Development of a novel, robust and cost-efficient process for valorizing dairy waste exemplified by ethanol production

Delactosed whey permeate (DWP) is a side stream of whey processing, which often is discarded as waste, despite of its high residual content of lactose, typically 10-20%. Microbial fermentation is one of the most promising approaches for valorizing nutrient rich industrial waste streams, including those generated by the dairies. Here we present a novel microbial platform specifically designed to generate useful compounds from dairy waste. As a starting point we use Corynebacterium glutamicum, an important workhorse used for production of amino acids and other important compounds, which we have rewired and complemented with genes needed for lactose utilization. To demonstrate the potential of this novel platform we produce ethanol from lactose in DWP. First, we introduced the lacSZ operon from Streptococcus thermophilus, encoding a lactose transporter and a β-galactosidase, and achieved slow growth on lactose. The strain could metabolize the glucose moiety of lactose, and galactose accumulated in the medium. After complementing with the Leloir pathway (galMKTE) from Lactococcus lactis, co-metabolization of galactose and glucose was accomplished. To further improve the growth and increase the sugar utilization rate, the strain underwent adaptive evolution in lactose minimal medium for 100 generations. The outcome was strain JS95 that grew fast in lactose mineral medium. Nevertheless, JS95 still grew poorly in DWP. The growth and final biomass accumulation were greatly stimulated after supplementation with NH4+, Mn2+, Fe2+ and trace minerals. In only 24 h of cultivation, a high cell density (OD600 of 56.8 ± 1.3) was attained. To demonstrate the usefulness of the platform, we introduced a plasmid expressing pyruvate decarboxylase and alcohol dehydrogenase, and managed to channel the metabolic flux towards ethanol. Under oxygen-deprived conditions, non-growing suspended cells could convert 100 g/L lactose into 46.1 ± 1.4 g/L ethanol in DWP, a yield of 88% of the theoretical. The resting cells could be re-used at least three times, and the ethanol productivities obtained were 0.96 g/L/h, 2.2 g/L/h, and 1.6 g/L/h, respectively. An efficient process for producing ethanol from DWP, based on C. glutamicum, was demonstrated. The results obtained clearly show a great potential for this newly developed platform for producing value-added chemicals from dairy waste.
Genetics of Lactococci

Lactococcus lactis is the best characterized species among the lactococci, and among the most consumed food-fermenting bacteria worldwide. Thanks to their importance in industrialized food production, lactococci are among the lead bacteria understood for fundamental metabolic pathways that dictate growth and survival properties. Interestingly, lactococci belong to the Streptococcaceae family, which includes food, commensal and virulent species. As basic metabolic pathways (e.g., respiration, metal homeostasis, nucleotide metabolism) are now understood to underlie virulence, processes elucidated in lactococci could be important for understanding pathogen fitness and synergy between bacteria. This chapter highlights major findings in lactococci and related bacteria, and covers five themes: distinguishing features of lactococci, metabolic capacities including the less known respiration metabolism in Streptococcaceae, factors and pathways modulating stress response and fitness, interbacterial dialogue via metabolites, and novel applications in health and biotechnology.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Section for Microbial and Chemical Ecology, Metabolic Signaling and Regulation, Department of Biotechnology and Biomedicine, Institut National de la Recherche Agronomique, Kitasato University
Corresponding author: Gruss, A.
Contributors: Gaudu, P., Yamamoto, Y., Jensen, P. R., Hammer, K., Lechardeur, D., Gruss, A.
Number of pages: 25
Publication date: 2019
Peer-reviewed: Yes
Harnessing biocompatible chemistry for developing improved and novel microbial cell factories

White biotechnology relies on the sophisticated chemical machinery inside living cells for producing a broad range of useful compounds in a sustainable and environmentally friendly way. However, despite the impressive repertoire of compounds that can be generated using white biotechnology, this approach cannot currently fully replace traditional chemical production, often relying on petroleum as a raw material. One challenge is the limited number of chemical transformations taking place in living organisms. Biocompatible chemistry, that is non-enzymatic chemical reactions taking place under mild conditions compatible with living organisms, could provide a solution. Biocompatible chemistry is not a novel invention, and has since long been used by living organisms. Examples include Fenton chemistry, used by microorganisms for degrading plant materials, and manganese or ketoacids dependent chemistry used for detoxifying reactive oxygen species. However, harnessing biocompatible chemistry for expanding the chemical repertoire of living cells is a relatively novel approach within white biotechnology, and it could potentially be used for producing valuable compounds which living organisms otherwise are not able to generate. In this mini review, we discuss such applications of biocompatible chemistry, and clarify the potential that lies in using biocompatible chemistry in conjunction with metabolically engineered cell factories for cheap substrate utilization, improved cell physiology, efficient pathway construction and novel chemicals production.

Sweet As Sugar-Efficient Conversion of Lactose into Sweet Sugars Using a Novel Whole-Cell Catalyst

Lactose, the sugar contained in milk, has a low sweetness. We have constructed an efficient whole-cell catalyst (WCC) that can be grown on dairy waste and that is able to convert lactose into a mixture of sugars as sweet as sucrose. The WCC is based on Corynebacterium glutamicum ATCC13032, which has been engineered to metabolize lactose, to express xylose and arabinose isomerase, and to eliminate byproduct formation. When introduced in concentrated cheese whey permeate, its content of 98 g/L lactose was completely hydrolyzed and the liberated sugars partially isomerized into 23.5 g/L fructose and 20.4 g/L tagatose, which corresponds to a 49% conversion of the glucose and a 44% conversion of galactose. The latter is similar to what can be obtained using purified enzymes. The new technology enables better resource utilization and allows for dairy waste to be converted into a valuable food sweetener with many potential uses.
Systems Biology – A Guide for Understanding and Developing Improved Strains of Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) are extensively employed in the production of various fermented foods, due to their safe status, ability to affect texture and flavor and finally due to the beneficial effect they have on shelf-life. More recently, LAB have also gained interest as production hosts for various useful compounds, particularly compounds with sensitive applications, such as food ingredients and therapeutics. As for all industrial microorganisms, it is important to have a good understanding of the physiology and metabolism of LAB in order to fully exploit their potential, and for this purpose, many systems biology approaches are available. Systems metabolic engineering, an approach that combines optimization of metabolic enzymes/pathways at the systems level, synthetic biology as well as in silico model simulation, has been used to build microbial cell factories for production of biofuels, food ingredients and biochemicals. When developing LAB for use in foods, genetic engineering is in general not an accepted approach. An alternative is to screen mutant libraries for candidates with desirable traits using high-throughput screening technologies or to use adaptive laboratory evolution to select for mutants with special properties. In both cases, by using omics data and data-driven technologies to scrutinize these, it is possible to find the underlying cause for the desired attributes of such mutants. This review aims to describe how systems biology tools can be used for obtaining both engineered as well as non-engineered LAB with novel and desired properties.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Colorado State University
Corresponding author: Solem, C.
Contributors: Liu, J., Chan, S. H. J., Chen, J., Solem, C., Jensen, P. R.
Number of pages: 19
Publication date: 2019
Peer-reviewed: Yes

Publication information
Journal: Frontiers in Microbiology
Volume: 10
Article number: 876
ISSN (Print): 1664-302X
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
Original language: English
Keywords: Food fermentation, Metabolic engineering, Strain development, Control analysis, Screening and selection
Electronic versions:
fmicb_10_00876.pdf
fmicb_10_00876.pdf
DOIs:
10.3389/fmicb.2019.00876
Source: PublicationPreSubmission
Source ID: 176331521
Research output: Contribution to journal › Journal article – Annual report year: 2019 › Research › peer-review

The SPI-19 encoded type-six secretion-systems (T6SS) of Salmonella enterica serovars Gallinarum and Dublin play different roles during infection
Salmonella Pathogenicity Islands 19 (SPI19) encodes a type VI secretion system (T6SS). SPI19 is only present in few serovars of S. enterica, including the host-adapted serovar S. Dublin and the host-specific serovar S. Gallinarum. The role of the SPI19 encoded T6SS in virulence in these serovar is not fully understood. Here we show that during infection of mice, a SPI19/T6SS deleted strain of S. Dublin 2229 was less virulent than the wild type strain after oral challenge, but not after IP challenge. The mutant strain also competed significantly poorer than the wild type strain when co-cultured with strains of E. coli, suggesting that this T6SS plays a role in pathogenicity by killing competing bacteria in the intestine. No significant difference was found between wild type S. Gallinarum G9 and its ΔSPI19/T6SS mutant in infection, whether chicken were challenged orally or by the IP route, and the S. Gallinarum G9 ΔSPI19/T6SS strain competed equally well as the wild type strain against strains of E. coli. However, contrary to what was observed with S. Dublin, the wild type G9
strains was significantly more cytotoxic to monocyte derived primary macrophages from hens than the mutant, suggesting
that SPI19/T6SS in S. Gallinarum mediates killing of eukaryotic cells. The lack of significant importance of SPI19/T6SS
after oral and systemic challenge of chicken was confirmed by knocking out SPI19 in a second strain, J91. Together the
results suggest that the T6SS encoded from SPI19 have different roles in the two serovars and that it is a virulence-factor
after oral challenge of mice in S. Dublin, while we cannot confirm previous results that SPI19/T6SS influence virulence
significantly in S. Gallinarum.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, University of
Copenhagen, Roskilde University
Corresponding author: Olsen, J. E.
Contributors: Schroll, C., Huang, K., Ahmed, S., Kristensen, B. M., Pors, S. E., Jelsbak, L., Lemire, S., Thomsen, L. E.,
Christensen, J. P., Jensen, P. R., Olsen, J. E.
Number of pages: 9
Pages: 23-31
Publication date: 2019
Peer-reviewed: Yes

