A Capture-SELEX Strategy for Multiplexed Selection of RNA Aptamers Against Small Molecules

In vitro selection of aptamers that recognize small organic molecules has proven difficult, in part due to the challenge of immobilizing small molecules on solid supports for SELEX (Systematic Evolution of Ligands by Exponential Enrichment). This study describes the implementation of RNA Capture-SELEX, a selection strategy that uses an RNA library to yield ligand-responsive RNA aptamers targeting small organic molecules in solution. To demonstrate the power of this method we selected several aptamers with specificity towards either the natural sweetener rebaudioside A or the food-coloring agent carminic acid. In addition, Bio-layer interferometry is used to screen clonal libraries of aptamer candidates and is used to interrogate aptamer affinity. The RNA-based Capture-SELEX strategy described here simplifies selection of RNA aptamers against small molecules by avoiding ligand immobilization, while also allowing selection against multiple candidate targets in a single experiment. This makes RNA Capture-SELEX particularly attractive for accelerated development of RNA aptamers targeting small metabolites for incorporation into synthetic riboswitches and for analytical biosensors.
Bacterial Genome Editing Strategy for Control of Transcription and Protein Stability

In molecular biology and cell factory engineering, tools that enable control of protein production and stability are highly important. Here, we describe protocols for tagging genes in *Escherichia coli* allowing for inducible degradation and transcriptional control of any soluble protein of interest. The underlying molecular biology is based on the two cross-kingdom tools CRISPRi and the N-end rule for protein degradation. Genome editing is performed with the CRMAGE technology and randomization of the translational initiation region minimizes the polar effects of tag insertion. The approach has previously been applied for targeting proteins originating from essential operon-located genes and has potential to serve as a universal synthetic biology tool.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Research Groups, Microbial Evolution and Synthetic Biology, Membrane Synthetic Biology Group, Department of Systems Biology, National Institute of Aquatic Resources, Bacterial Cell Factory Optimization
Authors: Lauritsen, I. (Intern), Martinez, V. (Intern), Ronda, C. (Intern), Nielsen, A. T. (Intern), Nørholm, M. H. H. (Intern)
Pages: 27-37
Publication date: 2018

Multiplex Genome Editing in *Escherichia coli*

Lambda Red recombineering is an easy and efficient method for generating genetic modifications in *Escherichia coli*. For gene deletions, lambda Red recombineering is combined with the use of selectable markers, which are removed through the action of, e.g., flippase (Flp) recombinase. This PCR-based engineering method has also been applied to a number of other bacteria. In this chapter, we describe a recently developed one plasmid-based method as well as the use of a strain with genomically integrated recombineering genes, which significantly speeds up the engineering of strains with multiple genomic alterations.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
Authors: Ingemann Jensen, S. (Intern), Nielsen, A. T. (Intern)
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Bacterial cells with improved tolerance to isobutyric acid

Bacterial cells genetically modified to improve their tolerance to certain commodity chemicals, such as isobutyric acid and related compounds, and methods of preparing and using such bacterial cells for production of isobutyric acid and related compounds.

Improved biological processes for the production of aryl sulfates

The present invention generally relates to the field of biotechnology as it applies to the production of aryl sulfates using recombinant host cells. More particularly, the present invention pertains to recombinant host cells comprising (e.g., expressing) a polypeptide having aryl sulfotransferase activity, wherein said recombinant host cells have been modified to have an increased uptake of sulfate compared to identical host cells that does not carry said modification. Further provided are processes for the production of aryl sulfates, such as zosteric acid, employing such recombinant host cells.
**Improved process for producing a fermentation product from a lignocellulose-containing material**

The present invention relates to the production of hydrolyzates from a lignocellulose-containing material, and to fermentation of the hydrolyzates. More specifically, the present invention relates to the detoxification of phenolic inhibitors and toxins formed during the processing of lignocellulose-containing material by sulfating the phenolic inhibitors and toxins using aryl sulfotransferase (EC 2.8.2.1) and sulfate transporter.

**General information**

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Authors: Jendresen, C. B. (Intern), Nielsen, A. T. (Intern)
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Publication: Research › Patent – Annual report year: 2017

**Bacterial cells with improved tolerance to polyamines**

Provided are bacterial cells genetically modified to improve their tolerance to certain commodity chemicals, such as polyamines, and methods of preparing and using such bacterial cells for production of polyamines and other compounds.

