Publications:

A bacterial cell factory for efficient production of ethanol from whey

The invention relates to a method for homo-ethanol production from lactose using a genetically modified lactic acid bacterium of the invention, where the cells are provided with a substrate comprising dairy waste supplemented with an amino nitrogen source (such as acid hydrolysed corn steep liquor). The invention further relates to genetically modified lactic acid bacterium and its use for homo-ethanol production from lactose in dairy waste. The lactic acid bacterium comprises both genes (lacABCD, LacEF, lacG) encoding enzymes catalysing the lactose catabolism pathway; and transgenes (pdc and adhB) encoding enzymes catalysing the conversion of pyruvate to ethanol. Additionally a number of genes (Idh, pta and adhE) are deleted in order to maximise homo-ethanol production as compared to production of lactate, acetoine and acetate production.
High-level production of diacetyl in a metabolically engineered lactic acid bacterium

The present invention provides a genetically modified lactic acid bacterium capable of producing diacetyl under aerobic conditions. Additionally, the invention provides a method for producing diacetyl using the genetically modified lactic acid bacterium under aerobic conditions in the presence of a source of iron-containing porphyrin and a metal ion selected from Fe3+, Fe2+ and Cu2+. The lactic acid bacterium is genetically modified by deletion of those genes in its genome that encode polypeptides having lactate dehydrogenase (E.C 1.1.1.27/E.C.1.1.1.28); α-acetolactate decarboxylase (E.C 4.1.1.5); water-forming NADH oxidase (E.C. 1.6.3.4); phosphotransacetylase (E.C.2.3.1.8) activity; and optionally devoid of or deleted for genes encoding polypeptides having diacetyl reductase ((R)-acetoin forming; EC: 1.1.1.303); D-acetoin reductase; butanediol dehydrogenase ((R,R)-butane-2,3-diol forming; E.C. 1.1.1.4/1.1.1.-) and alcohol dehydrogenase (E.C. 1.2.1.10) activity. The invention provides for use of the genetically modified lactic acid bacterium for the production of diacetyl and a food product.

A novel genetic tool for metabolic optimization of Corynebacterium glutamicum: efficient and repetitive chromosomal integration of synthetic promoter-driven expression libraries

Fine-tuning the expression level of multiple genes is usually pivotal for metabolic optimization. We have developed a tool for this purpose for the important industrial workhorse Corynebacterium glutamicum that allows for the introduction of synthetic promoter-driven expression libraries of arbitrary genes. We first devised a method for introducing genetic elements into the chromosome repeatedly, relying on site-specific recombinases and the vector pJS31 serving as the carrier. The pJS31 vector contains a synthetic cassette including a phage attachment site attP for integration, a bacterial attachment site attB for subsequent integration, a multiple cloning site, and two modified loxP sites to facilitate easy removal of undesirable vector elements. Meanwhile, we constructed a derivative of the wild-type strain ATCC 13032 carrying an attB site in its chromosome (JS34) and demonstrated that pJS31 readily could integrate into the attB site in this strain providing expression of the corresponding integrase. Subsequent expression of the Cre recombinase promoted recombination between the modified loxP sites, resulting in a strain only retaining the target insertions and an attB site. To simplify the procedure, non-replicating circular expression units for the phage integrase and the Cre recombinase were used. As a showcase, we used the tool to construct a battery of strains simultaneously expressing the two reporter genes, lacZ (encoding β-galactosidase) and gusA (encoding β-glucuronidase), to arbitrary levels. In principle, an unlimited number of genes, whether native, heterologous, or synthetic, can be introduced using the developed approach, and this should greatly facilitate metabolic optimization of this important platform organism.
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.
Finding the Needle in the Haystack—the Use of Microfluidic Droplet Technology to Identify Vitamin-Secreting Lactic Acid Bacteria

Efficient screening technologies aim to reduce both the time and the cost required for identifying rare mutants possessing a phenotype of interest in a mutagenized population. In this study, we combined a mild mutagenesis strategy with high-throughput screening based on microfluidic droplet technology to identify Lactococcus lactis variants secreting vitamin B2 (riboflavin). Initially, we used a roseoflavin-resistant mutant of L. lactis strain MG1363, JC017, which secreted low levels of riboflavin. By using fluorescence-activated droplet sorting, several mutants that secreted riboflavin more efficiently than JC017 were readily isolated from the mutagenesis library. The screening was highly efficient, and candidates with as few as 1.6 mutations per million base pairs (Mbp) were isolated. The genetic characterization revealed that riboflavin production was triggered by mutations inhibiting purine biosynthesis, which is surprising since the purine nucleotide GTP is a riboflavin precursor. Purine starvation in the mutants induced overexpression of the riboflavin biosynthesis cluster ribABGH. When the purine starvation was relieved by purine supplementation in the growth medium, the outcome was an immediate downregulation of the riboflavin biosynthesis cluster and a reduction in riboflavin production. Finally, by applying the new isolates in milk fermentation, the riboflavin content of milk (0.99 mg/liter) was improved to 2.81 mg/liter, compared with 0.66 mg/liter and 1.51 mg/liter by using the wild-type strain and the original roseoflavin-resistant mutant JC017, respectively. The results obtained demonstrate how powerful classical mutagenesis can be when combined with droplet-based microfluidic screening technology for obtaining microorganisms with useful attributes. IMPORTANCE The food industry prefers to use classical approaches, e.g., random mutagenesis followed by screening, to improve microorganisms used in food production, as the use of recombinant DNA technologies is still not widely accepted. Although modern automated screening platforms are widely accessible, screening remains as a bottleneck in strain development, especially when a mild mutagenesis approach is applied to reduce the chance of accumulating unintended mutations, which may cause unwanted phenotypic changes. Here, we incorporate a droplet-based high-throughput screening method into the strain development process and readily capture L. lactis variants with more efficient vitamin secretion from low-error-rate mutagenesis libraries. This study shows that useful mutants showing strong phenotypes but without extensive mutations can be identified with efficient screening technologies. It is therefore possible to avoid accumulating detrimental mutations while enriching beneficial ones through iterative mutagenesis screening. Due to the low mutation rates, the genetic determinants are also readily identified.

General information
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Harnessing the respiration machinery for high-yield production of chemicals in metabolically engineered Lactococcus lactis

When modifying the metabolism of living organisms with the aim of achieving biosynthesis of useful compounds, it is essential to ensure that it is possible to achieve overall redox balance. We propose a generalized strategy for this, based on fine-tuning of respiration. The strategy was applied on metabolically engineered Lactococcus lactis strains to optimize the production of acetoin and (R,R)−2,3-butanediol (R-BDO). In the absence of an external electron acceptor, a surplus of two NADH per acetoin molecule is produced. We found that a fully activated respiration was able to efficiently regenerate NAD+, and a high titer of 371 mM (32 g/L) of acetoin was obtained with a yield of 82% of the theoretical maximum. Subsequently, we extended the metabolic pathway from acetoin to R-BDO by introducing the butanediol dehydrogenase gene from Bacillus subtilis. Since one mole of NADH is consumed when acetoin is converted into R-BDO per mole, only the excess of NADH needs to be oxidized via respiration. Either by fine-tuning the respiration capacity or by using a dual-phase fermentation approach involving a switch from fully respiratory to non-respiratory conditions, we obtained 361 mM (32 g/L) R-BDO with a yield of 81% or 365 mM (33 g/L) with a yield of 82%, respectively. These results demonstrate the great potential in using finely-tuned respiration machineries for bio-production.
Respiration capacity, Hemin, Lactococcus lactis, Acetoin, (R,R)-2,3-butanediol
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Metabolic characterization and transformation of the non-dairy Lactococcus lactis strain KF147, for production of ethanol from xylose

The non-dairy lactic acid bacterium Lactococcus lactis KF147 can utilize xylose as the sole energy source. To assess whether KF147 could serve as a platform organism for converting second generation sugars into useful chemicals, we characterized growth and product formation for KF147 when grown on xylose. In a defined medium KF147 was found to co-metabolize xylose and arginine, resulting in bi-phasic growth. Especially at low xylose concentrations, arginine significantly improved growth rate. To facilitate further studies of the xylose metabolism, we eliminated arginine catabolism by deleting the arcA gene encoding the arginine deiminase. The fermentation product profile suggested two routes for xylose degradation, the phosphoketolase pathway and the pentose phosphate pathway. Inactivation of the phosphoketolase pathway redirected the entire flux through the pentose phosphate pathway whereas over-expression of phosphoketolase increased the flux through the phosphoketolase pathway. In general, significant amounts of the mixed-acid products, including lactate, formate, acetate and ethanol, were formed irrespective of xylose concentrations. To demonstrate the potential of KF147 for converting xylose into useful chemicals we chose to redirect metabolism towards ethanol production. A synthetic promoter library was used to drive the expression of codon-optimized versions of the Zymomonas mobilis genes encoding pyruvate decarboxylase and alcohol dehydrogenase, and the outcome was a strain producing ethanol as the sole fermentation product with a high yield corresponding to 83% of the theoretical maximum. The results clearly indicate the great potential of using the more metabolically diverse non-dairy L. lactis strains for bio-production based on xylose containing feedstocks.
Re-wiring of energy metabolism promotes viability during hyperreplication stress in E. coli

Chromosome replication in Escherichia coli is initiated by DnaA. DnaA binds ATP which is essential for formation of a DnaA-oriC nucleoprotein complex that promotes strand opening, helicase loading and replisome assembly. Following initiation, DnaA<sup>ATP</sup> is converted to DnaA<sup>ADP</sup> primarily by the Regulatory Inactivation of DnaA process (RIDA). In RIDA deficient cells, DnaA<sup>ATP</sup> accumulates leading to uncontrolled initiation of replication and cell death by accumulation of DNA strand breaks. Mutations that suppress RIDA deficiency either dampen overinitiation or permit growth despite overinitiation. We characterize mutations of the last group that have in common that distinct metabolic routes are rewired resulting in the redirection of electron flow towards the cytochrome bd-1. We propose a model where cytochrome bd-1 lowers the formation of reactive oxygen species and hence oxidative damage to the DNA in general. This increases the processivity of replication forks generated by overinitiation to a level that sustains viability.

General information
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Number of pages: 26
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Micro-organism for the production of stereo-specific s, s-2,3-butanediol

The invention relates to a genetically modified lactic acid bacterium capable of producing (S,S)-2,3-butanediol stereo specifically from glucose under aerobic conditions. Additionally the invention relates to a method for producing (S,S)-2,3-butanediol and L-acetoin using the genetically modified lactic acid bacterium, under aerobic conditions in the presence of a source of iron-containing porphyrin or a source of metal ions (Fe3+/Fe2+). The lactic acid bacterium is genetically modified to express heterologous genes encoding enzymes catalysing the stereo-specific synthesis of (S,S)-2,3-butanediol; and additionally a number of genes are deleted in order to maximise the production of (S,S)-2,3-butanediol as compared to other products of oxidative fermentation.
Acetoin and 2,3 butanediol isomers synthesis in metabolically engineered *Lactococcus lactis*
Harnessing the biosynthetic machinery of living cells is a common approach used for producing a broad range of useful chemicals. Here, we divert inherent metabolic routes in *L. lactis* to produce (3R)-acetoin and the derived 2,3 butanediol isomers. Efficient production of (3R)-acetoin was accomplished using a strain where the competing lactate, acetate and ethanol forming pathways had been blocked. By introducing different alcohol dehydrogenases into this strain, either EcBdh from *Enterobacter cloacae* or SadB from *Achromobacter xylosoxidans*, it was possible to achieve high-yield production of m-BDO or R-BDO respectively. To achieve biosustainable production of these chemicals from dairy waste, we transformed the above strains with the lactose plasmid pLP712. This enabled efficient production of (3R)-acetoin, m-BDO and R-BDO from processed whey waste, with titers of 27, 51, and 32.1g/L respectively. The corresponding yields obtained were 0.42, 0.47 and 0.40 g/g lactose, which is 82%, 89%, and 76% of maximum theoretical yield respectively. These results clearly demonstrate that *L. lactis* is an excellent choice as a cell factory for transforming lactose containing dairy waste into value added chemicals.

A novel cell factory for efficient production of ethanol from dairy waste
Sustainable and economically feasible ways to produce ethanol or other liquid fuels are becoming increasingly relevant due to the limited supply of fossil fuels and the environmental consequences associated with their consumption. Microbial production of fuel compounds has gained a lot of attention and focus has mostly been on developing bio-processes involving non-food plant biomass feedstocks. The high cost of the enzymes needed to degrade such feedstocks into its constituent sugars as well as problems due to various inhibitors generated in pretreatment are two challenges that have to be addressed if cost-effective processes are to be established. Various industries, especially within the food sector, often have waste streams rich in carbohydrates and/or other nutrients, and these could serve as alternative feedstocks for such bio-processes. The dairy industry is a good example, where large amounts of cheese whey or various processed forms thereof are generated. Because of their nutrient-rich nature, these substrates are particularly well suited as feedstocks for microbial production. We have generated a *Lactococcus lactis* strain which produces ethanol as its sole fermentation product from the lactose contained in residual whey permeate (RWP), by introducing lactose catabolism into a *L. lactis* strain CS4435 (MG1363 Δ(3) ldh, Δpta, ΔadhE, pCS4268), where the carbon flow has been directed toward ethanol instead of lactate. To achieve growth and ethanol production on RWP, we added corn steep liquor hydrolysate (CSLH) as
the nitrogen source. The outcome was efficient ethanol production with a titer of 41 g/L and a yield of 70% of the theoretical maximum using a fed-batch strategy. The combination of a low-cost medium from industrial waste streams and an efficient cell factory should make the developed process industrially interesting. A process for the production of ethanol using L. lactis and a cheap renewable feedstock was developed. The results demonstrate that it is possible to achieve sustainable bioconversion of waste products from the dairy industry (RWP) and corn milling industry (CSLH) to ethanol and the process developed shows great potential for commercial realization.

**General information**
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Authors: Liu, J. (Intern), Dantoft, S. H. (Intern), Würtz, A. (Ekstern), Jensen, P. R. (Intern), Solem, C. (Intern)
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Biofilm as a production platform for heterologous production of rhamnolipids by the non-pathogenic strain *Pseudomonas putida* KT2440

**Background**

Although a transition toward sustainable production of chemicals is needed, the physiochemical properties of certain biochemicals such as biosurfactants make them challenging to produce in conventional bioreactor systems. Alternative production platforms such as surface-attached biofilm populations could potentially overcome these challenges. Rhamnolipids are a group of biosurfactants highly relevant for industrial applications. However, they are mainly produced by the opportunistic pathogen *Pseudomonas aeruginosa* using hydrophobic substrates such as plant oils. As the biosynthesis is tightly regulated in *P. aeruginosa* a heterologous production of rhamnolipids in a safe organism can relieve the production from many of these limitations and alternative substrates could be used.

**Results**

In the present study, heterologous production of biosurfactants was investigated using rhamnolipids as the model compound in biofilm encased *Pseudomonas putida* KT2440. The rhlAB operon from *P. aeruginosa* was introduced into *P. putida* to produce mono-rhamnolipids. A synthetic promoter library was used in order to bypass the normal regulation of rhamnolipid synthesis and to provide varying expression levels of the rhlAB operon resulting in different levels of rhamnolipid production. Biosynthesis of rhamnolipids in *P. putida* decreased bacterial growth rate but stimulated biofilm formation by enhancing cell motility. Continuous rhamnolipid production in a biofilm was achieved using flow cell technology. Quantitative and structural investigations of the produced rhamnolipids were made by ultra performance liquid chromatography combined with high resolution mass spectrometry (HRMS) and tandem HRMS. The predominant rhamnolipid congener produced by the heterologous *P. putida* biofilm was mono-rhamnolipid with two C<sub>10</sub> fatty acids.

**Conclusion**

This study shows a successful application of synthetic promoter library in *P. putida* KT2440 and a heterologous biosynthesis of rhamnolipids in biofilm encased cells without hampering biofilm capabilities. These findings expands the possibilities of cultivation setups and paves the way for employing biofilm flow systems as production platforms for biochemicals, which as a consequence of physiochemical properties are troublesome to produce in conventional fermenter setups, or for production of compounds which are inhibitory or toxic to the production organisms.
Can microbes compete with cows for sustainable protein production - A feasibility study on high quality protein

An increasing population and their increased demand for high-protein diets will require dramatic changes in the food industry, as limited resources and environmental issues will make animal derived foods and proteins, gradually more unsustainable to produce. To explore alternatives to animal derived proteins, an economic model was built around the genome-scale metabolic network of E. coli to study the feasibility of recombinant protein production as a food source. Using a novel model, we predicted which microbial production strategies are optimal for economic return, by capturing the tradeoff between the market prices of substrates, product output and the efficiency of microbial production. A case study with the food protein, Bovine Alpha Lactalbumin was made to evaluate the upstream economic feasibilities. Simulations with different substrate profiles at maximum productivity were used to explore the feasibility of recombinant Bovine Alpha Lactalbumin production coupled with market prices of utilized materials. We found that recombinant protein production could be a feasible food source and an alternative to traditional sources.
Combining metabolic engineering and biocompatible chemistry for efficient production of food ingredients

Biocompatible chemistry, that is non-enzymatic chemical reactions compatible with living organisms, is gaining increasing attention because of its potential within biotechnology for expanding the repertoire of biological transformations carried out by enzymes. Here we demonstrate how biocompatible chemistry can be used for synthesizing valuable food ingredients as well as for linking metabolic pathways to achieve redox balance and rescued growth. By comprehensive rerouting of metabolism, activation of respiration, and finally metal ion catalysis, we successfully managed to convert the homolactic bacterium Lactococcus lactis into a homo-diacetyl producer with high titer (95 mM or 8.2 g/L) and high yield (87% of the theoretical maximum). Subsequently, the pathway was extended to (S,S)-2,3-butanediol (S-BDO) through efficiently linking two metabolic pathways via chemical catalysis. This resulted in efficient homo-S-BDO production with a titer of 74 mM (6.7 g/L) S-BDO and a yield of 82%. The diacetyl and S-BDO production rates and yields obtained are the highest ever reported, demonstrating the promising combination of metabolic engineering and biocompatible chemistry as well as the great potential of L. lactis as a new production platform.

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Combining metabolic engineering and biocompatible chemistry for high-yield production of homo-diacytely and homo-\((S,S)\)-2,3-butanediol

Biocompatible chemistry is gaining increasing attention because of its potential within biotechnology for expanding the repertoire of biological transformations carried out by enzymes. Here we demonstrate how biocompatible chemistry can be used for synthesizing valuable compounds as well as for linking metabolic pathways to achieve redox balance and rescued growth. By comprehensive rerouting of metabolism, activation of respiration, and finally metal ion catalysis, we successfully managed to convert the homolactic bacterium Lactococcus lactis into a homo-diacytely producer with high titer (95mM or 8.2g/L) and high yield (87% of the theoretical maximum). Subsequently, the pathway was extended to \((S,S)\)-2,3-butanediol (S-BDO) through efficiently linking two metabolic pathways via chemical catalysis. This resulted in efficient homo-S-BDO production with a titer of 74mM (6.7g/L) S-BDO and a yield of 82%. The diacytely and S-BDO production rates and yields obtained are the highest ever reported, demonstrating the promising combination of metabolic engineering and biocompatible chemistry as well as the great potential of L. lactis as a new production platform.

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Organisations: National Food Institute, Research Group for Microbial Biotechnology and Biorefining, University of Copenhagen, Korea Advanced Institute of Science & Technology
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Scopus rating (2011): SJR 3.124 SNIP 2.144 CiteScore 6.75
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Web of Science (2011): Indexed yes
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Scopus rating (2010): SJR 2.373 SNIP 1.802
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Scopus rating (2009): SJR 2.575 SNIP 1.421
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Draft Genome Sequence of Hymenobacter sp. Strain AT01-02, Isolated from a Surface Soil Sample in the Atacama Desert, Chile

Here, we report the 5.09-Mb draft genome sequence of Hymenobacter sp. strain AT01-02, which was isolated from a surface soil sample in the Atacama Desert, Chile. The isolate is extremely resistant to UV-C radiation and is able to accumulate high intracellular levels of Mn/Fe.

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Elucidation of the regulatory role of the fructose operon reveals a novel target for enhancing the NADPH supply in Corynebacterium glutamicum

The performance of Corynebacterium glutamicum cell factories producing compounds which rely heavily on NADPH has been reported to depend on the sugar being metabolized. While some aspects of this phenomenon have been elucidated, there are still many unresolved questions as to how sugar metabolism is linked to redox and to the general metabolism. We here provide new insights into the regulation of the metabolism of this important platform organism by systematically characterizing mutants carrying various lesions in the fructose operon. Initially, we found that a strain where the dedicated fructose uptake system had been inactivated (KO-ptsF) was hampered in growth on sucrose minimal medium, and suppressor mutants appeared readily. Comparative genomic analysis in conjunction with enzymatic assays revealed that suppression was linked to inactivation of the pfkB gene, encoding a fructose-1-phosphate kinase. Detailed characterization of KO-ptsF, KO-pfkB and double knock-out (DKO) derivatives revealed a strong role for sugar-phosphates, especially fructose-1-phosphate (F1P), in governing sugar as well as redox metabolism due to effects on transcriptional regulation of key genes. These findings allowed us to propose a simple model explaining the correlation between sugar phosphate concentration, gene expression and ultimately the observed phenotype. To guide us in our analysis and help us identify bottlenecks in metabolism we debugged an existing genome-scale model onto which we overlaid the transcriptome data. Based on the results obtained we managed to enhance the NADPH supply and transform the wild-type strain into delivering the highest yield of lysine ever obtained on sucrose and fructose, thus providing a good example of how regulatory mechanisms can be harnessed for bioproduction.
Integrating biocompatible chemistry and manipulating cofactor partitioning in metabolically engineered *Lactococcus lactis* for fermentative production of (3S)-acetoin

Biocompatible chemistry (BC), i.e. non-enzymatic chemical reactions compatible with living organisms, is increasingly used in conjunction with metabolically engineered microorganisms for producing compounds that do not usually occur naturally. Here we report production of one such compound, (3S)-acetoin, a valuable precursor for chiral synthesis, using a metabolically engineered *Lactococcus lactis* strain growing under respiratory conditions with ferric iron serving as a BC component. The strain used has all competing product pathways inactivated, and an appropriate cofactor balance is achieved by fine-tuning the respiratory capacity indirectly via the hemin concentration. We achieve high-level (3S)-acetoin production with a final titer of 66 mM (5.8 g/L) and a high yield (71% of the theoretical maximum). To the best of our knowledge, this is the first report describing production of (3S)-acetoin from sugar by microbial fermentation, and the results obtained confirm the potential that lies with BC for producing useful chemicals.
The extensive use of fossil fuels has a severe influence on the environment. In order to reduce the dependency on these limited resources and to protect the environment substantial effort is being made to implement renewable resources. One
part of this transition is to develop methods for sustainable production of chemicals, which can be achieved by microbial cell factories. The work presented in this PhD thesis elucidates the application of Pseudomonas putida as a microbial cell factory for production of the biosurfactant rhamnolipid. The rhamnolipid production was achieved by heterologous expression of the rhlAB operon from Pseudomonas aeruginosa using a synthetic promoter library in P. putida. Since rhamnolipid production is associated with difficulties in conventional bioreactors we have used biofilm encased P. putida to circumvent these problems. We show that biofilm can be used as a production platform for continuous production of rhamnolipids. A method for quantitative and qualitative analysis of the produced rhamnolipids was developed based on ultra performance liquid chromatography combined with high resolution mass spectrometry. This enabled detection of low levels of rhamnolipids. The applicability of glycerol as a substrate was also investigated. Since glycerol is a poor substrate adaptive evolution was made in order to improve the capabilities of P. putida to proliferate on glycerol. The evolved lineages all had significantly increased growth rate, enhanced cell density and reduced lag phase. The genomic alterations were identified by genome sequencing and revealed parallel evolution. Glycerol was also shown to be able to support biofilm growth and as a result of this it can be used as an alternative substrate for producing biochemicals in conventional and biofilm reactors. The use of biofilm as a production platform and the usage of glycerol as a feedstock show the potential of using microbial cell factories in the transition toward sustainable production of chemicals. Particularly, the applicability of biofilm as a production platform can emerge as a promising alternative for producing toxic biochemicals and for producing biochemicals which are difficult to cope in conventional bioreactors.

