner

DTU-lønnet stipendie
01/02/2005 → 21/11/2008
Award relations: Discovery and molecular biology of the abortive infection phage resistance system AbiV from Lactococcus lactis
Project: PhD

Characterization of the Molecular and Genetic Regulation of Nucleotide Metabolism in Lactococcus Lactis
Jendresen, C. B., PhD Student, Novo Nordisk Foundation Center for Biosustainability
Martinussen, J., Main Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Supervisor, Department of Biotechnology and Biomedicine
Jarmer, H. Ø., Examiner, Department of Biotechnology and Biomedicine
Ingmer, H., Examiner
Kok, J., Examiner

DTU-lønnet stipendie
Award relations: Characterization of the Molecular and Genetic Regulation of Nucleotide Metabolism in Lactococcus Lactis
Project: PhD

Funfsjonel Biodiversitet i Komplekse Mikrobielle Populationer
Lausten, T., PhD Student, Department of Systems Biology
Ahring, B. K., Main Supervisor, Department of Systems Biology
Westermann, P., Supervisor, Department of Systems Biology
Kilstrup, M., Examiner
Lund Nielsen, J., Examiner
Priemé, A., Examiner
DTU-lønnet stipendie
01/02/2001 → 30/09/2005
Award relations: Funfsjonel Biodiversitet i Komplekse Mikrobielle Populationer
Project: PhD

Lactic Acid Bacteria as a new platform for sustainable production of biochemicals
Boguta, A. M., PhD Student
Martinussen, J., Main Supervisor
Jensen, P. R., Supervisor
Kilstrup, M., Examiner
Holo, H., Examiner
Serensen, K., Examiner
Institutt stipendie (DTU)
15/12/2011 → 29/09/2016
Award relations: Lactic Acid Bacteria as a new platform for sustainable production of biochemicals
Project: PhD

Regulation of Pigment Production in Fungi
Rasmussen, K. B., PhD Student, Department of Systems Biology
Thrane, U., Main Supervisor
Mortensen, U. H., Supervisor
Kilstrup, M., Examiner
Olsson, S., Examiner
Stadler, M., Examiner
Institutt stipendie (DTU)
01/08/2011 → 02/12/2015
Award relations: Regulation of Pigment Production in Fungi
Project: PhD

Liquid Chromatography-mass spectrometric based metabolomics
Magdenoska, O., PhD Student, Department of Systems Biology
Nielsen, K. F., Main Supervisor
Thykær, J., Supervisor, Department of Systems Biology
Kilstrup, M., Examiner
Christensen, K. B., Examiner, Department of Systems Biology
Seifar, R. M., Examiner
Institutt stipendie (DTU)
01/06/2011 → 02/09/2015
Award relations: Liquid Chromatography-mass spectrometric based metabolomics
Project: PhD

Systems biology investigations of Pseudomonas aeruginosa evolution in association with human airway infections
Pedersen, S. D., PhD Student, Department of Systems Biology
Molin, S., Main Supervisor, Department of Biotechnology and Biomedicine
Jelsbak, L., Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
de Lorenzo, V., Examiner
Schneider, D., Examiner
DTU, Samfinansiering
01/03/2010 → 03/07/2013
Award relations: Systems biology investigations of Pseudomonas aeruginosa evolution in association with human airway infections
The potential of Lactic Acid Bacteria as microbial factory for pentanol isomer production
Starlit, K. I., PhD Student
Jensen, P. R., Main Supervisor
Jørgensen, S. T., Supervisor
Kebmann, B., Supervisor, Department of Systems Biology
Martinussen, J., Supervisor
Kilstrup, M., Examiner
Kok, J., Examiner
Mijakovic, I., Examiner
Industrial PhD
01/06/2012 → 18/04/2018
Award relations: The potential of Lactic Acid Bacteria as microbial factory for pentanol isomer production
Project: PhD