**General information**

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Research Groups, iLoop, Bacterial Cell Factory Optimization, Global Econometric Modeling, Department of Biotechnology and Biomedicine, Bacterial Synthetic Biology, ALE Technology & Software Development
Authors: Lennen, R. (Intern), Nielsen, A. T. (Intern), Herrgaard, M. (Intern), Sommer, M. O. A. (Intern), Feist, A. (Intern), Tharwat Tolba Mohamed, E. (Intern)
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Publication: Research › Patent – Annual report year: 2017
Applying thermostable β-galactosidase, BgaB, from Geobacillus stearothermophilus as a versatile reporter under anaerobic and aerobic conditions: Alternative title; Application of the thermostable beta-galactosidase, BgaB, from Geobacillus stearothermophilus as a versatile reporter under anaerobic and aerobic conditions

Use of thermophilic organisms has a range of advantages, but the significant lack of engineering tools limits their applications. Here we show that β-galactosidase from Geobacillus stearothermophilus (BgaB) can be applicable in a range of conditions, including different temperatures and oxygen concentrations. This protein functions both as a marker, promoting colony color development in the presence of a lactose analogue S-gal, and as a reporter enabling quantitative measurement by a simple colorimetric assay. Optimal performance was observed at 70 °C and pH 6.4. The gene was introduced into G. thermoglucosidans. The combination of BgaB expressed from promoters of varying strength with S-gal produced distinct black colonies in aerobic and anaerobic conditions at temperatures ranging from 37 to 60 °C. It showed an important advantage over the conventional β-galactosidase (LacZ) and substrate X-gal, which were inactive at high temperature and under anaerobic conditions. To demonstrate the versatility of the reporter, a promoter library was constructed by randomizing sequences around −35 and −10 regions in a wild type groES promoter from Geobacillus sp. GHH01. The library contained 28 promoter variants and encompassed fivefold variation. The experimental pipeline allowed construction and measurement of expression levels of the library in just 4 days. This β-galactosidase provides a promising tool for engineering of aerobic, anaerobic, and thermophilic production organisms such as Geobacillus species.

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CRISPR/Cas9-based genome editing for simultaneous interference with gene expression and protein stability
Interference with genes is the foundation of reverse genetics and is key to manipulation of living cells for biomedical and biotechnological applications. However, classical genetic knockout and transcriptional knockdown technologies have
different drawbacks and offer no control over existing protein levels. Here, we describe an efficient genome editing approach that affects specific protein abundances by changing the rates of both RNA synthesis and protein degradation, based on the two cross-kingdom control mechanisms CRISPRi and the N-end rule for protein stability. In addition, our approach demonstrates that CRISPRi efficiency is dependent on endogenous gene expression levels. The method has broad applications in e.g. study of essential genes and antibiotics discovery.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Membrane Synthetic Biology Group, Research Groups, Microbial Evolution and Synthetic Biology, Bacterial Cell Factory Optimization
Authors: Martínez, V. (Intern), Lauritsen, I. (Intern), Hobel, T. (Intern), Li, S. (Intern), Nielsen, A. T. (Intern), Nørholm, M. (Intern)
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BFI (2014): BFI-level 2
Scopus rating (2014): SJR 6.576 SNIP 2.568 CiteScore 8.74
Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 6.582 SNIP 2.266 CiteScore 8.46
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Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
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ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 5.758 SNIP 2.172 CiteScore 7.86
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 5.24 SNIP 2.034
Web of Science (2010): Indexed yes
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Scopus rating (2009): SJR 5.571 SNIP 1.869
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Scopus rating (2008): SJR 4.641 SNIP 1.557
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 4.86 SNIP 1.787
Web of Science (2007): Indexed yes
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Detection of p-coumaric acid from cell supernatant using surface enhanced Raman scattering