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Authors: Wigneswaran, V. (Intern), Jelsbak, L. (Intern), Folkesson, A. (Intern), Jensen, P. R. (Intern)
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Lactic Acid Bacteria as a new platform for sustainable production of fuels and chemicals
The diminishing natural resources and environmental issues lead us to consider other ways of producing materials, chemicals and energy to satisfy the ever-increasing needs of our society. Lignocellulosic biomass is the most abundant type of substrate in the world; it is also cheap and renewable which makes it a perfect candidate substrate for production of value added products. The second generation biorefineries, employing microorganisms for conversion of lignocellulosic feedstocks into value added products, are not yet employed commercially in a large scale. To increase the economic feasibility of the process, robust microbial catalysts are necessary, both having a broad substrate utilization range and being tolerant to the common inhibitors generated during the lignocellulose pretreatment. Even though many microorganisms are already well characterized and commercially employed in 1st generation biorefineries, the conversion of lignocellulose is a more complex process; thus, the pursue for a suitable microbe continues. In this PhD study, a wide collection of Lactic Acid Bacteria was systematically screened for the strains’ tolerance levels towards various inhibitors coming from the pretreatment of lignocellulosic biomass, as well as for their capabilities to utilize various sugar substrates, including both pentoses and hexoses. Almost 300 strains were tested, including 141 different isolates of Lactobacillus plantarum, L. paraplanarum, L. pentosus, L. brevis, L. buchneri and L. paracasei, and all available Lactobacillus and Pediococcus type strains. Five most promising strains were subjected to further studies; these included L. pentosus LMG 17672, L. pentosus LMG 17673, L. pentosus 10-16, P. pentosaceous ATCC 25745 and P. acidilactici DSM 20284. The strains were tested in growth experiments with increased concentrations of the key inhibitors, such as furfural and HMF, as well as with the presence of the most common combinations of inhibitors, mimicking real-life lignocellulosic feedstocks: sugarcane bagasse, wheat straw and soft wood. The two most promising strains were selected; these were L. pentosus LMG 17673 and P. acidilactici DSM 20284. They were not only found highly resistant to the key inhibitors, but they were also demonstrated to utilize pentoses, xylose and arabinose. For one of the selected most promising strains, P. acidilactici DSM 20284, a chemically defined medium was developed and optimized. The resulting Pediococcus Defined Medium (PDM) proved to support the growth of a variety of other species as well, including all Pediococcus species and several fastidious Lactobacilli. Thus, the PDM medium appears to be superior to the previously published defined media, and can therefore be suitable for physiological, biochemical or nutritional investigations in other LAB species. An efficient transformation procedure is necessary for strain’s rational genetic engineering. To ease strategies for further strain improvement, a transformation procedure was developed and optimized for P. acidilactici DSM 20284, increasing the
transformation efficiency by 2 log units. An optimized method allows for the transformation with an efficiency of 2.8·10³ transformants per μg DNA, permitting the genetic modification of this strain. In order to even further enhance the P. acidilactici DSM 20284 tolerance to furfural, an adaptation experiment was performed by continuous serial-transfer method. After 408 generations, an adapted strain A28 was isolated and showed an increased growth rate on the rich MRS medium with addition of furfural; yet, it also demonstrated a 27% better growth in MRS medium alone. A whole genome resequencing analysis revealed 62 mutations in the genome of the adapted strain compared to the wild-type. The mutations were mainly single nucleotide polymorphisms, but there were also 12 single insertions identified. More than half of the mutations were non-synonymous substitutions, leading to an amino acid change. Two transcriptional regulators, HrcA and CtsR, were affected by non-synonymous substitutions within the protein or the Shine-Dalgarno sequence, respectively. Several membrane proteins as well as proteins involved in the cell redox homeostasis were also mutated. Purine biosynthesis, salvage and transport related genes were also affected by mutations, likely having an influence on the intracellular nucleotide pool sizes, thereby allowing for an increased growth rate. The analysis of the transcriptomic profiles of the wild-type P. acidilactici DSM 20284 and the adapted strain A28 revealed that the applied furfural concentration did not induce the stress response neither in the wildtype nor in the adapted strain. This finding indicates that both strains are already well adapted to furfural; thereby during the adaptive laboratory evolution experiment the strain adapted towards a faster and more efficient growth on the medium rather than towards furfural resistance. However, several genes related to exopolysaccharide biosynthesis or encoding membrane proteins were induced in the adapted strain, indicating that the cell wall structure might be important for the cell’s protection against furfural. The higher growth rate on the other hand, occurred to be enabled by an optimization of the purine and pyrimidine biosynthesis and salvage pathways, up-regulation of the folic acid biosynthesis as well as several enzymes involved in glycolysis. Finally, the study confirmed the remarkable potential of LAB for their use as microbial cell factories for conversion of lignocellulosic substrates into value-added products.
Stimulation of acetoin production in metabolically engineered Lactococcus lactis by increasing ATP demand

Having a sufficient supply of energy, usually in the form of ATP, is essential for all living organisms. In this study, however, we demonstrate that it can be beneficial to reduce ATP availability when the objective is microbial production. By introducing the ATP hydrolyzing F1-ATPase into a Lactococcus lactis strain engineered into producing acetoin, we show that production titer and yield both can be increased. At high F1-ATPase expression level, the acetoin production yield could be increased by 10%, however, because of the negative effect that the F1-ATPase had on biomass yield and growth, this increase was at the cost of volumetric productivity. By lowering the expression level of the F1-ATPase, both the volumetric productivity and the final yield could be increased by 5% compared to the reference strain not overexpressing the F1-ATPase, and in batch fermentation, it was possible to convert 176 mM (32 g/L) of glucose into 146.5 mM (12.9 g/L) acetoin with a yield of 83% of the theoretical maximum. To further demonstrate the potential of the cell factory developed, we complemented it with the lactose plasmid pLP712, which allowed for growth and acetoin production from a dairy waste stream, deproteinized whey. Using this cheap and renewable feedstock, efficient acetoin production with a titer of 157 mM (14 g/L) acetoin was accomplished.
Synthesis of (3R)-acetoin and 2,3-butanediol isomers by metabolically engineered Lactococcus lactis

The potential that lies in harnessing the chemical synthesis capabilities inherent in living organisms is immense. Here we demonstrate how the biosynthetic machinery of Lactococcus lactis can be diverted to make (3R)-acetoin and the derived 2,3-butanediol isomers meso-(2,3)-butanediol (m-BDO) and (2R,3R)-butanediol (R-BDO). Efficient production of (3R)-acetoin was accomplished using a strain where the competing lactate, acetate and ethanol forming pathways had been blocked. By introducing different alcohol dehydrogenases into this strain, either EcBDH from Enterobacter cloacae or SadB from Achromobacter xylosoxidans, it was possible to achieve high-yield production of m-BDO or R-BDO respectively. To achieve biosustainable production of these chemicals from dairy waste, we transformed the above strains with the lactose plasmid pLP712. This enabled efficient production of (3R)-acetoin, m-BDO and R-BDO from processed whey waste, with titers of 27, 51, and 32 g/L respectively. The corresponding yields obtained were 0.42, 0.47 and 0.40 g/g lactose, which is 82%, 89%, and 76% of maximum theoretical yield respectively. These results clearly demonstrate that L. lactis is an excellent choice as a cell factory for transforming lactose containing dairy waste into value added chemicals.

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Adaptation of Lactococcus lactis to high growth temperature leads to a dramatic increase in acidification rate

*Lactococcus lactis* is essential for most cheese making, and this mesophilic bacterium has its growth optimum around 30 °C. We have, through adaptive evolution, isolated a mutant TM29 that grows well up to 39 °C, and continuous growth at 40 °C is possible if pre-incubated at a slightly lower temperature. At the maximal permissive temperature for the wild-type, 38 °C, TM29 grows 33% faster and has a 12% higher specific lactate production rate than its parent MG1363, which results in fast lactate accumulation. Genome sequencing was used to reveal the mutations accumulated, most of which were shown to affect thermal tolerance. Of the mutations with more pronounced effects, two affected expression of single proteins (chaperone; riboflavin transporter), two had pleiotropic effects (RNA polymerase) which changed the gene expression profile, and one resulted in a change in the coding sequence of CDP-diglyceride synthase. A large deletion containing 10 genes was also found to affect thermal tolerance significantly. With this study we demonstrate a simple approach to obtain non-GMO derivatives of the important *L. lactis* that possess properties desirable by the industry, e.g. thermal robustness and increased rate of acidification. The mutations we have identified provide a genetic basis for further investigation of thermal tolerance.

General information

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A New Type of YumC-Like Ferredoxin (Flavodoxin) Reductase Is Involved in Ribonucleotide Reduction

The trxB2 gene, which is annotated as a thioredoxin reductase, was found to be essential for growth of Lactococcus lactis in the presence of oxygen. The corresponding protein (TrxB2) showed a high similarity with Bacillus subtilis YumC (E value = 4.0E-88), and YumC was able to fully complement the ΔtrxB2 mutant phenotype. YumC represents a novel type of ferredoxin (flavodoxin) reductase (FdR) with hitherto-unknown biological function. We adaptively evolved the ΔtrxB2 mutant under aerobic conditions to find suppressor mutations that could help elucidate the involvement of TrxB2 in aerobic growth. Genome sequencing of two independent isolates, which were able to grow as well as the wild-type strain under aerated conditions, revealed the importance of mutations in nrdI, encoding a flavodoxin involved in aerobic ribonucleotide reduction. We suggest a role for TrxB2 in nucleotide metabolism, where the flavodoxin (NrdI) serves as its redox partner, and we support this hypothesis by showing the beneficial effect of deoxynucleosides on aerobic growth of the ΔtrxB2 mutant. Finally, we demonstrate, by heterologous expression, that the TrxB2 protein functionally can substitute for YumC in B. subtilis but that the addition of deoxynucleosides cannot compensate for the lethal phenotype displayed by the B. subtilis yumC knockout mutant. Ferredoxin (flavodoxin) reductase (FdR) is involved in many important reactions in both eukaryotes and prokaryotes, such as photosynthesis, nitrate reduction, etc. The recently identified bacterial YumC-type FdR belongs to a novel type, the biological function of which still remains elusive. We found that the YumC-like FdR (TrxB2) is essential for aerobic growth of Lactococcus lactis. We suggest that the YumC-type FdR is involved in the ribonucleotide reduction by the class Ib ribonucleotide reductase, which represents the workhorse for the bioconversion of nucleotides to deoxynucleotides in many prokaryotes and eukaryotic pathogens under aerobic conditions. As the partner of the flavodoxin (NrdI), the key FdR is missing in the current model describing the class Ib system in Escherichia coli. With this study, we have established a role for this novel type of FdR and in addition found the missing link needed to explain how ribonucleotide reduction is carried out under aerobic conditions.
High-level ethanol production by metabolically engineered Lactococcus lactis using economically renewable feedstocks

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Increased expression of pyruvate carboxylase and biotin protein ligase increases lysine production in a biotin prototrophic Corynebacterium glutamicum strain
Corynebacterium glutamicum, a Gram-positive bacterium used for the production of various biochemicals, is naturally a biotin auxotroph. We introduced the biotin genes from Bacillus subtilis on a plasmid, pBIO, into a lysine-producing derivative (termed AHP-3) that has been described previously, and achieved biotin prototrophy. We found that AHP-3, containing pBIO, was able to produce lysine in a medium lacking biotin and that the lysine yield on glucose was similar to what is obtained when using a medium containing biotin. However, there was a decrease in specific growth rate of 20% when the strain was cultivated without biotin, indicating a suboptimal intracellular concentration of biotin. In an attempt to locate the potential bottleneck, we added pimelic acid, an early biotin precursor, and found that growth rate could be restored fully, which demonstrates that the bottleneck is in pimeloyl-CoA (or pimeloyl-Acyl Carrier Protein [ACP]) formation. Pyruvate carboxylase (pycA), a biotin-dependent enzyme needed for lysine biosynthesis and biotin ligase (birA), which is responsible for attaching biotin to pyruvate carboxylase, were overexpressed by replacing the native promoters with the strong superoxide dismutase (sod) promoter, to see whether growth could be restored. Neither pycA nor birA overexpression, whether alone or in combination, had an effect on specific growth rate, but they did have a positive effect on lysine yield, which increased by 55% in the strain overexpressing both enzymes.

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Authors: Wang, Z. (Intern), Moslehi-Jenabian, S. (Ekstern), Solem, C. (Intern), Jensen, P. R. (Intern)
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Web of Science (2015): Indexed yes
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Web of Science (2014): Indexed yes
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Acetate Kinase Isozymes Confer Robustness in Acetate Metabolism

Acetate kinase (ACK) (EC no: 2.7.2.1) interconverts acetyl-phosphate and acetate to either catabolize or synthesize acetyl-CoA dependent on the metabolic requirement. Among all ACK entries available in UniProt, we found that around 45% are multiple ACKs in some organisms including more than 300 species but surprisingly, little work has been done to clarify whether this has any significance. In an attempt to gain further insight we have studied the two ACKs (AckA1, AckA2) encoded by two neighboring genes conserved in Lactococcus lactis (L. lactis) by analyzing protein sequences, characterizing transcription structure, determining enzyme characteristics and effect on growth physiology. The results show that the two ACKs are most likely individually transcribed. AckA1 has a much higher turnover number and AckA2 has a much higher affinity for acetate in vitro. Consistently, growth experiments of mutant strains reveal that AckA1 has a higher capacity for acetate production which allows faster growth in an environment with high acetate concentration. Meanwhile, AckA2 is important for fast acetate-dependent growth at low concentration of acetate. The results demonstrate that the two ACKs have complementary physiological roles in L. lactis to maintain a robust acetate metabolism for fast growth at different extracellular acetate concentrations. The existence of ACK isozymes may reflect a common evolutionary strategy in bacteria in an environment with varying concentrations of acetate.
Development of droplets-based microfluidic systems for single-cell high-throughput screening

High-throughput screening (HTS) plays an important role in the development of microbial cell factories. One of the most popular approaches is to use microplates combined with the application of robotics, liquid handling and sophisticated detection methods. However, these workstations require large investment, and a logarithmic increase to screen large combinatorial libraries over the decades also makes it gradually out of depth. Here, we are trying to develop a feasible high-throughput system that uses microfluidics to compartmentalize a single cell for propagation and analysis in monodisperse picoliter aqueous droplets surround by an immiscible fluorinated oil phase. Our aim is to use this system to facilitate the screening process for both the biotechnology and food industry.

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**Elucidating Flux Regulation of the Fermentation Modes of Lactococcus lactis: A Multilevel Study**

The long history of application to the dairy industry has established Lactococcus lactis (L. lactis), the lactic acid bacterium, as one of the most extensively characterized low GC organisms. The relatively simple metabolism of L. lactis has also made it an attractive target for metabolic engineering for the production of non-food related chemicals. Moreover, the status of being the first genetically modified organism to deliver immunoproteins alive to human has brought L. lactis considerable fame in biomedical research. Beside the exceptional industrial relevance of L. lactis, it is also an important subject for basic research in cellular metabolism because L. lactis exhibits an interesting metabolic shift. Under anaerobic conditions, on fast fermentable sugars, L. lactis produces lactate as the primary product, known as homolactic fermentation but on slowly fermentable sugars, significant amounts of formate, acetate and ethanol are formed, known as mixed-acid fermentation. This shift is termed the mixed-acid shift. This type of shift between a low-yield and a high-yield metabolism has drawn a lot of research focus and has similarly been observed in other bacteria, yeast and even tumor cells. Efforts have been put to find out the mechanism regulating the mixed-acid shift as well as to answer questions such as why L. lactis prefers such a switch. Until now, some pieces of evidence have been reported and several factors and models have been proposed as the keys to regulating the shift, including the expression level of certain genes in glycolysis and fermentation pathways, the levels of the cofactors NADH, NAD⁺, ATP and ADP, the balance between catabolism and anabolism, etc. In this project, we studied the mixed-acid fermentation of L. lactis by (i) examining the roles of the enzymes in the mixed-acid fermentation pathway under different growth conditions; (ii) testing the predicted effect of the cofactors NADH, NAD⁺ on the mixed-acid shift proposed in previous studies; (iii) looking into the connection between amino acid metabolism and the mixed-acid shift; and (iv) contrasting the difference regarding the mixed-acid shift between two widely studied laboratory strains of L. lactis, MG1363 that shifts significantly and IL1403 that does not shift. We have measured the promoter activities of several mixed-acid genes which suggested that the regulatory elements governing the transcriptional regulation of the mixed-acid genes in MG1363 and IL1403 were different. This led us to performing experimental control analysis of the role of pyruvate formate-lyase (PFL) in MG1363 and IL1403. The expression of PFL in MG1363 and IL1403 was probably detrimental. The two homologous acetate kinases in MG1363 were also chacterized with respect to the transcription and enzyme activities. The isozymes were found to have complementary physiological roles that became important in acetate-producing or acetate-assimilating conditions respectively. The proposed roles of NADH and NAD⁺ on the mixed-acid shift were tested by perturbation via introducing activities of 2,3-butanediol dehydrogenase and supplying extracellular acetoin as an oxidizing agent. The additional NAD⁺ regenerating activities allowed a faster growth of MG1363 on maltose by shifting ethanol production into acetate production and also stimulated formate and acetate production in IL1403. Dependence of the mixed-acid fermentation of MG1363 on amino acid availability was observed and the impact of individual amino acids could differ significantly. Meanwhile, a computational method for combining metabolic flux analysis and elementary mode analysis was developed and applied to analyse a case of amino acid metabolism of L. lactis.

**General information**

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**Estimating biological elementary flux modes that decompose a flux distribution by the minimal branching property**

**MOTIVATION:** Elementary flux mode (EFM) is a useful tool in constraint-based modeling of metabolic networks. The property that every flux distribution can be decomposed as a weighted sum of EFMs allows certain applications of EFMs to studying flux distributions. The existence of biologically infeasible EFMs and the non-uniqueness of the decomposition, however, undermine the applicability of such methods. Efforts have been made to find biologically feasible EFMs by incorporating information from transcriptional regulation and thermodynamics. Yet, no attempt has been made to distinguish biologically feasible EFMs by considering their graphical properties. A previous study on the transcriptional...
regulation of metabolic genes found that distinct branches at a branch point metabolite usually belong to distinct metabolic pathways. This suggests an intuitive property of biologically feasible EFMs, i.e. minimal branching.

RESULTS: We developed the concept of minimal branching EFM and derived the minimal branching decomposition (MBD) to decompose flux distributions. Testing in the core Escherichia coli metabolic network indicated that MBD can distinguish branches at branch points and greatly reduced the solution space in which the decomposition is often unique. An experimental flux distribution from a previous study on mouse cardiomyocyte was decomposed using MBD. Comparison with decomposition by a minimum number of EFMs showed that MBD found EFMs more consistent with established biological knowledge, which facilitates interpretation. Comparison of the methods applied to a complex flux distribution in Lactococcus lactis similarly showed the advantages of MBD. The minimal branching EFM concept underlying MBD should be useful in other applications.
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. trpB/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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Identification of potential drug targets in *Salmonella enterica* sv. Typhimurium using metabolic modelling and experimental validation

*Salmonella enterica* sv. *Typhimurium* is an established model organism for Gram-negative, intracellular pathogens. Owing to the rapid spread of resistance to antibiotics among this group of pathogens, new approaches to identify suitable target proteins are required. Based on the genome sequence of *S. Typhimurium* and associated databases, a genome-scale metabolic model was constructed. Output was based on an experimental determination of the biomass of *Salmonella* when growing in glucose minimal medium. Linear programming was used to simulate variations in the energy demand while growing in glucose minimal medium. By grouping reactions with similar flux responses, a subnetwork of 34 reactions responding to this variation was identified (the catabolic core). This network was used to identify sets of one and two reactions that when removed from the genome-scale model interfered with energy and biomass generation. Eleven such sets were found to be essential for the production of biomass precursors. Experimental investigation of seven of these showed that knockouts of the associated genes resulted in attenuated growth for four pairs of reactions, whilst three single reactions were shown to be essential for growth.

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Microbial production of lysine from sustainable feedstock

Lysine is produced in a fermentation process using Corynebacterium glutamicum. And even though production strains have been improved for decades, there is still room for further optimization.

Polyamines are essential for virulence in Salmonella enterica serovar Gallinarum despite evolutionary decay of polyamine biosynthesis genes

Serovars of Salmonella enterica exhibit different host-specificities where some have broad host-ranges and others, like S. Gallinarum and S. Typhi, are host-specific for poultry and humans, respectively. With the recent availability of whole genome sequences it has been reported that host-specificity coincides with accumulation of pseudogenes, indicating adaptation of host-restricted serovars to their narrow niches. Polyamines are small cationic amines and in Salmonella they can be synthesized through two alternative pathways directly from L-ornithine to putrescine and from L-arginine via agmatine to putrescine. The first pathway is not active in S. Gallinarum and S. Typhi, and this prompted us to investigate the importance of polyamines for virulence in S. Gallinarum. Bioinformatic analysis of all sequenced genomes of Salmonella revealed that pseudogene formation of the speC gene was exclusive for S. Typhi and S. Gallinarum and happened through independent events. The remaining polyamine biosynthesis pathway was found to be essential for oral infection with S. Gallinarum since single and double mutants in speB and speE, encoding the pathways from agmatine to putrescine and from putrescine to spermidine, were attenuated. In contrast, speB was dispensable after intraperitoneal challenge, suggesting that putrescine was less important for the systemic phase of the disease. In support of this hypothesis, a ΔspeE;ΔpotCD mutant, unable to synthesize and import spermidine, but with retained ability to import and synthesize putrescine, was attenuated after intraperitoneal infection. We therefore conclude that polyamines are essential for virulence of S. Gallinarum. Furthermore, our results point to distinct roles for putrescine and spermidine during systemic infection.
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Processing of biowaste for sustainable products in developing countries
The modern global society faces great challenges in supply of energy, feed, food, and other products in a sustainable way. One way to mitigate the negative effects of providing these local eco-services is to convert biomass – instead of petroleum or natural gas – into a variety of food, feed, biomaterials, energy and fertilizer, maximizing the value of the biomass and minimizing the waste. This integrated approach corresponds to the biorefinery concept and is gaining attention in many parts of the world (Kam & Kam 2004). Energy, food and feed production is the driver for development in this area, but as biorefineries become more and more sophisticated with time, other products will be developed. Today, almost all organic chemicals - and also fertilizer - are produced from crude oil and petroleum and technologies with are driven by fossil energy, thus referred to as petro-chemicals and fossil fertilizer. It is generally anticipated that white biotechnology, the use of fermentation and enzymatic processes will play a key role for future cleaner production of bulk chemicals, energy carriers as well as fertilizer from biomass sources by saving resources and reduce negative environmental impacts from the chemical production. In order to replace fossil based energy carriers, chemicals and fertilizer, cost is the critical challenge for success. Thus, easily accessible and low costs biomass feedstock is a prerequisite for making bio-based production economically feasible. Industrial, agriculture and municipal biowastes have the potential to be that resource. However, it is of great importance to be aware of how to utilize the different sources of biowaste and for which purpose. In October 2012, a new EU project, funded under the FP7 programme was launched with partners from the EU, Africa and Malaysia. The objective of the proposed project is to show and demonstrate the technical roadmap - a strategy - for efficient technological utilization of selected significant biowaste in five African countries - Morocco, Egypt, Ghana, South Africa, and Kenya- derived from both the industrial and agricultural sector, thus, turning biowaste into a new resource for sustainable products. Our group is involved in developing strains and microbial fermentation processes for these bioconversions.

Screening of lactic acid bacteria for their potential as microbial cell factories for bioconversion of lignocellulosic feedstocks
Background: The use of fossil carbon sources for fuels and petrochemicals has serious impacts on our environment and is unable to meet the demand in the future. A promising and sustainable alternative is to substitute fossil carbon sources with microbial cell factories converting lignocellulosic biomass into desirable value added products. However, such bioprocesses require availability of suitable and efficient microbial biocatalysts, capable of utilizing C5 sugars and tolerant to inhibitory compounds generated during pretreatment of biomass. In this study, the performance of a collection of lactic acid bacteria was evaluated regarding their properties with respect to the conversion of lignocellulosic feedstocks. The strains were examined for their ability to utilize xylose and arabinose as well as their resistance towards common inhibitors from pretreated lignocellulosic biomass (furan derivatives, phenolic compounds, weak acids). Results: Among 296 tested Lactobacillus and Pediococcus strains, 3 L. pentosus, 1 P. acidilactici and 1 P. pentosaceus isolates were found to be both capable of utilizing xylose and arabinose and highly resistant to the key inhibitors from chemically pretreated lignocellulosic biomass. When tested in broth with commonly found combinations of inhibitors, the selected strains showed merely 4%, 1% and 37% drop in growth rates for sugarcane bagasse, wheat straw and soft wood representatives, respectively, as compared to Escherichia coli MG1655 showing decreased growth rates by 36%, 21% and 90%, respectively, under the same conditions. Conclusion: The study showed that some strains of Lactobacilli and Pediococci have the potential to be used as production platforms for value-added products from pretreated lignocellulosic biomass. Selected Lactobacilli and Pediococci strains were able to tolerate the key inhibitors in higher concentrations compared to E. coli; in addition, as these isolates were also capable of fermenting xylose and arabinose, they constitute good candidates for efficient lignocellulosic feedstock bioconversions.
sugarcane bagasse, wheat straw, Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Gram-Positive Cocci [07700] Pediococcus pentosaceus species Pediococcus acidilactici species, Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Regular Nonsporing Gram-Positive Rods [07830] Lactobacillus pentosus species fermentation agent, Facultatively Anaerobic Gram-Negative Rods Eubacteria Bacteria Microorganisms
Synthetic promoter libraries for Corynebacterium glutamicum

The ability to modulate gene expression is an important genetic tool in systems biology and biotechnology. Here, we demonstrate that a previously published easy and fast PCR-based method for modulating gene expression in lactic acid bacteria is also applicable to Corynebacterium glutamicum. We constructed constitutive promoter libraries based on various combinations of a previously reported C. glutamicum -10 consensus sequence (gngnTA(c/t)aaTgg) and the Escherichia coli -35 consensus, either with or without an AT-rich region upstream. A promoter library based on consensus sequences frequently found in low-GC Gram-positive microorganisms was also included. The strongest promoters were found in the library with a -35 region and a C. glutamicum -10 consensus, and this library also represents the largest activity span. Using the alternative -10 consensus TATAAT, which can be found in many other prokaryotes, resulted in a weaker but still useful promoter library. The upstream AT-rich region did not affect promoter strength in C. glutamicum. In addition to the constitutive promoters, a synthetic inducible promoter library, based on the E. coli lac-promoter, was constructed by randomizing the 17-bp spacer between -35 and -10 consensus sequences and the sequences surrounding these. The inducible promoter library was shown to result in β-galactosidase activities ranging from 284 to 1,665 Miller units when induced by IPTG, and the induction fold ranged from 7–59. We find that the synthetic promoter library (SPL) technology is convenient for modulating gene expression in C. glutamicum and should have many future applications, within basic research as well as for optimizing industrial production organisms.
Transforming Lactococcus lactis into a microbial cell factory

Biological conversion of lignocellulosic biomass to biofuels and -chemicals is a promising technology to reduce dependency on fossil fuels. This is important considering the environmental problems associated with consumption of the fossil fuels together with the fact that the reserves are limited and will be depleted if the increasing demand continues. However, one of the main challenges in the biological conversion is the identification of suitable platform organisms that can convert all the sugars present in the lignocellulosic biomass, including xylose. The aim of this PhD project was to
investigate the potential of Lactococcus lactis as a platform organism for production of biofuels and chemicals with a focus on characterization and optimization of the xylose metabolism. The plant isolate L. lactis KF147 was selected as the potential platform organism due to its natural ability to utilize both the pentose sugars xylose and arabinose. One of the desirable traits of a good platform organism is that it is easy to manipulate genetically. Since genetic manipulation usually involves introducing exogenous DNA, it is important that suitable methods are available. For this reason a standard protocol for preparing competent L. lactis KF147 cells was optimized resulting in a 100-fold increase in the transformation efficiency. Tools for introducing genes are likewise important. To expand the repertoire of genetic engineering tools available for L. lactis a novel tool named Repetitive Marker-Free Site-Specific Integration was developed. This tool facilitates repetitive rounds of site-specific integration of genes into the chromosome of L. lactis without leaving behind undesired vector elements. The site-specific integration is based on elements from the temperate lactococcal phage TP901-1, whereas excision of undesirable elements relies on a modified Cre-loxP system and 5-fluoroorotate mediated counter-selection. The plasmid used for the site-specific integration was termed pKV6 and when it is used for integrating genes, a new attachment site, attBmin, is likewise introduced, which can subsequently be used for the next round of integration. The xylose metabolism in L. lactis KF147 was characterized in a defined medium supplemented with 0.2%, 1%, or 3% (w/v) xylose. The defined medium contains free arginine, and it was found that L. lactis KF147 co-metabolizes the arginine through the arginine deiminase pathway. To simplify further analysis arcA encoding the arginine deiminase was deleted, thus eliminating the arginine catabolism. We found that in L. lactis KF147 xylose is metabolized through two pathways namely the phosphoketolase pathway and the non-oxidative part of the pentose phosphate pathway. The only products formed were lactate, formate, acetate and ethanol, and the composition of the products depended on the xylose concentration. As xylose concentration increased, the proportion of xylose metabolized through the pentose phosphate pathway also increased. The effect from deleting and over-expressing the phosphoketolase pathway was also studied. L. lactis KF147 was also grown in rich M17 medium with xylose which showed that product composition strongly depends on the growth medium as the yield of lactate per xylose increase significantly. The pentose phosphate pathway present in L. lactis KF147 and other genome sequenced L. lactis strains is, however, a modified version of the known pentose phosphate pathway as no transaldolase gene is present in any of the these strains. A codon optimized version of the transaldolase gene ywjh from B. subtilis 168 was introduced in the phosphoketolase deficient strain. The effect of the introduced transaldolase gene was investigated in defined medium with 0.2% and 3% (w/v) xylose; however, no significant effects on either the growth rates or product formation were observed; even though expression of the introduced transaldolase was confirmed. The xylT gene, predicted to encode a D-xylose/H+-linked symporter, was deleted and the effect on growth evaluated in defined medium with 0.2% and 1% (w/v) xylose. Only at the low xylose concentration was a reduced growth rate observed for the ΔxylT strain compared to the parent strain. Based on this it was deduced that more than one transport system for xylose is present in L. lactis KF147. An adaptive evolution experiment was carried out with the goal of isolating mutants with improved growth on xylose. For this purpose the phosphoketolase deficient strain was applied. Of the 44 evolved strains screened for improved growth on xylose, three were selected for more detailed studies where specific growth rate and product formation were determined. These three strains underwent whole genome sequencing as well. The most interesting evolved strain was AD29 that, as the only of the investigated strains, exhibited both a pronounced accelerated growth on xylose (62.5% faster), and a changed fermentation profile with a clear increase in lactate production and corresponding drop in the production of formate, acetate, and ethanol. Three adaptive mutations were identified in AD29. Two is by all accounts involved in regulatory mechanisms either to stress (ybfB) or more globally (ygfF), and the last facilitate improved uptake of xylose (ptnC). Based on the above findings we conclude that L. lactis KF147 possesses many of the features a platform organism need, however whether the industry will find it attractive remains to be seen.