Publication information
Journal: Veterinary Microbiology
Volume: 230
ISSN (Print): 0378-1135
Ratings:
BFI (2019): BFI-level 2
Web of Science (2019): Indexed yes
Original language: English
Keywords: Cytotoxicity, Dublin, Gallinarum, Oral infection, Salmonella, Systemic infection, T6SS
DOIs: 10.1016/j.vetmic.2019.01.006
Source: Scopus
Source ID: 85059821123
Research output: Contribution to journal › Journal article – Annual report year: 2019 › Research › peer-review

Alterations in the transcription factors GntR1 and RamA enhance the growth and central metabolism of Corynebacterium
glutamicum
Evolution, i.e. the change in heritable characteristics of biological populations over successive generations, has created
the diversity of life that exists today. In this study we have harnessed evolution to create faster growing mutants of
Corynebacterium glutamicum, to debottleneck growth rate of this highly important industrial workhorse. After
approximately 1500 generations of Adaptive Laboratory Evolution (ALE) in defined minimal medium with glucose, we
obtained faster growing mutants with specific growth rate as high as 0.64h-1 as compared with 0.45h-1 for the wild type,
and this 42% improvement is the highest reported for C. glutamicum to date. By genome resequencing and inverse
metabolic engineering, we were able to pinpoint two mutations contributing to most of the growth improvement, and these
resided in the transcriptional regulators GntR1 (gntR1-E70K) and RamA (ramA-A52V). We confirmed that the two
mutations lead to alteration rather than elimination of function, and their introduction in the wild-type background resulted
in a specific growth rate of 0.62h-1. The glycolytic and pentose phosphate pathway fluxes had both increased significantly,
and a transcriptomic analyses supported this to be associated with increased capacity. Interestingly, the observed fast
growth phenotype was not restricted to glucose but was also observed on fructose, sucrose and xylose, however, the
effect of the mutations could only be seen in minimal medium, and not rich BHI medium, where growth was already fast.
We also found that the mutations could improve the performance of resting cells, under oxygen-deprived conditions,
where an increase in sugar consumption rate of around 30% could be achieved. In conclusion, we have demonstrated that
it is feasible to reprogram C. glutamicum into growing faster and thus enhance its industrial potential.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Hamburg University of
Technology, Technical University of Denmark
Corresponding author: Solem, C.
Contributors: Wang, Z., Liu, J., Chen, L., Zeng, A., Solem, C., Jensen, P. R.
Pages: 1-12
Publication date: 2018
Peer-reviewed: Yes

Publication information
Journal: Metabolic Engineering
Strain development is frequently used to improve the performance and functionality of industrially important microbes. As traditional mutagenesis screen is especially utilized by the food industry to improve strains used in food fermentation, high-throughput and cost-effective screening tools are important in mutant selection. The emerging droplet-based microfluidics technology miniaturizes the volume for cell cultivation and phenotype interrogation down to the pico-liter scales, which facilitates screening of microbes for improved phenotypical properties tremendously. In this mini-review, we present recent application of the droplet-based microfluidics in microbial strain improvement with a focus on its potential use in the screening of lactic acid bacteria.

Inactivation of TCA cycle enhances Staphylococcus aureus persister cell formation in stationary phase

Persisters cells constitute a small subpopulation of bacteria that display remarkably high antibiotic tolerance and for pathogens such as Staphylococcus aureus are suspected as culprits of chronic and recurrent infections. Persisters formed during exponential growth are characterized by low ATP levels but less is known of cells in stationary phase. By enrichment from a transposon mutant library in S. aureus we identified mutants that in this growth phase displayed enhanced persister cell formation. We found that inactivation of either sucA or sucB, encoding the subunits of the alpha-ketoglutarate dehydrogenase of the tricarboxylic acid cycle (TCA cycle), increased survival to lethal concentrations of...
ciprofloxacin by 10-100 fold as did inactivation of other TCA cycle genes or atpA encoding a subunit of the F1F0 ATPase. In S. aureus, TCA cycle activity and gene expression are de-repressed in stationary phase but single cells with low expression may be prone to form persisters. While ATP levels were not consistently affected in high persister mutants they commonly displayed reduced membrane potential, and persistence was enhanced by a protein motive force inhibitor. Our results show that persister cell formation in stationary phase does not correlate with ATP levels but is associated with low membrane potential.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, University of Copenhagen, University of Tübingen
Corresponding author: Ingmer, H.
Contributors: Wang, Y., Bojer, M. S., George, S. E., Wang, Z., Jensen, P. R., Wolz, C., Ingmer, H.
Number of pages: 13
Publication date: 2018
Peer-reviewed: Yes

Publication information
Journal: Scientific Reports
Volume: 8
Issue number: 1
Article number: 10849
ISSN (Print): 2045-2322
Ratings:
BFI (2018): BFI-level 1
Scopus rating (2018): CiteScore 4.29 SJR 1.414 SNIP 1.24
Web of Science (2018): Indexed yes
Original language: English
Electronic versions:
s41598_018_29123_0.pdf
DOIs:
10.1038/s41598-018-29123-0
Source: FindIt
Source ID: 2437761893
Research output: Contribution to journal » Journal article – Annual report year: 2018 » Research » peer-review

Protein from green biomass as a food resource

General information
Publication status: Published
Organisations: Research group for Microbial Biotechnology and Biorefining, National Food Institute
Contributors: Nørgaard, D. S., Stærmose, M. D., Jensen, P. R.
Number of pages: 1
Publication date: 2018

Host publication information
Title of host publication: Sustain Conference 2018 : Creating Technology for a Sustainable Society
Place of publication: Lyngby, Denmark
Publisher: Technical University of Denmark (DTU)
Editors: C. M., K. M.
Article number: P-1
URLs:
http://www.sustain.dtu.dk/
Research output: Chapter in Book/Report/Conference proceeding » Conference abstract in proceedings – Annual report year: 2018 » Research » peer-review

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, acetoin and acetate production.

**General information**

Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining
Contributors: Jensen, P. R., Liu, J., Solem, C., Dantoft, S. H.
Publication date: 31 Aug 2017

**Publication information**

IPC: C12N 15/ 75 A I
Patent number: WO2017144672
Filing date: 31/08/2017
Priority date: 25/02/2016
Priority number: EP20160157325
Original language: English
Electronic versions:
WO2017144672A1.pdf
Source: espacenet
Source ID: WO2017144672
Research output: Patent › Patent – Annual report year: 2017 › Research

**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.

**General information**

Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining
Contributors: Solem, C., Jensen, P. R., Liu, J.
Publication date: 13 Apr 2017

**Publication information**

IPC: C12N 15/ 74 A I
Patent number: WO2017060455
Filing date: 13/04/2017
Priority date: 25/02/2016
Priority number: EP20160157443
Original language: English
Electronic versions:
WO2017060455.pdf
Source: espacenet
Source ID: WO2017060455
Research output: Patent › Patent – Annual report year: 2017 › Research

**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.
Engineering Lactococcus lactis into a cell factory for production of butanol isomers

Finding the Needle in the Haystack-the Use of Microfluidic Droplet Technology to Identify Vitamin-Secreting Lactic Acid Bacteria
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.
Lipid Biotechnology and Biochemistry

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Aarhus University
Contributors: Anankanbil, S., Suo, F., Jensen, P. R., Guo, Z.
Pages: 779-824
Publication date: 2017

Host publication information
Title of host publication: Lipid Biotechnology and Biochemistry
Place of publication: Boca Raton
Publisher: CRC Press
Edition: 4
ISBN (Print): 978-1-4987-4485-0
ISBN (Electronic): 978-1-4987-4487-4
Food Lipids: Chemistry, Nutrition, and Biotechnology.
DOI: 10.1201/9781315151854-32
Source: FindIt
Source ID: 2356863483
Research output: Chapter in Book/Report/Conference proceeding ▶ Book chapter – Annual report year: 2017 ▶ Research ▶ peer-review

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.

General information
Publication status: Published
Organisations: Department of Systems Biology, National Food Institute, Research group for Microbial Biotechnology and Biorefining, Department of Biotechnology and Biomedicine, Metabolic Signaling and Regulation
Contributors: Petersen, K. V., Liu, J., Chen, J., Martinussen, J., Jensen, P. R., Solem, C.
Number of pages: 12
Publication date: 2017
Peer-reviewed: Yes

Publication information
Journal: Biotechnology Journal
Volume: 12
Issue number: 8
Article number: 1700171
ISSN (Print): 1860-6768
Ratings:
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.12
Web of Science (2017): Impact factor 3.507
Protein from green biomass as a food resource

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Technical University of Denmark
Contributors: Nørgaard, D. S., Duvier Stærmose, M., Bang-Berthelsen, C. H., Jensen, P. R.
Number of pages: 1
Publication date: 2017

Host publication information
Title of host publication: Book of Abstracts Sustain 2017
Place of publication: Kgs. Lyngby, Denmark
Publisher: Technical University of Denmark (DTU)
Article number: Sustain Abstract F-3
Electronic versions:
ABSTRACT BOOK
SustainAbstracts2017c.compressed_61.pdf

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, DnaAATP is converted to DnaAADP primarily by the Regulatory Inactivation of DnaA process (RIDA). In RIDA deficient cells, DnaAAATP 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
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, University of Copenhagen, Copenhagen University Hospital Frederiksberg and Bispebjerg
Contributors: Charbon, G., Campion, C., Chan, S. H. J., Bjørn, L., Weimann, A., da Silva, L., Jensen, P. R., Løbner-Olesen, A.
Number of pages: 26
Publication date: 2017
Peer-reviewed: Yes

Publication information
Journal: PLoS Genetics
Volume: 13
Issue number: 1
Article number: e1006590
ISSN (Print): 1553-7390
Ratings:
BFI (2017): BFI-Level 2
Scopus rating (2017): CiteScore 5.57 SJR 4.829 SNIP 1.398
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.