Isoprenoid production in yeast
Carlsen, S., PhD Student, Department of Systems Biology
Kielland-Brandt, M., Main Supervisor
Eilasson Lantz, A., Supervisor
Nielsen, M. L., Supervisor, Department of Systems Biology
Stephanopoulos, G., Supervisor
Kilstrup, M., Examiner
Arneborg, N., Examiner
Pronk, J., Examiner
Institut stipendie (DTU)
01/10/2008 → 17/12/2012
Award relations: Isoprenoid production in yeast
Project: PhD

Structure-function relationship investigations and protein engineering of carbohydrate binding surface sites of barley amylases and potato starch binding module family 45
Andersen, J. M., PhD Student, Department of Biotechnology and Biomedicine
Svensson, B., Main Supervisor, Department of Biotechnology and Biomedicine
Abou Hachem, M., Supervisor, Department of Biotechnology and Biomedicine
Barrangou, R., Supervisor
Klaenhammer, T., Supervisor
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Coutinho, P. M., Examiner
Mirza, O. A., Examiner
Institut stipendie (DTU) Samf.
01/02/2009 → 30/09/2012
Award relations: Structure-function relationship investigations and protein engineering of carbohydrate binding surface sites of barley amylases and potato starch binding module family 45
Project: PhD

Brug af Bacillus Subtilis til Poduktion af et naturligt aromastof
Hansen, M., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
Hansen, E. B., Supervisor
Mijakovic, I., Supervisor
Kilstrup, M., Examiner
Kuipers, O. P., Examiner
Mascher, T., Examiner
1/3 DTU-stip, 2/3 FUR/andet
01/02/2005 → 23/09/2009
Award relations: Brug af Bacillus Subtilis til Poduktion af et naturligt aromastof
Project: PhD

Stabilization of plant derived food pigments by microbial conversion
Coumou, H., PhD Student, Department of Systems Biology
Mortensen, U. H., Main Supervisor
Microbial interactions and evolutionary dynamics in a multispecies community
Khademi, S. M. H., PhD Student, Department of Biotechnology and Biomedicine
Jelsbak, L., Main Supervisor, Department of Biotechnology and Biomedicine
Molin, S., Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Feil, E. J., Examiner
Winstanley, C., Examiner
Feil, E. J., Examiner
Winstanley, C., Examiner
Forskningsrådsfinansiering
01/10/2013 → 17/03/2010
Award relations: Microbial interactions and evolutionary dynamics in a multispecies community
Project: PhD

Nano-Platforms for Coordinating Enzymatic Pathways in Cell Factories
Buron, L. D., PhD Student, Department of Systems Biology
Mortensen, U. H., Main Supervisor
Kilstrup, M., Examiner
Brodelius, E. P., Examiner
Møller, B. L., Examiner
Institut stipendie (DTU)
01/12/2013 → 28/02/2018
Award relations: Nano-Platforms for Coordinating Enzymatic Pathways in Cell Factories
Project: PhD

Isolation and characterization of bacteriaophages with therapeutic potential
Villarroel, J., PhD Student, Department of Bio and Health Informatics
Nielsen, M., Main Supervisor, Department of Health Technology
Kilstrup, M., Supervisor, Department of Biotechnology and Biomedicine
Larsen, M. V., Supervisor, Department of Bio and Health Informatics
Nielsen, H., Examiner, Department of Health Technology
Lavigne, R., Examiner
Nielsen, D. S., Examiner
Lavigne, R., Examiner
Institut stipendie (DTU)
01/12/2013 → 28/02/2018
Award relations: Isolation and characterization of bacteriaophages with therapeutic potential
Project: PhD

Persister cells in Listeria monocytogenes
Curtis, T., PhD Student, Department of Systems Biology
Gram, L., Main Supervisor
Knudsen, G. M., Supervisor, Department of Systems Biology
Kilstrup, M., Examiner
Donegan, N. P., Examiner
Frees, D., Examiner
Institut stipendie (DTU)
01/11/2013 → 23/01/2017
Award relations: Persister cells in Listeria monocytogenes
Project: PhD

Thiol Redox Control Systems in Lactic Acid Bacteria
Skjoldager, N., PhD Student
Heat shock in Lactococcus: Regulation and mutants in stress response