**General information**

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Characterization of Lactococcus lactis mutants with improved performance at high temperatures and potential dairy applications

*Lactococcus lactis* (L. lactis) is a Gram-positive mesophile, which has considerable importance in the dairy industry for production of cheese and butter milk, and which carries the “GRAS” (generally recognized as safe) designation. Temperature has a great impact on dairy fermentation processes through its effect on the starter culture. The optimum and maximum temperature for most *L. lactis* strains are approximately 30°C and 38°C, respectively. Increasing the fermentation temperature could have several beneficial effects, e.g. reduce bacteriophage attacks and increase acidification rate, the latter because the increased energy consumption at high temperatures potentially could stimulate glycolysis. However in many cases the fitness is affected and mostly negative effects on productivity are observed.

In this study, the non-GMO approach, experimental adaptation, was employed for isolating thermo-tolerant *L. lactis*. The adaptation was carried out using a serial-transfer regime at steadily increasing temperatures, and the strain used was *L. lactis* subsp. cremoris MG1363, which is a well-characterized dairy isolate. After exposure to increasing temperatures over 900 generations, one mutant (TM29) capable of growing at 40°C was successfully isolated. By determining the temperature dependent growth rate profile, a shift of the optimum temperature from 30°C to 36°C was observed for TM29. Meanwhile, metabolic flux analysis revealed that TM29 was able to hold higher glucose consumption and lactate production rates when compared to MG1363 at high temperatures.

Whole genome re-sequencing identified 13 SNPs, one DIP and one large deletion in TM29, and additional sequencing of the isolated intermediates indicated dynamic accumulation of mutations with rising fitness in a temporal order. DNA microarray analysis revealed apparent differences in the transcriptional response to heat between the mutant and parent. It was found that SNPs preceding gene *groESL* and *ribU* resulted in over-expression of chaperone proteins GroES-GroEL, and membrane associated riboflavin transporter protein RibU in TM29, respectively. Moreover, a large deletion in TM29 caused the inactivation of 10 genes (*llmg_1349*-*llmg_1358*).

Through allelic replacement and gene knockout followed by fitness assessment, four main positive mutations were eventually discovered. The SNP preceding *groESL* and deletion of *llmg_1349*-*llmg_1358* contributed to 30% and 10% increase in the growth rate of MG1363 at 38°C, respectively. The over-expression that was caused by the SNP preceding *ribU* relieved FAD starvation, which results in insufficient pyruvate dehydrogenase and NADH oxidase at high temperatures. Through replacing the mutated *rpoC* allele, that encodes the β’ subunit of the RNA polymerase, MG1363 exhibited extended maximum growth temperature with concomitant phenotypic changes, e.g. with respect to morphology and cellular fatty acid composition.

At last, acidification capability in milk was compared at different temperatures, for the endpoint mutant TM29 and the wild-type MG1363. It was found that TM29 was able acidify milk at 40°C, whereas MG1363 could not. Tradeoffs, as a consequence of using a synthetic medium for the evolution, were also observed. TM29 acidified milk slower than MG1363 at 36°C, which was not in accordance with growth in synthetic medium.

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**Complete Genome Sequence of Pediococcus pentosaceus Strain SL4**

Pediococcus pentosaceus SL4 was isolated from a Korean fermented vegetable product, kimchi. We report here the whole-genome sequence (WGS) of *P. pentosaceus* SL4. The genome consists of a 1.79-Mb circular chromosome (G+C content of 37.3%) and seven distinct plasmids ranging in size from 4 kb to 50 kb.

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Engineering strategies aimed at control of acidification rate of lactic acid bacteria

The ability of lactic acid bacteria to produce lactic acid from various sugars plays an important role in food fermentations. Lactic acid is derived from pyruvate, the end product of glycolysis and thus a fast lactic acid production rate requires a high glycolytic flux. In addition to lactic acid, alternative end products - ethanol, acetic acid and formic acid - are formed by many species. The central role of glycolysis in lactic acid bacteria has provoked numerous studies aiming at identifying potential bottleneck(s) since knowledge about flux control could be important not only for optimizing food fermentation processes, but also for novel applications of lactic acid bacteria, such as cell factories for the production of green fuels and chemicals. With respect to the control and regulation of the fermentation mode, some progress has been made, but the question of which component(s) control the main glycolytic flux remains unanswered. © 2012 Elsevier Ltd.

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Investigation of glycerol assimilation and cofactor metabolism in Lactococcus lactis

The production of biodiesel has been steadily increasing during the last decade, and with it crude glycerol as a byproduct. Despite being rich in glycerol, the increased supply has saturated the demand for glycerol, making purification a non-viable option. The background for this project was to investigate the suitability of lactic acid bacteria as production organisms for the production of biofuels and biochemicals. Specifically, the goal was to adapt the model organism Lactococcus lactis to convert crude glycerol, to value-added fuels or chemicals. Work was divided between four main areas: life cycle assessment of the GLYFINERY project, screening of L. lactis spp. for glycerol utilization, engineering of glycerol metabolism in L. lactis and finally an investigation into perturbation of energy metabolism in L. lactis.

The work from the life cycle assessment resulted in two reports, detailing the technological requirements for the GLYFINERY processes. These have been included in the appendix (section A).

The screening did not reveal any L. lactis strains capable of assimilating glycerol nor did it reveal any conditions favorable to glycerol dissimilation in L. lactis. The conditions evaluated were: anaerobic, aerobic and respiration permissive growth in combination with either glycerol as a sole substrate or with co-metabolism of glycerol with common sugar substrates. Although no growth on glycerol was seen, both positive and detrimental effects were observed from cultures with glycerol supplementation.

Supplementation of nucleosides to the growth medium or increased substrate concentration were found to counteract the inhibitory effects and improve the growth rate, though not completely to the level of the reference strain. The fact that this effect was predominantly observed while utilizing xylose implicates the involvement of the pentose phosphate pathway. A
Optimization of lysine metabolism in Corynebacterium glutamicum

Commercial pig and poultry production use the essential amino acid lysine as a feed additive with the purpose of optimizing the feed utilization. Lysine is produced by a fermentation process involving either Corynebacterium glutamicum or Escherichia coli. The global annual production is around 1,000,000 tons. The aim of this project is to optimize the yield of lysine in C. glutamicum using metabolic engineering strategies.

According to a genome scale model of C. glutamicum, theoretically there is much room for increasing the lysine yield (Kjeldsen and Nielsen 2009). Lysine synthesis requires NADPH, and increased NADPH availability is therefore a potential way to enhance lysine production. The generation of NADPH is mainly located in the pentose phosphate pathway (PPP).
Using the genome scale model the phosphoglucoisomerase enzyme (PGI) has been identified as a possible bottleneck in the metabolism, which the project intends to eliminate. PGI catalyzes the conversion of alpha-D-glucose-6-phosphate to fructose-6-phosphate just downstream of the branch in the glycolysis, but it also catalyzes the reverse reaction. It is unknown whether up- or down-regulation of the pgi is required to increase the flux through the PPP, increasing the NADPH synthesis and enabling increased lysine production.

Synthetic promoter libraries (SPL) enable fine tuning of the expression of genes. To test the feasibility of SPL in C. glutamicum four constitutive SPLs and one inducible SPL were constructed. The libraries were placed in front of a β-galactosidase gene and the activity of the enzyme was measured to quantify the different promoter strengths in the libraries. The results demonstrate that the SPL technology is an applicable technique for metabolic engineering in C. glutamicum.

SPL was used to both up- and down-regulate the expression of pgi, and it was measured how the regulations affected the lysine production. In this thesis it is shown that under the applied growth conditions it is possible to increase the lysine yield by a factor of 1.6 from 0.0086 mole lysine ∙ mole glucose⁻¹ to 0.0138 mole lysine ∙ mole glucose⁻¹.

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**Oxidative Stress at High Temperatures in Lactococcus lactis Due to an Insufficient Supply of Riboflavin.**
Lactococcus lactis MG1363 was found to be unable to grow at temperatures above 37°C in a defined medium without riboflavin, and the cause was identified to be dissolved oxygen introduced during preparation of the medium. At 30°C, growth was unaffected by dissolved oxygen and oxygen was consumed quickly. Raising the temperature to 37°C resulted in severe growth inhibition and only slow removal of dissolved oxygen. Under these conditions, an abnormally low intracellular ratio of [ATP] to [ADP] (1.4) was found (normally around 5), which indicates that the cells are energy limited. By adding riboflavin to the medium, it was possible to improve growth and oxygen consumption at 37°C, and this also normalized the [ATP]-to-[ADP] ratio. A codon-optimized redox-sensitive green fluorescent protein (GFP) was introduced into L. lactis and revealed a more oxidized cytoplasm at 37°C than at 30°C. These results indicate that L. lactis suffers from heat-induced oxidative stress at increased temperatures. A decrease in intracellular flavin adenine dinucleotide (FAD), which is derived from riboflavin, was observed with increasing growth temperature, but the presence of riboflavin made the decrease smaller. The drop was accompanied by a decrease in NADH oxidase and pyruvate dehydrogenase activities, both of which depend on FAD as a cofactor. By overexpressing the riboflavin transporter, it was possible to improve FAD biosynthesis, which resulted in increased NADH oxidase and pyruvate dehydrogenase activities and improved fitness at high temperatures in the presence of oxygen.

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Repetitive, Marker-Free, Site-Specific Integration as a Novel Tool for Multiple Chromosomal Integration of DNA

We present a tool for repetitive, marker-free, site-specific integration in Lactococcus lactis, in which a nonreplicating plasmid vector (pKV6) carrying a phage attachment site (attP) can be integrated into a bacterial attachment site (attB).
The novelty of the tool described here is the inclusion of a minimal bacterial attachment site (attBmin), two mutated loxP sequences (lox66 and lox71) allowing for removal of undesirable vector elements (antibiotic resistance marker), and a counterselection marker (oroP) for selection of loxP recombination on the pKV6 vector. When transformed into L. lactis expressing the phage TP901-1 integrase, pKV6 integrates with high frequency into the chromosome, where it is flanked by attL and attR hybrid attachment sites. After expression of Cre recombinase from a plasmid that is not able to replicate in L. lactis, loxP recombinants can be selected for by using 5-fluoroorotic acid. The introduced attBmin site can subsequently be used for a second round of integration. To examine if attP recombination was specific to the attB site, integration was performed in strains containing the attB, attL, and attR sites or the attL and attR sites only. Only attP-attB recombination was observed when all three sites were present. In the absence of the attB site, a low frequency of attP-attL recombination was observed. To demonstrate the functionality of the system, the xylose utilization genes (xylABR and xylT) from L. lactis strain KF147 were integrated into the chromosome of L. lactis strain MG1363 in two steps.

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Rewiring Lactococcus lactis for Ethanol Production

Lactic acid bacteria (LAB) are known for their high tolerance toward organic acids and alcohols (R. S. Gold, M. M. Meagher, R. Hutkins, and T. Conway, J. Ind. Microbiol. 10:45–54, 1992) and could potentially serve as platform organisms for production of these compounds. In this study, we attempted to redirect the metabolism of LAB model organism Lactococcus lactis toward ethanol production. Codon-optimized Zymomonas mobilis pyruvate decarboxylase (PDC) was introduced and expressed from synthetic promoters in different strain backgrounds. In the wild-type L. lactis strain MG1363 growing on glucose, only small amounts of ethanol were obtained after introducing PDC, probably due to a low native alcohol dehydrogenase activity. When the same strains were grown on maltose, ethanol was the major product and lesser amounts of lactate, formate, and acetate were formed. Inactivating the lactate dehydrogenase genes ldhX, ldhB, and ldh and introducing codon-optimized Z. mobilis alcohol dehydrogenase (ADHB) in addition to PDC resulted in high-yield ethanol formation when strains were grown on glucose, with only minor amounts of by-products formed. Finally, a strain with ethanol as the sole observed fermentation product was obtained by further inactivating the phosphotransacetylase (PTA) and the native alcohol dehydrogenase (ADHE).

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Stochastic Differential Equations in Artificial Pancreas Modelling

Type 1 diabetes accounts for approximately 5% of the total diabetes population. It is caused by the destruction of insulin producing β-cells in the pancreas. Various treatment strategies are available today, some of which include advanced technological devices such as an insulin pump and a continuous glucose monitor (CGM). Despite these technological advances in the treatment of type 1 diabetes, the disease still poses an enormous and constant challenge for the patients. To obtain tight glucose control the patients are required to assess how much they will eat prior to the meal. They have to assess the timing, intensity and duration of physical exercise in advance, to adjust the insulin dose accordingly. Additionally, several uncontrollable and unpredictable factors such as stress, hormonal cycles and sickness changing the metabolic state make this task even more difficult.

The development of the insulin pump and the CGM has paved the way for a fully automatic treatment regime, the artificial pancreas. The idea is to connect the CGM with the insulin pump via a control algorithm running on e.g. the patients smart phone. The CGM observations are sent to the smart phone and based on this information, the control algorithm computes the optimal dose adjustment and sends instructions to the insulin pump.

To develop control algorithms, mathematical models of the physiological dynamics are needed. They attempt to describe the significant dynamics of the system and hence they approximate the system behavior. However, uncertainty in the model occurs due to the nature of physiological systems and due to the presence of unknown disturbances. An attractive approach to deal with this uncertainty is to use stochastic differential equations (SDEs). In a model based on SDEs, the noise is separated into two terms: 1) a diffusion term occurring from model misspecifications, effects of unknown disturbances, or just true stochastic behavior of the system and 2) a measurement noise term representing the serially uncorrelated error occurring due to the imperfect analysing equipment. The diffusion term affects the evolution of the system directly.

The purpose of this PhD-project was to investigate the potential of SDEs in the artificial pancreas development. Especially, the emerging continuous monitoring of glucose levels makes SDEs highly applicable to this field. The current thesis aims at demonstrating and discussing the benefits and challenges by using SDEs compared to traditional methods on the basis of the results of the project.

First of all, we designed a clinical study to obtain high quality data from type 1 diabetes patients to identify the models from. The study included the main factors influencing the glucose level: insulin boluses, meals, and exercise. A modelling study showed that using SDEs in model development can be advantageous in several ways. We were able to pinpoint model deficiencies in a well-known model and to track parameter variation probably caused by a differences in meal type. This information could be added to the model to improve the fit. The study was limited by the lack of a software capable of handling SDE models of population effects instead of single-subject effects. A prototype of this type of software was developed parallel to the end of the project. Thus, we could finally identify a population model of the effect of exercise on the insulin absorption rate. The small amount of observations made it impossible to use SDEs to track parameter variation. Instead, we formulated a model structure with showed to be significantly better than the base model with a constant rate.

Two studies specifically related to the CGM observations were performed during the project. In the first study, we showed that SDEs could be used to tune a control algorithm for overnight glucose control on the basis of CGM observations. The tuned algorithm improved the controller performance in a subsequent clinical study. Further attempts to deal with the problems related to the CGM included a Bayesian estimation scheme. By incorporating prior knowledge about the uncertainty in the CGM observations into the estimation method, we succeeded in predicting the plasma glucose level with acceptable confidence from the CGM observations only.

Overall, the project confirms that SDEs have a large potential within this field. However, future modeling requires a robust software capable of handling the nonlinear population SDE models. When this is available, larger modeling studies can be initiated and the impact of SDEs would be expected to increase.

General information
State: Published
Organisations: Department of Applied Mathematics and Computer Science, Dynamical Systems, Department of Systems Biology, Systems Biotechnology
Authors: Duun-Henriksen, A. K. (Intern), Madsen, H. (Intern), Jensen, P. R. (Intern)
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Bioconversion of crude glycerol feedstocks into ethanol by Pachysolen tannophilus

Glycerol, the by-product of biodiesel production, is considered as a waste by biodiesel producers. This study demonstrated the potential of utilising the glycerol surplus through conversion to ethanol by the yeast Pachysolen tannophilus (CBS4044). This study demonstrates a robust bioprocess which was not sensitive to the batch variability in crude glycerol dependent on raw materials used for biodiesel production. The oxygen transfer rate (OTR) was a key factor for ethanol production, with lower OTR having a positive effect on ethanol production. The highest ethanol production was 17.5 g/L on 5% (v/v) crude glycerol, corresponding to 56% of the theoretical yield. A staged batch process achieved 28.1 g/L ethanol, the maximum achieved so far for conversion of glycerol to ethanol in a microbial bioprocess. The fermentation physiology has been investigated as a means to designing a competitive bioethanol production process, potentially improving economics and reducing waste from industrial biodiesel production.
Conversion of the biodiesel by-product glycerol by the non-conventional yeast Pachysolen tannophilus

The focus on developing new renewable energy in the transportation sector by the EU has boosted the production of biodiesel from rapeseed and other vegetable oils in Europe. This has led to an immense increase in the production of glycerol, which is an inevitable byproduct from the biodiesel production process. Since the volume of the glycerol by-product has exceeded the current market need, biodiesel producers are looking for new methods for sustainable glycerol management and improving the competitiveness of the biodiesel industries. The EU Commission funded GLYFINERY project is one initiative targeted to development of a novel technology based on biological conversion of the glycerol feedstocks into known and new advanced liquid biofuels, bioenergy and valuable green chemicals in an integrated biorefinery concept.

As part of the GLYFINERY project, the objective of this PhD project was to develop a process for bioconversion of waste glycerol into biofuel ethanol, characterize and optimize the process. The present thesis comprises of eight chapters. The project background, scope and aims are introduced in Chapter 1. Besides, the related background knowledge for better understanding the studies in the following chapters is also introduced in this chapter. Chapter 2-7 are comprised of the experimental results obtained during the whole PhD study.

The well characterized yeast Saccharomyces cerevisiae has been used for fermentation of alcoholic beverages throughout thousands of years of human history, and is applied in many areas of modern biotechnology. In this project the interest was in investigating nonconventional yeasts which had the capability of conversion of glycerol primarily to liquid biofuels. Chapter 2 is about the initial results for screening of the potential candidates for glycerol fermentation. Two candidates Pachia pastoris and Pachysolen tannophilus were shown to be capable of producing ethanol with glycerol as the sole carbon source. After growth comparison on glycerol and tests for extracellular metabolites in agitated flasks, P. tannophilus was selected as the object of further studies for conversion of glycerol to ethanol.

In chapter 3, physiology studies in lab scale fermentation of the ethanol production process with P. tannophilus were investigated on glycerol. The effect of aeration, pH and nitrogen source was studied for improving the ethanol production and yield and designing a competitive ethanol production process. The ethanol tolerance of P. tannophilus on glycerol was studied for further characterizing the ethanol production process. A growth comparison on crude glycerol and pure glycerol was performed to test if the impurities in the crude glycerol inhibit the growth of P. tannophilus and affect product formation. Based on optimized parameters, 28.1 g/L ethanol was produced by a staged batch process, which was the maximum achieved so far for conversion of glycerol to ethanol by a microbial bioprocess.

The physiology study of ethanol tolerance of P. tannophilus showed that the ethanol tolerance of this strain was relatively low. The low ethanol tolerance of P. tannophilus might be the factor which inhibits further improvement of ethanol production process. Chapter 4 describes adaptive evolution studies performed to enhance the ethanol tolerance of P. tannophilus on glycerol. The adapted strains isolated during the evolution process were characterised according to the ethanol tolerance, growth rate on glycerol, ethanol production and growth profile on glycerol.

For better understanding the genetic background, the genomic DNA of P. tannophilus CBS4044 was isolated and sequenced. The draft genome sequencing results of P. tannophilus are summarized in chapter 5. Raw data of short reads
from genome sequencing results were assembled together. The protein-coding genes were identified and the putative amino acid sequences were analysed for the gene function annotation. Pulsed field gel electrophoresis was performed to predict the chromosome numbers and approximate chromosome sizes in *P. tannophilus*. For the purpose of further improving the yields and production levels of ethanol produced, it would be beneficial if *P. tannophilus* could be genetically engineered and the ethanol synthesis pathway in *P. tannophilus* could be investigated. The whole-genome sequencings of *P. tannophilus* also makes it possible to perform genetic engineering of this strain. Chapter 6 describes the attempts to set up the transformation system in *P. tannophilus* in order to know more about the genetic background and further improve the ethanol production process. The commonly applied methods using antibiotic resistance and auxotrophic markers URA3 were used for transformation selection. Since the genome of *P. tannophilus* CBS4044 was sequenced and the mechanism behind glycerol metabolism is poorly understood in this strain. In chapter 7 focusses on studying the genes involved in glycerol metabolism in *P. tannophilus*, which were predicted by blasting with the sequences of genes known to have these functions in *S. cerevisiae*. Quantitative realtime PCR was performed to unveil the expression pattern of the genes during growth on glycerol. The glycerol metabolism and pathways in *P. tannophilus* are discussed. The genes involved in glycerol transport in *P. tannophilus* have been cloned and expressed in *S. cerevisiae* (CEN.PK 113-5D) strains to validate the function of the predicted glycerol transporter genes. Finally, the most relevant results from all the studies during the PhD are summarised and future perspectives for continuing these studies are presented in Chapter 8.

**General information**

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Organisations: Department of Systems Biology, Fungal Physiology and Biotechnology, Systems Biotechnology
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**Draft Genome Sequence of the Yeast Pachysolen tannophilus CBS 4044/NRRL Y-2460**

A draft genome sequence of the yeast *Pachysolen tannophilus* CBS 4044/NRRL Y-2460 is presented. The organism has the potential to be developed as a cell factory for biorefineries due to its ability to utilize waste feedstocks. The sequenced genome size was 12,238,196 bp, consisting of 34 scaffolds. A total of 4,463 genes from 5,346 predicted open reading frames were annotated with function.