General information
Publication status: Published
Organisations: Systems Biotechnology, National Food Institute, Research group for Microbial Biotechnology and Biorefining
Contributors: Solem, C., Jensen, P. R., Chen, J., Liu, J.
Publication date: 23 Jun 2016

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 xylosooxidans, 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 32.1 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.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Systems Biotechnology
Contributors: Kandasamy, V., Liu, J., Dantoft, S. H., Solem, C., Jensen, P. R.
Number of pages: 1
Publication date: 2016
Peer-reviewed: Yes
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.

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 C10 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 biochemistrys, 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.

General information
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.
(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 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.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, University of Copenhagen, Korea Advanced Institute of Science and Technology
Contributors: Liu, J., Chan, S. H. J., Brock-Nannestad, T., Chen, J., Lee, S. Y., Solem, C., Jensen, P. R.
Pages: 57-67
Publication date: 2016
Peer-reviewed: Yes

Publication information
Journal: Metabolic Engineering
Volume: 36
ISSN (Print): 1096-7176
Ratings:
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.33 SJR 3.626 SNIP 1.888
Web of Science (2016): Impact factor 8.142
Web of Science (2016): Indexed yes
Original language: English
Keywords: Metabolic engineering, Biocompatible chemistry, Homo-diacetyl, Homs-(S,S)2,3 butanediol, Lactococcus lactis
DOIs:
10.1016/j.ymben.2016.02.008
Source: Findit
Source ID: 2298610426
Research output: Contribution to journal > Journal article – Annual report year: 2016 > Research > peer-review

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.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, NASA Ames Research Center
Number of pages: 2
Publication date: 2016
Peer-reviewed: Yes

Publication information
Journal: Genome Announcements
Volume: 4
Issue number: 1
Article number: e01701-15
ISSN (Print): 2169-8287
Ratings:
Scopus rating (2016): CiteScore 0.41 SJR 0.583 SNIP 0.469
Web of Science (2016): Indexed yes
Original language: English
Electronic versions:
Genome_Announc._2016_Holm_Hansen_.pdf
DOIs:
10.1128/genomeA.01701-15
Source: Findit
Source ID: 2291980764
Research output: Contribution to journal > Journal article – Annual report year: 2016 > Research > peer-review
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.
Microbial population heterogeneity versus bioreactor heterogeneity: evaluation of Redox Sensor Green as an exogenous metabolic biosensor

Microbial heterogeneity in metabolic performances has attracted a lot of attention, considering its potential impact on industrial bioprocesses. However, little is known about the impact of extracellular perturbations (i.e. bioreactor heterogeneity) on cell-to-cell variability in metabolic performances (i.e. microbial population heterogeneity). In this work, we have evaluated the relevance of Redox Sensor Green (RSG) as an exogenous biosensor of metabolic activity at the single cell level. RSG signal is proportional to the activity of the electron transport chain and its signal is strongly affected by metabolic burden, availability of electron final acceptor and side metabolisms (i.e. overflow and mixed acid fermentation). RSG can also be used for the estimation of the impact of scale-down conditions on microbial metabolic robustness. The relationship linking averaged RSG activity and its cell-to-cell variability (noise) has been highlighted but seems unaffected by environmental perturbations.
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.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Arla Foods
Contributors: Liu, J., Kandasamy, V., Würtz, A., Jensen, P. R., Solem, C.
Number of pages: 9
Pages: 9509-9517
Publication date: 2016
Peer-reviewed: Yes

Publication information
Journal: Applied Microbiology and Biotechnology
Volume: 100
Issue number: 2
ISSN (Print): 0175-7598
Ratings:
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.57 SJR 1.2 SNIP 1.194
Web of Science (2016): Impact factor 3.42
Web of Science (2016): Indexed yes
Original language: English
Keywords: Additional ATP consumption, F1-ATPASE, Acetoin, Lactococcus lactis
DOIs:
10.1007/s00253-016-7687-1
Source: FindIt
Source ID: 2306103895
Research output: Contribution to journal › Journal article – Annual report year: 2016 › Research › peer-review

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.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining
Contributors: Kandasamy, V., Liu, J., Dantoft, S. H., Solem, C., Jensen, P. R.
Number of pages: 9
Publication date: 2016
Peer-reviewed: Yes

Publication information
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.
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.

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining
Contributors: Chen, J., Shen, J., Solem, C., Jensen, P. R.
Number of pages: 8
Publication date: 2015
Peer-reviewed: Yes

Publication information
Journal: mBio (Online)
Volume: 6
Issue number: 6
Article number: e01132-15
ISSN (Print): 2150-7511
Ratings:
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 4.93
Web of Science (2015): Impact factor 6.975
Web of Science (2015): Indexed yes
Original language: English
Electronic versions:
mBio_2015_Chen_.pdf
DOI:
10.1128/mbio.01132-15
Source: FindIt
Source ID: 2287613041
Research output: Contribution to journal › Journal article – Annual report year: 2015 › Research › peer-review

High-level ethanol production by metabolically engineered Lactococcus lactis using economically renewable feedstocks

General information
Publication status: Published
Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Systems Biotechnology
Contributors: Liu, J., Solem, C., Jensen, P. R.
Number of pages: 1
Publication date: 2015

Host publication information
Title of host publication: Book of Abstracts. DTU's Sustain Conference 2015
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.

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 surrounded 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.

General information
Publication status: Published
Organisations: Systems Biotechnology, National Food Institute, Division of Industrial Food Research, Department of Micro- and Nanotechnology, Fluidic Array Systems and Technology, ESPCI Laboratoire LBC
Contributors: Chen, J., Jensen, T. G., Godina, A., Solem, C., Dufva, M., Jensen, P. R.
Number of pages: 1
Publication date: 2014

Host publication information
Title of host publication: Abstract Book - DTU Sustain Conference 2014
Place of publication: Kgs. Lyngby
Publisher: Technical University of Denmark (DTU)
Research output: Chapter in Book/Report/Conference proceeding – Annual report year: 2014 > Research > peer-review

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
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.
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.

**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.
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.

General information
Publication status: Published
Organisations: Systems Biotechnology, National Food Institute, Division of Industrial Food Research
Contributors: Wang, Z., Grishkova, M., Solem, C., Jensen, P. R.
Number of pages: 1
Publication date: 2014

General information
Publication status: Published
Organisations: Systems Biotechnology, National Food Institute, Division of Industrial Food Research
Contributors: Wang, Z., Grishkova, M., Solem, C., Jensen, P. R.
Number of pages: 1
Publication date: 2014

Host publication information
Title of host publication: Abstract Book - DTU Sustain Conference 2014
Place of publication: Kgs. Lyngby
Publisher: Technical University of Denmark (DTU)
Research output: Chapter in Book/Report/Conference proceeding › Conference abstract in proceedings – Annual report year: 2014 › Research › peer-review

Publication information
Journal: Veterinary Microbiology
Volume: 170
Issue number: 1-2
ISSN (Print): 0378-1135
Ratings:
BFI (2014): BFI-level 2
Scopus rating (2014): CiteScore 2.54 SJR 1.291 SNIP 1.251
Web of Science (2014): Impact factor 2.511
Web of Science (2014): Indexed yes
Original language: English
Keywords: Salmonella Gallinarum, Polyamines, Virulence, Host-specificity, Evolution
Electronic versions:
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.
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 (ggnnTA(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 appear to 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.
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.

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.
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.
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.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

General information
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Petersen, K. V., Martinussen, J., Jensen, P. R., Solem, C.
Pages: 3563-3569
Publication date: 2013
Peer-reviewed: Yes

Publication information
Journal: Applied and Environmental Microbiology
Volume: 79
Issue number: 12
ISSN (Print): 0099-2240
Ratings:
BFI (2013): BFI-level 2
Scopus rating (2013): CiteScore 4.25 SJR 1.899 SNIP 1.414
Web of Science (2013): Impact factor 3.952
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Original language: English
Electronic versions:
Repetitive_Marker_Free.pdf
DOIs:
10.1128/AEM.00346-13
Source: dtu
Source ID: n:oai:DTIC-ART:highwire/387284184::28741
Research output: Contribution to journal › Journal article – Annual report year: 2013 › Research › peer-review

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 idhX, idhB, and idh 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).
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.
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
Publication status: Published
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
Contributors: Liu, X., Kaas, R. S., Jensen, P. R., Workman, M.
Pages: 827
Publication date: 2012
Peer-reviewed: Yes

Publication Information
Journal: Eukaryotic Cell (Online Edition)
Volume: 11
Issue number: 6
ISSN (Print): 1535-9786
Ratings:
Scopus rating (2012): CiteScore 3.81 SJR 2.162 SNIP 0.993
Web of Science (2012): Impact factor 3.586
ISI indexed (2012): ISI indexed no
Original language: English
Electronic versions:
PDFB7.pdf
DOIs:
10.1128/EC.00114-12

Bibliographical note
This work was funded by the European Community's 7th Framework Research Programme under grant agreement number 213506 (project GLYFINERY), providing financial support to X.L. and M.W.

Overnight Control of Blood Glucose in People with Type 1 Diabetes

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.

General information
Publication status: Published
Organisations: Department of Informatics and Mathematical Modeling, Scientific Computing, Mathematical Statistics, Department of Systems Biology, Center for Systems Microbiology, Center for Energy Resources Engineering, Horus ApS, Copenhagen University Hospital
Contributors: Boiroux, D., Duun-Henriksen, A. K., Schmidt, S., Nørgaard, K., Madsbad, S., Skyggebjerg, O., Jensen, P. R., Poulsen, N. K., Madsen, H., Jørgensen, J. B.
Pages: 73-78
Publication date: 2012

Host publication information
Title of host publication: Biological and Medical Systems
Volume: 8
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.
between gene sequence and functional protein in living cells, but out of these the most straight-forward 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.