Bacteria respond to stress by induction of specific proteins in order to be able to grow or at least to increase survival. During a temperature upshift, Lactococcus lactis is subjected to a classical heat shock with increased synthesis of the two major chaperone complexes DnaK-GrpE-DnaJ and GroES-GroEL, as well as ATP-dependent proteases. The main task of the chaperones is to recognize non-native polypeptides and prevent their aggregation by assisting the correct folding into the native state. The expression of these chaperones is controlled by the negative regulator HrcA which binds to inverted repeats (CIRCE-elements) located in front of the chaperone genes. The purpose of the project is by genetical and physiological analysis of a selected set of general stress genes to achieve basic knowledge of the physiological roles of the corresponding proteins during both growth and stress conditions in Lactococcus lactis. Temperature, salt, acid, and survival in stationary phase are the general stress parameters used in the analysis - conditions which are all of industrial importance. The project also includes analysis of the regulatory circuits involved in the stress responses. We aim to isolate a range of mutants with different phenotypes, which are important as tools for the physiological and genetical analyses of stress in Lactococcus. These include 2D-gel analysis, reporter gene fusions to individual stress genes, mRNA analysis by slot blotting, in vitro protein/DNA interactions, and several other molecular biology techniques. Some of the mutants may be used for construction of improved starter cultures.

Hammer, K., Project Manager, Department of Microbiology
Nielsen, A. K., Project Participant, Department of Microbiology
Abdul-Al, A. A. K., Project Participant, Department of Microbiology
Kilstrup, M., Project Participant, Department of Microbiology
Vogensen, F. K., Project Participant, Den Kongelige Veterinær og Landbohøjskole

Ukendt: DKK2,709,150.00
01/01/1998 → 31/12/2000

Collaborators: University of Copenhagen, Center for Advanced Food Studies, Danish Dairy Research Foundation, Den Kongelige Veterinær og Landbohøjskole

Award relations: Heat shock in Lactococcus: Regulation and mutants in stress response

Project: Research
Stress Response in Bacteria
Bacteria often experience stressing conditions and adapt to these by changing their pattern of gene expression. An understanding of the cellular mechanisms by which they adapt and survive during periods of changing environmental conditions is crucial to our use of such organisms to produce food and to our efforts to inhibit or eliminate spoilage or pathogenic microorganisms. The current project is planned to provide a better understanding of some of the fundamental aspects of stress regulation in food relevant bacteria, and towards the possible influence of stress stimuli on virulence of food borne pathogens. The project is structured in five subprojects, dealing specifically with:

Hammer, K., Project Manager, Department of Microbiology
Breuner, A., Project Participant, Department of Microbiology
Kilstrup, M., Project Participant, Department of Microbiology
Molin, S., Project Participant, Department of Microbiology
Hansen, M. C., Project Participant, Department of Microbiology
Tolker-Nielsen, T., Project Participant, Department of Microbiology
Ingmer, H., Project Participant, Den Kongelige Veterinær og Landbohøjskole
Knöchel, S., Project Participant, Den Kongelige Veterinær og Landbohøjskole
Olsen, J. E., Project Participant, Den Kongelige Veterinær og Landbohøjskole

Ukendt: DKK3,604,000.00
01/03/1998 → 31/12/2001
Collaborators: University of Copenhagen, Center for Advanced Food Studies, Den Kongelige Veterinær og Landbohøjskole
Award relations: Stress Response in Bacteria
Project: Research

Purine regulation in Lactococcus lactis
Kilstrup, M., Project Manager, Department of Microbiology
Pulka-Amin, M., Project Participant, Department of Microbiology
Nilsson, D., Project Participant, Chr. Hansen AS