**General information**

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Organisations: Department of Systems Biology, Center for Microbial Biotechnology, Center for Systems Microbiology, National Food Institute, Division of Epidemiology and Microbial Genomics, Division of Microbiology and Risk Assessment
Authors: Liu, X. (Intern), Kaas, R. S. (Intern), Jensen, P. R. (Intern), Workman, M. (Intern)
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BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.878 SNIP 0.858 CiteScore 3.12
In this paper, we develop and test a Model Predictive Controller (MPC) for overnight stabilization of blood glucose in people with type 1 diabetes. The controller uses glucose measurements from a continuous glucose monitor (CGM) and its decisions are implemented by a continuous subcutaneous insulin infusion (CSII) pump. Based on a priori patient information, we propose a systematic method for computation of the model parameters in the MPC. Safety layers improve the controller robustness and reduce the risk of hypoglycemia. The controller is evaluated in silico on a cohort of 100 randomly generated patients with a representative intersubject variability. This cohort is simulated overnight with realistic variations in the insulin sensitivities and needs. Finally, we provide results for the first tests of this controller in a real clinic.
Polyamines Are Required for Virulence in Salmonella enterica Serovar Typhimurium

Sensing and responding to environmental cues is a fundamental characteristic of bacterial physiology and virulence. Here we identify polyamines as novel environmental signals essential for virulence of Salmonella enterica serovar Typhimurium, a major intracellular pathogen and a model organism for studying typhoid fever. Central to its virulence are two major virulence loci Salmonella Pathogenicity Island 1 and 2 (SPI1 and SPI2). SPI1 promotes invasion of epithelial cells, whereas SPI2 enables S. Typhimurium to survive and proliferate within specialized compartments inside host cells. In this study, we show that an S. Typhimurium polyamine mutant is defective for invasion, intracellular survival, killing of the nematode Caenorhabditis elegans and systemic infection of the mouse model of typhoid fever. Virulence of the mutant could be restored by genetic complementation, and invasion and intracellular survival could, as well, be complemented by the addition of exogenous putrescine and spermidine to the bacterial cultures prior to infection. Interestingly, intracellular survival of the polyamine mutant was significantly enhanced above the wild type level by the addition of exogenous putrescine and spermidine to the bacterial cultures prior to infection, indicating that these polyamines function as an environmental signal that primes S. Typhimurium for intracellular survival. Accordingly, experiments addressed at elucidating the roles of these polyamines in infection revealed that expression of genes from both of the major virulence loci SPI1 and SPI2 responded to exogenous polyamines and was reduced in the polyamine mutant. Together our data demonstrate that putrescine and spermidine play a critical role in controlling virulence in S. Typhimurium most likely through stimulation of expression of essential virulence loci. Moreover, our data implicate these polyamines as key signals in S. Typhimurium virulence.

General information
State: Published
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Tunable promoters in synthetic and systems biology.

Synthetic and systems biologists need standardized, modular and orthogonal tools yielding predictable functions in vivo. In systems biology such tools are needed to quantitatively analyze the behavior of biological systems while the efficient engineering of artificial gene networks is central in synthetic biology. A number of tools exist to manipulate the steps in between gene sequence and functional protein in living cells, but out of these the most straightforward approach is to alter the gene expression level by manipulating the promoter sequence. Some of the promoter tuning tools available for accomplishing such altered gene expression levels are discussed here along with examples of their use, and ideas for new tools are described. The road ahead looks very promising for synthetic and systems biologists as tools to achieve just about anything in terms of tuning and timing multiple gene expression levels using libraries of synthetic promoters now exist.

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Organisations: Department of Systems Biology, Center for Systems Microbiology
Authors: Dehli, T. (Intern), Solem, C. (Intern), Jensen, P. R. (Intern)
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Tuning of Controller for Type I Diabetes Treatment with Stochastic Differential Equations

People with type 1 diabetes need several insulin injections every day to keep their blood glucose level in the normal range and thereby avoiding the acute and long term complications of diabetes. One of the recent treatments consists of a pump injecting insulin into the subcutaneous layer combined with a continuous glucose monitor (CGM) frequently observing the glucose level. Automatic control of the insulin pump based on CGM observations would ease the burden of constant diabetes treatment and management. We have developed a controller designed to keep the blood glucose level in the normal range by adjusting the size of insulin infusions from the pump based on model predictive control (MPC). A clinical pilot study to test the performance of the MPC controller overnight was performed. The conclusion was that the controller relied too much on the local trend of the blood glucose level which is a problem due to the noise corrupted observations from the CGM. In this paper we present a method to estimate the optimal Kalman gain in the controller based on stochastic differential equation modeling. With this model type we could estimate the process noise and observation noise separately based on data from the first clinical pilot study. In doing so we obtained a more robust control algorithm which is less sensitive to fluctuations in the CGM observations and rely more on the global physiological trend of the blood glucose level. Finally, we present the promising results from the second pilot study testing the improved controller.
Bacillus subtilis Two-Component System Sensory Kinase DegS Is Regulated by Serine Phosphorylation in Its Input Domain

Bacillus subtilis two-component system DegS/U is well known for the complexity of its regulation. The cytosolic sensory kinase DegS does not receive a single predominant input signal like most two-component kinases, instead it integrates a wide array of metabolic inputs that modulate its activity. The phosphorylation state of the response regulator DegU also does not confer a straightforward "on/off" response; it is fine-tuned and at different levels triggers different sub-regulons. Here we describe serine phosphorylation of the DegS sensing domain, which stimulates its kinase activity. We demonstrate that DegS phosphorylation can be carried out by at least two B. subtilis Hanks-type kinases in vitro, and this stimulates the phosphate transfer towards DegU. The consequences of this process were studied in vivo, using phosphomimetic (Ser76Asp) and non-phosphorylatable (Ser76Ala) mutants of DegS. In a number of physiological assays focused on different processes regulated by DegU, DegS S76D phosphomimetic mutant behaved like a strain with intermediate levels of DegU phosphorylation, whereas DegS S76A behaved like a strain with lower levels of DegU phosphorylation. These findings suggest a link between DegS phosphorylation at serine 76 and the level of DegU phosphorylation, establishing this post-translational modification as an additional trigger for this two-component system.

General information
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Organisations: Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Department of Systems Biology, Center for Microbial Biotechnology, Center for Systems Microbiology, AgroParisTech
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BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.11 SJR 1.201 SNIP 1.092
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
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ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.369 SNIP 1.23 CiteScore 4.58
ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.631 SNIP 1.161
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
The construction of a library of synthetic promoters revealed some specific features of strong Streptomyces promoters

Streptomyces are bacteria of industrial interest whose genome contains more than 73% of bases GC. In order to define, in these GC-rich bacteria, specific sequence features of strong promoters, a library of synthetic promoters of various sequence composition was constructed in Streptomyces. To do so, the sequences located upstream, between and downstream of the −35 and −10 consensus promoter sequences were completely randomized and some variability was introduced in the −35 (position 6) and −10 (positions 3, 4 and 5) hexamers recognized by the major vegetative sigma factor HrdB. The synthetic promoters were cloned into the promoter-probe plasmid pIJ487 just upstream of the promoter-less aphII gene that confers resistance to neomycin. This synthetic promoter library was transformed into Streptomyces lividans, and the resulting transformants were screened for their ability to grow in the presence of different concentrations of neomycin (20, 50, and 100 μg ml⁻¹). Promoter strengths varied up to 12-fold, in small increments of activity increase, as determined by reverse transcriptase-PCR. This collection of promoters of various strengths can be useful for the fine-tuning of gene expression in genetic engineering projects. Thirty-eight promoters were sequenced, and the sequences of the 14 weakest and 14 strongest promoters were compared using the WebLogo software with small sample correction. This comparison revealed that the −10 box, the −10 extended motif as well as the spacer of the strong Streptomyces promoters are more G rich than those of the weak promoters.
Towards a quantitative prediction of the fluxome from the proteome

The promise of proteomics and fluxomics is limited by our current inability to integrate these two levels of cellular organization. Here we present the derivation, experimental parameterization, and appraisal of flux functions that enable the quantitative prediction of changes in metabolic fluxes from changes in enzyme levels. We based our derivation on the hypothesis that, in the determination of steady-state flux changes, the direct proportionality between enzyme concentrations and reaction rates is principal, whereas the complexity of enzyme–metabolite interactions is secondary and can be described using an approximate kinetic format. The quality of the agreement between predicted and experimental fluxes in Lactococcus lactis, supports our hypothesis and demonstrates the need and usefulness of approximative descriptions in the study of complex biological systems. Importantly, these flux functions are scalable to genome-wide networks, and thus drastically expand the capabilities of flux prediction for metabolic engineering efforts beyond those conferred by the currently used constraints-based models.
Protein-Tyrosine Phosphorylation in Bacillus Subtilis Signal Transduction

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Organisations: Center for BioProcess Engineering, Department of Chemical and Biochemical Engineering, Department of Systems Biology, Center for Systems Microbiology
Authors: Jers, C. (Intern), Mijakovic, I. (Intern), Jensen, P. R. (Intern)
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Bacillus subtilis BY-kinase PtkA controls enzyme activity and localization of its protein substrates

Bacillus subtilis BY-kinase PtkA was previously shown to phosphorylate, and thereby regulate the activity of two classes of protein substrates: UDP-glucose dehydrogenases and single-stranded DNA-binding proteins. Our recent phosphoproteome study identified nine new tyrosine-phosphorylated proteins in B. subtilis. We found that the majority of these proteins could be phosphorylated by PtkA in vitro. Among these new substrates, single-stranded DNA exonuclease YorK, and aspartate semialdehyde dehydrogenase Asd were activated by PtkA-dependent phosphorylation. Because enzyme activity was not affected in other cases, we used fluorescent protein tags to study the impact of PtkA on localization of these proteins in vivo. For several substrates colocalization with PtkA was observed, and more importantly, the localization pattern of the proteins enolase, YjoA, YnfE, YvyG, Ugd and SsbA was dramatically altered in Delta ptkA background. Our results confirm that PtkA can control enzyme activity of its substrates in some cases, but also reveal a new mode of action for PtkA, namely ensuring correct cellular localization of its targets.

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Metabolic and Transcriptional Response to Cofactor Perturbations in Escherichia coli

Metabolic cofactors such as NADH and ATP play important roles in a large number of cellular reactions, and it is of great interest to dissect the role of these cofactors in different aspects of metabolism. Toward this goal, we overexpressed NADH oxidase and the soluble F1-ATPase in Escherichia coli to lower the level of NADH and ATP, respectively. We used a global interaction network, comprising of protein interactions, transcriptional regulation, and metabolic networks, to integrate data from transcription profiles, metabolic fluxes, and the metabolite levels. We identified high-scoring networks for the two strains. The results revealed a smaller, but denser network for perturbations of ATP level, compared with that
of NADH level. The action of many global transcription factors such as ArcA, Fnr, CRP, and IHF commonly involved both NADH and ATP, whereas others responded to either ATP or NADH. Overexpressing NADH oxidase invokes response in widespread aspects of metabolism involving the redox cofactors (NADH and NADPH), whereas ATPase has a more focused response to restore ATP level by enhancing proton translocation mechanisms and repressing biosynthesis. Interestingly, NADPH played a key role in restoring redox homeostasis through the concerted activity of isocitrate dehydrogenase and UdhA transhydrogenase. We present a reconciled network of regulation that illustrates the overlapping and distinct aspects of metabolism controlled by NADH and ATP. Our study contributes to the general understanding of redox and energy metabolism and should help in developing metabolic engineering strategies in E. coli.
Phosphoglycerate Mutase Is a Highly Efficient Enzyme without Flux Control in Lactococcus lactis

The glycolytic enzyme phosphoglycerate mutase (PGM), which catalyzes the conversion of 3-phosphoglycerate to 2-phosphoglycerate, was examined in Lactococcus lactis with respect to its function, kinetics and glycolytic flux control. A library of strains with PGM activities ranging between 15-465% of the wild-type level was constructed by replacing the native promoter of pgm with synthetic promoters of varying strengths. The specific growth rate and glucose flux were found to be maximal at the wild-type level at which PGM had no flux control. Low flux control of PGM was found on mixed acid fluxes at highly reduced PGM activities. At the wild-type level PGM operated very far from V-max. Consequently, in a strain with only 15% PGM activity, the catalytic rate of PGM was almost six times higher than in the wildtype. K-m of PGM for 3-phosphoglycerate was 1.0 m M and k(cat) was 3,200 s(-1). The L. lactis PGM was dependent on 2,3-bisphosphoglyceric acid for activity, which showed that the enzyme is of the dPGM type in accordance with its predicted homology to dPGM enzymes from other organisms. In conclusion, PGM from L. lactis is a highly efficient catalyst, which partially explains why this enzyme has limited control in wild-type L. lactis.

General information
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Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Solem, C. (Intern), Petranovic, D. (Ekstern), Købmann, B. (Intern), Mijakovic, I. (Intern), Jensen, P. R. (Intern)
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Scopus rating (2016): SJR 0.749 SNIP 0.509 CiteScore 1.62
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.963 SNIP 0.574 CiteScore 1.84
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.95 SNIP 0.739 CiteScore 1.84
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.702 SNIP 0.542 CiteScore 1.62
The MG1363 and IL1403 Laboratory Strains of Lactococcus lactis and Several Dairy Strains Are Diploid

Bacteria are normally haploid, maintaining one copy of their genome in one circular chromosome. We have examined the cell cycle of laboratory strains of Lactococcus lactis, and, to our surprise, we found that some of these strains were born with two complete nonreplicating chromosomes. We determined the cellular content of DNA by flow cytometry and by radioactive labeling of the DNA. These strains thus fulfill the criterion of being diploid. Several dairy strains were also found to be diploid while a nondairy strain and several other dairy strains were haploid in slow-growing culture. The diploid and haploid strains differed in their sensitivity toward UV light, in their cell size, and in their D period, the period between termination of DNA replication and cell division.

General information
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Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Michelsen, O. (Intern), Hansen, F. G. (Intern), Albrechtsen, B. (Ekstern), Jensen, P. R. (Intern)
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Control analysis of the purine biosynthesis in Lactococcus lactis
Control analysis of the purine biosynthesis in Lactococcus lactis

General information
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Organisations: Department of Systems Biology, Center for Systems Microbiology
Authors: Jessing, S. G. (Intern), Haaber, J. B. B. (Intern), Jendresen, C. B. (Intern), Jensen, P. R. (Intern), Kilstrup, M. (Intern)
Publication date: 2009
Event: Abstract from 3rd Congress of European Microbiologists, Goteborg, Sweden.
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Publication: Research - peer-review › Poster – Annual report year: 2009

Engineering of Bacillus subtilis 168 for increased nisin resistance

Nisin is a natural bacteriocin produced commercially by Lactococcus lactis and widely used in the food industry as a preservative because of its broad host spectrum. Despite the low productivity and troublesome fermentation of L. lactis, no alternative cost-effective host has yet been found. Bacillus subtilis had been suggested as a potential host for the biosynthesis of nisin but was discarded due to its sensitivity to the lethal action of nisin. In this study, we have reevaluated the potential of B. subtilis as a host organism for the heterologous production of nisin. We applied transcriptome and proteome analyses of B. subtilis and identified eight genes upregulated in the presence of nisin. We demonstrated that the overexpression of some of these genes boosts the natural defenses of B. subtilis, which allows it to sustain higher levels of nisin in the medium. We also attempted to overcome the nisin sensitivity of B. subtilis by introducing the nisin resistance genes nisFEG and nisl from L. lactis under the control of a synthetic promotore library.

General information
State: Published
Organisations: Department of Systems Biology, Division of Toxicology and Risk Assessment, National Food Institute
Authors: Hansen, M. (Intern), Wangari, R. (Intern), Hansen, E. B. (Intern), Mijakovic, I. (Intern), Jensen, P. R. (Intern)
Pages: 6688-6695
Publication date: 2009
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 75
Issue number: 21
ISSN (Print): 0099-2240

Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.043 SNIP 1.647
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.054 SNIP 1.602
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.074 SNIP 1.653
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 2.108 SNIP 1.648
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 2.097 SNIP 1.821
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 2.046 SNIP 1.754
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.989 SNIP 1.736
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.957 SNIP 1.758
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 2.3 SNIP 1.732

Original language: English

DOIs:
10.1128/AEM.00943-09
Co-factor engineering in lactobacilli: Effects of uncoupled ATPase activity on metabolic fluxes in Lactobacillus (L.) plantarum and L. sakei

The hydrolytic F-1-part of the F1F0-ATPase was over-expressed in Lactobacillus (L.) plantarum NC8 and L. sakei Lb790x during fermentation of glucose or ribose, in order to study how changes in the intracellular levels of ATP and ADP affect the metabolic fluxes. The uncoupled ATPase activity resulted in a decrease in intracellular energy level (ATP/ADP ratio), biomass yield and growth rate. Interestingly, the glycolytic and ribolytic flux increased in L. plantarum with uncoupled ATPase activity compared to the reference strain by up to 20% and 50%, respectively. The ATP demand was estimated to have approximately 80% control on both the glycolytic and ribolytic flux in L. plantarum under these conditions. In contrast, the glycolytic and ribolytic flux decreased in L. sakei with uncoupled ATPase activity. (C) 2008 Elsevier Inc. All rights reserved.

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Rud, I. (Ekstern), Solem, C. (Intern), Jensen, P. R. (Intern), Axelsson, L. (Ekstern), Naterstad, K. (Ekstern)
Pages: 207-215
Publication date: 2008
Main Research Area: Technical/natural sciences

Publication information
Journal: Metabolic Engineering
Volume: 10
Issue number: 5
ISSN (Print): 1096-7176
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.33 SJR 3.54 SNIP 1.864
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.611 SNIP 1.822 CiteScore 8.2
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.381 SNIP 2.034 CiteScore 7.23
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 4.004 SNIP 2.185 CiteScore 8.43
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.032 SNIP 1.858 CiteScore 6.72
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 3.124 SNIP 2.144 CiteScore 6.75
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.373 SNIP 1.802
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.575 SNIP 1.421
Web of Science (2009): Indexed yes
Control analysis of the role of triosephosphate isomerase in glucose metabolism in Lactococcus lactis

Triosephosphate isomerase (TPI), which catalyses the conversion of dihydroxyacetone phosphate (DHAP) to glyceraldehyde-3-phosphate (G3P), was studied for its control on glycolysis and mixed acid production in L. lactis subspecies lactis IL1403 and L. lactis subspecies cremoris MG1363. Strains in which the TPI activity was modulated from 3%-225% (IL1403) or 13%-103% (MG1363) of the wild-type level were constructed by changing the expression of the tpiA gene. The enzyme was found to be present in high excess in the wild-type cells and 10% TPI activity still supported more than 70% of the wild-type glycolytic flux in both strains. Homolactic product formation was preserved throughout the range of TPI activities studied, although a slight increase in the amount of acetate and formate production was observed in the strains with strongly reduced TPI activity for both IL1403 and MG1363. The upstream metabolites glucose-6-phosphate, fructose-1,6-bisphosphate and DHAP in the IL1403 derivatives were essentially unchanged for TPI activities from 26% to 225%. At a TPI activity of 3%, the level of DHAP increased four times. The finding that an increased level of DHAP coincides with an increase in formate production is surprising and indicates that pyruvate formate lyase is not inhibited by DHAP under these conditions.

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Solem, C. (Intern), Købmann, B. J. (Intern), Jensen, P. R. (Intern)
Pages: 64-72
Publication date: 2008
Main Research Area: Technical/natural sciences
Escherichia coli strains with promoter libraries constructed by Red/ET recombination pave the way for transcriptional fine tuning

System-oriented applications of genetic engineering, such as metabolic engineering, often require the serial optimization of enzymatic reaction steps, which can be achieved by transcriptional, fine-tuning. However, approaches to changing gene expression are usually limited to deletion and/or strong overexpression and rarely match the desired optimal transcript levels. A solution to this all-or-nothing approach has been the use of a synthetic promoter library (SPL) that is based on randomized sequences flanking the consensus -10 and -35 promoter regions and allows for fine-tuning of bacterial gene expression. Red/ET recombination perfectly complements SPL technology, since it enables easy modification of the Escherichia Coli genome and can be accomplished with linear DNA (i.e., the SPL). To demonstrate the synergistic use of Red/ET and SPL for metabolic engineering applications, we replaced the native promoter of a genomic localized phosphoglucose isomerase (pgi)-lacZ reporter construct by all SPL. Using these technologies together we were able to rapidly identify synthetic promoter sequences that resulted in activity range of 25% to 570% of the native pgi-promoter.

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Braatsch, S. (Ekstern), Helmark, S. (Intern), Kranz, H. (Ekstern), Købmann, B. J. (Intern), Jensen, P. R. (Intern)
Pages: 335-337
Publication date: 2008
Main Research Area: Technical/natural sciences

Publication information
Journal: BioTechniques
Volume: 45
Issue number: 3
ISSN (Print): 0736-6205
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 1.123 SNIP 0.578 CiteScore 1.16
BFI (2015): BFI-level 1
Increased biomass yield of Lactococcus lactis during energetically limited growth and respiratory conditions

Lactococcus lactis is known to be capable of respiration under aerobic conditions in the presence of haemin. In the present study the effect of respiration on ATP production during growth on different sugars was examined. With glucose as the sole carbon source, respiratory conditions in L. lactis MG1363 resulted in only a minor increase, 21%, in biomass yield. Since ATP production through substrate-level phosphorylation was essentially identical with and without respiration, the increased biomass yield was a result of energy-saving under respiratory conditions estimated to be 0.4 mol of ATP/mol of glucose. With maltose as the energy source, the increase in biomass yield amounted to 51% compared with an aerobic culture that lacked haemin. This higher ATP yield was obtained by redirecting pyruvate metabolism from lactate to acetate production, and from savings through respiration. However, even after subtracting these contributions, approx. 0.3 mol of ATP/mol of glucose remained unaccounted for. A similar response to respiratory conditions (0.2 mol of ATP/mol of...
glucose) was observed in a mutant that had a decreased glucose uptake rate during growth on glucose caused by
disruption of the PTSmannose (glucose/mannose-specific phosphotransferase system). Amino acid catabolism could be
excluded as the source of the additional ATP. Since mutants without a functional H+-ATPase produced less ATP under
sugar starvation and respiratory conditions, the additional ATP yield appears to come partly from energy saved on proton
pumping through the H+-ATPase due to respiration and partly from a reversed function of the H+-ATPase towards
oxidative phosphorylation. These results may contribute to the design and implementation of carbon-efficient high-cell-
density cultures of this industrially important species of bacterium.

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Authors: Købmann, B. J. (Intern), Blank, L. M. (Ekstern), Solem, C. (Intern), Petranovic, D. (Intern), Nielsen, L. K.
(Ekstern), Jensen, P. R. (Intern)
Pages: 25-33
Publication date: 2008
Main Research Area: Technical/natural sciences

Publication information
Journal: Biotechnology and Applied Biochemistry
Volume: 50
ISSN (Print): 0885-4513
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.418 SNIP 0.439 CiteScore 1.22
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.406 SNIP 0.466 CiteScore 1.23
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.525 SNIP 0.567 CiteScore 1.45
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.46 SNIP 0.735 CiteScore 1.69
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.479 SNIP 0.67 CiteScore 1.64
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.617 SNIP 0.707 CiteScore 1.78
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.592 SNIP 0.59
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.566 SNIP 0.773
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.443 SNIP 0.507
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 0.607 SNIP 0.832
Scopus rating (2006): SJR 0.66 SNIP 0.979
Scopus rating (2005): SJR 0.659 SNIP 1.126
Scopus rating (2004): SJR 0.478 SNIP 0.691
Scopus rating (2003): SJR 0.494 SNIP 0.847
Scopus rating (2002): SJR 0.478 SNIP 0.864
Scopus rating (2001): SJR 0.715 SNIP 1.239
Scopus rating (2000): SJR 0.426 SNIP 0.762
Scopus rating (1999): SJR 0.562 SNIP 0.798
Original language: English
DOIs:
Online diagnosticer af bacteriofag-inficerede celler

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Michelsen, O. (Intern), Jensen, P. R. (Intern)
Pages: 80-83
Publication date: 2008
Main Research Area: Technical/natural sciences

Publication information
Journal: Mælkeritidende
Volume: 4
ISSN (Print): 0024-9645
Ratings:
ISI indexed (2013): ISI indexed no
ISI indexed (2012): ISI indexed no
ISI indexed (2011): ISI indexed no
Original language: Danish
Source: orbit
Source-ID: 233188
Publication: Communication › Journal article – Annual report year: 2008

Plasmid pCS1966, a new selection/counterselection tool for strain construction in Lactic Acid Bacteria based on the oroP gene encoding an orotate transporter from Lactococcus lactis

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Solem, C. (Intern), Defoor, E. M. C. (Intern), Jensen, P. R. (Intern), Martinussen, J. (Intern)
Pages: 4772-4775
Publication date: 2008
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 74
Issue number: 15
ISSN (Print): 0099-2240
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
The extent of co-metabolism of glucose and galactose by L. lactis changes with the expression of the lacSZ operon from Streptococcus thermophilus

The lactose transporter and β-galactosidase from Streptococcus thermophilus, encoded by the lacSZ operon, were introduced into the lactose-negative strain Lactococcus lactis MG1363 and the expression of the lacSZ operon was modulated by substitution of the native promoter with randomized synthetic promoters. A series of strains with various expression levels of lacSZ were examined for their fermentation of lactose. Strains with a high expression level were found to metabolize lactose in a similar manner to S. thermophilus, i.e. the galactose moiety of lactose was excreted to the growth medium and only glucose was metabolized in glycolysis. Interestingly, strains with low expression of the operon showed a mixed acid metabolism and co-metabolism of galactose and glucose. The lactose flux increased gradually with increasing expression of the lacSZ operon until an optimum was observed at intermediate β-galactosidase activities of 2000-3000 Miller units. At higher expression levels, the flux decreased. These strains had a glycolytic flux comparable with those of reference strains with the standard lactococcal PTSlac (lactose phosphotransferase transport system) lactose transporter, which indicates that lactose transport is not rate-limiting for glycolysis in Lactococcus. Finally, an additional ATP drain was introduced into the fastest growing strain, CS2004, to test whether the ATP demand controlled glycolysis under these conditions, but in fact no increase in glycolytic flux was observed. © 2008 Portland Press Ltd.
The Ser/Thr/Tyr phosphoproteome of Lactococcus lactis IL1403 reveals multiple phosphorylated proteins
Bacillus subtilis strain deficient for the protein-tyrosine kinase PtkA exhibits impaired DNA replication

Bacillus subtilis has recently come into the focus of research on bacterial protein-tyrosine phosphorylation, with several protein kinases, phosphatases and their substrates identified in this Gram-positive model organism. B. subtilis protein-tyrosine phosphorylation system PtkA/PtpZ was previously shown to regulate the phosphorylation state of UDP-glucose dehydrogenases and single-stranded DNA-binding proteins. This promiscuity towards substrates is reminiscent of eukaryal kinases and has prompted us to investigate possible physiological effects of ptkA and ptpZ gene inactivations in this study. We were unable to identify any striking phenotypes related to control of UDP-glucose dehydrogenases, natural competence and DNA lesion repair; however, a very strong phenotype of ΔptkA emerged with respect to DNA replication and cell cycle control, as revealed by flow cytometry and fluorescent microscopy. B. subtilis cells lacking the kinase PtkA accumulated extra chromosome equivalents, exhibited aberrant initiation mass for DNA replication and an unusually long D period.
Detection of bacteriophage-infected cells of Lactococcus lactis using flow cytometry

Bacteriophage infection in dairy fermentation constitutes a serious problem worldwide. We have studied bacteriophage infection in Lactococcus lactis by using the flow cytometer. The first effect of the infection of the bacterium is a change from cells in chains toward single cells. We interpret this change as a consequence of a cease in cell growth, while the ongoing cell divisions leave the cells as single cells. Late in the infection cycle, cells with low-density cell walls appear, and these cells can be detected on cytograms of light scatter versus, for instance, fluorescence of stained DNA. We describe a new method for detection of phage infection in Lactococcus lactis dairy cultures. The method is based on flow cytometric detection of cells with low-density cell walls. The method allows fast and early detection of phage-infected bacteria, independently of which phage has infected the culture. The method can be performed in real time and therefore increases the chance of successful intervention in the fermentation process.