**General information**
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Dehli, T., Solem, C., Jensen, P. R.
Pages: 181-201
Publication date: 2012
Peer-reviewed: Yes

**Publication information**
Journal: Sub-cellular biochemistry.
Volume: 64
ISSN (Print): 0306-0225
Ratings:
Scopus rating (2012): CiteScore 2 SJR 1.737 SNIP 0.91
Web of Science (2012): Indexed yes
Original language: English
Source: dtu
Source ID: n::oai:DTIC-ART:pubmed/372303956::20488
Research output: Contribution to journal › Journal article – Annual report year: 2012 › Research › peer-review

**Tuning of Controller for Type 1 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 rst 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.

**General information**
Publication status: Published
Organisations: Department of Informatics and Mathematical Modeling, Scientific Computing, Mathematical Statistics, Department of Systems Biology, Center for Systems Microbiology, Center for Energy Resources Engineering, Horus ApS, Copenhagen University Hospital
Contributors: Duun-Henriksen, A. K., Boiroux, D., Schmidt, S., Skyggebjerg, O., Madsbad, S., Jensen, P. R., Jørgensen, J. B., Poulsen, N. K., Nørgaard, K., Madsen, H.
Pages: 46-51
Publication date: 2012

**Host publication information**
Title of host publication: Biological and Medical Systems
Volume: 8
ISBN (Print): 978-3-902823-10-6
(IFAC Proceedings Volumes (IFAC-PapersOnline) ).
Electronic versions:
DunnHenriksen_BMS2012_0083_FI.pdf
DOIs:
10.3182/20120829-3-HU-2029.00083
Research output: Chapter in Book/Report/Conference proceeding › Article in proceedings – Annual report year: 2012 › Research › peer-review
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 theDegS 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
Publication status: Published
Organisations: Department of Chemical and Biochemical Engineering, Center for BioProcess Engineering, Department of Systems Biology, Center for Microbial Biotechnology, Center for Systems Microbiology, AgroParisTech
Contributors: Jers, C., Kobir, A., Søndergaard, E. O., Jensen, P. R., Mijakovic, I.
Pages: e14653
Publication date: 2011
Peer-reviewed: Yes

Publication information
Journal: P L o S One
Volume: 6
Issue number: 2
ISSN (Print): 1932-6203
Ratings:
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 4.58 SJR 2.425 SNIP 1.252
Web of Science (2011): Impact factor 4.092
ISI indexed (2011): ISI indexed no
Web of Science (2011): Indexed yes
Original language: English
Electronic versions:
journal.pone.0014653.pdf
DOI:
10.1371/journal.pone.0014653
URLs:
http://www.plosone.org/home.action
Source: orbit
Source ID: 277406
Research output: Contribution to journal › Journal article – Annual report year: 2011 › Research › peer-review

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 pJ487 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.
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.

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Jers, C., Pedersen, M. M., Paspaliari, D. K., Schutz, W., Johnsson, C., Soufi, B., Macek, B., Jensen, P. R., Mijakovic, I.
Pages: 287-299
Publication date: 2010
Peer-reviewed: Yes

Publication information
Journal: Molecular Microbiology
Volume: 77
Issue number: 2
ISSN (Print): 0950-382X
Ratings:
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 3.678 SNIP 1.201
Web of Science (2010): Impact factor 4.819
Web of Science (2010): Indexed yes
Original language: English
DOIs:
10.1111/j.1365-2958.2010.07227.x
Source: orbit
Source ID: 277835
Research output: Contribution to journal › Journal article – Annual report year: 2011 › Research › peer-review

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.

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology, Systems Biotechnology
Contributors: Holm, A. K., Blank, L., Oldiges, M., Schmid, A., Solem, C., Jensen, P. R., Vemuri, G.
Pages: 17498-17506
Publication date: 2010
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.

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**
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Michelsen, O., Hansen, F. G., Albrechtsen, B., Jensen, P. R.
Pages: 1058-1065
Publication date: 2010
Peer-reviewed: Yes

**Publication information**
Journal: Journal of Bacteriology
Volume: 192
Issue number: 4
ISSN (Print): 0021-9193
Ratings:
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.64 SNIP 1.148
Web of Science (2010): Impact factor 3.726
Web of Science (2010): Indexed yes
Original language: English
DOIs: 10.1128/JB.00900-09
Source: orbit
Source ID: 262738
Research output: Contribution to journal › Journal article – Annual report year: 2010 › Research › peer-review

**Control analysis of the purine biosynthesis in Lactococcus lactis**

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

**Control analysis of the purine biosynthesis in Lactococcus lactis**

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

**DiaCon: an interdisciplinary approach to diabetes control**

**General information**
Publication status: Published
Organisations: Scientific Computing, Department of Informatics and Mathematical Modeling, Mathematical Statistics, Center for Systems Microbiology, Department of Systems Biology
Contributors: Finan, D. A., Duun-Christensen, A. K., Schmidt, S., Boiroux, D., Jørgensen, J. B., Nørgaard, K., Jensen, P. R., Poulsen, N. K., Madsen, H.
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 nisI from L. lactis under the control of a synthetic promoter library.

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 under these conditions. In contrast, the glycolytic and ribolytic flux decreased in L. sakei 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. (C) 2008 Elsevier Inc. All rights reserved.
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.

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.
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 PTS-mannose (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.
Online diagnosticering af bacteriofag-inficerede celler

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Michelsen, O., Jensen, P. R.
Pages: 80-83
Publication date: 2008
Peer-reviewed: Unknown

Publication information
Journal: Mælkeritidende
Volume: 4
ISSN (Print): 0024-9645
Original language: Danish
Source: orbit
Source ID: 233188
Research output: Contribution to journal › Journal article – Annual report year: 2008 › Communication

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
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Solem, C., Defoor, E. M. C., Jensen, P. R., Martinussen, J.
Pages: 4772-4775
Publication date: 2008
Peer-reviewed: Yes

Publication information
Journal: Applied and Environmental Microbiology
Volume: 74
Issue number: 15
ISSN (Print): 0099-2240
Ratings:
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 2.156 SNIP 1.572
Web of Science (2008): Indexed yes
Original language: English
DOIs:
10.1128/AEM.00134-08
Source: orbit
Source ID: 233161
Research output: Contribution to journal › Journal article – Annual report year: 2008 › Research › peer-review

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 proteins 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.

The las enzymes control pyruvate metabolism in Lactococcus lactis during growth on maltose

The las enzymes control pyruvate metabolism in Lactococcus lactis during growth on maltose

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology, Universidad Autónoma de Madrid
Contributors: Michelsen, O., Cuesta-Dominguez, Á., Albrectsen, B., Jensen, P. R.
Pages: 7575-81
Publication date: 2007
Peer-reviewed: Yes
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.

Control analysis of the importance of phosphoglycerate enolase for metabolic fluxes in Lactococcus lactis subsp. lactis IL1403.

Genetics of Lactococci
Lactococcus lactis - traditional and GMO strains

General information
Publication status: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Contributors: Jensen, P. R., Købmann, B. J., Solem, C.
Pages: S317-S317
Publication date: 2006
Peer-reviewed: Yes

Publication information
Journal: Toxicology Letters
Volume: 164
ISSN (Print): 0378-4274
Ratings:
Scopus rating (2006): SJR 1.018 SNIP 1.278
Web of Science (2006): Indexed yes
Original language: English
Source: orbit
Source ID: 201843
Research output: Contribution to journal › Conference abstract in journal – Annual report year: 2006 › Research › peer-review

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
Publication status: Published
Organisations: Department of Systems Biology
Contributors: Hammer, K., Mijakovic, I., Jensen, P. R.
Pages: 53-55
Publication date: 2006
Peer-reviewed: Yes

Publication information
Journal: Trends in Biotechnology
Volume: 24
Issue number: 2
ISSN (Print): 0167-7799
Ratings:
Scopus rating (2006): SJR 2.176 SNIP 2.433
Web of Science (2006): Indexed yes
Original language: English
DOIs:
10.1016/j.tibtech.2005.12.003
Source: orbit
Source ID: 190420
Research output: Contribution to journal › Journal article – Annual report year: 2006 › Research › peer-review
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.

General information
Publication status: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, Systems Biotechnology
Contributors: Mijakovic, I., Musumeci, L., Tautz, L., Petranovic, D., Edwards, R. A., Jensen, P. R., Mustelin, T., Deutscher, J., Bottini, N.
Pages: 3384-3390
Publication date: 2005
Peer-reviewed: Yes

Publication Information
Journal: Journal of Bacteriology
Volume: 187
Issue number: 10
ISSN (Print): 0021-9193
Ratings:
Scopus rating (2005): SJR 2.66 SNIP 1.158
Web of Science (2005): Indexed yes
Original language: English
DOIs:
Source: orbit
Source ID: 184030
Research output: Contribution to journal › Journal article – Annual report year: 2005 › Research › peer-review

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.

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Michelsen, O., Hansen, F. G., Jensen, P. R.
Publication date: 2005
Peer-reviewed: No
Event: Poster session presented at Symposium on lactic acid bacterium : Genetics, Metabolism and applications, Egmond aan Zee, The Netherlands, .
Source: orbit
Source ID: 184385
Research output: Contribution to conference › Poster – Annual report year: 2005 › Research

Lactococcus lactis is diploid
As part of a collaboration with Danish Dairy Research Foundation we are interested in the DNA replication of Lactococcus lactis. For that we implemented flowcytometric analysis for these studies. The L. lactis does not respond to inhibition by rifampicin by finishing ongoing replication forks. We therefore turned to slow growing cultures in order to obtain information about the DNA replication in the cell cycle. From these studies we have obtained evidence that suggest that slow growing L. lactis are born with two chromosomes in contrast to other studied bacteria, which are born with one chromosome. This unexpected result has been confirmed by radioactive labelling of slow growing cultures of Lactococcus lactis, which also
showed the presence of two chromosomes. We therefore conclude that Lactococcus lactis is the first diploid bacterium found.