A standard protocol for analysis of microbial factories requires the screening of several populations in order to find the best performing ones. Standard analytical methods usually include high performance liquid chromatography (HPLC), thin layer chromatography (TLC) or spectrophotometry, which are expensive and time-consuming processes. Surface Enhanced Raman Spectroscopy (SERS), instead, is a highly sensitive spectroscopic technique for specific, fast and real-time sensing of biological samples. Here we demonstrate the use of SERS to discriminate between two different bacterial populations based on detection of p-coumaric acid (pHCA) in cell supernatant. SERS active substrates, based on leaning gold-capped silicon nanopillars, were used for detection. They were successfully used to detect culture medium spiked with pHCA, and the effect of medium dilution was studied. For analysis of biological production of pHCA, triplicate cultures of E. coli strains expressing a pHCA-forming enzyme (P) as well as of a non-producing strain (C) were grown. Then, supernatant samples were collected and their pHCA content was measured using SERS and HPLC for comparison. The intensity of the pHCA Raman mode at 1169 cm⁻¹ (CH-rocking motion) showed different trends for P and C strains, similar to the results obtained using the HPLC method. Results illustrate that SERS can be used for quick and semiquantitative discrimination of pHCA concentrations in cell supernatant medium.
Development of an intracellular glycolytic flux sensor for high throughput applications in E. coli

The aim of this PhD project was to construct, test and apply an intracellular, growth-independent and direct measureable glycolytic flux biosensor in E. coli.

Studying the metabolic flux of bacterial cells is of growing interest as it is of fundamental importance to bacterial physiology as well as for in silico modeling and metabolic engineering. The metabolic flux contains information about how efficiently a bacterium can utilize a given carbon source and in which extend it is directed towards the different central metabolic pathways. The knowledge of these fluxes can contribute to the development of efficient production pathways and the identification of possible accumulation points in the engineered pathway. Furthermore it can give information about regulatory networks within the cell.

The developed biosensor is based on the transcription factor Cra and links the metabolic flux to the expression of green fluorescent protein (GFP). The dynamic range of the final biosensor construct covers the whole range of natural, intracellular glycolytic fluxes, induced by different carbon sources and it could also be shown that it is even capable of monitoring a further flux increase.

The sensor was applied to study the flux-altering effects of gene knockouts in E. coli at the single cell level in a vastly parallelized and high-throughput manner. After growth for several generations in rich and minimal media, 2126 gene knockouts, mainly outside of the core metabolism, could be screened. 3 gene knockouts with a high flux and 158 with a low flux phenotype were found, comprising many flagella and phage related genes as well as many so far uncharacterized proteins.

Taken together, the glycolytic flux biosensor offers a tool to screen for metabolic flux changes in an efficient, fast and parallelizable way, opening up for novel screening approaches that enhance our understanding of microbial physiology and can be applied to improve microbial cell factories.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Department of Biotechnology and Biomedicine, Bacterial Synthetic Biology, Research Groups, Bacterial Cell Factory Optimization
Authors: Lehning, C. E. (Intern), Sommer, M. O. A. (Intern), Nielsen, A. T. (Intern)
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Publication: Research › Ph.D. thesis – Annual report year: 2017

Exploiting the potential of gas fermentation
The use of gas fermentation for production of chemicals and fuels with lower environmental impact is a technology that is gaining increasing attention. Over 38 Gt of CO2 is annually being emitted from industrial processes, thereby contributing significantly to the concentration of greenhouse gases in the atmosphere. Together with the gasification of biomass and different waste streams, these gases have the potential for being utilized for production of chemicals through fermentation processes. Acetogens are among the most studied organisms capable of utilizing waste gases. Although engineering of heterologous production of higher value compounds has been successful for a number of acetogens, the processes are challenging due to the redox balance and the lack of efficient engineering tools. In this review, we address the availability of different gaseous feedstock and gasification processes, and we focus on the advantages of alternative fermentation scenarios, including thermophilic production strains, multi-stage fermentations, mixed cultures, as well as mixotrophy. Such processes have the potential to significantly broaden the product portfolio, increase the product concentrations and yields, while enabling the exploitation of alternative and mixed feedstocks. The reviewed processes also have the potential to address challenges associated with product inhibition and may contribute to reducing the costs of downstream processing. Given the widespread availability of gases, such processes will likely significantly impact the transition towards a more sustainable society.
Genetic toolbox for controlled expression of functional proteins in Geobacillus spp.

Species of genus Geobacillus are thermophilic bacteria and play an ever increasing role as hosts for biotechnological applications both in academia and industry. Here we screened a number of Geobacillus strains to determine which industrially relevant carbon sources they can utilize. One of the strains, G. thermoglucosidasius C56-YS93, was then chosen to develop a toolbox for controlled gene expression over a wide range of levels. It includes a library of semi-synthetic constitutive promoters (76-fold difference in expression levels) and an inducible promoter from the xylA gene. A library of synthetic in silico designed ribosome binding sites was also created for further tuning of translation. The $P_{xylA}$ was further used to successfully express native and heterologous xylanases in G. thermoglucosidasius. This toolbox enables fine-tuning of gene expression in Geobacillus species for metabolic engineering approaches in production of biochemicals and heterologous proteins.