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology, Universidad Autónoma de Madrid
Authors: Michelsen, O. (Intern), Cuesta-Dominguez, Á. (Ekstern), Albrektsen, B. (Intern), Jensen, P. R. (Intern)
Pages: 7575-81
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 73
Issue number: 23
ISSN (Print): 0099-2240
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
The las enzymes control pyruvate metabolism in Lactococcus lactis during growth on maltose

General information
State: Published
Organisations: Department of Systems Biology
Authors: Solem, C. (Intern), Købmann, B. J. (Intern), Yang, F. (Ekstern), Jensen, P. R. (Intern)
Pages: 6727-30
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Bacteriology
Volume: 189
Issue number: 18
ISSN (Print): 0021-9193
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.08 SJR 1.908 SNIP 0.884
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.151 SNIP 0.959 CiteScore 2.84
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.069 SNIP 0.937 CiteScore 2.72
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 2.136 SNIP 1.018 CiteScore 3
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 2.103 SNIP 1.092 CiteScore 3.42
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.444 SNIP 1.158 CiteScore 3.83
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.6 SNIP 1.147
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.675 SNIP 1.18
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.591 SNIP 1.092
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.626 SNIP 1.151
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.632 SNIP 1.133
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.611 SNIP 1.167
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 2.449 SNIP 1.185
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 2.669 SNIP 1.145
The serine/threonine/tyrosine phosphoproteome of the model bacterium Bacillus subtilis

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Macek, B. (Ekstern), Mijakovic, I. (Intern), Olsen, J. V. (Ekstern), Gnad, F. (Ekstern), Kumar, C. (Ekstern), Jensen, P. R. (Intern), Mann, M. (Ekstern)
Pages: 697-707
Publication date: 2007
Main Research Area: Technical/natural sciences

Publication information
Journal: Molecular and Cellular Proteomics
Issue number: 6
ISSN (Print): 1535-9476
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 6.3
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 5.78
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 5.12
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 5.4
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 6
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 7.9
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
BFI (2009): BFI-level 2
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Web of Science (2008): Indexed yes
A synthetic promoter library for constitutive gene expression in Lactobacillus plantarum

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Rud, I. (Ekstern), Jensen, P. R. (Intern), Naterstad, K. (Ekstern), Axelsson, L. (Ekstern)
Pages: 1011-1019
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information
Journal: Microbiology
Volume: 152
Issue number: 4
Original language: English
Source-ID: 194772
Publication: Research - peer-review › Journal article – Annual report year: 2006

Bacterial single-stranded DNA-binding proteins are phosphorylated on tyrosine.
Single-stranded DNA-binding proteins (SSBs) are required for repair, recombination and replication in all organisms. Eukaryotic SSBs are regulated by phosphorylation on serine and threonine residues. To our knowledge, phosphorylation of SSBs in bacteria has not been reported. A systematic search for phosphotyrosine-containing proteins in Streptomyces griseus by immunoaffinity chromatography identified bacterial SSBs as a novel target of bacterial tyrosine kinases. Since genes encoding protein-tyrosine kinases (PTKs) have not been recognized in streptomycetes, and SSBs from Streptomyces coelicolor (ScSSB) and Bacillus subtilis (BsSSB) share 38.7% identity, we used a B. subtilis protein-tyrosine kinase YwqD to phosphorylate two cognate SSBs (BsSSB and YwpH) in vitro. We demonstrate that in vivo phosphorylation of B. subtilis SSB occurs on tyrosine residue 82, and this reaction is affected antagonistically by kinase YwqD and phosphatase YwqE. Phosphorylation of B. subtilis SSB increased binding almost 200-fold to single-stranded DNA in vitro. Tyrosine phosphorylation of B. subtilis, S. coelicolor and Escherichia coli SSBs occurred while they were expressed in E. coli, indicating that tyrosine phosphorylation of SSBs is a conserved process of post-translational modification in taxonomically distant bacteria.

General information
State: Published
Organisations: Department of Systems Biology, Center for Microbial Biotechnology, Department of Physics
Pages: 1588-1596
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information
Journal: Nucleic Acids Research
Volume: 34
Issue number: 5
ISSN (Print): 0305-1048
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 9.28 SJR 7.397 SNIP 2.657
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 7.239 SNIP 2.639 CiteScore 9.48
Control analysis of the importance of phosphoglycerate enolase for metabolic fluxes in Lactococcus lactis subsp. lactis IL1403.

**General information**

State: Published

Organisations: Center for Microbial Biotechnology, Department of Systems Biology

Authors: Købmann, B. J. (Intern), Solem, C. (Intern), Jensen, P. R. (Intern)

Pages: 346-349

Publication date: 2006

Main Research Area: Technical/natural sciences
Genetics of Lactococci

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Gaudu, P. (Ekstern), Yamamoto, Y. (Ekstern), Jensen, P. R. (Intern), Hammer, K. (Intern), Gruss, A. (Ekstern)
Pages: 356-368
Publication date: 2006

Host publication information
Title of host publication: Gram-positive Pathogens
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 190453
Publication: Research - peer-review › Book chapter – Annual report year: 2006

Lactococcus lactis - traditional and GMO strains

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Jensen, P. R. (Intern), Købmann, B. J. (Intern), Solem, C. (Intern)
Pages: S317-S317
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information
Journal: Toxicology Letters
Volume: 164
ISSN (Print): 0378-4274
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.83 SJR 1.25 SNIP 1.204
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.298 SNIP 1.126 CiteScore 3.62
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.143 SNIP 1.165 CiteScore 3.45
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.106 SNIP 1.212 CiteScore 3.56
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Synthetic promoter libraries- tuning of gene expression.

The study of gene function often requires changing the expression of a gene and evaluating the consequences. In principle, the expression of any given gene can be modulated in a quasi-continuum of discrete expression levels but the traditional approaches are usually limited to two extremes: gene knockout and strong overexpression. However, applications such as metabolic optimization and control analysis necessitate a continuous set of expression levels with only slight increments in strength to cover a specific window around the wildtype expression level of the studied gene; this requirement can be met by using promoter libraries. This approach generally consists of inserting a library of promoters in front of the gene to be studied, whereby the individual promoters might deviate either in their spacer sequences or bear slight deviations from the consensus sequence of a vegetative promoter. Here, we describe the two different methods for obtaining promoter libraries and compare their applicability.

General information

State: Published
Organisations: Department of Systems Biology
Authors: Hammer, K. (Intern), Mijakovic, I. (Intern), Jensen, P. R. (Intern)
Pages: 53-55
Publication date: 2006
Main Research Area: Technical/natural sciences

Publication information

Journal: Trends in Biotechnology
Volume: 24
Control analysis as a tool to understand the formation of the las operon in Lactococcus lactis

General information
Control analysis as a tool to understand the formation of the las operon in Lactococcus lactis
In Lactococcus lactis the enzymes phosphofructokinase (PFK), pyruvate kinase (PK) and lactate dehydrogenase (LDH) are uniquely encoded in the las operon and we here apply Metabolic Control Analysis to study the role of this organisation. Earlier work showed that LDH at wildtype level has zero control on glycolysis and growth rate but high negative control on formate production. We find that PFK and PK have zero control on glycolysis and growth rate at the wildtype enzyme level but both enzymes exert strong positive control on the glycolytic flux at reduced activities. PK has high positive control on formate and acetate production, whereas PFK has no control on these fluxes. Decreased expression of the entire las operon resulted in a strong decrease in growth rate and the glycolytic flux; at 53% expression of the las operon the glycolytic flux was reduced to 44% and the flux control coefficient increased towards 3. Increased las expression resulted in a slight decrease in the glycolytic flux. At the wildtype level the control was close to zero on both glycolysis and the pyruvate branches. The sum of control coefficients for the three enzymes individually was comparable to the control coefficient found for the entire operon; the strong positive control by PK almost cancels out the negative control by LDH on formate production. The analysis suggests that co-regulation of PFK and PK provides a very efficient way to regulate glycolysis, and co-regulating PK and LDH allows the cells to maintain homolactic fermentation during regulation of glycolysis.

In vitro characterization of the Bacillus subtilis protein tyrosine phosphatase YwqE.
Both gram-negative and gram-positive bacteria possess protein tyrosine phosphatases (PTPs) with a catalytic Cys residue. In addition, many gram-positive bacteria have acquired a new family of PTPs, whose first characterized member was CpsB from Streptococcus pneumoniae. Bacillus subtilis contains one such CpsB-like PTP, YwqE, in addition to two class II Cys-based PTPs, YwIE and YfkJ. The substrates for both YwIE and YfkJ are presently unknown, while YwqE was shown to dephosphorylate two phosphotyrosine-containing proteins implicated in UDP-glucuronate biosynthesis, YwqD and YwqF. In this study, we characterize YwqE, compare the activities of the three B. subtilis PTPs (YwqE, YwIE, and YfkJ), and demonstrate that the two B. subtilis class II PTPs do not dephosphorylate the physiological substrates of YwqE.
Lactococcus lactis - a diploid bacterium.
In contrast to higher eukaryotes, bacteria are haploid, i.e. they store their genetic information in a single chromosome, which is then duplicated during the cell cycle. If the growth rate is sufficiently low, the bacterium is born with only a single copy of the chromosome, which gets duplicated before the bacterium divides. Fast-growing bacteria have overlapping rounds of replication, and can contain DNA corresponding to more than four genome equivalents. However, the terminus region of the chromosome is still present in just one copy after division, and is not duplicated until right before the next division. Thus, the regions of the chromosome that are the last to be replicated are haploid even in fast-growing bacteria. In contrast to this general rule for bacteria, we found that Lactococcus lactis, a bacterium which has been exploited for thousands of years for the production of fermented milk products, is born with two complete non-replicating chromosomes. L. lactis therefore remain diploid throughout its entire life cycle.

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
Authors: Kilstrup, M. (Intern), Hammer, K. (Intern), Jensen, P. R. (Intern), Martinussen, J. (Intern)
Pages: 555-590
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: F E M S Microbiology Reviews
Volume: 29
ISSN (Print): 0168-6445
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 13.54
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): CiteScore 13.38
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 12.9
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 13.78
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): CiteScore 12.18
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 11.87
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Web of Science (2008): Indexed yes
Web of Science (2007): Indexed yes
Web of Science (2005): Indexed yes
Web of Science (2004): Indexed yes
Web of Science (2003): Indexed yes
Web of Science (2002): Indexed yes
Web of Science (2001): Indexed yes
Web of Science (2000): Indexed yes
Original language: English
Source: orbit
Source-ID: 184082
Publication: Research - peer-review ➜ Journal article – Annual report year: 2005
Protein-Tyrosine Phosphorylation in Bacillus subtilis.

In recent years bacterial protein-tyrosine kinases have been found to phosphorylate a growing number of protein substrates, including RNA polymerase sigma factors, UDP-glucose dehydrogenases and single-stranded DNA-binding proteins. The activity of these protein substrates was affected by tyrosine phosphorylation, indicating that this post-translational modification could regulate physiological processes ranging from stress response and exopolysaccharide synthesis to DNA metabolism. Some interesting work in this field was done in Bacillus subtilis, and we here present the current state of knowledge on protein-tyrosine phosphorylation in this gram-positive model organism. With its two kinases, two kinase modulators, three phosphatases and at least four different tyrosine-phosphorylated substrates, B. subtilis is the bacterium with the highest number of presently known participants in the global network of protein-tyrosine phosphorylation. We discuss the approaches currently used to chart this network: ranging from studies of substrate specificity and the physiological role of tyrosine phosphorylation of individual enzymes to the global approaches at the level of systems biology.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Mijakovic, I. (Intern), Petranovic, D. (Intern), Bottini, N. (Ekstern), Deutscher, J. (Ekstern), Jensen, P. R. (Intern)
Pages: 189–197
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Molecular Microbiology and Biotechnology
Volume: 9
ISSN (Print): 1464-1801
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.749 SNIP 0.509 CiteScore 1.62
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.963 SNIP 0.574 CiteScore 1.84
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.95 SNIP 0.739 CiteScore 1.84
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.702 SNIP 0.542 CiteScore 1.62
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.893 SNIP 0.767 CiteScore 1.87
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.067 SNIP 0.928 CiteScore 2.44
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.076 SNIP 0.999
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.582 SNIP 0.817
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.424 SNIP 0.613
Scopus rating (2007): SJR 1.425 SNIP 0.859
Scopus rating (2006): SJR 1.317 SNIP 0.507
Scopus rating (2005): SJR 1.578 SNIP 0.769
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.314 SNIP 0.682
Triosephosphate isomerase has no control on the glycolytic flux and metabolic shift in Lactococcus lactis IL1403

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Solem, C. (Intern), Købmann, B. J. (Intern), Jensen, P. R. (Intern)
Pages: S158-S158
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Biotechnology
Volume: 118
ISSN (Print): 0168-1656
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.88 SJR 0.978 SNIP 0.937
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.068 SNIP 0.987 CiteScore 2.87
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.113 SNIP 1.144 CiteScore 2.95
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.173 SNIP 1.188 CiteScore 3.22
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.255 SNIP 1.312 CiteScore 3.4
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.157 SNIP 1.064 CiteScore 2.87
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.126 SNIP 1.18
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.216 SNIP 1.235
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.136 SNIP 1.265
Web of Science (2008): Indexed yes
Tunable promoters in systems biology.

The construction of synthetic promoter libraries has represented a major breakthrough in systems biology, enabling the subtle tuning of enzyme activities. A number of tools are now available that allow the modulation of gene expression and the detection of changes in expression patterns. But, how does one choose the correct promoter and what are the appropriate methods for reading promoter strength? Furthermore, how fine should the tuning of gene expression be for some specific applications and how can the simultaneous and individual tuning of multiple genes be achieved? Some recent studies have helped us to find answers to many of these questions.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, Systems Biotechnology
Authors: Mijakovic, I. (Intern), Petranovic, D. (Intern), Jensen, P. R. (Intern)
Pages: 329-335
Publication date: 2005
Main Research Area: Technical/natural sciences

Publication information
Journal: Current Opinion in Biotechnology
Volume: 16
Issue number: 3
ISSN (Print): 0958-1669
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.55 SJR 3.331 SNIP 2.1
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.113 SNIP 2.143 CiteScore 7.99
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.271 SNIP 2.068 CiteScore 7.45
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.322 SNIP 2.198 CiteScore 7.93
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Experimental modulation of gene expression

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Købmann, B. J. (Intern), Tornøe, J. (Ekstern), Johansson, B. (Ekstern), Jensen, P. R. (Intern)
Pages: 155-179
Publication date: 2004

Host publication information
Title of host publication: Metabolic engineering in the post genomic era
Place of publication: Oxford
Publisher: BIOS Scientific Publishers Ltd
Editor: Kholodenko, B.
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 155281
Publication: Research › Book chapter – Annual report year: 2004

Expression of the pyrG gene determines the pool sizes of CTP and dCTP in Lactococcus lactis
The pyrG gene from Lactococcus lactis encodes CTP synthase (EC 6.4.3.2), an enzyme converting UTP to CTP. A series of strains were constructed with different levels of pyrG expression by insertion of synthetic constitutive promoters with different strengths in front of pyrG. These strains expressed pyrG levels in a range from 3 to 665% relative to the wild-type
expression level. Decreasing the level of CTP synthase to 43% had no effect on the growth rate, showing that the capacity of CTP synthase in the cell is in excess in a wild-type strain. We then studied how pyrG expression affected the intracellular pool sizes of nucleotides and the correlation between pyrG expression and nucleotide pool sizes was quantified using metabolic control analysis in terms of inherent control coefficients. At the wild-type expression level, CTP synthase had full control of the CTP concentration with a concentration control coefficient close to one and a negative concentration control coefficient of -0.28 for the UTP concentration. Additionally, a concentration control coefficient of 0.49 was calculated for the dCTP concentration. Implications for the homeostasis of nucleotide pools are discussed.

**General information**

State: Published

Organisations: Center for Microbial Biotechnology, Department of Systems Biology

Authors: Jørgensen, C. (Ekstern), Hammer, K. (Intern), Jensen, P. R. (Intern), Martinussen, J. (Intern)

Pages: 2438-2445

Publication date: 2004

Main Research Area: Technical/natural sciences

**Publication information**

Journal: European Journal of Biochemistry

Volume: 271

ISSN (Print): 0014-2956

Ratings:

- BFI (2017): BFI-level 1
- BFI (2016): BFI-level 1
- Scopus rating (2016): CiteScore 4.06
- BFI (2015): BFI-level 1
- Scopus rating (2015): CiteScore 3.92
- BFI (2014): BFI-level 1
- Scopus rating (2014): CiteScore 3.94
- BFI (2013): BFI-level 1
- Scopus rating (2013): CiteScore 4.02
- ISI indexed (2013): ISI indexed yes
- BFI (2012): BFI-level 1
- Scopus rating (2012): CiteScore 3.84
- ISI indexed (2012): ISI indexed yes
- BFI (2011): BFI-level 2
- Scopus rating (2011): CiteScore 3.36
- ISI indexed (2011): ISI indexed yes
- BFI (2010): BFI-level 2
- BFI (2009): BFI-level 2
- BFI (2008): BFI-level 1
- Web of Science (2004): Indexed yes
- Web of Science (2003): Indexed yes
- Web of Science (2002): Indexed yes
- Web of Science (2001): Indexed yes
- Web of Science (2000): Indexed yes

Original language: English

Source: orbit

Source-ID: 155280

Publication: Research - peer-review › Journal article – Annual report year: 2004

**Hvad kontrollorer syriningsfærdigthed af den primære starter?**

**General information**

State: Published

Organisations: Microbial Physiology and Genetics, Department of Systems Biology

Authors: Kebmann, B. J. (Intern), Solem, C. (Intern), Jensen, P. R. (Intern)

Pages: 55-64

Publication date: 2004

Main Research Area: Technical/natural sciences
Kontrolanalyse af glykosen i mikrobielle systemer

**General information**
State: Published
Organisations: Microbial Physiology and Genetics, Department of Systems Biology
Authors: Købmann, B. J. (Intern), Solem, C. (Intern), Jensen, P. R. (Intern)
Pages: 22-26
Publication date: 2004
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Mælkeritidende
Volume: 3
Original language: Danish
Source: orbit
Source-ID: 155282
Publication: Research › Journal article – Annual report year: 2004

Transformation of Leuconostoc carnosum 4010 and evidence for natural competence of the organism

Plasmid transformation in Leuconostoc carnosum 4010 was analyzed. A successful transformation protocol for L. carnosum was established by modifying an existing protocol for Lactococcus lactis. Several parameters, including the number of generations that the cells had grown at the time of harvest, glycine concentration, the time of incubation for phenotypic expression, and the electrical field strength, were investigated and proved to have influence on the transformation frequency. Electrocompetence was found to be transient and to peak in the early exponential growth phase. Optimized conditions resulted in transformation frequencies of up to $6.7 \times 10^5$ transformants per microgram of plasmid DNA. A total of five plasmids in L. carnosum were successfully introduced and maintained. Interestingly, we discovered that DNA uptake was of a frequency of $3 \times 10^{-6}$ to $19 \times 10^{-6}$ transformants per CFU in the absence of an applied electrical field. We concluded that L. carnosum is naturally competent.

**General information**
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Helmark, S. (Intern), Hansen, M. E. (Intern), Jelle, B. (Ekstern), Sørensen, K. (Ekstern), Jensen, P. R. (Intern)
Pages: 3695-3699
Publication date: 2004
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Applied and Environmental Microbiology
Volume: 70
Issue number: 6
ISSN (Print): 0099-2240
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Experimental control analysis of glycolysis in Lactococcus lactis
Glyceraldehyde-3-phosphate dehydrogenase has no control over glycolytic flux in Lactococcus lactis MG1363

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has previously been suggested to have almost absolute control over the glycolytic flux in Lactococcus lactis (B. Poolman, B. Bosman, J. Kiers, and W. N. Konings, J. Bacteriol. 169:5887-5890, 1987). Those studies were based on inhibitor titrations with iodoacetate, which specifically inhibits GAPDH, and the data suggested that it should be possible to increase the glycolytic flux by overproducing GAPDH activity. To test this hypothesis, we constructed a series of mutants with GAPDH activities from 14 to 210% of that of the reference strain MG1363. We found that the glycolytic flux was unchanged in the mutants overproducing GAPDH. Also, a decrease in the GAPDH activity had very little effect on the growth rate and the glycolytic flux until 25% activity was reached. Below this activity level, the glycolytic flux decreased proportionally with decreasing GAPDH activity. These data show that GAPDH activity has no control over the glycolytic flux (flux control coefficient = 0.0) at the wild-type enzyme level and that the enzyme is present in excess capacity by a factor of 3 to 4. The early experiments by Poolman and coworkers were performed with cells resuspended in buffer, i.e., nongrowing cells, and we therefore analyzed the control by GAPDH under similar conditions. We found that the glycolytic flux in resting cells was even more insensitive to changes in the GAPDH activity; in this case GAPDH was also present in a large excess and had no control over the glycolytic flux.
Precise determinations of C and D periods by flow cytometry in Escherichia coli K-12 and B/r

The C and D cell cycle periods of seven Escherichia coli K-12 strains and three E. coli B/r strains were determined by computer simulation of DNA histograms obtained by flow cytometry of batch cultures grown at several different generation times. To obtain longer generation times two of the K-12 strains were cultivated at several different dilution rates in glucose-limited chemostats. The replication period (C period) was found to be similar in K-12 and B/r strains grown at similar generation times. At generation times below 60 min the C period was constant; above 60 min it increased linearly with increasing generation time. The period from termination of replication to cell division (D period) was more variable. It was much shorter in B/r than in K-12 strains. Like the C period it was relatively constant at generation times below 60 min and it increased with increasing generation times at longer generation times. In glucose-limited chemostats good correlation was found between D periods and generation times, whereas batch cultures exhibited carbon-source-dependent variations. Chemostat cultures showed cell cycle variations very similar to those obtained in batch cultures. These flow cytometric determinations of cell cycle periods confirm earlier determinations of the C period and establish that the D period also varies with generation time in slowly growing cultures. In addition they extend the range of growth rates at which cell cycle periods have been determined in E. coli K-12.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Michelsen, O. (Intern), de Mattos, M. (Ekstern), Jensen, P. R. (Intern), Hansen, F. G. (Intern)
Pages: 1001-1010
Publication date: 2003
Main Research Area: Technical/natural sciences

Publication information
The level of glucose-6-phosphate dehydrogenase activity strongly influences xylose fermentation and inhibitor sensitivity in recombinant Saccharomyces cerevisiae strains

Disruption of the ZWF1 gene encoding glucose-6-phosphate dehydrogenase (G6PDH) has been shown to reduce the xylitol yield and the xylose consumption in the xylose-utilizing recombinant Saccharomyces cerevisiae strain TMB3255. In the present investigation we have studied the influence of different production levels of G6PDH on xylose fermentation. We used a synthetic promoter library and the copper-regulated CUP1 promoter to generate G6PDH-activities between 0% and 179% of the wildtype level. G6PDH-activities of 1% and 6% of the wild-type level resulted in 2.8- and 5.1-fold increase in specific xylose consumption, respectively, compared with the ZWF1-disrupted strain. Both strains exhibited decreased xylitol yields (0.13 and 0.19 g/g xylose) and enhanced ethanol yields (0.36 and 0.34 g/g xylose) compared with the control strain TMB3001 (0.29 g xylitol/g xylose, 0.31 g ethanol/g xylose). Cytoplasmic transhydrogenase (TH) from Azotobacter vinelandii has previously been shown to transfer NADPH and NAD(+) into NADP(+) and NADH, and TH-overproduction resulted in lower xylitol yield and enhanced glycerol yield during xylose utilization. Strains with low G6PDH-activity grew slower in a lignocellulose hydrolysate than the strain with wild-type G6PDH-activity, which suggested that the availability of intracellular NADPH correlated with tolerance towards lignocellulose-derived inhibitors. Low G6PDH-activity strains were also more sensitive to H2O2 than the control strain TMB3001.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Jeppsson, M. (Ekstern), Johansson, B. (Ekstern), Jensen, P. R. (Intern), Hahn-Hagerdal, B. (Ekstern), Gorwa-Grauslund, M. (Ekstern)
Pages: 1263-1272
Publication date: 2003
Main Research Area: Technical/natural sciences

Publication information
Journal: Yeast
Volume: 20
Issue number: 15
ISSN (Print): 0749-503X
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.816 SNIP 0.811 CiteScore 1.87
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.962 SNIP 0.745 CiteScore 2.01
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.875 SNIP 0.792 CiteScore 1.67
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.232 SNIP 0.72 CiteScore 2.09
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.197 SNIP 0.762 CiteScore 2.05
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.063 SNIP 0.701 CiteScore 1.77
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.049 SNIP 0.835
Web of Science (2010): Indexed yes
A turbo engine with automatic transmission? How to many chemicomotion to the subtleties and robustness of life

Most genomes are much more complex than required for the minimum chemistry of life. Evolution has selected sophistication more than life itself. Could this also apply to bioenergetics? We first examine mechanisms through which bioenergetics could deliver sophistication. We illustrate possible benefits of the turbo-charging of catabolic pathways, of loose coupling, low-gear catabolism, automatic transmission in energy coupling, and of homeostasis. Mechanisms for such phenomena may reside at the level of individual proton pumps, or consist of rerouting of electrons over parallel pathways. The mechanisms may be confined to preexisting components, or involve the plasticity of gene expression that is so characteristic of most living organisms. These possible benefits lead us to the conjecture that also bioenergetics has evolved more for sophistication than for necessity. We next discuss a hitherto unresolved enigma, i.e., bioenergetics does not seem to be critical for the physiological state. To decide on how critical bioenergetics is, we quantified the control exerted by catabolism on important physiological functions such as growth rate and growth yield. We also determined whether a growth inhibition mostly affected bioenergetics (catabolism) or anabolism; if ATP increases with growth rate, then growth should be considered energy (catabolism) limited. The experimental results for Escherichia coli pinpoint the enigma: its energy metabolism (catabolism) is not critical for growth rate. These results might suggest that because it has no direct control over cell function, bioenergetics is unimportant. Paradoxically however, in biology, highly important mechanisms tend to have little control on cell function, precisely because of that importance. Sophistication in terms of homeostatic mechanisms has evolved to guarantee robustness of the most important functions: The most important mechanisms are redundant in biology. Bioenergetics may be an excellent example of this paradox, in line with the above conjecture. It may be highly important and sophisticated. We then discuss work that has begun to focus on the sophistication of bioenergetics. Homeostasis of the energetics of DNA structure in E. coli is extensive. It relies both on preexisting components and on responsive gene expression. The vastly parallel electron-transfer network of Paracoccus denitrificans engages in sophisticated dynamic and hierarchical regulation. The growth yield of the organism can depend on which terminal oxidases are active. Effective proton translocation may vary due to rerouting of electrons. We conclude that much sophistication of bioenergetics will be discovered in this era of functional genomics.