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.

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.
Triosephosphate isomerase has no control on the glycolytic flux and metabolic shift in Lactococcus lactis IL1403

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.
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.
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.

Experimental control analysis of glycolysis in Lactococcus lactis

General information
Publication status: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Contributors: Helmark, S., Hansen, M. E., Jelle, B., Sørensen, K., Jensen, P. R.
Pages: 3695-3699
Publication date: 2004
Peer-reviewed: Yes

Publication information
Journal: Applied and Environmental Microbiology
Volume: 70
Issue number: 6
ISSN (Print): 0099-2240
Ratings:
Scopus rating (2004): SJR 2.108 SNIP 1.648
Web of Science (2004): Indexed yes
Original language: English
DOI: 10.1128/aem.70.6.3695-3699.2004
Source: orbit
Source ID: 155279
Research output: Contribution to journal › Journal article – Annual report year: 2004 › Research › peer-review
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.
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.

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. that 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.
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 1, 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 1 (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).

General information
Publication status: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology
Contributors: Snoep, J., van der Weijden, C., Andersen, H., Westerhoff, H., Jensen, P. R.
Pages: 1662-1669
Publication date: 2002
Peer-reviewed: Yes

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
Publication status: Published
Organisations: Center for Microbial Biotechnology, Department of Systems Biology, Bacterial Physiology and Genetics Group
Contributors: Købmann, B. J., Andersen, H. W., Solem, C., Jensen, P. R.
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.

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
Publication status: Published
Organisations: Bacterial Physiology and Genetics Group, Department of Systems Biology, Center for Microbial Biotechnology
Contributors: Hammer, K., Jensen, P. R.
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.

General information
Publication status: Published
Organisations: Department of Systems Biology
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 [ATP]/[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.

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.
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.
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)J=-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.

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.

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
Publication status: Published
Organisations: Department of Biotechnology
Contributors: Boe, L., Danielsen, M., Knudsen, S., Petersen, J. B., Maymann, J., Jensen, P. R.
Pages: 47-55
Publication date: 2000
Peer-reviewed: Yes

Publication information
Journal: Mutation Research
Volume: 448
Issue number: 1
Original language: English
Source: orbit
Source ID: 173956
Research output: Contribution to journal – Journal article – Annual report year: 2000 › Research › peer-review

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

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Købmann, B. J., Nilsson, D., Kuipers, O. P., Jensen, P. R.
Pages: 4738-4743
Publication date: 2000
Peer-reviewed: Yes

Publication information
Journal: Journal of Bacteriology
Volume: 182
Issue number: 17
ISSN (Print): 0021-9193
Ratings:
Scopus rating (2000): SJR 2.469 SNIP 1.168
Web of Science (2000): Indexed yes
Original language: English
Source: orbit
Source ID: 177531
Research output: Contribution to journal – Journal article – Annual report year: 2000 › Research › peer-review

What controls the growth rate of Escherichia coli? Is it transport after all?

General information
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Boogerd, F. C., Ibiyemi, D. I. A., Michelsen, O., Jensen, P. R.
Pages: 19-24
Publication date: 2000

Host publication information
Title of host publication: Animating the Cellular Map
Source: orbit
Extensive regulation compromises the extent to which DNA gyrase controls DNA supercoiling and growth rate of Escherichia coli

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology, Division of Microbiology and Risk Assessment, National Food Institute, Vrije Universiteit Amsterdam
Contributors: Jensen, P. R., van der Weijden, C. C., Jensen, L. B., Westerhoff, H. V., Snoep, J. L.
Pages: 865-877
Publication date: 1999
Peer-reviewed: Yes

Publication information
Journal: European Journal of Biochemistry
Volume: 266
Issue number: 3
ISSN (Print): 0014-2956
Original language: English
DOIs: 10.1046/j.1432-1327.1999.00921.x
Source: orbit
Source ID: 173841
Research output: Contribution to journal › Journal article – Annual report year: 1999 › Research › peer-review

Metode til begrænsning af mikroorganismers produktdannelse

General information
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Jensen, P. R.
Publication date: 1999

Publication information
Patent number: PA 1999 01304
Original language: Danish
Source: orbit
Source ID: 173988
Research output: Patent › Patent – Annual report year: 1999 › Research

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 (Fβ1?) of the membrane bound (Fβ0?Fβ1? type) H?+-ATPase or a portion of Fβ1? exhibiting ATPase activity. The organism from which the Fβ1? ATPase or portions thereof is derived, or in which the Fβ1? ATPase or portions thereof is expressed, may be selected from prokaryotes and eukaryotes. In particular the DNA encoding Fβ1? 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 Fβ1? subunit $g(b)$ or a portion thereof and various combinations of said gene or portion with the genes encoding the other Fβ1? 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.

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Jensen, P. R., Snoep, J. L., Westerhoff, H. V. A.
Publication date: 1998
Artificial promoter libraries for selected organisms and promoters derived from such libraries
An artificial promoter library for a selected organism or group of organisms is constructed as a mixture of double stranded DNA fragments, the sense strands of which comprise at least two consensus sequences of efficient promoters from said organism or group of organisms, or parts thereof comprising at least half of each, and surrounding or intermediate nucleotide sequences (spacers) of variable length in which at least 7 nucleotides are selected randomly among the nucleobases A, T, C and G. The sense strands of the double stranded DNA fragments may also include a regulatory DNA sequence imparting a specific regulatory feature, such as activation by a change in the growth conditions, to the promoters of the library. Further, they may have a sequence comprising one or more recognition sites for restriction endonucleases added to one of or both their ends. The selected organism or group of organisms may be selected from prokaryotes and from eukaryotes; and in prokaryotes the consensus sequences to be retained most often will comprise the -35 signal (-35 to -30): TTGACA and the -10 signal (-12 to -7): TATAAT or parts of both comprising at least 3 conserved nucleotides of each, while in eukaryotes said consensus sequences should comprise a TATA box and at least one upstream activation sequence (UAS). Such artificial promoter libraries can be used i.a. for optimizing the expression of specific genes in various selected organisms.

Artificial Promoters for Metabolic Optimization
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.

Artificial Promoters for Metabolic Optimization
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.
atp mutants of Escherichia coli fail to grow on succinate due to a transport deficiency

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.

General information
Publication status: Published
Organisations: Department of Microbiology, Vrije Universiteit Amsterdam
Contributors: Boogerd, F., Boe, L., Michelsen, O., Jensen, P. R.
Pages: 5855-5859
Publication date: 1998
Peer-reviewed: Yes

Experimental strategies to determine the control of glycolysis in Lactococcus lactis

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

Growth of Escherichia coli on C4-dicarboxylates is significantly controlled by the C4-dicarboxylate transporter
Hierarchical control of DNA supercoiling

Hierarchical Control of the H+-ATPase on cytochrome expression in Escherichia coli

Modeling of free-energy metabolism in Lactococcus lactis
Synthetic promoters for experimental control analysis

General information
Publication status: 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
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology
Contributors: Kebmand, B. J., Andersen, H. W., Schurmann, R. Å., Hammer, K., Jensen, P. R.
Pages: 42-43
Publication date: 1998

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

General information
Publication status: Published
Organisations: Center for Systems Microbiology, Department of Systems Biology, Chr. Hansen AS, Vrije Universiteit Amsterdam, University of Gothenburg
Pages: 205-210
Publication date: 1998
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
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam
Contributors: Westerhoff, H. V., Jensen, P. R., Snoep, J. L.
Pages: 111-120
Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: Thermochimica Acta
Volume: 309
Issue number: 1-2
ISSN (Print): 0040-6031
Original language: English
DOI:
10.1016/S0040-6031(97)00353-5
Source: orbit
Source ID: 171696
Research output: Contribution to journal › Journal article – Annual report year: 1998 › Research › peer-review

The sequence of spacers between the consensus sequences modulates the strength of procaryotic promoters

General information
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Jensen, P. R., Hammer, K.
Pages: 82-87
Publication date: 1998
Peer-reviewed: Yes

Publication information
Journal: Applied and Environmental Microbiology
Volume: 64
Issue number: 1
Original language: English
Source: orbit
Hierarchical control of electron-transfer

General information
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam, Lomonosov Moscow State University
Contributors: Westerhoff, H. V., Jensen, P. R., Egger, L., van Heeswijk, W. C., van Spanning, R., Kholodenko, B. N., Snoep, J. L.
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
Source: orbit
Source ID: 171965

A method of converting ATP into ADP in a living cell

General information
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology
Contributors: Jensen, P. R.
Publication date: 1996

Publication information
Original language: English
Source: orbit
Source ID: 164773

Changes in the cellular energy state affect the activity of the bacterial phosphotransferase system

General information
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, University of Amsterdam
Contributors: Rohwer, J., Jensen, P. R., Shinohara, Y., Postma, P., Westerhoff, H.
Pages: 225-230
Publication date: 1996
Peer-reviewed: Yes

Publication information
Journal: European Journal of Biochemistry
Volume: 235
ISSN (Print): 0014-2956
Original language: English
Source: orbit
Source ID: 165188

Control of DNA supercoiling in the procaryotic cell.

General information
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam, Danish Veterinary Laboratory
DNA supercoiling depends on the phosphorylation potential in *Escherichia coli*.

**General information**
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Netherlands Cancer Institute, Vrije Universiteit Amsterdam
Contributors: Van Workum, M., van Dooren, S., Oldenburg, N., Molenaar, D., Jensen, P. R., Snoep, J., Westerhoff, H.
Pages: 351-360
Publication date: 1996
Peer-reviewed: Yes

**Publication information**
Journal: Molecular Microbiology
Volume: 20
Issue number: 2
ISSN (Print): 0950-382X
Original language: English
Source: orbit
Source ID: 165189
Research output: Contribution to journal › Journal article – Annual report year: 1996 › Research › peer-review

Low control by proton leak on the efficiency of oxidative phosphorylation in *E. coli*.