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Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.74 SNIP 1.147 CiteScore 3.94
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
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ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
Genome-wide identification of tolerance mechanisms towards p-coumaric acid in Pseudomonas putida

The soil bacterium Pseudomonas putida KT2440 has gained increasing biotechnological interest due to its ability to tolerate different types of stress. Here, the tolerance of P. putida KT2440 towards eleven toxic chemical compounds was investigated. P. putida was found to be significantly more tolerant towards three of the eleven compounds when compared to Escherichia coli. Increased tolerance was for example found towards p-coumaric acid, an interesting precursor for polymerization with a significant industrial relevance. The tolerance mechanism was therefore investigated using the genome-wide approach, Tn-seq. Libraries containing a large number of miniTn5-Km transposon insertion mutants were grown in the presence and absence of p-coumaric acid, and the enrichment or depletion of mutants was quantified by high-throughput sequencing. Several genes, including the ABC transporter Ttg2ABC and the cytochrome c maturation system (ccm), were identified to play an important role in the tolerance towards p-coumaric acid of this bacterium. Most of the identified genes were involved in membrane stability, suggesting that tolerance towards p-coumaric acid is related to transport and membrane integrity. This article is protected by copyright. All rights reserved.
Increased production of L-serine in Escherichia coli through Adaptive Laboratory Evolution

L-serine is a promising building block biochemical with a high theoretical production yield from glucose. Toxicity of L-serine is however prohibitive for high-titer production in E. coli. Here, E. coli lacking L-serine degradation pathways was evolved for improved tolerance by gradually increasing L-serine concentration from 3 to 100 g/L using adaptive laboratory evolution (ALE). Genome sequencing of isolated clones revealed multiplication of genetic regions, as well as mutations in thrA, thereby showing a potential mechanism of serine inhibition. Other mutations were evaluated by MAGE combined with amplicon sequencing, revealing role of rho, lrp, pykF, eno, and rpoB on tolerance and fitness in minimal medium. Production using the tolerant strains resulted in 37 g/L of L-serine with a 24% mass yield. The resulting titer is similar to the highest production reported for any organism thereby highlighting the potential of ALE for industrial biotechnology.

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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.381 SNIP 2.034 CiteScore 7.23
Web of Science (2014): Indexed yes
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ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.032 SNIP 1.858 CiteScore 6.72
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 3.124 SNIP 2.144 CiteScore 6.75
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.373 SNIP 1.802
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.575 SNIP 1.421
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.757 SNIP 1.028
Lab-on-a-disc platform for screening of genetically modified E. coli cells via cell-free electrochemical detection of p-Coumaric acid

We present a robust easy to use lab-on-a-disc (LoD) device with integrated sample pre-treatment and electrochemical detection system for cell-free detection of a secondary metabolite, p-Coumaric acid (pHCA), produced by genetically modified E. coli. In the LoD device, which incorporates eight filtration and electrochemical detection units, the sample filtration was performed by rotating the disc using a programmable closed-loop stepper motor. The electrodes, patterned on plastic substrate, were connected through a printed circuit board to the slip ring using a robust magnetic clamping system that enables easy assembly and robust electrical connections. pHCA was quantified in a linear range from 0.125 up to 2 mM using square wave voltammetry. The platform was successfully used for the quantification of pHCA produced by two genetically modified E. coli strains after 24 h of cell culture. The data obtained from the electrochemical measurements showed good correlation with high performance liquid chromatographic analysis. The developed LoD system offers fast and easy detection of pHCA, enabling screening of genetically modified organisms based on the quantity of produced secondary metabolites.
Lactobacilli and pediococci as versatile cell factories - Evaluation of strain properties and genetic tools