General information

State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Koefoed, S. (Ekstern), Otten, M. (Ekstern), Købmann, B. J. (Intern), Bruggeman, F. (Ekstern), Bakker, B. (Ekstern), Snoep, J. (Ekstern), Krab, K. (Ekstern), van Spanning, R. (Ekstern), van Verseveld, H. (Ekstern), Jensen, P. R. (Intern), Koster, J. (Ekstern), Westerhoff, H. (Ekstern)
Bacteriophage resistance of a Delta thyA mutant of Lactococcus lactis blocked in DNA replication

The thyA gene, which encodes thymidylate synthase (TS), of Lactococcus lactis CHCC373 was sequenced, including the upstream and downstream regions. We then deleted part of thyA by gene replacement. The resulting strain, MBP71 DeltathyA, was devoid of TS activity, and in media without thymidine, such as milk, there was no detectable d’ITP pool in the cells. Hence, DNA replication was abolished, and acidification by MBP71 was completely unaffected by the presence of nine different phages tested at a multiplicity of infection (MOI) of 0.1. Nonreplicating MBP71 must be inoculated at a higher level than CHCC373 to achieve a certain pH within a specified time. For a pH of 5.2 to be reached in 6 h, the inoculation level of MBP71 must be 17-fold higher than for CHCC373. However, by adding a limiting amount of thymidine this could be lowered to just 5-fold the normal amount, while acidification was unaffected with MBP71 up to an MOI of 0.01. It was found that nonreplicating MBP71 produced largely the same products as CHCC373, though the acetaldehyde production of the former was higher.

General information
State: Published
Organisations: Bacterial Physiology and Genetics Group, Department of Systems Biology
Authors: Pedersen, M. (Ekstern), Jensen, P. R. (Intern), Janzen, T. (Ekstern), Nilsson, D. (Ekstern)
Pages: 3010-3023
Publication date: 2002
Main Research Area: Technical/natural sciences
DNA supercoiling in Escherichia coli is under tight and subtle homeostatic control, involving gene-expression and metabolic regulation of both topoisomerase I and DNA gyrase

DNA of prokaryotes is in a nonequilibrium structural state, characterized as 'active' DNA supercoiling. Alterations in this state affect many life processes and a homeostatic control of DNA supercoiling has been suggested [Menzel, R. & Gellert, M. (1983) Cell 34, 105-113]. We here report on a new method for quantifying homeostatic control of the high-energy state of in vivo DNA. The method involves making small perturbation in the expression of topoisomerase I, and measuring the effect on DNA supercoiling of a reporter plasmid and on the expression of DNA gyrase. In a separate set of experiments the expression of DNA gyrase was manipulated and the control on DNA supercoiling and topoisomerase I expression was measured [part of these latter experiments has been published in Jensen, P.R., van der Weijden, C.C., Jensen, L.B., Westerhoff, H.V. & Snoep, J.L. (1999) Eur. J. Biochem. 266, 865-877]. Of the two regulatory mechanisms via which homeostasis is conferred, regulation of enzyme activity or regulation of enzyme expression, we quantified the first to be responsible for 72% and the latter for 28%. The gene expression regulation could be dissected to DNA gyrase (21%) and to topoisomerase I (7%). On a scale from 0 (no homeostatic control) to 1 (full homeostatic control) we quantified the homeostatic control of DNA supercoiling at 0.87. A 10% manipulation of either topoisomerase I or DNA gyrase activity results in a 1.3% change of DNA supercoiling only. We conclude that the homeostatic regulation of the nonequilibrium DNA structure in wild-type Escherichia coli is almost complete and subtle (i.e. involving at least three regulatory mechanisms).
Experimental determination of control of glycolysis in Lactococcus lactis

The understanding of control of metabolic processes requires quantitative studies of the importance of the different enzymatic steps for the magnitude of metabolic fluxes and metabolite concentrations. An important element in such studies is the modulation of enzyme activities in small steps above and below the wild-type level. We review a genetic approach that is well suited for both Metabolic Optimization and Metabolic Control Analysis and studies on the importance of a number of glycolytic enzymes for metabolic fluxes in Lactococcus lactis. The glycolytic enzymes phosphofructokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), pyruvate kinase (PYK) and lactate dehydrogenase (LDH) are shown to have no significant control on the glycolytic flux in exponentially growing cells of L. lactis MG1363. Introduction of an uncoupled ATPase activity results in uncoupling of glycolysis from biomass production. With MG1363 growing in defined medium supplemented with glucose, the ATP demanding processes do not have a significant control on the glycolytic flux; it appears that glycolysis is running at maximal rate. It is likely that the flux control is distributed over many enzymes in L. lactis, but it cannot yet be excluded that one of the remaining glycolytic steps is a rate-limiting step for the glycolytic flux.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, Bacterial Physiology and Genetics Group
Authors: Købmann, B. J. (Intern), Andersen, H. W. (Intern), Solem, C. (Intern), Jensen, P. R. (Intern)
Pages: 237-248
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
Journal: Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology
Volume: 82
Issue number: 1-4
ISSN (Print): 0003-6072
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 1.86 SJR 0.818 SNIP 0.833
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.965 SNIP 0.845 CiteScore 1.99
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.773 SNIP 0.8 CiteScore 1.99
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.824 SNIP 1.083 CiteScore 2.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.081 SNIP 0.917 CiteScore 2.02
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.903 SNIP 0.999 CiteScore 2.17
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.822 SNIP 0.834
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.943 SNIP 0.829
Expression of genes encoding F-1-ATPase results in uncoupling of glycolysis from biomass production in Lactococcus lactis

We studied how the introduction of an additional ATP-consuming reaction affects the metabolic fluxes in Lactococcus lactis. Genes encoding the hydrolytic part of the F-1 domain of the membrane-bound (F1F0) H+-ATPase were expressed from a range of synthetic constitutive promoters. Expression of the genes encoding F-1-ATPase was found to decrease the intracellular energy level and resulted in a decrease in the growth rate. The yield of biomass also decreased, which showed that the incorporated F-1-ATPase activity caused glycolysis to be uncoupled from biomass production. The increase in ATPase activity did not shift metabolism from homolactic to mixed-acid fermentation, which indicated that a low energy state is not the signal for such a change. The effect of uncoupled ATPase activity on the glycolytic flux depended on the growth conditions. The uncoupling stimulated the glycolytic flux threefold in nongrowing cells resuspended in buffer, but in steadily growing cells no increase in flux was observed. The latter result shows that glycolysis occurs close to its maximal capacity and indicates that control of the glycolytic flux under these conditions resides in the glycolytic reactions or in sugar transport.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Købmann, B. J. (Intern), Solem, C. (Intern), Pedersen, M. (Ekstern), Nilsson, D. (Ekstern), Jensen, P. R. (Intern)
Pages: 4274-4282
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 68
Issue number: 9
ISSN (Print): 0099-2240
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Generation of a synthetic mammalian promoter library by modification of sequences spacing transcription factor binding sites

The development of a set of synthetic mammalian promoters with different specific activities is described. The library is based on a synthetic promoter, JeT, constructed as a 200 bp chimeric promoter built from fragments of the viral SV40 early promoter and the human beta-actin and ubiquitin C promoters. The JeT promoter was made by separating the included consensus boxes by the same distances in base pairs as found in the wild-type promoters, thus preserving transcription factor interaction. The resulting promoter was shown to drive reporter expression to high levels in enhanced green fluorescent protein and secreted alkaline phosphatase reporter assays. By replacing sequences separating the transcription factor binding sites with randomized sequences of the same length, sets of new promoters with different strengths, spanning a 10-fold range of transcriptional activity in cell culture, was obtained. The measured activity of each promoter in the library was highly specific and reproducible when tested in HiB5 and ARPE-19 cell culture.
Increasing acidification of nonreplicating Lactococcus lactis Delta thyA mutants by incorporating ATPase activity

Lactococcus lactis MBP71 Delta thyA (thymidylate synthase) cannot synthesize dTTP de novo, and DNA replication is dependent on thymidine in the growth medium. In the nonreplicating state acidification by MBP71 was completely insensitive to bacteriophages (M. B. Pedersen, P. R. Jensen, T. Janzen, and D. Nilsson, Appl. Environ. Microbiol. 68:3010-3023, 2002). For nonreplicating MBP71 the biomass increased 3.3-fold over the first 3.5 h, and then the increase stopped. The rate of acidification increased 2.3-fold and then started to decrease. Shortly after inoculation the lactic acid flux was 60% of that of exponentially growing MBP71. However, when nonspecific ATPase activity was incorporated into MBP71, the lactic acid flux was restored to 100% but not above that point, indicating that control over the flux switched from ATP demand to ATP supply (i.e., to sugar transport and glycolysis). As determined by growing nonreplicating cells with high ATPase activity on various sugar sources, it appeared that glycolysis exerted the majority of the control. ATPase activity also stimulated the rate of acidification by nonreplicating MBP71 growing in milk, and pH 5.2 was reached 40% faster than it was without ATPase activity. We concluded that ATPase activity is a functional means of increasing acidification by nonreplicating L. lactis.
Kontrol af Metabolisk Flux igennem glykosen hos Laktokokker I

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Hammer, K. (Intern), Jensen, P. R. (Intern)
Pages: 481-483
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
Journal: Mælkeritidende
Issue number: 20
Original language: Danish
Source: orbit
Source-ID: 116733
Publication: Communication › Journal article – Annual report year: 2002

Kontrol af Metabolisk Flux igennem glykosen hos Laktokokker II

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Hammer, K. (Intern), Jensen, P. R. (Intern)
Pages: 582
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
Journal: Mælkeritidende
Modulation of gene expression made easy

A new approach for modulating gene expression, based on randomization of promoter (spacer) sequences, was developed. The method was applied to chromosomal genes in Lactococcus lactis and shown to generate libraries of clones with broad ranges of expression levels of target genes. In one example, overexpression was achieved by introducing an additional gene copy into a phage attachment site on the chromosome. This resulted in a series of strains with phosphofructokinase activities from 1.4 to 11 times the wild-type activity level. In this example, the pfk gene was cloned upstream of a gusA gene encoding beta-glucuronidase, resulting in an operon structure in which both genes are transcribed from a common promoter. We show that there is a linear correlation between the expressions of the two genes, which facilitates screening for mutants with suitable enzyme activities. In a second example, we show that the method can be applied to modulating the expression of native genes on the chromosome. We constructed a series of strains in which the expression of the las operon, containing the genes pfk, pyk, and ldh, was modulated by integrating a truncated copy of the pfk gene. Importantly, the modulation affected the activities of all three enzymes to the same extent, and enzyme activities ranging from 0.5 to 3.5 times the wild-type level were obtained.
The extent to which ATP demand controls the glycolytic flux depends strongly on the organism and conditions for growth. Using molecular genetics we have introduced uncoupled ATPase activity in two different bacterial species, Escherichia coli and Lactococcus lactis, and determined the elasticities of the growth rate and glycolytic flux towards the intracellular $[\text{ATP}]/[\text{ADP}]$ ratio. During balanced growth in batch cultures of E. coli the ATP demand was found to have almost full control on the glycolytic flux (FCC=0.96) and the flux could be stimulated by 70%. In contrast to this, in L. lactis the control by ATP demand on the glycolytic flux was close to zero. However, when we used non-growing cells of L. lactis (which have a low glycolytic flux) the ATP demand had a high flux control and the flux could be stimulated more than two fold. We suggest that the extent to which ATP demand controls the glycolytic flux depends on how much excess capacity of glycolysis is present in the cells.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
The glycolytic flux in Escherichia coli is controlled by the demand for ATP

The nature of the control of glycolytic flux is one of the central, as-yet-uncharacterized issues in cellular metabolism. We developed a molecular genetic tool that specifically induces ATP hydrolysis in living cells without interfering with other aspects of metabolism. Genes encoding the F-1 part of the membrane-bound (F1F0) H+-ATP synthase were expressed in steadily growing Escherichia coli cells, which lowered the intracellular [ATP]/[ADP] ratio. This resulted in a strong stimulation of the specific glycolytic flux concomitant with a smaller decrease in the growth rate of the cells. By optimizing additional ATP hydrolysis, we increased the flux through glycolysis to 1.7 times that of the wild-type flux. The results demonstrate why attempts in the past to increase the glycolytic flux through overexpression of glycolytic enzymes have
been unsuccessful: the majority of flux control (> 75%) resides not inside but outside the pathway, i.e., with the enzymes that hydrolyze ATP. These data further allowed us to answer the question of whether catabolic or anabolic reactions control the growth of E. coli. We show that the majority of the control of growth rate resides in the anabolic reactions, i.e., the cells are mostly "carbon" limited. Ways to increase the efficiency and productivity of industrial fermentation processes are discussed.
Totalt regulerbare promotorer i skræddersyede starterkulturer

General information
State: Published
Organisations: Department of Systems Biology
Authors: Johansen, A. H. (Intern), Andersen, H. W. (Intern), Solem, C. (Intern), Jensen, P. R. (Intern)
Pages: 274-277
Publication date: 2002
Main Research Area: Technical/natural sciences

Hemin reconstitutes proton extrusion in an H+-ATPase-negative mutant of Lactococcus lactis

H+-ATPase is considered essential for growth of Lactococcus lactis. However, media containing hemin restored the aerobic growth of an H+-ATPase-negative mutant, suggesting that hemin complements proton extrusion. We show that inverted membrane vesicles prepared from hemin-grown L. lactis cells are capable of coupling NADH oxidation to proton translocation.

General information
State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Blank, L. (Ekstern), Købmann, B. J. (Intern), Michelsen, O. (Intern), Nielsen, L. (Ekstern), Jensen, P. R. (Intern)
Pages: 6707-6709
Publication date: 2001
Main Research Area: Technical/natural sciences

Journal: Journal of Bacteriology
Volume: 183
Issue number: 22
ISSN (Print): 0021-9193
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
Lactate dehydrogenase has no control on lactate production but has a strong negative control on formate production in Lactococcus lactis

A series of mutant strains of Lactococcus lactis were constructed with lactate dehydrogenase (LDH) activities ranging from below 1% to 133% of the wild-type activity level. The mutants with 59% to 133% of lactate dehydrogenase activity had growth rates similar to the wild-type and showed a homolactic pattern of fermentation. Only after lactate dehydrogenase
activity was reduced ninefold compared to the wild-type was the growth rate significantly affected, and the ldh mutants started to produce mixed-acid products (formate, acetate, and ethanol in addition to lactate). Flux control coefficients were determined and it was found that lactate dehydrogenase exerted virtually no control on the glycolytic flux at the wild-type enzyme level and also not on the flux catalyzed by the enzyme itself, i.e., on the lactate production. As expected, the flux towards the mixed-acid products was strongly enhanced in the strain deleted for lactate dehydrogenase. What is more surprising is that the enzyme had a strong negative control ($C_{LDH(F1)} = -1.3$) on the flux to formate at the wild-type level of lactate dehydrogenase. Furthermore, we showed that L. lactis has limited excess of capacity of lactate dehydrogenase, only 70% more than needed to catalyze the lactate flux in the wild-type cells.

**General information**

State: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Authors: Andersen, H. (Ekstern), Pedersen, M. (Ekstern), Hammer, K. (Intern), Jensen, P. R. (Intern)
Pages: 6379-6389
Publication date: 2001
Main Research Area: Technical/natural sciences

**Publication information**

Journal: European Journal of Biochemistry
Volume: 268
Issue number: 24
ISSN (Print): 0014-2956
Ratings:
BFI (2017): BFI-level 1
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 4.06
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.92
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.94
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 4.02
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.84
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 3.36
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
BFI (2009): BFI-level 2
BFI (2008): BFI-level 1
Web of Science (2004): Indexed yes
Web of Science (2003): Indexed yes
Web of Science (2002): Indexed yes
Web of Science (2001): Indexed yes
Web of Science (2000): Indexed yes
Original language: English
Source: orbit
Source-ID: 45804
Publication: Research - peer-review › Journal article – Annual report year: 2001

**Twofold reduction of phosphofructokinase activity in Lactococcus lactis results in strong decreases in growth rate and in glycolytic flux**

Two mutant strains of Lactococcus lactis in which the promoter of the las operon, harboring pfk, pyk, and ldh, were replaced by synthetic promoters were constructed. These las mutants had an approximately twofold decrease in the activity of phosphofructokinase, whereas the activities of pyruvate kinase and lactate dehydrogenase remained closer to the wild-type level. In defined medium supplemented with glucose, the growth rate of the mutants was reduced to 57 to 70% of wild-type levels and the glycolytic flux was reduced to 62 to 76% of wild-type levels. In complex medium growth was even further reduced. Surprisingly, the mutants still showed homolactic fermentation, which indicated that the limitation was different from standard glucose-limited conditions. One explanation could be that the reduced activity of
phosphofructokinase resulted in the accumulation of sugar-phosphates. Indeed, when one of the mutants was starved for glucose in glucose-limited chemostat, the growth rate could gradually be increased to 195% of the growth rate observed in glucose-saturated batch culture, suggesting that phosphofructokinase does affect the concentration of upstream metabolites. The pools of glucose-6-phosphate and fructose-6-phosphate were subsequently found to be increased two- to fourfold in the las mutants, which indicates that phosphofructokinase exerts strong control over the concentration of these metabolites.
Hemin reconstitutes the growth of an H+-ATPase negative mutant of Lactococcus lactis.

Investigation of flux control by the demand for ATP on the glycolytic flux in Escherichia coli and Lactococcus lactis

Is the glycolytic flux in Lactococcus lactis controlled by glycolysis itself?
The B, C and D Cell Cycle periods increase with increasing generation time in slowly growing cultures of Escherichia coli.
The Frequency of Mutators in Populations of Escherichia coli

General information
State: Published
Organisations: Department of Biotechnology
Authors: Boe, L. (Intern), Danielsen, M. (Ekstern), Knudsen, S. (Intern), Petersen, J. B. (Ekstern), Maymann, J. (Ekstern), Jensen, P. R. (Intern)
Pages: 47-55
Publication date: 2000
Main Research Area: Technical/natural sciences

Publication information
Journal: Mutation Research
Volume: 448
Issue number: 1
Original language: English
Source: orbit
Source-ID: 174886
Publication: Research - peer-review › Journal article – Annual report year: 2000

The membrane-bound H+-ATPase complex is essential for growth of Lactococcus lactis

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Købmann, B. J. (Intern), Nilsson, D. (Ekstern), Kuipers, O. P. (Ekstern), Jensen, P. R. (Intern)
Pages: 4738-4743
Publication date: 2000
Main Research Area: Technical/natural sciences

Publication information
Journal: Journal of Bacteriology
Volume: 182
Issue number: 17
ISSN (Print): 0021-9193
Ratings:
BFI (2017): BFI-level 1
What controls the growth rate of Escherichia coli? Is it transport after all?
Extensive regulation compromises the extent to which DNA gyrase controls DNA supercoiling and growth rate of Escherichia coli

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology, Division of Microbiology and Risk Assessment, National Food Institute, Free University
Authors: Jensen, P. R. (Intern), van der Weijden, C. C. (Ekstern), Jensen, L. B. (Intern), Westerhoff, H. V. (Ekstern), Snoep, J. L. (Ekstern)
Pages: 865-877
Publication date: 1999
Main Research Area: Technical/natural sciences

Publication information
Journal: European Journal of Biochemistry
Volume: 266
Issue number: 3
ISSN (Print): 0014-2956
Ratings:
BFI (2017): BFI-level 1
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 4.06
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 3.92
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 3.94
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 4.02
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 3.84
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): CiteScore 3.36
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
BFI (2009): BFI-level 2
BFI (2008): BFI-level 1
Web of Science (2004): Indexed yes
Web of Science (2003): Indexed yes
Web of Science (2002): Indexed yes
Web of Science (2001): Indexed yes
Web of Science (2000): Indexed yes
Original language: English
DOI: 10.1046/j.1432-1327.1999.00921.x
A METHOD OF IMPROVING THE PRODUCTION OF BIOMASS OR A DESIRED PRODUCT FROM A CELL

The production of biomass or a desired product from a cell can be improved by inducing conversion of ATP to ADP without primary effects on other cellular metabolites or functions which is achieved by expressing an uncoupled ATPase activity in said cell and incubating the cell with a suitable substrate to produce said biomass or product. This is conveniently done by expressing in said cell the soluble part (F\text{\textbeta}1?) of the membrane bound (F\text{\textbeta}0\text{\textbeta}1? type) H\textsuperscript{+}\textsuperscript{-} ATPase or a portion of F\text{\textbeta}1? exhibiting ATPase activity. The organism from which the F\text{\textbeta}1? ATPase or portions thereof is derived, or in which the F\text{\textbeta}1? ATPase or portions thereof is expressed, may be selected from prokaryotes and eukaryotes. In particular the DNA encoding F\text{\textbeta}1? or a portion thereof may be derived from bacteria and eukaryotic microorganisms such as yeasts, other fungi and cell lines of higher organisms and be selected from the group consisting of the gene encoding the F\text{\textbeta}1? subunit $g(b)$ or a portion thereof and various combinations of said gene or portion with the genes encoding the other F\text{\textbeta}1? subunits or portions thereof. The method can be used i.a. for optimizing the formation of biomass or a desired product by a cell by expressing different levels of uncoupled ATPase activity in the cell, incubating the cell on a suitable substrate, measuring the conversion rate of substrate into biomass or the desired product at each level of ATPase expression, and choosing a level of ATPase expression at which the conversion rate is optimized.
In this article, we review some of the expression systems that are available for Metabolic Control Analysis and Metabolic Engineering, and examine their advantages and disadvantages in different contexts. In a recent approach, artificial promoters for modulating gene expression in micro-organisms were constructed using synthetic degenerated oligonucleotides. From this work, a promoter library was obtained for Lactococcus lactis, containing numerous individual promoters and covering a wide range of promoter activities. Importantly, the range of promoter activities was covered in small steps of activity change. Promoter libraries generated by this approach allow for optimization of gene expression and for experimental control analysis in a wide range of biological systems by choosing from the promoter library promoters giving, e.g., 25%, 50%, 200%, and 400% of the normal expression level of the gene in question. If the relevant variable (e.g., the flux or yield) is then measured with each of these constructs, then one can calculate the control coefficient and determine the optimal expression level. One advantage of the method is that the construct which is found to have the optimal expression level is then, in principle, ready for use in the industrial fermentation process; another advantage is that the system can be used to optimize the expression of different enzymes within the same cell. (C) 1998 John Wiley & Sons, Inc.
Escherichia coli atp mutants, which lack a functional Hf-ATPase complex, are capable of growth on glucose but not on succinate or other C-4-dicarboxylates (Suc(-) phenotype). Suc(+) revertants of an atp deletion strain were isolated which were capable of growth on succinate even though they lack the entire Hf-ATPase complex. Complementation in trans with the yhiF gene suppressed the growth of the Suc(+) mutants on succinate, which implicates the yhiF gene product in the regulation of C-4-dicarboxylate metabolism. Indeed, when the E. coli C-4-dicarboxylate transporter (encoded by the dctA gene) was expressed in trans, the Suc(-) phenotype of the atp deletion strain reverted to Suc(+), which shows that the reason why the E. coli atp mutant is unable to grow aerobically on C-4-dicarboxylates is insufficient transport capacity for these substrates.
Experimental strategies to determine the control of glycolysis in Lactococcus lactis

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, University of Gothenburg
Pages: 261-267
Publication date: 1998

Host publication information
Title of host publication: Biothermokinetics In the Post Genomic Era
Place of publication: Göteborg
Publisher: Chalmers Reproservice
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 171681
Publication: Research - peer-review › Journal article – Annual report year: 1998

Growth of Escherichia coli on C4-dicarboxylates is significantly controlled by the C4-dicarboxylate transporter

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam, University of Gothenburg
Authors: Boogerd, F. C. (Ekstern), Boe, L. (Intern), Michelsen, O. (Intern), Jensen, P. R. (Intern), Larsson, C. (ed.) (Ekstern), Påhlman, I. (ed.) (Ekstern), Gustafsson, L. (ed.) (Ekstern)
Pages: 117-122
Publication date: 1998

Host publication information
Title of host publication: Biothermokinetics In The Post Genomic Era
Place of publication: Göteborg
Publisher: Chalmers Reproservice
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 171702
Publication: Research - peer-review › Article in proceedings – Annual report year: 1998

Hierarchical control of DNA supercoiling

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam, University of Gothenburg
Authors: Snoep, J. L. (Ekstern), van der Weijden, C. (Ekstern), Andersen, H. W. (Intern), Jensen, P. R. (Intern), Westerhoff, H. V. (Ekstern), Larsson, C. (ed.) (Ekstern), Påhlman, I. (ed.) (Ekstern), Gustafsson, L. (ed.) (Ekstern)
Pages: 157-162
Publication date: 1998

Host publication information
Title of host publication: Biothermokinetics In The Post Genomic Era
Hierarchical Control of the H+-ATPase on cytochrome expression in Escherichia coli

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam, University of Gothenburg
Authors: Koefoed, S. (Ekstern), Jensen, P. R. (Intern), Egger, L. (Ekstern), Westerhoff, H. V. (Ekstern), Snoep, J. L. (Ekstern), Larsson, C. (ed.) (Ekstern), Påhlman, I. (ed.) (Ekstern), Gustafsson, L. (ed.) (Ekstern)
Pages: 171-174
Publication date: 1998

Modeling of free-energy metabolism in Lactococcus lactis

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, University of Gothenburg
Pages: 215-220
Publication date: 1998

Synthetic promoters for experimental control analysis

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, University of Gothenburg
Pages: 11-17
Publication date: 1998
Synthetic promoters for Metabolic Engineering

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Authors: Købmand, B. J. (Intern), Andersen, H. W. (Intern), Schurmann, R. Å. (Intern), Hammer, K. (Intern), Jensen, P. R. (Intern)
Pages: 42-43
Publication date: 1998

Host publication information
Title of host publication: Streptococcal genetics; Genetics of the Streptococci, Enterococci and Lactococci
Place of publication: Washington
Publisher: American Society for Microbiology (ASM)
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 172039
Publication: Research - peer-review › Article in proceedings – Annual report year: 1998

The glycolytic flux in E. coli appears to be controlled by the demand for ATP

General information
State: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology, Chr. Hansen A/S, Vrije Universiteit Amsterdam, University of Gothenburg
Authors: Købmand, B. J. (Intern), Nilsson, D. (Ekstern), Snoep, J. L. (Ekstern), Westerhoff, H. V. (Ekstern), Jensen, P. R. (Intern), Larsson, C. (ed.) (Ekstern), Påhlman, I. (ed.) (Ekstern), Gustafsson, L. (ed.) (Ekstern)
Pages: 205-210
Publication date: 1998

Host publication information
Title of host publication: Biothermokinetics In The Post Genomic Era
Place of publication: Göteborg
Publisher: Chalmers Reproservice
Main Research Area: Technical/natural sciences
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Thermodynamics of complexity: The live Cell
Thermodynamics has always been a remarkable science in that it studies macroscopic properties that are only partially determined by the properties of individual molecules. Entropy and free energy only exist in constellations of more than a single molecule (degree of freedom). They are the so-called emergent properties. Tendency towards increased entropy is an essential determinant for the behaviour of ideal gas mixtures, showing that even in the simplest physical/chemical systems, (dys)organisation of components is crucial for the behaviour of systems. This presentation aims at illustrating the thesis that the aforesaid holds a fortiori for the living cell: Much of the essence of the live state depends more on the manner in which the molecules are organised than on the properties of single molecules.