**General information**
Publication status: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, Vrije Universiteit Amsterdam
Contributors: Jensen, P. R., Westerhoff, H. E. A. (ed.)
Pages: 35-40
Publication date: 1996

**Host publication information**
Title of host publication: Biothermokinetik of the living cell
Place of publication: Amsterdam
Publisher: Biothermokinetic Press
Source: orbit
Source ID: 165192
Research output: Chapter in Book/Report/Conference proceeding › Article in proceedings – Annual report year: 1996 › Research › peer-review

Energy buffering of DNA structure fails when *Escherichia coli* runs out of substrate

To study how changes in the (ATP)/(ADP) ratio affect the level of DNA supercoiling in *Escherichia coli*, the cellular content of H+-ATPase was modulated around the wild-type level. A relatively large drop in the (ATP)/(ADP) ratio from the normal ratio resulted in a small increase in the linking number of our reporter plasmid (corresponding to a small decrease in negative supercoiling). However, when cells depleted their carbon and energy source, the ensuing drop in energy state was accompanied by a strong increase in linking number. This increase was not due to reduced transcription of the DNA in the absence of growth substrate, since rifampin had virtually no effect on the plasmid linking number. To examine whether DNA supercoiling depends more strongly on the cellular energy state at low (ATP)/(ADP) ratios than at high ratios, we used cells that were already at a low energy state after substrate depletion; after the addition of an uncoupler to these cells, the (ATP)/(ADP) ratio decreased further, which resulted in a strong increase in plasmid linking number. Our results suggest that the strong thermodynamic control of DNA supercoiling takes over at low (ATP)/(ADP) ratios, whereas at high ratios homeostatic control mechanisms attenuate thermodynamic control.
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., highATPADP ratio or H+ ion gradient. Although maintenance of a sufficiently high ATPADP 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.
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 the catabolic fluxes, 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.

General information

Publication status: Published
Organisations: Department of Microbiology
Contributors: Jensen, P. R., Michelsen, O., Westerhoff, H. V.
Number of pages: 12
Pages: 543-554
Publication date: 1995
Peer-reviewed: Yes
Molecular biology for flux control

**General information**
Publication status: Published
Organisations: Department of Microbiology
Contributors: Jensen, P. R., Snoep, J., Molenaar, D., Vanheeswijk, W., Kholodenko, B., Vandergugten, A., Westerhoff, H.
Number of pages: 4
Pages: 367-370
Publication date: 1995
Peer-reviewed: Yes

**Publication information**
Journal: Biochemical Society. Transactions
Volume: 23
Issue number: 2
ISSN (Print): 0300-5127
Original language: English
Keywords: 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, ESCHERICHIA-COLI, MITOCHONDRIAL RESPIRATION, ENZYMES, PATHWAY, STRENGTH, SYSTEMS, YEAST, STEPS, GENETICS, HYDROGEN-ATPASE, MATHEMATICAL MODEL, MEETING PAPER, METABOLIC CONTROL ANALYSIS, Facultatively Anaerobic Gram-Negative Rods Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Enterobacteriaceae [06702] Escherichia coli, ATPASE 9000-83-3, 00520, General biology - Symposia, transactions and proceedings, 04500, Mathematical biology and statistical methods, 10064, Biochemistry studies - Proteins, peptides and amino acids, 10506, Biophysics - Molecular properties and macromolecules, 10515, Biophysics - Biocybernetics, 10808, Enzymes - Physiological studies, 31000, Physiology and biochemistry of bacteria, 31500, Genetics of bacteria and viruses, Biochemistry and Molecular Biophysics, Computational Biology, Enzymology, Genetics, Mathematical Biology, Models and Simulations, Physiology

**DOIs:**
10.1042/bst0230367

Source: FindIt
Source ID: 57595314

Research output: Contribution to journal › Conference article – Annual report year: 1995 › Research › peer-review

---

Structure and partitioning of bacterial DNA: determined by a balance of competition 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**
Publication status: Published
Organisations: Department of Microbiology
Contributors: Woldringh, C. L., Jensen, P. R., Westerhoff, H. V.
How to determine control of growth rate in a chemostat. Using metabolic control analysis to resolve the paradox

The chemostat makes it possible to study microbial physiology at steady state. However, because growth rate in a chemostat is set by the experimenter, it seems impossible to employ the chemostat to study the control of microbial growth by processes within the microorganism. In this paper we show how, paradoxically, one can determine control of growth rate, of growth yield and of other fluxes in a chemostat. We develop metabolic control analysis for the chemostat. This analysis does not depend on the particular way in which specific growth rate varies with the concentration of the growth limiting substrate.

General information
Publication status: Published
Organisations: Department of Microbiology
Contributors: Snoep, J. L., Jensen, P. R., Groeneveld, P., Molenaar, D., Kholodenko, B. N., Westerhoff, H. V.
Number of pages: 10
Pages: 1023-1032
Publication date: 1994
Peer-reviewed: Yes

Publication information
Journal: Biochemistry and Molecular Biology International
Volume: 33
Issue number: 5
ISSN (Print): 1039-9712
Original language: English
Keywords: Bacteria, Biomass, Culture Media, Fungi, Kinetics, Metabolism, Models, Biological, ANALYTICAL METHOD, GROWTH LIMITING SUBSTRATE ANALYSIS, MICROBIAL PHYSIOLOGY, Microorganisms (Microorganisms) - Microorganisms [01000] microorganisms, 10060, Biochemistry studies - General, 13002, Metabolism - General metabolism and metabolic pathways, 32000, Microbiological apparatus, methods and media, 32500, Tissue culture, apparatus, methods and media, Methods and Techniques
Source: FindIt
Source ID: 62398604
Research output: Contribution to journal › Journal article – Annual report year: 1994 › Research › peer-review

Modelling of Oxidative Phosphorylation in E. coli

General information
Publication status: Published
Organisations: Department of Microbiology
Contributors: Jensen, P. R., Rohwer, J., Michelsen, O., Westerhoff, H. V.
Pages: 218-220
Publication date: 1994

Host publication information
Title of host publication: Modern Trends in Biothermokinetics 3
Publisher: Innsbruck Univ. Press
Editor: Gneiger et al.
Research output: Chapter in Book/Report/Conference proceeding › Book chapter – Annual report year: 1994 › Research › peer-review

Control analysis of the dependence of Escherichia coli physiology on the H⁺-ATPase

The H⁺-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 (H⁺-ATPase) exerts virtually no control on growth rate (VBRC VBR ≤ 0.01), a minor positive control on growth yield (C = 0.15), and a small but negative control on respiration rate (C = -0.25). The control the enzyme exerts on the consumption rate of the carbon and free-energy substrate is negative (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 H⁺-ATPase on growth rate and growth yield increased slightly; the control on growth rate passed through a maximum (C = 0.15) 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 H⁺-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 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 lt 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 O₂ 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 \( \mu = 0.3 \text{ h}^{-1} \). With 19 amino acids added, the growth rate increased to \( \mu = 0.7 \text{ h}^{-1} \) 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.

Modulation of cellular energy state and DNA supercoiling in E. coli

Multiplicity of control
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 tac 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.

General information
Publication status: Published
Organisations: Department of Microbiology
Contributors: Jensen, P. R., Michelsen, O.
Number of pages: 7
Pages: 7635-7641
Publication date: 1992
Peer-reviewed: Yes

Publication information
Journal: Journal of Bacteriology
Volume: 174
Issue number: 23
ISSN (Print): 0021-9193
Original language: English
Keywords: Adenosine Diphosphate, Adenosine Triphosphate, Carbon, Citric Acid Cycle, Cytochrome b Group, Energy Metabolism, Escherichia coli, Gene Deletion, Glycolysis, Membrane Potentials, Oxidation-Reduction, Oxygen Consumption, Proton-Translocating ATPases, 61D2G4IYVH Adenosine Diphosphate, 7440-44-0 Carbon, 8L70Q75FXE Adenosine Triphosphate, EC 3.6.3.14 Proton-Translocating ATPases, ACETATE, ATP SYNTHESIS, GLYCOLYSIS, GROWTH RATE, GROWTH YIELD, MEMBRANE-BOUND PROTON TRANSLOCATING ATPASE, OXIDATIVE PHOSPHORYLATION, RESPIRATION, TRICARBOXYLIC ACID PATHWAY, TYPE B CYTOCHROMES, Facultatively Anaerobic Gram-Negative Rods Eubacteria Bacteria Microorganisms (Bacteria, Eubacteria, Microorganisms) - Enterobacteriaceae [06702] Escherichia coli, ACETATE 71-50-1, ATP 56-65-5Q, 42530-29-0Q, 94587-45-8Q, 111839-44-2Q, ATPASE 9000-83-3, CARBON 7440-44-0, 10060, Biochemistry studies - General, 10062, Biochemistry studies - Nucleic acids, purines and pyrimidines, 10064, Biochemistry studies - Proteins, peptides and amino acids, 10068, Biochemistry studies - Carbohydrates, 10508, Biophysics - Membrane phenomena, 10510, Biophysics - Bioenergetics: electron transport and oxidative phosphorylation, 10802, Enzymes - General and comparative studies: coenzymes, 10808, Enzymes - Physiological studies, 13002, Metabolism - General metabolism and metabolic pathways, 13003, Metabolism - Energy and respiratory metabolism, 13004, Metabolism - Carbohydrates, 13014, Metabolism - Nucleic acids, purines and pyrimidines, 25508, Development and Embryology - Morphogenesis, 31000, Physiology and biochemistry of bacteria, 31500, Genetics of bacteria and viruses, Biochemistry and Molecular Biophysics, Bioenergetics, Enzymology, Genetics, Metabolism, Physiology

DOIs:
Source: FindIt
Source ID: 59350856
Research output: Contribution to journal › Journal article – Annual report year: 1993 › Research › peer-review
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 hydroxymethylulinulin. 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.