01/01/1996 → 31/01/1999
Collaborators: Chr. Hansen AS
Project: Research

Bacterial stress responses, with relations to food production and food safety
Bacteria respond to stress by induction of specific proteins (stress proteins) in order to continue growth or to increase survival. Because of this, exposure to sublethal stress conditions usually increase the tolerance to additional stress levels. Using a hierarchical regulatory model as a tool in the unraveling of the regulatory networks we define a stimulon as the class of proteins, which are regulated by a specific stress condition. The stimulon can be subdivided into regulons, which are classes of proteins that share a common regulatory pathway. The lowest level of specification is the target, which is a single protein whose level is regulated in response to the stress condition. A target may belong to many regulons, and a regulon may belong to many stimulons. The goal of the project is to identify stimulons, regulons, targets and regulatory networks involved in dairy related stress responses, and their role in the generation of stress resistance. The focus of the project is heat and osmotic stress together with the physiological stress resulting from shortage of purines, phosphate and glucose. The latter three conditions were chosen due to their generation of temperature stress cross-protection. We have selected the well characterized lactic acid bacterium Lactococcus lactis as a model organism for gram-positive dairy related bacteria. The methodologies used include physiological analysis (survival and growth rate), proteomics (2D-GE of proteins coupled to mass spectrometry), Transcriptomics (analysis of mRNA levels from single or multible genes, with plans to include gene array techniques), & mutant construction and analysis (mutated in stress genes or regulatory genes). Currently we are undertaking a detailed molecular analysis of the heat stress repressor, HrcA, and the purine specific activator, PurR, together with a proteomic, and transcriptional analysis of their respective regulons using regulatory mutants.

Kilstrup, M., Project Manager, Department of Microbiology
Pulka-Amin, M., Project Participant, Department of Microbiology
Nielsen, A. K., Project Participant, Department of Microbiology
Hammer, K., Project Participant, Department of Microbiology

01/02/1998 → 31/12/2000
Project: Research

Heat shock in Lactococcus. Physiology and genetics.
Bacteria respond to stress by induction of specific proteins in order to be able to grow or at least to increase survival. Exposure to sublethal stress conditions therefore usually increase the tolerance of additional stress. Heat and salt stress
are encountered during cheese making and the aim of this project is to investigate how these conditions affect the physiology of lactococcus lactis. The major milestones of this project are to identify and clone the genes being induced by heat and salt, and to construct and characterize mutants in some of these genes. The effect of the stress stimulus was monitored on the protein as well as on the mRNA level. This was achieved by developing methods for labelling, extraction and 2 dimensional gel electrophoresis of lactococcal proteins. The identification of relevant protein spots is performed by western blots or by N-terminal amino acid sequencing. Also better methods for RNA extraction from lactococci have been developed, and used for isolation of heat induced genes by subtractive hybridization. Other genes were isolated by PCR techniques and the DnaK operon was a gift from M. Gasson (UK). In total the stress group at DTU and KVL now have 13 genes available, all isolated from the same lactococcal strain MG1363. Apart from the general chaperones dnaK and groELS, these genes include three different clp genes. Furthermore genes from a cell division operon have been isolated. Insertion mutants in several of these genes have been isolated and are being investigated.

Hammer, K., Project Manager, Department of Microbiology
Sørensen, K., Project Participant, Department of Microbiology
Sørensen, L., Project Participant, Department of Microbiology
Kilstrup, M., Project Participant, Department of Microbiology
Appel, K. F., Project Participant, Department of Microbiology
Jensen, K. B., Project Participant, Department of Microbiology
Jacobsen, S., Project Participant, Department of Biochemistry and Nutrition
Vogensen, F. K., Project Participant, University of Copenhagen
Ingmer, H., Project Participant, University of Copenhagen
Arnau, J., Project Participant, University of Copenhagen
Koch, B., Project Participant, University of Copenhagen

Ukendt: DKK2,700,000.00
01/11/1993 → 28/02/1997
Collaborators: University of Copenhagen
Award relations: Heat shock in Lactococcus. Physiology and genetics.
Project: Research

DNA-baseret identifikation af mikroorganismer i vanddrukke arkeologiske genstande
Kilstrup, M., Project Manager, Department of Systems Biology, Bacterial Physiology and Genetics Group
Project ID: 45644
Forskningsprojekter - Andre ministerier og styrelser: DKK282,500.00
01/01/2003 → 31/12/2005
Award relations: DNA-baseret identifikation af mikroorganismer i vanddrukke arkeologiske genstande
Project: Research