This is due to the phenomenon of 'Complexity'. BioComplexity is defined here as the phenomenon that the behaviour of two functionally interacting biological components (molecules, protein domains, pathways, organelles) differs from the behaviour these components would exhibit in isolation from one another, where the difference should be essential for the maintenance and growth of the living state. For a true understanding of this BioComplexity, modern thermodynamic concepts and methods (nonequilibrium thermodynamics, metabolic and hierarchical control analysis) will be needed.

We shall propose to redefine nonequilibrium thermodynamics as: The science that aims at understanding the behaviour of nonequilibrium systems by taking into account both the molecular properties and the emergent properties that are due to (dys)organisation. This redefinition will free nonequilibrium thermodynamics from the limitations imposed by earlier near-
equilibrium assumptions, resolve the duality with kinetics, and bridge the apparent gap with metabolic control analysis. Subsequently, the complexity of the control of the energy metabolism of E. coli will be analysed in detail. New control theorems will be derived for newly defined control coefficients. It will become transparent that molecular genetic experimentation will allow one to penetrate into the mechanisms of the complex regulation of energy metabolism. (C) 1998 Elsevier Science B.V.

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam
Authors: Westerhoff, H. V. (Ekstern), Jensen, P. R. (Intern), Snoep, J. L. (Ekstern)
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BFI (2015): BFI-level 1
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Web of Science (2015): Indexed yes
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Scopus rating (2014): SJR 0.916 SNIP 1.489 CiteScore 2.56
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.628 SNIP 1.513 CiteScore 2.33
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.75 SNIP 1.424 CiteScore 2.1
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.588 SNIP 1.286 CiteScore 1.99
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.572 SNIP 1.246
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.778 SNIP 1.289
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.613 SNIP 1.245
Scopus rating (2007): SJR 0.801 SNIP 1.215
Scopus rating (2006): SJR 0.576 SNIP 1.119
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.623 SNIP 1.142
Scopus rating (2004): SJR 0.553 SNIP 1.091
Scopus rating (2003): SJR 0.759 SNIP 0.968
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 0.708 SNIP 0.965
The sequence of spacers between the consensus sequences modulates the strength of procaryotic promoters

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Authors: Jensen, P. R. (Intern), Hammer, K. (Intern)
Pages: 82-87
Publication date: 1998
Main Research Area: Technical/natural sciences

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Journal: Applied and Environemental Microbiology
Volume: 64
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Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Hierarchical control of electron-transfer

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam, Moscow State University
Authors: Westerhoff, H. V. (Ekstern), Jensen, P. R. (Intern), Egger, L. (Ekstern), van Heeswijk, W. C. (Ekstern), van Spanning, R. (Ekstern), Kholodenko, B. N. (Ekstern), Snoep, J. L. (Ekstern)
Publication date: 1997

Host publication information
Title of host publication: Biological Electron-transfer chains: genetics, composition and mode of operation
Place of publication: The Netherlands
Publisher: Kluwer Academic Publishers - Nato series
Main Research Area: Technical/natural sciences
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Publication: Research - peer-review » Article in proceedings – Annual report year: 1997

A method of converting ATP into ADP in a living cell

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Authors: Jensen, P. R. (Intern)
Publication date: 1996

Publication information
Original language: English
Main Research Area: Technical/natural sciences
Source: orbit
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Publication: Research » Patent – Annual report year: 1996
Changes in the cellular energy state affect the activity of the bacterial phosphotransferase system

Control of DNA supercoiling in the procaryotic cell.
DNA supercoiling depends on the phosphorylation potential in Escherichia coli.

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Netherlands Cancer Institute, Vrije Universiteit Amsterdam
Authors: Van Workum, M. (Ekstern), van Dooren, S. (Ekstern), Oldenburg, N. (Ekstern), Molenaar, D. (Ekstern), Jensen, P. R. (Intern), Snoep, J. (Ekstern), Westerhoff, H. (Ekstern)
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Main Research Area: Technical/natural sciences

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Volume: 20
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ISSN (Print): 0950-382X
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): SJR 2.543 SNIP 0.99 CiteScore 3.7
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.942 SNIP 1.087 CiteScore 3.95
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 3.176 SNIP 1.223 CiteScore 4.25
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 3.631 SNIP 1.303 CiteScore 4.9
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 3.405 SNIP 1.269 CiteScore 4.78
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 3.439 SNIP 1.293 CiteScore 4.72
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 3.63 SNIP 1.206
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 3.938 SNIP 1.331
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 3.89 SNIP 1.225
Web of Science (2008): Indexed yes
Web of Science (2007): Indexed yes
Low control by proton leak on the efficiency of oxidative phosphorylation in E. coli.

Energy buffering of DNA structure fails when Escherichia coli runs out of substrate
Energy, control and DNA structure in the living cell

Maintenance (let alone growth) of the highly ordered living cell is only possible through the continuous input of free energy. Coupling of energetically downhill processes (such as catabolic reactions) to uphill processes is essential to provide this free energy and is catalyzed by enzymes either directly or via "storage" in an intermediate high energy form, i.e., highATP/ADP ratio or H+ ion gradient. Although maintenance of a sufficiently high ATP/ADP ratio is essential to overcome the thermodynamic burden of uphill processes, it is not clear to what degree enzymes that control this ratio also control cell physiology. Indeed, in the living cell homeostatic control mechanisms might exist for the free-energy transduction pathways so as to prevent perturbation of cellular function when the Gibbs energy supply is compromised. This presentation addresses the extent to which the intracellular ATP level is involved in the control of cell physiology, how the elaborate control of cell function may be analyzed theoretically and quantitatively, and if this can be utilized selectively to affect certain cell types.

General information

State: Published
Organisations: Department of Microbiology, University of Amsterdam
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Publication information

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Volume: 55
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Ratings:
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BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.784 SNIP 0.78 CiteScore 2.39
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.894 SNIP 0.876 CiteScore 2.29
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.946 SNIP 0.811 CiteScore 2.03
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.037 SNIP 1.009 CiteScore 2.51
ISI indexed (2013): ISI indexed yes
Experimental determination of control by the H\(^+\)-ATPase in Escherichia coli

Strains carrying deletions in the atp genes, encoding the H+-ATPase, were unable to grow on nonfermentable substrates such as succinate, whereas with glucose as the substrate the growth rate of an atp deletion mutant was surprisingly high (some 75-80% of wild-type growth rate). The rate of glucose and oxygen consumption of these mutants was increased compared to the wild-type rates. In order to analyze the importance of the H+-ATPase at its physiological level, the cellular concentration of H+-ATPase was modulated around the wild-type level, using genetically manipulated strains. The control coefficient by the H+-ATPase with respect to growth rate and catabolic fluxes was measured. Control on growth rate was absent at the wildtype concentration of H+-ATPase, independent of whether the substrate for growth was glucose or succinate. Control by the H+-ATPase on catabolism, including respiration, was negative at the wild-type H+-ATPase level. Moreover, the turnover number of the individual H+-ATPase enzymes increased as the H+-ATPase concentration was lowered. The negative control by the H+-ATPase on catabolism may thus be involved in a homeostatic control of ATP synthesis and, to some extent, explain the zero control by the H+-ATPase on E. coli growth rate.
Hierarchies in control

The living cell functions by virtue of an enormous number of different processes. It is one of the most difficult challenges of modern biology to elucidate how all those processes are coordinated quantitatively so as to lead to a viable system with optimal responses to various changes in the environment. The biochemical and biophysical processes of the living cell do not constitute a network with random connections. In this paper we shall discuss that cell function is organized in hierarchical substructures. We will briefly touch on the topics of (i) metabolic control and regulated gene expression, (ii) time dependent metabolism in intact yeast cells, and (iii) metabolite channelling.
Molecular biology for flux control

General information
State: Published
Organisations: Department of Microbiology
Authors: Jensen, P. R. (Intern), Snoep, J. (Ekstern), Molenaar, D. (Ekstern), Vanheeswijk, W. (Ekstern), Kholodenko, B. (Ekstern), Vangrugten, A. (Ekstern), Westerhoff, H. (Ekstern)
Number of pages: 4
Pages: 367-370
Publication date: 1995
Main Research Area: Technical/natural sciences

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Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.99 SJR 1.825 SNIP 0.628
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.96 SNIP 0.714 CiteScore 3.04
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.086 SNIP 0.783 CiteScore 3.23
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 2.011 SNIP 0.77 CiteScore 3.23
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 2.063 SNIP 0.788 CiteScore 3.33
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 2.573 SNIP 0.915 CiteScore 3.89
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.469 SNIP 0.945
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.298 SNIP 0.809
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 2.207 SNIP 0.783
Scopus rating (2007): SJR 2.358 SNIP 0.786
Scopus rating (2006): SJR 2.166 SNIP 0.787
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.922 SNIP 0.791
Scopus rating (2004): SJR 1.829 SNIP 0.676
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.41 SNIP 0.604
Scopus rating (2002): SJR 1.175 SNIP 0.481
Scopus rating (2001): SJR 0.549 SNIP 0.248
Scopus rating (2000): SJR 0.361 SNIP 0.188
Scopus rating (1999): SJR 0.371 SNIP 0.202
Original language: English

Enzymes, Escherichia coli, Homeostasis, Kinetics, Models, Biological, Molecular Biology, Proton-Translocating ATPases, EC 3.6.3.14 Proton-Translocating ATPases, METABOLIC CONTROL-THEORY, OXIDATIVE-PHOSPHORYLATION,
Structure and partitioning of bacterial DNA: determined by a balance of compaction and expansion forces?
The mechanisms that determine chromosome structure and chromosome partitioning in bacteria are largely unknown. Here we discuss two hypotheses: (i) the structure of the Escherichia coli nucleoid is determined by DNA binding proteins and DNA supercoiling, representing a compaction force on the one hand, and by the coupled transcription/translation/translocation of plasma membrane and cell wall proteins, representing an expansion force on the other hand; (ii) the two forces are important for the partitioning process of chromosomes.

General information
State: Published
Organisations: Department of Microbiology
Authors: Woldringh, C. L. (Ekstern), Jensen, P. R. (Intern), Westerhoff, H. V. (Ekstern)
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Main Research Area: Technical/natural sciences

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Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 1.76 SJR 0.747 SNIP 0.597
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.131 SNIP 0.752 CiteScore 2.08
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BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.122 SNIP 0.767 CiteScore 2.17
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.043 SNIP 0.72 CiteScore 2.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.069 SNIP 0.817 CiteScore 2.25
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.096 SNIP 0.761 CiteScore 2.26
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.07 SNIP 0.756
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.11 SNIP 0.835
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.067 SNIP 0.827
Scopus rating (2007): SJR 1.095 SNIP 0.859
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.091 SNIP 0.851
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 0.984 SNIP 0.798
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 0.989 SNIP 0.723
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.004 SNIP 0.87
Scopus rating (2002): SJR 0.94 SNIP 0.788
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 0.943 SNIP 0.75
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 0.88 SNIP 0.737
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 0.965 SNIP 0.753

Original language: English
Chromosomes, Bacterial, DNA Replication, DNA, Bacterial, DNA, Superhelical, DNA-Binding Proteins, Escherichia coli, Models, Genetic, Nucleic Acid Conformation, MICROBIOLOGY, ESCHERICHIA-COLI, GENE-EXPRESSION, TOPOISOMERASE-I, CELL-DIVISION, CHROMOSOME, PROTEIN, TRANSCRIPTION, MUTANTS, EXPORT, CYCLE, DNA SUPERCOILING, NUCLEOID STRUCTURE, COUPLED TRANSCRIPTION TRANSLATION, PROTEIN TRANSLOCATION, DNA supercoiling, Nucleoid structure, Coupled transcription/translation, Protein translocation, CELL WALL, CHROMOSOME STRUCTURE, DNA BINDING PROTEINS, PLASMA MEMBRANE, SUPERCOILING, TRANSLATION, TRANSLOCATION, Facultatively Anaerobic Gram-Negative Rods Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Enterobacteriaceae [06702] Escherichia coli, 10010, Comparative biochemistry, 10052, Biochemistry methods - Nucleic acids, purines and pyrimidines, 10062, Biochemistry studies - Nucleic acids, purines and pyrimidines, 10064, Biochemistry studies - Proteins, peptides and amino acids, 10300, Replication, transcription, translation, 10504, Biophysics - Methods and techniques, 10506, Biophysics - Molecular properties and macromolecules, 13012, Metabolism - Proteins, peptides and amino acids, 13014, Metabolism - Nucleic acids, purines and pyrimidines, 30500, Morphology and cytology of bacteria, 31000, Physiology and biochemistry of bacteria, 31500, Genetics of bacteria and viruses, 32300, Microbiological ultrastructure, Biochemistry and Molecular Biophysics, Cell Biology, Genetics, Metabolism, Methods and Techniques, Molecular Genetics, Physiology

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Control and Regulation: The best of two worlds

General information
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Organisations: Department of Microbiology
Authors: Westerhoff, H. V. (Ekstern), Jensen, P. R. (Intern), Rohwer, J. (Ekstern), Kholodenco, B. N. (Ekstern)
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Publisher: Innsbruck Univ. Press
Control of Dynamics and Steady state; theory and applications to multidrug resistance

How to determine control of growth rate in a chemostat. Using metabolic control analysis to resolve the paradox

How to determine control of growth rate in a chemostat. Using metabolic control analysis to resolve the paradox

Modelling of Oxidative Phosphorylation in E. coli
Control analysis of the dependence of Escherichia coli physiology on the $\text{H}^+\text{-ATPase}$

The $\text{H}^+\text{-ATPase}$ plays a central role in Escherichia coli free-energy transduction and hence in E. coli physiology. We here investigate the extent to which this enzyme also controls the growth rate, growth yield, and respiratory rate of E. coli. We modulate the expression of the atp operon and determine the effect on said properties. When quantified in terms of control coefficients, we find that, in the wild-type cell growing on glucose in minimal medium, this key enzyme ($\text{H}^+\text{-ATPase}$) exerts virtually no control on growth rate ($\text{VBR C} \lt 0.01$), a minor positive control on growth yield ($\text{C} = 0.15$), and a small but negative control on respiration rate ($\text{C} = -0.25$). The control the enzyme exerts on the consumption rate of the carbon and free-energy substrate is negative ($\text{C} = -0.15$). We also studied how the control coefficients themselves vary with the expression of the atp operon. As the level of expression of the atp operon was reduced, the control exerted by the $\text{H}^+\text{-ATPase}$ on growth rate and growth yield increased slightly; the control on growth rate passed through a maximum ($\text{C} = 0.1$) and disappeared when the atp operon was not expressed at all, reflecting that with this substrate there are alternative routes for ATP synthesis. At elevated levels of the $\text{H}^+\text{-ATPase}$ compared to the wild type, the control exerted by the enzyme on growth rate became negative. The evolutionary context of the absence of control by the atp operon on growth rate is discussed.
Excess capacity of $\text{H}^+$ ATPase and inverse respiratory control in Escherichia coli

With succinate as free-energy source, Escherichia coli generating virtually all ATP by oxidative phosphorylation might be expected heavily to tax its ATP generating capacity. To examine this the H+-ATPase (ATP synthase) was modulated over a 30-fold range. Decreasing the amount of H+-ATPase reduced the growth rate much less than proportionally; the H+-ATPase controlled growth rate by <10%. This lack of control reflected excess capacity: the rate of ATP synthesis per H+-ATPase (the turnover number) increased by 60% when the number of enzymes was decreased by 40%. At 15% H+-ATPase, the enzyme became limiting and its turnover was increased even further, due to an increased driving force caused by a reduction in the total flux through the enzymes. At smaller reductions of (H+-ATPase) the total flux was not reduced, revealing a second cause for increased turnover number through increased membrane potential: respiration was increased showing that in E. coli, respiration and ATP synthesis are, in part, inversely coupled. Indeed, growth yield per O$_2$ decreased, suggesting significant leakage or slip at the high respiration rates and membrane potential found at low H+-ATPase concentrations, and explaining that growth yield may be increased by activating the H+-ATPase.
Minimal requirements for exponential growth of *Lactococcus lactis*

A minimal growth medium containing glucose, acetate, vitamins, and eight amino acids allowed for growth of *Lactococcus lactis* subsp. *lactis*, with a specific growth rate in batch culture of $\mu = 0.3 \, h^{-1}$. With 19 amino acids added, the growth rate increased to $\mu = 0.7 \, h^{-1}$ and the exponential growth phase proceeded until high cell concentrations were reached. We show that morpholinepropanesulfonic acid (MOPS) is a suitable buffer for *L. lactis* and may be applied in high concentrations.
Scopus rating (2009): SJR 1.972 SNIP 1.528
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 2.043 SNIP 1.647
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 2.054 SNIP 1.602
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 2.074 SNIP 1.653
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 2.108 SNIP 1.648
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 2.097 SNIP 1.821
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 2.046 SNIP 1.754
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.989 SNIP 1.736
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.957 SNIP 1.758
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Scopus rating (1999): SJR 2.3 SNIP 1.732
Original language: English
ACETATE, AMINO ACIDS, GLUCOSE, GROWTH MEDIUM, MORPHOLINEPROPANESULFONIC ACID BUFFER,
VITAMINS, Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Gram-Positive Cocci [07700]
gram-positive cocci Lactococcus lactis, ACETATE 71-50-1, GLUCOSE 50-99-7Q, 58367-01-4Q, 10060, Biochemistry
studies - General, 10063, Biochemistry studies - Vitamins, 10064, Biochemistry studies - Proteins, peptides and amino
acids, 10068, Biochemistry studies - Carbohydrates, 31000, Physiology and biochemistry of bacteria, 32000,
Microbiological apparatus, methods and media, Biochemistry and Molecular Biophysics, Methods and Techniques,
Physiology, BACTERIA, LACTOCOCCUS, MEDIA, MICROBIOLOGICAL TECHNIQUES, Food sciences
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Modulation of cellular energy state and DNA supercoiling in E. coli

General information
State: Published
Organisations: Department of Microbiology, National Food Institute
Authors: Jensen, P. R. (Intern), Oldenburg, N. (Ekstern), Petra, B. (Ekstern), Michelsen, O. (Intern), Westerhoff, H.
(Ekstern)
Number of pages: 6
Pages: 391-396
Publication date: 1993

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Title of host publication: Modern trends in biothermokinetics : 5th International Meeting on Biothermokinetics
Publisher: Plenum
Editor: Schuster et al.
ISBN (Print): 0306445794
Main Research Area: Technical/natural sciences
PHYSIOLOGY, ENDOCRINOLOGY, CYTOLOGY, BIOPHYSICS
Source: FindIt
Source-ID: 147346643
Publication: Research - peer-review › Article in proceedings – Annual report year: 1993

Multiplicity of control

General information
The use of lac-type promoters in control analysis

For control analysis, it is necessary to modulate the activity of an enzyme around its normal level and measure the changes in steady-state fluxes or concentrations. We describe an improved method for effecting the modulation, as elaborated for Escherichia coli. The chromosomal gene, encoding the enzyme of interest, is put under the control of a lacUV5 or a tacI promoter. The alternative use of the two promoters leads to an expression range which should make it suitable for the use in control analysis of many enzymes. The lacUV5 promoter should be used when the wild-type expression level is low, the tacI promoter when the latter is high. The endogenous lac operon is placed under the control of a second copy of the lacUV5 promoter and a lacYam mutation (eliminating lactose permease, the transport system for the inducer isopropyl-thio-beta-D-galactoside) is introduced. The method was demonstrated experimentally by constructing E. coli strains, in which the chromosomal atp operon is transcribed from the lacUV5 and the tacI promoter. We measured the concentration of the c subunit of H+-ATPase, and found that the expression of this enzyme could be modulated between non-detectable levels and up to five times the wild-type level. Thus, in the absence of inducer, no expression of atp genes could be detected when the atp operon was controlled by the lacUV5 promoter, and we estimate that the expression was less than 0.0025 times the wild-type level. We show that the introduction of a lac Y mutation facilitated the attainment of steady induction levels of partially induced cells. The mutation also reduced positive cooperativity in the dependence of expression on the concentration of isopropyl-thio-beta-D-galactoside (the inducer) and shifted the concentration of inducer needed for half maximum induction to higher values. These properties should facilitate the experimental modulation of the enzyme activity by varying the concentration of the inducer.
Carbon and energy metabolism of atp mutants of Escherichia coli

The membrane-bound H+-ATPase plays a key role in free-energy transduction of biological systems. We report how the carbon and energy metabolism of Escherichia coli changes in response to deletion of the atp operon that encodes this enzyme. Compared with the isogenic wild-type strain, the growth rate and growth yield were decreased less than expected for a shift from oxidative phosphorylation to glycolysis alone as a source of ATP. Moreover, the respiration rate of a atp deletion strain was increased by 40% compared with the wild-type strain. This result is surprising, since the atp deletion strain is not able to utilize the resulting proton motive force for ATP synthesis. Indeed, the ratio of ATP concentration to ADP concentration was decreased from 19 in the wild type to 7 in the atp mutant, and the membrane potential of the atp deletion strain was increased by 20%, confirming that the respiration rate was not controlled by the magnitude of the opposing membrane potential. The level of type b cytochromes in the mutant cells was 80% higher than the level in the wild-type cells, suggesting that the increased respiration was caused by an increase in the expression of the respiratory genes. The atp deletion strain produced twice as much by-product (acetate) and exhibited increased flow through the tricarboxylic acid cycle and the glycolytic pathway. These three changes all lead to an increase in substrate level phosphorylation; the first two changes also lead to increased production of reducing equivalents. We interpret these data
as indicating that E. coli makes use of its ability to respire even if it cannot directly couple this ability to ATP synthesis; by respiring away excess reducing equivalents E. coli enhances substrate level ATP synthesis.
Uncoupler resistance in E. coli Tuv and Cuv is due to the exclusion of uncoupler by the outer membrane

The uncoupler resistant bacterial strains E. coli Tuv and Cuv share the high deoxycholate sensitivity of the parent strain, Doc S. However, both Tuv and Cuv show greater resistance than Doc S to other detergents. Measurement of the periplasmic volume indicates that the outer membrane of Doc S is freely permeable to both TPP+ and hydroxymethylinulin. Tuv and Cuv are able to exclude these compounds. EDTA treatment was necessary prior to measuring membrane potentials in Tuv and Cuv. Under conditions where Δψ could be measured, uncouplers acted to dissipate Δψ with equal potency in all strains. Uncoupler resistant proline uptake in Tuv and Cuv was abolished by EDTA treatment. Transduction experiments with phage P1 showed that uncoupler resistance could be transferred from Tuv to Doc S. Such transductants were no longer sensitive to novobiocin. The gene for uncoupler resistance cotransduced with the gene pyrE (82 min). Plating efficiency experiments with P1 suggests that detergent sensitivity in Doc S arises from an rfa (81 min) mutation. This mutation is no longer present in Tuv.
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 4.79
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 4.94
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 4.79
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 5.17
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.56
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
BFI (2009): BFI-level 1
BFI (2008): BFI-level 2
Web of Science (2004): Indexed yes
Web of Science (2002): Indexed yes
Original language: English
CCCP carbonylcyanide-m-chlorophenylhydrazone, TTFB 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole, TPP + tetraphenylphosphonium cation, SDS sodium dodecyl sulphate
Source: FindIt
Source-ID: 78453662
Publication: Research - peer-review › Journal article – Annual report year: 1990

Projects:

**Improving the thermotolerance of the mesophilic starter**

National Food Institute
Period: 01/06/2017 → 31/05/2020
Number of participants: 3
Phd Student:
Dorau, Robin (Ekstern)
Supervisor:
Jensen, Peter Ruhdal (Intern)
Main Supervisor:
Solem, Christian (Intern)

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

**Improving the thermotolerance of the mesophilic starter**

National Food Institute
Period: 01/06/2017 → 31/05/2020
Number of participants: 3
Phd Student:
Dorau, Robin (Intern)
Supervisor:
Jensen, Peter Ruhdal (Intern)
Main Supervisor:
Solem, Christian (Intern)

**Financing sources**
Source: Internal funding (public)
Name of research programme: Samfinansieret - Andet
**ALLEVIATE - A novel strategy for food allergy prevention and treatment**

Food allergy is an adverse effect to otherwise harmless proteins in the food, whereas oral tolerance is the default result from ingestion of food proteins. Food allergy is a major health problem of growing concern, affecting ~5-8% of young children and 2-4% of adults. No reliable strategy exists for prevention and treatment of food allergy, and strict avoidance of the offending food is presently the only viable management option. Living with food avoidance has a huge impact on the quality of life of food allergic patients, with daily fear of serious or even fatal reactions. The need for efficient methods for prevention and treatment is therefore evident and urgent.