This review discusses opportunities and bottlenecks for cell factory development of Lactic Acid Bacteria (LAB), with an emphasis on lactobacilli and pediococci, their metabolism and genetic tools. In order to enable economically feasible bio-based production of chemicals and fuels in a biorefinery, the choice of product, substrate and production organism is important. Currently, the most frequently used production hosts include Escherichia coli and Saccharomyces cerevisiae, but promising examples are available of alternative hosts such as LAB. Particularly lactobacilli and pediococci can offer benefits such as thermostolerance, an extended substrate range and increased tolerance to stresses such as low pH or high alcohol concentrations. This review will evaluate the properties and metabolism of these organisms, and provide an overview of their current biotechnological applications and metabolic engineering. We substantiate the review by including experimental results from screening various lactobacilli and pediococci for transformability, growth temperature range and ability to grow under biotechnologically relevant stress conditions. Since availability of efficient genetic engineering tools is
a crucial prerequisite for industrial strain development, genetic tool development is extensively discussed. A range of genetic tools exist for Lactococcus lactis, but for other species of LAB like lactobacilli and pediococci such tools are less well developed. Whereas lactobacilli and pediococci have a long history of use in food and beverage fermentation, their use as platform organisms for production purposes is rather new. By harnessing their properties such as therotolerance and stress resistance, and by using emerging high-throughput genetic tools, these organisms are very promising as versatile cell factories for biorefinery applications.
Quantification of a bacterial secondary metabolite by SERS combined with SLM extraction for bioprocess monitoring

During the last few decades, great advances have been reached in high-throughput design and building of genetically engineered microbial strains, leading to a need for fast and reliable screening methods. We developed and optimized a microfluidic supported liquid membrane (SLM) extraction device and combined it with surface enhanced Raman scattering (SERS) sensing for the screening of a biological process, namely for the quantification of a bacterial secondary metabolite, p-coumaric acid (pHCA), produced by Escherichia coli. The microfluidic device proved to be robust and reusable, enabling efficient removal of interfering compounds from the real samples, reaching more than 13-fold up-concentration of the donor at 10 μL min⁻¹ flow rate. With this method, we quantified pHCA directly from the bacterial supernatant, distinguishing between various culture conditions based on the pHCA production yield. The obtained data showed good correlation with HPLC analysis.
Surface Enhanced Raman Scattering for Quantification of p-Coumaric Acid Produced by Escherichia coli

The number of newly developed genetic variants of microbial cell factories for production of biochemicals has been rapidly growing in recent years, leading to an increased need for new screening techniques. We developed a method based on surface-enhanced Raman scattering (SERS) coupled with liquid-liquid extraction (LLE) for quantification of p-coumaric acid (pHCA) in the supernatant of genetically engineered Escherichia coli (E. coli) cultures. pHCA was measured in a dynamic range from 1 μM up to 50 μM on highly uniform SERS substrates based on leaning gold-capped nanopillars, which showed an in-wafer signal variation of only 11.7%. LLE using dichloromethane as organic phase was combined with the detection in order to increase selectivity and sensitivity by decreasing the effect of interfering compounds from the analytes of interest. The difference in pHCA production yield between three genetically engineered E. coli strains was successfully evaluated using SERS and confirmed with high-performance liquid chromatography. As this novel approach has potential to be automated and parallelized, it can be considered for high-throughput screening in metabolic engineering.

General information
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Authors: Morelli, L. (Intern), Zor, K. (Intern), Jendresen, C. B. (Intern), Rindzevicius, T. (Intern), Schmidt, M. S. (Intern), Nielsen, A. T. (Intern), Boisen, A. (Intern)
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Thermodynamics and economic feasibility of acetone production from syngas using the thermophilic production host *Moorella thermoacetica*

**Background:** Syngas fermentation is a promising option for the production of biocommodities due to its abundance and compatibility with anaerobic fermentation. Using thermophilic production strains in a syngas fermentation process allows recovery of products with low boiling point from the off-gas via condensation. Results: In this study we analyzed the production of acetone from syngas with the hypothetical production host derived from *Moorella thermoacetica* in a bubble column reactor at 60 degrees C with respect to thermodynamic and economic feasibility. We determined the cost of syngas production from basic oxygen furnace (BOF) process gas, from natural gas, and from corn stover and identified BOF gas as an economically interesting source for syngas. Taking gasliquid mass transfer limitations into account, we applied a thermodynamics approach to derive the CO to acetone conversion rate under the process conditions. We estimated variable costs of production of 389 $/t acetone for a representative production scenario from BOF gas with costs for syngas as the main contributor. In comparison, the variable costs of production from natural gas-and corn stover-derived syngas were determined to be higher due to the higher feedstock costs (1724 and 2878 $/t acetone, respectively). Conclusion: We applied an approach of combining thermodynamic and economic assessment to analyze a hypothetical bioprocess in which the volatile product acetone is produced from syngas with a thermophilic microorganism. Our model allowed us to identify process metrics and quantify the variable production costs for different scenarios. Economical production of bulk chemicals is challenging, making rigorous thermodynamic/economic modeling critical before undertaking an experimental program and as an ongoing guide during the program. We intend this study to give an incentive to apply the demonstrated approach to other bioproduction processes.
Genetically modified microorganisms having improved tolerance towards L-serine.