General information
Publication status: Published
Organisations: Department of Microbiology
Contributors: Haworth, R. S., Jensen, P. R., Michelsen, O., Wyatt, J. A., Brealey, C. J., Beechey, R. B.
Number of pages: 6
Pages: 67-72
Publication date: 1990
Peer-reviewed: Yes

Publication information
Journal: BBA Bioenergetics
Volume: 1019
Issue number: 1
ISSN (Print): 0005-2728
Original language: English
Keywords: CCCPCarbonylcyanide-m-chlorophenylhydrazone, TTFB4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole, TPP+tetraphenylphosphonium cation, SDSsodium dodecyl sulphate
Source: FindIt
Source ID: 78453662
Research output: Contribution to journal › Journal article – Annual report year: 1990 › Research › peer-review

Projects:

Characterization of novel lantibiotics in actinobacteria
Zhao, G., PhD Student, National Food Institute
Jensen, P. R., Main Supervisor
Solem, C., Supervisor
01/01/2019 → 31/12/2021
Project: PhD

Production of therapeutic proteins in Lactococcus lactis
Xiao, H., PhD Student, National Food Institute
Jensen, P. R., Main Supervisor
Bang-Berthelsen, C. H., Supervisor
Solem, C., Supervisor
Stipendie fra udlandet
01/12/2017 → 30/11/2020
Award relations: Production of therapeutic proteins in Lactococcus lactis
Project: PhD

Improving the thermotolerance of the mesophilic starter
Dorau, R., PhD Student, National Food Institute
Solem, C., Main Supervisor
Jensen, P. R., Supervisor
Samfinansieret - Andet
01/06/2017 → 31/05/2020
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.

Bøgh, K. L., Project Manager, National Food Institute, Research Group for Gut Microbiology and Immunology
Madsen, C. B., Project Participant, National Food Institute, Research Group for Gut Microbiology and Immunology
Kryger, K., Project Participant, National Food Institute
Qvortrup, K., Project Participant, Department of Chemistry, Organic Chemistry
Jensen, P. R., Project Participant, National Food Institute, Research group for Microbial Biotechnology and Biorefining
Bang-Berthelsen, C. H., Project Participant, National Food Institute, Research group for Microbial Biotechnology and Biorefining
Ottesen, P. C., Project Participant, Office for Innovation & Sector Services
Bang-Berthelsen, I., Project Manager, National Food Institute
Sancho Vega, A. I., Project Participant, National Food Institute, Research Group for Gut Microbiology and Immunology

Keywords: Food Allergy, Immunotherapy, Infant formula, Allergy, Milk allergy, Peanut allergy
Collaborators: University of Leeds, Arla Foods, Medical University of Vienna, University of Toronto
Project: Research
Solem, C., Supervisor
Kilstrup, M., Examiner
Poolman, B., Examiner
Sørensen, K., Examiner
Technical University of Denmark
01/11/2011 → 28/03/2014
Award relations: Characterization of a high-temperature adaptive Lactococcus lactis mutant and its application in milk fermentation
Project: PhD

Lactic Acid Bacteria as cell factories
Liu, J., PhD Student, National Food Institute
Solem, C., Main Supervisor
Jensen, P. R., Supervisor
Hansen, E. B., Examiner
Kleerebezem, M., Examiner
Zeng, A., Examiner
Technical University of Denmark
01/06/2014 → 30/09/2017
Award relations: Lactic Acid Bacteria as cell factories
Project: PhD

Protein production in Gram-positive bacteria under adverse conditions
Vestergaard, M., PhD Student, National Food Institute
Solem, C., Main Supervisor
Bang, D. D., Examiner
Mijakovic, I., Examiner
Joensson, H. N., Examiner
Technical University of Denmark
01/04/2014 → 30/09/2017
Award relations: Protein production in Gram-positive bacteria under adverse conditions
Project: PhD

Udvikling af bakterielle strukturer med universel resistens mod bakteriofag infektion
Pedersen, M. B., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
Hassing, H., Supervisor
Martinussen, J., Examiner
Lillevang, S., Examiner
Pedersen, P. A., Examiner
Nilsson, D., Supervisor
Innovationsfonden
01/02/1999 → 26/11/2002
Award relations: Udvikling af bakterielle strukturer med universel resistens mod bakteriofag infektion
Project: PhD

Generation and evaluation of artificial mammalian promoters for in vivo expression of therapeutic genes
Tornøe, J., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
Kusk, P., Supervisor
Løbner-Ølesen, A., Examiner
Nerlov, C., Examiner
Okkels, J. S., Examiner
Innovationsfonden
01/01/1999 → 01/07/2002
Award relations: Generation and evaluation of artificial mammalian promoters for in vivo expression of therapeutic genes
Project: PhD

Kontrolanalyse af ethanol produktion i Saccharomyces Cerevisiae
Helmark, S., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
PhD

**Metabolisk kontrol-analyse af glykolysen i lactococcus lactis**

Andersen, H. W., PhD Student, Department of Systems Biology  
Jensen, P. R., Main Supervisor  
Mouritzen, J., Examiner  
Snoep, J. L., Examiner  
Hammer, K., Supervisor  

Blandet Finansiering - SU  
01/02/1996 → 18/09/2000  

Award relations: Metabolisk kontrol-analyse af glykolysen i lactococcus lactis  
Project: PhD

**Improvement of the ethanol yield of hemicellulose degrading bacteria**

Clausen, A., PhD Student, Department of Systems Biology  
Ahring, B. K., Main Supervisor  
Kristjánsson, J. K., Examiner  
Jensen, P. R., Examiner  
Sommer, P., Examiner  

Program-stipendium  
01/06/1997 → 13/02/2002  

Award relations: Improvement of the ethanol yield of hemicellulose degrading bacteria  
Project: PhD

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

Glendorf, T., PhD Student, Technical University of Denmark  
Jensen, P. R., Main Supervisor  
Kjeldsen, T., Supervisor  
Gammeltoft, S., Examiner  
Mortensen, U. H., Examiner  
Siddle, K., Examiner  
Glendorf, T., PhD Student, Department of Systems Biology  
ErhvervsPhD-ordningen VTU  

Award relations: Analysis of insulin binding by systematic amino acid scanning mutagenesis Importance of insulin B chain residues for receptor isoform binding  
Project: PhD

**Kontrol af Biofilmdannelse**

Hansen, S. K., PhD Student, Department of Systems Biology  
Molin, S., Main Supervisor  
Jensen, P. R., Examiner  
Ramos, J. L., Examiner  
Singh, P. K., Examiner  

DTU-lønnet stipendie  
01/03/2001 → 24/01/2006  

Award relations: Kontrol af Biofilmdannelse  
Project: PhD

**Metabolic optimization of Corynebacterium glutamicum for enhanced lysine production**

Wang, Z., PhD Student, National Food Institute  
Solem, C., Main Supervisor  
Jensen, P. R., Supervisor  
Hobley, T. J., Examiner
Kalinowski, J., Examiner
Mijakovic, I., Examiner
Technical University of Denmark
15/12/2012 → 21/04/2016
Award relations: Metabolic optimization of Corynebacterium glutamicum for enhanced lysine production
Project: PhD

Lactic Acid Bacteria as a new platform for sustainable production of biochemicals
Boguta, A. M., PhD Student, Department of Systems Biology
Martinussen, J., Main Supervisor
Jensen, P. R., Supervisor
Kilstrup, M., Examiner
Sørensen, K., Examiner
Holo, H., Examiner
Technical University of Denmark
15/12/2011 → 29/09/2016
Award relations: Lactic Acid Bacteria as a new platform for sustainable production of biochemicals
Project: PhD

Transforming Lactococcus lactis into a microbial cell factory
Petersen, K. V., PhD Student, Department of Systems Biology
Solem, C., Main Supervisor
Jensen, P. R., Supervisor
Martinussen, J., Supervisor
Mijakovic, I., Examiner
Kok, J., Examiner
Jørgensen, S. T., Examiner
Technical University of Denmark
01/01/2011 → 30/09/2014
Award relations: Transforming Lactococcus lactis into a microbial cell factory
Project: PhD

Pseudomonas species as a platform for biofuels and biochemicals
Wigneswaran, V., PhD Student, Department of Systems Biology
Jelsbak, L., Main Supervisor
Folkesson, A., Supervisor
Jensen, P. R., Supervisor
Molin, S., Examiner
Burmølle, M., Examiner
Segura, A., Examiner
Technical University of Denmark
01/10/2010 → 26/05/2016
Award relations: Pseudomonas species as a platform for biofuels and biochemicals
Project: PhD

Development of Model Systems for the Biodegradation of Glycerol
Liu, X., PhD Student, Department of Systems Biology
Workman, M., Main Supervisor
Jensen, P. R., Supervisor
Eliasson Lantz, A., Examiner
Jørgensen, H., Examiner
Walker, G. M., Examiner
EU-finansieret
01/03/2009 → 20/08/2012
Award relations: Development of Model Systems for the Biodegradation of Glycerol
Project: PhD

The potential of Lactic Acid Bacteria as microbial factory for pentanol isomer production
Jensen, P. R., Main Supervisor, Department of Biotechnology and Biomedicine
Jørgensen, S. T., Supervisor
Kebmann, B., Supervisor, Department of Systems Biology
Martinussen, J., Supervisor, Department of Biotechnology and Biomedicine
Kilstrup, M., Examiner, Department of Biotechnology and Biomedicine
Mijakovic, I., Examiner, Department of Biotechnology and Biomedicine
Starlit, K. I., PhD Student, Department of Systems Biology
Kok, J., Examiner
Industrial PhD
01/06/2012 → 18/04/2018
Award relations: The potential of Latic Acid Bacteria as microbial factory for pentanol isomer production
Project: PhD