The purpose of the project is to develop methods to prevent and treat food allergy using a novel strategy, recently invented. Our vision is to overcome limitations in current strategies for food allergy prevention and treatment; being efficient without inducing allergic reactions.

The specific goals of the project are:
1) To develop protein ingredients for a new generation of hypoallergenic (HA) infant formulas (IF) for cow’s milk allergy (CMA) prevention
2) To develop a drug candidate for use in immunotherapy (IT) for peanut allergy (PA) treatment

These products would have the capacity to enhance the quality of life for millions of patients in risk of developing CMA and of patients with an already established PA. The market potential is great for both product categories. In addition, the newly developed strategy may form the basis for prevention, treatment and diagnostic products targeting other food allergies.

National Food Institute
Research Group for Gut Microbiology and Immunology
Department of Chemistry
Organic Chemistry
Research Group for Microbial Biotechnology and Biorefining
Office for Innovation & Sector Services
Medical University of Vienna
University of Toronto
University of Leeds

Arla Foods Ingredients Group P/S
Period: 01/01/2017 → 31/12/2020
Number of participants: 7
Food Allergy, Immunotherapy, Infant formula, Allergy, Milk allergy, Peanut allergy
Acronym: ALLEVIATE
Project participant:
- Madsen, Charlotte Bernhard (Intern)
- Kryger, Karsten (Intern)
- Qvortrup, Katrine (Intern)
- Jensen, Peter Ruhdal (Intern)
- Bang-Berthelsen, Claus Heiner (Intern)
- Hulgaard, Egil (Intern)

Project Manager, academic:
- Bøgh, Katrine Lindholm (Intern)

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**Biofuels of the future - Development of a Lactic Acid Bacteria platform for sustainable production of higher alcohols**

National Food Institute
Period: 01/11/2015 → 31/10/2018
Number of participants: 4
Phd Student:
- Mar, Mette Jurlander (Intern)

Supervisor:
- Kandasamy, Vijayalakshmi (Intern)

Solem, Christian (Intern)
Main Supervisor:
- Jensen, Peter Ruhdal (Intern)
Bioconversion of Lignocellulose to Free Fatty Acids Using Yeast

National Food Institute
Period: 01/12/2014 → 01/12/2018
Number of participants: 4
Phd Student: Suo, Fan (Intern)
Supervisor: Dantoft, Shruti Harnal (Intern)
Pedersen, Per Amstrup (Ekstern)
Main Supervisor: Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Stipendie fra udlandet
Project: PhD

Lactic Acid Bacteria as cell factories

National Food Institute
Period: 01/06/2014 → 30/09/2017
Number of participants: 6
Phd Student: Liu, Jianming (Intern)
Supervisor: Jensen, Peter Ruhdal (Intern)
Main Supervisor: Solem, Christian (Intern)
Examiner: Hansen, Egon Bech (Intern)
Kleerebezem, Michiel (Ekstern)
Zeng, An-Ping (Ekstern)

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

Lysine production in Gram-positive bacteria

National Food Institute
Period: 01/06/2014 → 17/02/2015
Number of participants: 3
Phd Student: Grishkova, Maria (Intern)
Supervisor: Solem, Christian (Intern)
Main Supervisor: Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD
Protein production in Gram-positive bacteria under adverse conditions
National Food Institute
Period: 01/04/2014 → 30/09/2017
Number of participants: 6
Phd Student:
Vestergaard, Mike (Intern)
Supervisor:
Solem, Christian (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Bang, Dang Duong (Intern)
Jönsson, Håkan N. (Ekstern)
Mijakovic, Ivan (Intern)

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

Development of new diagnostic technologies
National Food Institute
Period: 01/08/2013 → 22/12/2016
Number of participants: 7
Phd Student:
Chin, Wai Hoe (Intern)
Supervisor:
Sun, Yi (Intern)
Wolff, Anders (Intern)
Main Supervisor:
Bang, Dang Duong (Intern)
Examiner:
Jensen, Peter Ruhdal (Intern)
Ingmer, Hanne (Ekstern)
Sjöback, Robert (Ekstern)

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

Metabolic optimization of Corynebacterium glutamicum for enhanced lysine production
National Food Institute
Period: 15/12/2012 → 21/04/2016
Number of participants: 6
Phd Student:
Wang, Zhihao (Intern)
Supervisor:
Jensen, Peter Ruhdal (Intern)
Main Supervisor:
Solem, Christian (Intern)
Examiner:
Hobley, Timothy John (Intern)
Kalinowski, Jörn (Ekstern)
Mijakovic, Ivan (Intern)
Production of organic acids in Gram - positive bacteria

National Food Institute
Period: 01/12/2012 → 03/05/2018
Number of participants: 3
Phd Student:
Shen, Jing (Intern)
Supervisor:
Jensen, Peter Ruhdal (Intern)
Main Supervisor:
Solem, Christian (Intern)

Improving second generation biorefinery processes using clues from stress response in Lactococcus lactis

Department of Systems Biology
Period: 15/10/2012 → 15/03/2017
Number of participants: 6
Phd Student:
Hviid, Anne-Mette Meisner (Intern)
Supervisor:
Jensen, Peter Ruhdal (Intern)
Main Supervisor:
Kilstrup, Mogens (Intern)
Examiner:
Gram, Lone (Intern)
Kok, Jan (Ekstern)
Neves, Ana Rute (Intern)

The potential of Lactic Acid Bacteria as microbial factory for pentanol isomer production

Department of Systems Biology
Period: 01/06/2012 → 05/06/2017
Number of participants: 5
Phd Student:
Starlit, Karen Imbæk (Ekstern)
Supervisor:
Jørgensen, Sten Troels (Ekstern)
Købmann, Brian (Intern)
Martinussen, Jan (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD
Lactic Acid Bacteria as a new platform for sustainable production of biochemicals

Department of Systems Biology
Number of participants: 6
Phd Student:
Boguta, Anna Monika (Intern)
Supervisor:
Jensen, Peter Ruhdal (Intern)
Main Supervisor:
Martinussen, Jan (Intern)
Examiner:
Kilstrup, Mogens (Intern)
Holo, Helge (Ekstern)
Sørensen, Kim (Intern)

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

Relations
Publications:
Lactic Acid Bacteria as a new platform for sustainable production of fuels and chemicals
Project: PhD

Elucidating and comparing flux regulation across bacterial species

Department of Systems Biology
Period: 15/11/2011 → 27/05/2015
Number of participants: 6
Phd Student:
Chan, Siu Hung Joshua (Intern)
Supervisor:
Solem, Christian (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Hobley, Timothy John (Intern)
Molenaar, Douwe (Ekstern)
Snoep, Jacob Leendert (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD

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

Department of Systems Biology
Period: 01/11/2011 → 28/03/2014
Number of participants: 6
Phd Student:
Chen, Jun (Intern)
Supervisor:
Solem, Christian (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Kilstrup, Mogens (Intern)
Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD

Transforming Lactococcus lactis into a microbial cell factory

Department of Systems Biology
Period: 01/01/2011 → 30/09/2014
Number of participants: 7
Phd Student:
Petersen, Kia Vest (Intern)
Supervisor:
Jensen, Peter Ruhdal (Intern)
Martinussen, Jan (Intern)
Main Supervisor:
Solem, Christian (Intern)
Examiner:
Mijakovic, Ivan (Intern)
Jørgensen, Sten Troels (Ekstern)
Kok, Jan (Ekstern)

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

Biofuels production in yeast

National Food Institute
Period: 01/12/2010 → 29/05/2017
Number of participants: 5
Phd Student:
Phadnavis, Ambareesh Govind (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Solem, Christian (Intern)
Blank, Lars M. (Ekstern)
Pedersen, Per Amstrup (Ekstern)

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

Enzyme Immobilisation and Bioprocessing

National Food Institute
Period: 15/11/2010 → 04/06/2014
Number of participants: 6
Phd Student:
Alftrén, Johan (Intern)
Supervisor:
Koski, Mařa (Intern)
Main Supervisor:
Hobley, Timothy John (Intern)
A Microbial Platform based on Non-Conventional Yeasts

Department of Systems Biology
Period: 01/11/2010 → 14/06/2013
Number of participants: 4
Phd Student: Holt, Philippe (Intern)
Supervisor: Jensen, Peter Ruhdal (Intern)
Thykaer, Jette (Intern)
Main Supervisor: Workman, Mhairi (Intern)

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

Pseudomonas species as a platform for biofuels and biochemicals

Department of Systems Biology
Period: 01/10/2010 → 26/05/2016
Number of participants: 7
Phd Student: Wigneswaran, Vinoth (Intern)
Supervisor: Folkesson, Anders (Intern)
Jensen, Peter Ruhdal (Intern)
Main Supervisor: Jelsbak, Lars (Intern)
Examiner: Molin, Søren (Intern)
Burmølle, Mette (Ekstern)
Segura, Ana (Ekstern)

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

Comparative Systems Biology

Department of Systems Biology
Period: 01/01/2010 → 18/12/2013
Number of participants: 6
Phd Student: Dehli, Tore Ibsen (Intern)
**Modningsforløbet i ostekorn, analyseret for lactococcus stammer med flere kromosomer**

*Department of Systems Biology*
*Period: 01/10/2009 → 30/06/2014*
*Number of participants: 2*
*Phd Student: Pedersen, Malene Mejer (Intern)*
*Main Supervisor: Jensen, Peter Ruhdal (Intern)*

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

**A systematic approach to identify new targets for control of pathogenic bacteria**

*Department of Systems Biology*
*Period: 15/06/2009 → 29/10/2010*
*Number of participants: 3*
*Phd Student: Hartman, Hassan (Ekstern)*
*Supervisor: Jensen, Peter Ruhdal (Intern)*
*Main Supervisor: Kilstrup, Mogens (Intern)*

**Financing sources**
*Source: Internal funding (public)*
*Name of research programme: Forskningsrådsfinansiering*
*Project: PhD*

**Stokastisk dynamisk modellering til kort-tidsregulering af glukose/insulin-metabolismen**

*Department of Informatics and Mathematical Modeling*
*Period: 01/06/2009 → 12/12/2013*
*Number of participants: 6*
*Phd Student: Duun-Henriksen, Anne Katrine (Intern)*
*Supervisor: Jensen, Peter Ruhdal (Intern)*
*Main Supervisor: Madsen, Henrik (Intern)*
*Examiner: Christiansen, Lasse Engbo (Intern)*
*Andreassen, Steen (Ekstern)*
*Gabrielsson, Johan (Ekstern)*

**Financing sources**
*Source: Internal funding (public)*
*Name of research programme: Forskningsrådsfinansiering*
*Project: PhD*
Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD

Development of Model Systems for the Biodegradation of Glycerol
Department of Systems Biology
Period: 01/03/2009 → 20/08/2012
Number of participants: 6
Phd Student:
Liu, Xiaoying (Intern)
Supervisor:
Jensen, Peter Ruhdal (Intern)
Main Supervisor:
Workman, Mhairi (Intern)
Examiner:
Eliasson Lantz, Anna (Intern)
Jørgensen, Henning (Intern)
Walker, Graeme M. (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Anden EU-finansiering
Project: PhD

Optimering af fermenteringsprocessen til lysin produktion
Department of Systems Biology
Period: 15/12/2008 → 28/03/2014
Number of participants: 5
Phd Student:
Rytter, Jakob Vang (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Martinussen, Jan (Intern)
Blank, Lars Mathias (Ekstern)
Pedersen, Per Amstrup (Ekstern)

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

Udvikling af mikroorganismer til biobrændselsproduktion
Department of Systems Biology
Period: 01/12/2008 → 01/03/2013
Number of participants: 7
Phd Student:
Hansen, Anders Cai Holm (Intern)
Supervisor:
Solem, Christian (Intern)
Workman, Mhairi (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Kilstrup, Mogens (Intern)
Købmann, Brian (Intern)
**Protein-Tyrosine Phosphorylation in Bacillus Subtilis Signal Transduction**

Department of Systems Biology  
Period: 15/03/2007 → 22/09/2010  
Number of participants: 6  
Phd Student: Jers, Carsten (Intern)  
Main Supervisor: Mijakovic, Ivan (Intern)  
Examiners: Jelsbak, Lars (Intern), Grangeasse, Christophe (Ekstern), Stülke, Jörg Michael (Ekstern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Anden EU-finansiering  
Project: PhD

**Analysis of insulin binding by systematic amino acid scanning mutagenesis importance of insulin B chain residues for receptor isoform binding**

Department of Systems Biology  
Number of participants: 6  
Phd Student: Glendorf, Tine (Ekstern)  
Main Supervisor: Jensen, Peter Ruhdal (Intern)  
Examiners: Gammeltoft, Steen (Ekstern), Mortensen, Uffe Hasbro (Intern), Siddle, Kenneth (Ekstern)  

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

**Brug af Bacillus Subtilis til Produktion af et naturligt aromastof**

Department of Systems Biology  
Period: 01/02/2005 → 23/09/2009  
Number of participants: 7  
Phd Student: Hansen, Mette (Intern)  
Main Supervisor: Hansen, Egon Bech (Intern)  
Examiners: Mijakovic, Ivan (Intern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: ErhvervsPhD-ordningen VTU  
Project: PhD
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Kilstrup, Mogens (Intern)
Kuipers, Oscar Paul (Ekstern)
Mascher, Thorsten (Ekstern)

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

Kontrolanalyse af ethanol produktion i Saccharomyces Cerevisiae
Department of Systems Biology
Period: 01/03/2004 → 29/04/2009
Number of participants: 6
Phd Student:
Helmark, Søren (Intern)
Supervisor:
Købmann, Brian (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Martinussen, Jan (Intern)
Hartmann-Petersen, Rasmus (Ekstern)
Mijakovic, Ivan (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Forskningsrådsfinansiering
Project: PhD

Kontrol analyse af ethanol produktion i Saccharomyces cerevisiae
Bacterial Physiology and Genetics Group
Department of Systems Biology
Period: 01/01/2004 → 12/07/2008
Number of participants: 1
Project Manager, organisational:
Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Forskningsrådene - STVF
Name of research programme: Forskningsrådene - STVF
Amount: 1,700,000.00 Danish Kroner
Project

The role of post-transitional modifications in the control of carbon metabolism of Gram positive bacteria
Bacterial Physiology and Genetics Group
Department of Systems Biology
Period: 01/01/2004 → 31/12/2004
Number of participants: 1
Project Manager, organisational:
Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Forskningsrådene - SNF
Name of research programme: Forskningsrådene - STVF
Amount: 487,862.00 Danish Kroner
Mælk - diagnosticering af bakteriofaginficerede celler i syrningsprocesser
Bacterial Physiology and Genetics Group
Department of Systems Biology
Period: 01/08/2003 → 31/07/2006
Number of participants: 1
Project Manager, organisational:
Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Forskningsprojekter - Fødevareministeriet
Name of research programme: Forskningsprojekter - Fødevareministeriet
Amount: 3,429,851.00 Danish Kroner

Mælk, syrningsaktivitet af den primære starter
Bacterial Physiology and Genetics Group
Department of Systems Biology
Period: 01/01/2003 → 31/12/2006
Number of participants: 1
Project ID: 93S 24FH Å02 00016
Project Manager, organisational:
Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Forskningsprojekter - Fødevareministeriet
Name of research programme: Forskningsprojekter - Fødevareministeriet
Amount: 5,577,954.00 Danish Kroner

Kontrol af Biofilmdannelse
Department of Systems Biology
Period: 01/03/2001 → 24/01/2006
Number of participants: 5
Phd Student:
Hansen, Susse Kirkelund (Intern)
Main Supervisor:
Molin, Søren (Intern)
Examiner:
Jensen, Peter Ruhdal (Intern)
Ramos, Juan L. (Ekstern)
Singh, Pradeep K. (Ekstern)

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

Energimetabolismen i mælkesyrebakterier
Department of Systems Biology
Period: 01/08/2000 → 30/11/2001
Number of participants: 2
Phd Student:
Solem, Christian (Intern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
**Start cultures with universal resistance against bacteriophages**
The project is a collaboration between DTU and Chr. Hansen A/S, supported by the Danish Academy for Technical Sciences (ATV/ Martin B. Pedersen)

Department of Microbiology
Department of Systems Biology

**Udvikling af bakterielle strukturer med universel resistens mod bakteriofag infektion**

Department of Systems Biology

**Generation and evaluation of artificial mammalian promoters for in vivo expression of therapeutic genes**

Department of Systems Biology
Energy metabolism and stress in Lactococcus lactis
In this project we study the control and regulation of free-energy metabolism in the bacterium Lactococcus lactis. This is a relatively simple model system, because these cells only make ATP through substrate level phosphorylation. Experimentally, we modulate the expression of the genes encoding the H+-ATPase and the genes encoding the enzymes in and surrounding the glycolytic pathway. In order to understand, in a quantitative way, the control and regulation in this system, we use biomathematical tools such as metabolic control analysis and computer modelling.

Department of Microbiology
Department of Systems Biology
Period: 01/03/1998 → 01/01/9999
Number of participants: 3
Project participant:
Schürmann, Regina Åris (Intern)
Købmann, Brian (Intern)
Jensen, Peter Ruhdal (Intern)

Acidification by Lactic Acid Bacteria
The project is a collaboration with Chr. Hansen A/S and the project content is confidential
Department of Microbiology
Department of Systems Biology
Chr. Hansen A/S
Period: 01/06/1997 → 31/05/2000
Number of participants: 2
Project participant:
Købmann, Brian (Intern)
Project Manager, organisational:
Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Unknown
Name of research programme: Ukendt
Amount: 597,000.00 Danish Kroner
Source: Unknown
Name of research programme: Ukendt
Amount: 375,000.00 Danish Kroner

Forøget laktat dannelse i mælkesyrebakterier
Department of Systems Biology
Period: 01/06/1997 → ...
Number of participants: 6
Phd Student:
Købmann, Brian (Intern)
Supervisor:
Nilsson, Dan (Ekstern)
Main Supervisor:
Jensen, Peter Ruhdal (Intern)
Examiner:
Lillevang, Søren (Ekstern)
Pedersen, Per Amstrup (Ekstern)
Løbner-Olesen, Anders (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Erhvervsforskerordningen
Improvement of the ethanol yield of hemicellulose degrading bacteria

Department of Systems Biology

Period: 01/06/1997 → 13/02/2002

Number of participants: 5

Phd Student:
Clausen, Anders (Intern)

Main Supervisor:  
Ahring, Birgitte Kiær (Intern)

Examiner:
Kristjánsson, Jakob K. (Ekstern)
Jensen, Peter Ruholal (Intern) 
Sommer, Peter (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Program Stipendium-SU, Eksp
Project: PhD

Control of Metabolic flux through glycolysis in L. lactis

This project is a collaboration between the Department of Microbiology (DTU), Department of Biotechnology (DTU) and The danish dairy research foundation (MFF). The project consists of three subprojects as listed below. The aim of the overall project is to describe the control by individual glycolytic enzymes on the flux through glycolysis in L. lactis. The approach is: 1) to study the concentration of glycolytic intermediates in chemostat experiments and design a mathematical model that describes the observations , 2) to modulate the expression of genes encoding glycolytic enzymes around their wildtype level and study the effect on glycolytic flux, the level of glycolytic intermediate levels and endproducts, 3) to introduce mutations in the genes of interest and study the effect on glycolytic flux, intermediates and endproducts. The three subprojects have the following titles: A. Mathematic Modelling of glycolysis in Lactococcus lactis. B. Metabolic Control Analysis of glycolysis in Lactococcus lactis C. Isolation and characterization of glycolytic mutants

Department of Microbiology
Department of Biotechnology

Danish Dairy Research Foundation

Center for Advanced Food Studies

Period: 01/02/1996 → 31/08/1999

Number of participants: 8

Project participant:
Willemoes, Martin (Intern)
Andersen, Heidi Winterberg (Intern)
Madsen, Katrine (Intern)
Jensen, Peter Ruholal (Intern)
Jochumsen, Kirsten Væver (Intern)
Viladsen, John (Intern)
Hansen, Kianoush Kangarlou (Intern)

Project Manager, organisational:
Hammer, Karin (Intern)

Financing sources
Source: Unknown
Name of research programme: Uekndt
Amount: 4,028,400.00 Danish Kroner
Project

Metabolisk kontrol-analyse af glykolsylen i lactococcus lactis

Department of Systems Biology
Synthetic promoters for control analysis and metabolic engineering

In this project we use a new method for creating libraries of synthetic promoters for a range of microorganisms, bacteria as well as yeast, and more recently for higher eukaryotic organisms. The promoters differ only slightly in strength but together they cover a broad range of promoter activities, including very efficient promoters. The strategy used here was to maintain the consensus sequence essentially constant and randomize the spacer sequences between the consensus sequences.

Department of Microbiology
Department of Systems Biology
Period: 01/05/1995 → 01/01/9999
Number of participants: 7
Project participant:
Piskur, Jure (Intern)
Hammer, Karin (Intern)
Schürmann, Regina Áris (Intern)
Andersen, Heidi Winterberg (Intern)
Hahn-Hagerdal, B. (Ekstern)
Johansen, T. E. (Ekstern)

Project Manager, organisational:
Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Unknown
Name of research programme: Ukendt
Amount: 100,000.00 Danish Kroner

Control and Regulation of DNA supercoiling in E. coli

This project is a collaboration with professor Westerhoffs and Dr. Snoeps group at the Free University, Amsterdam, The Netherlands. Using genetic engineering, we have constructed E. coli mutants that allow us to modulate the expression of the genes involved in DNA supercoiling, i.e. the genes encoding DNA gyrase and topoisomerase I and to determine the control by these enzymes on DNA supercoiling and expression of various genes in E. coli

Department of Microbiology
Department of Systems Biology
Period: 01/03/1994 → 01/01/9999
Number of participants: 3
Project participant:
Andersen, Heidi Winterberg (Intern)
Schürmann, Regina Áris (Intern)

Project Manager, organisational:
Jensen, Peter Ruhdal (Intern)

Financing sources
Hierarchical Control Analysis of free-energy metabolism in E. coli
This project is a collaboration with professor Westerhoffs and Dr. Snoeps group at the Free University, Amsterdam, The Netherlands. Hierarchical and Metabolic Control Analysis is used to determine the relative importance of the components of E. coli free-energy metabolism, and of the genetic feed-back loops that attenuates the control by some of these components.

Department of Microbiology
Department of Systems Biology
Period: 01/03/1994 → 01/01/9999
Number of participants: 3
Project participant:
Michelsen, Ole (Intern)
Schürmann, Regina Åris (Intern)

Jensen, Peter Ruhdal (Intern)

Financing sources
Source: Unknown
Name of research programme: Ukendt
Amount: 49,200.00 Danish Kroner

DNA supercoiling and Nucleoid structure in E. coli
This project is a collaboration with Dr. Conrad Woldringh, Institute for Molecular Cell Biology, Biocentrum, University of Amsterdam, Holland. Using strains with tunable expression of the topoisomerase genes, we modulate the level of DNA supercoiling and study the effects hereof on compaction and expansion of the bacterial nucleoid.

Department of Microbiology
Department of Systems Biology
Period: 01/01/1993 → 01/01/9999
Number of participants: 1
Project Manager, organisational:
Jensen, Peter Ruhdal (Intern)

E. coli cell cycle in chemostat cultures
Collaboration with Dr. Joost Teixeira de Mattos, Department of Microbiology, University of Amsterdam, Amsterdam, The Netherlands. In this project we analyse how the B (period of preparation), C (period of DNA synthesis) and D (period of preparation for division) periods in the E. coli cell cycle vary with growth rate in slow growing glucose limited chemostat cultures.

Department of Microbiology
Department of Systems Biology
Period: 01/01/1993 → 01/01/9999
Number of participants: 3
Project participant:
Jensen, Peter Ruhdal (Intern)
Tjell, Vibeke (Intern)
Michelsen, Ole (Intern)