The present invention generally relates to the microbiological industry, and specifically to the production of L-serine or L-serine derivatives using genetically modified bacteria. The present invention provides genetically modified microorganisms, such as bacteria, wherein the expression of genes encoding for enzymes involved in the degradation of L-serine is attenuated, such as by inactivation, which makes them particularly suitable for the production of L-serine at higher yield. The present invention also provides means by which the microorganism, and more particularly a bacterium, can be made tolerant towards higher concentrations of serine. The present invention also provides methods for the production of L-serine or L-serine derivative using such genetically modified microorganisms.
Method for the production of L-serine using genetically engineered microorganisms deficient in serine degradation pathways.
The present invention generally relates to the microbiological industry, and specifically to the production of L-serine using genetically modified bacteria. The present invention provides genetically modified microorganisms, such as bacteria, wherein the expression of genes encoding for enzymes involved in the degradation of L-serine is attenuated, such as by inactivation, which makes them particularly suitable for the production of L-serine at higher yield. The present invention also provides means by which the microorganism, and more particularly a bacterium, can be made tolerant towards higher concentrations of serine. The present invention also provides methods for the production of L-serine or L-serine derivative using such genetically modified microorganisms.

A two-cassette reporter system for assessing target gene translation and target gene product inclusion body formation
The present invention relates to a dual cassette reporter system capable of assessing target gene translation and target gene product folding. The present invention further relates to vectors and host cells comprising the dual cassette reporter system. In addition the invention relates to the use of the dual cassette reporter system for assessing target gene translation and target gene product folding.
A process for producing a fermentation product from a lignocellulose-containing material.

The present invention relates to the production of hydrolyzates from a lignocellulose-containing material, and to fermentation of the hydrolyzates. More specifically, the present invention relates to the detoxification of phenolic inhibitors and toxins formed during the processing of lignocellulose-containing material by enzymatically sulfating the phenolic inhibitors and toxins using aryl sulfotransferases.

Biological processes for the production of aryl sulfates.

The present invention generally relates to the field of biotechnology as it applies to the production of aryl sulfates using polypeptides or recombinant cells comprising said polypeptides. More particularly, the present invention pertains to polypeptides having aryl sulfotransferase activity, recombinant host cells expressing same and processes for the production of aryl sulfates employing these polypeptides or recombinant host cells.
Processes for the production of hydroxycinnamic acids using polypeptides having tyrosine ammonia lyase activity.

The present invention generally relates to the field of biotechnology as it applies to the production of hydroxycinnamic acids using polypeptides having tyrosine ammonia lyase activity. More particularly, the present invention pertains to polypeptides having tyrosine ammonia lyase activity and high substrate specificity towards tyrosine, which makes them particularly suitable in the production of p-coumaric acid and other hydroxycinnamic acids. The present invention thus provides processes for the production of p-coumaric acid and other hydroxycinnamic acids employing these polypeptides as well as recombinant host cells expressing same.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups, Bacterial Synthetic Biology, Applied Metabolic Engineering
Authors: Jendresen, C. B. (Intern), Siedler, S. (Intern), Stahlhut, S. G. (Intern), Nielsen, A. T. (Intern)
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Publication information
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Date: 21/01/2016
Priority date: 14/07/2014
Priority number: EP20140176975
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Electronic versions:
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Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: WO2016008886
Publication: Research › Patent – Annual report year: 2016

Broad host range ProUSER vectors enable fast characterization of inducible promoters and optimization of p-coumaric acid production in Pseudomonas putida KT2440