Stokastisk dynamisk modellering til kort-tidsregulering af glukose/insulin-metabolismen
Duun-Henriksen, A. K., PhD Student, Department of Informatics and Mathematical Modeling
Madsen, H., Main Supervisor
Jensen, P. R., Supervisor
Christiansen, L. E., Examiner
Gabrielsen, J., Examiner
Andreassen, S., Examiner
Forskningsrådsfinansiering
01/06/2009 → 12/12/2013
Award relations: Stokastisk dynamisk modellering til kort-tidsregulering af glukose/insulin-metabolismen
Project: PhD

Comparative Systems Biology
Dehli, T. I., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
Solem, C., Supervisor
Mijakovic, I., Examiner
Axelsson, L., Examiner
Westermann, P., Examiner
Technical University of Denmark
01/01/2010 → 18/12/2013
Award relations: Comparative Systems Biology
Project: PhD

Optimering af fermenteringsprocessen til lysin produktion
Rytter, J. V., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
Martinussen, J., Examiner
Blank, L. M., Examiner
Pedersen, P. A., Examiner
Institut, samfinansiering
15/12/2008 → 28/03/2014
Award relations: Optimering af fermenteringsprocessen til lysin produktion
Project: PhD

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

Protein-Tyrosine Phosphorylation in Bacillus Subtilis Signal Transduction
Jers, C., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
Mijakovic, I., Supervisor
Jelsbak, L., Examiner
Grangeasse, C., Examiner
Stülke, J. M., Examiner
DTU-lønned stipendie
15/03/2007 → 22/09/2010
Award relations: Protein-Tyrosine Phosphorylation in Bacillus Subtilis Signal Transduction
Project: PhD

Elucidating and comparing flux regulation across bacterial species
Chan, S. H. J., PhD Student, Department of Systems Biology
Jensen, P. R., Main Supervisor
Solem, C., Supervisor
Hobley, T. J., Examiner
Snoep, J. L., Examiner
Molenaar, D., Examiner
Forskningsrådsfinansiering
15/11/2011 → 27/05/2015
Award relations: Elucidating and comparing flux regulation across bacterial species
Project: PhD

Development of new diagnostic technologies
Chin, W. H., PhD Student, National Food Institute
Bang, D. D., Main Supervisor
Sun, Y., Supervisor
Wolff, A., Supervisor
Jensen, P. R., Examiner
Sjöback, R., Examiner
Ingmer, H., Examiner
Technical University of Denmark
01/08/2013 → 22/12/2016
Award relations: Development of new diagnostic technologies
Project: PhD

Enzyme Immobilisation and Bioprocessing
Alftrén, J., PhD Student, National Food Institute
Hobley, T. J., Main Supervisor
Koski, M., Supervisor
Jensen, P. R., Examiner
Felby, C., Examiner
Øyaas, K., Examiner
Technical University of Denmark
15/11/2010 → 04/06/2014
Award relations: Enzyme Immobilisation and Bioprocessing
Project: PhD

Production of organic acids in Gram - positive bacteria
Shen, J., PhD Student, National Food Institute
Solem, C., Main Supervisor
Jensen, P. R., Supervisor
Martinussen, J., Examiner
Mijakovic, I., Examiner
Zeng, A., Examiner
Technical University of Denmark
01/12/2012 → 21/02/2019
Award relations: Production of organic acids in Gram - positive bacteria
Project: PhD

Improving second generation biorefinery processes using clues from stress response in Lactococcus lactis
Hviid, A. M., PhD Student, Department of Systems Biology
Kilstrup, M., Main Supervisor
Jensen, P. R., Supervisor
Gram, L., Examiner
Neves, A. R., Examiner
Kok, J., Examiner
Technical University of Denmark
15/10/2012 → 15/03/2017
Award relations: Improving second generation biorefinery processes using clues from stress response in Lactococcus lactis
Project: PhD

Biofuels of the future - Development of a Lactic Acid Bacteria platform for sustainable production of higher alcohols
Mar, M. J., PhD Student, National Food Institute
Jensen, P. R., Main Supervisor
Kandasamy, V., Supervisor
Solem, C., Supervisor
Samfinansiert - Andet
01/11/2015 → 05/11/2019
Award relations: Biofuels of the future - Development of a Lactic Acid Bacteria platform for sustainable production of higher alcohols
Project: PhD

Acidification by Lactic Acid Bacteria
The project is a collaboration with Chr. Hansen A/S and the project content is confidential
Jensen, P. R., Project Manager, Department of Microbiology
Købmann, B., Project Participant, Department of Microbiology
Ukendt: DKK597,000.00, Ukendt: DKK375,000.00
01/06/1997 → 31/05/2000
Collaborators: Chr. Hansen AS
Award relations: Acidification by Lactic Acid Bacteria, Acidification by Lactic Acid Bacteria
Project: Research

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.
Jensen, P. R., Project Manager, Department of Microbiology
Schürmann, R. Å., Project Participant, Department of Microbiology
Købmann, B., Project Participant, Department of Microbiology
01/03/1998 → 01/01/9999
Project: Research

Starter 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)
Jensen, P. R., Project Manager, Department of Microbiology
01/02/1999 → 31/01/2002
Project: Research

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 microorganism, 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.
Jensen, P. R., Project Manager, Department of Microbiology
Piskur, J., Project Participant, Department of Microbiology
Hammer, K., Project Participant, Department of Microbiology
Schürmann, R. Å., Project Participant, Department of Microbiology
Andersen, H. W., Project Participant, Department of Microbiology
Hahn-Hagerdal, B., Project Participant, Unknown
Johansen, T. E., Project Participant, Unknown
Ukendt: DKK100,000.00
01/05/1995 → 01/01/9999
Collaborators: Unknown
Award relations: Synthetic promoters for control analysis and metabolic engineering
Project: Research

**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.
Michelsen, O., Project Manager, Department of Microbiology
Jensen, P. R., Project Participant, Department of Microbiology
Tjell, V., Project Participant, Department of Microbiology
01/01/1993 → 01/01/9999
Project: Research

**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.
Michelsen, O., Project Participant, Department of Microbiology
Jensen, P. R., Project Manager, Department of Microbiology
Schürmann, R. Å., Project Participant, Department of Microbiology
Ukendt: DKK49,200.00
01/03/1994 → 01/01/9999
Award relations: Hierarchical Control Analysis of free-energy metabolism in E. coli
Project: Research

**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.
Jensen, P. R., Project Manager, Department of Microbiology
01/01/1993 → 01/01/9999
Project: Research

**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
Jensen, P. R., Project Manager, Department of Microbiology
Andersen, H. W., Project Participant, Department of Microbiology
Schürmann, R. Å., Project Participant, Department of Microbiology
Ukendt: DKK0.00
01/03/1994 → 01/01/9999
Award relations: Control and Regulation of DNA supercoiling in E. coli
Project: Research

**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
Hammer, K., Project Manager, Department of Microbiology
Project Participants:
- Willemoes, M., Department of Microbiology
- Andersen, H. W., Department of Microbiology
- Madsen, K., Department of Microbiology
- Jensen, P. R., Department of Microbiology
- Jochumsen, K. V., Department of Biotechnology
- Villadsen, J., Department of Biotechnology
- Hansen, K. K., Department of Biotechnology

Ukendt: DKK4,028,400.00
01/02/1996 → 31/08/1999

Collaborators: Danish Dairy Research Foundation, Center for Advanced Food Studies

Award relations: Control of Metabolic flux through glycolysis in L. lactis

Project: Research

Mælk - diagnosticering af bakteriofaginficerede celler i syrningsprocesser

Jensen, P. R., Project Manager, Department of Systems Biology, Bacterial Physiology and Genetics Group

Forskningsprojekter - Fødevareministeriet: DKK3,429,851.00
01/08/2003 → 31/07/2006

Award relations: Mælk - diagnosticering af bakteriofaginficerede celler i syrningsprocesser

Project: Research

Mælk, syrningsaktivitet af den primære starter

Jensen, P. R., Project Manager, Department of Systems Biology, Bacterial Physiology and Genetics Group

Project ID: 93S 24FH Å02 00016
Forskningsprojekter - Fødevareministeriet: DKK5,577,954.00
01/01/2003 → 31/12/2006

Award relations: Mælk, syrningsaktivitet af den primære starter

Project: Research

The role of post-transitional modifications in the control of carbon metabolism of Gram positive bacteria

Jensen, P. R., Project Manager, Department of Systems Biology, Bacterial Physiology and Genetics Group

Statens Teknisk Videnskabelige Forskningsråd: DKK487,862.00
01/01/2004 → 31/12/2004

Award relations: The role of post-transitional modifications in the control of carbon metabolism of Gram positive bacteria

Project: Research

Kontrol analyse af ethanol produktion i Saccharomyces cerevisiae

Jensen, P. R., Project Manager, Department of Systems Biology, Bacterial Physiology and Genetics Group

Statens Teknisk Videnskabelige Forskningsråd: DKK1,700,000.00
01/01/2004 → 12/07/2008

Award relations: Kontrol analyse af ethanol produktion i Saccharomyces cerevisiae

Project: Research

Press clippings:

Græsprotein
Peter Ruhdal Jensen
25/01/2018
National Food Institute, Research group for Microbial Biotechnology and Biorefining

Media coverage (1)

Græsprotein
25/01/2018
DR P4 Sjælland (Regional), Denmark, Radio
Anne-Marie Dynes Møller
Peter Ruhdal Jensen
Research group for Microbial Biotechnology and Biorefining, National Food Institute

Press/Media: Press / Media