Pseudomonas putida KT2440 has gained increasing interest as a host for the production of biochemicals. Because of the lack of a systematic characterization of inducible promoters in this strain, we generated ProUSER broad-host-expression plasmids that facilitate fast uracil-based cloning. A set of ProUSER-reporter vectors was further created to characterize different inducible promoters. The PrhaB and Pm promoters were orthogonal and showed titratable, high, and homogeneous expression. To optimize the production of p-coumaric acid, P. putida was engineered to prevent degradation of tyrosine and p-coumaric acid. Pm and PrhaB were used to control the expression of a tyrosine ammonia lyase or AroG* and TyrA* involved in tyrosine production, respectively. Pathway expression was optimized by modulating inductions, resulting in small-scale p-coumaric acid production of 1.2 mM, the highest achieved in Pseudomonads under comparable conditions. With broad-host-range compatibility, the ProUSER vectors will serve as useful tools for optimizing gene expression in a variety of bacteria.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
Authors: Calero Valdayo, P. (Intern), Ingemann Jensen, S. (Intern), Nielsen, A. T. (Intern)
Number of pages: 13
Pages: 741-753
Publication date: 2016
Main Research Area: Technical/natural sciences
One of the great challenges facing society is how to sustainably produce food, chemicals and other commodities required to maintain and develop our current life style. To compete with and ultimately replace existing petrochemical-based manufacturing processes, the development of innovative and effective solutions is needed.

In this project we have explored the possibility of using designed consortiums for the covalorization of the main carbon sources in lignocellulosic biomass (xylose, glucose, arabinose, and acetic acid). In one study we have used preprocessing simulations, constraint-based modelling, and state-of-the art metabolic engineering tools to develop
consortium of cells capable of efficient valorization of synthetic hemicellulosic hydrolysate. Stable co-existence and effective covalorization was achieved through niche-differentiation, auxotrophy, and adaptive evolution. In another study, stable consortia based fermentation was achieved through niche partitioning, syntrophy (auxotrophy combined with removal of inhibitory side product), and CRISPRi mediated gene silencing. The achieved results demonstrate that consortium based approaches for valorizing complex biomass and waste related carbon sources can be an attractive alternative to the design of a so-called “superbug” and can thereby add significant value to biorefineries.

**General information**

State: Published

Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Global Econometric Modeling, iLoop, Systems Environmental Microbiology, CHO Cell Line Engineering and Design, Research Groups

Authors: Ingemann Jensen, S. (Intern), Sukumara, S. (Intern), Özdemir, E. (Intern), Schneider, K. (Intern), Calero Valdayo, P. M. (Intern), Li, S. (Intern), Ronda, C. (Intern), Nielsen, A. T. (Intern)

Number of pages: 1

Publication date: 2016


Main Research Area: Technical/natural sciences

Links:

http://www.sustain.dtu.dk/

**Bibliographical note**

Sustain Abstract I-2

Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2016

**CRMAGE: CRISPR Optimized MAGE Recombineering**

A bottleneck in metabolic engineering and systems biology approaches is the lack of efficient genome engineering technologies. Here, we combine CRISPR/Cas9 and λ Red recombineering based MAGE technology (CRMAGE) to create a highly efficient and fast method for genome engineering of Escherichia coli. Using CRMAGE, the recombineering efficiency was between 96.5% and 99.7% for gene recoding of three genomic targets, compared to between 0.68% and 5.4% using traditional recombineering. For modulation of protein synthesis (small insertion/RBS substitution) the efficiency was increased from 6% to 70%. CRMAGE can be multiplexed and enables introduction of at least two mutations in a single round of recombineering with similar efficiencies. PAM-independent loci were targeted using degenerate codons, thereby making it possible to modify any site in the genome. CRMAGE is based on two plasmids that are assembled by a USER-cloning approach enabling quick and cost efficient gRNA replacement. CRMAGE furthermore utilizes CRISPR/Cas9 for efficient plasmid curing, thereby enabling multiple engineering rounds per day. To facilitate the design process, a web-based tool was developed to predict both the λ Red oligos and the gRNAs. The CRMAGE platform enables highly efficient and fast genome editing and may open up promising prospective for automation of genome-scale engineering.

**General information**

State: Published

Organisations: Bacterial Cell Factories, Novo Nordisk Foundation Center for Biosustainability, iLoop, Bacterial Synthetic Biology, Research Groups, Bacterial Cell Factory Optimization

Authors: Ronda, C. (Intern), Pedersen, L. E. (Intern), Sommer, M. O. A. (Intern), Nielsen, A. T. (Intern)

Number of pages: 11

Publication date: 2016

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BFI (2016): BFI-level 1

Scopus rating (2016): CiteScore 4.63 SJR 1.625 SNIP 1.401

Web of Science (2016): Indexed yes

BFI (2015): BFI-level 1

Scopus rating (2015): SJR 2.057 SNIP 1.684 CiteScore 5.3

Web of Science (2015): Indexed yes
Decoupling of Growth from Production of Biochemicals and Proteins

With increasing awareness of sustainability in our current society, alternative approaches to produce fuels and petro-derived chemicals are required. Biofuels and biochemicals produced from microbial cell factories provide an alternative to current fossil based chemicals. Meanwhile, microbial cell factories have found their applications for producing a wide range of products, such as food additives, pharmaceuticals and industrial enzymes. However, for most of current microbial cell factories, the performance is far from sufficient to be used in an economic industrial fermentation process. One general problem for these cell factories is the un-controlled accumulation of biomass during fermentation, which significantly reduces the fraction of substrate that can be converted into products. Therefore, a method for controlling cell growth during production becomes desirable in order to improve the production of a range of interesting molecules. This thesis has aimed at developing various methods for controlling cell growth as well as to maintain their capacity for production, in order to improving the performance of microbial cell factories. Using the popular cell factory organism, E. coli, as our model system, three different strategies have been employed to explore the desired growth controlling methods. First, the cell growth was controlled by limiting nutrients or adding inhibitory chemicals in cultivation medium. Significant improvement of production yield and specific productivity, of both tyrosine and mevalonate, was achieved through this type of growth limitation. Second, rationally designed genetic growth switches, based on CRISPR interference (CRISPRi) systems, have been developed. By switching off cell growth during production, the production of biochemicals and proteins, exemplified by mevalonate and GFP, has been improved. Finally, a CRISPRi library, designed to search through the whole genome of E. coli, has been applied. Several novel target genes were identified to be efficient for growth control as well as maintaining protein production. These results demonstrate the possibility of decoupling cell growth from production, and they provide alternative and widely applicable approaches for improving the performance of cell factories. The library approach provides a novel strategy to identify targets for growth switches without prior knowledge, and the method can be applied to a range of other cell factory host cell organisms.
Developing Lactic Acid Bacteria for the conversion of brown macroalgae into green chemicals and fuels

Microbial conversion of biomass plays a major role in establishing a bio-based economy, which aims at replacing fossil resources with renewable substrates for the production of fuels and chemicals. Current efforts in using non-edible ('second generation') biomass rather than food-derived sugars focus on lignocellulosic materials such as crop residues and non-edible plants. However, lignin is often toxic to the production organism and hard to eliminate, and economically feasible conversion of cellulose and hemicellulose is still challenging. An attractive alternative includes brown macroalgae or seaweed, which do not contain lignin, do not require fresh water, are not a major food source, and contain a higher sugar fraction. The main sugars are mannitol, laminarin (glucose) and alginate (guluronate and mannnuronate). We will use metabolic engineering and laboratory evolution of Lactic Acid Bacteria (LAB) for the conversion of brown macroalgae into green chemicals and fuels. To select the best-suited production platform, we are screening Lactobacillus and Pediococcus strains for traits like genetic accessibility, substrate utilization and several stress tolerances. Most microorganisms, including LAB, do not naturally utilize alginates and hence the introduction of these pathways will be the first step in engineering the selected strain, after which further efforts will focus on co-utilization of the different sugar fractions and establishment of product pathways.

General information
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Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
Authors: Bosma, E. F. (Intern), Nielsen, A. T. (Intern)
Number of pages: 1
Publication date: 2016
Main Research Area: Technical/natural sciences
Links:
http://www.sustain.dtu.dk/

Bibliographical note
Sustain Abstract B-5
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2016

Engineering of High Yield Production of L-serine in Escherichia coli

L-serine is a widely used amino acid that has been proposed as a potential building block biochemical. The high theoretical yield from glucose makes a fermentation based production attractive. In order to achieve this goal, serine degradation to pyruvate and glycine in E. coli MG1655 was prevented by deletion of three L-serine deaminases sdaA, sdaB, and tdcG, as well as serine hydroxyl methyl transferase (SHMT) encoded by glyA. Upon overexpression of the serine production pathway, consisting of a feedback resistant version of serA along with serB and serC, this quadruple deletion strain showed a very high serine production yield (0.45 g/g glucose) during small-scale batch fermentation in minimal medium. Serine, however, was found to be highly toxic even at low concentrations to this strain, which lead to slow growth and production during fed batch fermentation, resulting in a serine production of 8.3 g/L. The production strain was therefore evolved by random mutagenesis to achieve increased tolerance towards serine. Additionally, overexpression of eamA, a cysteine/homoserine transporter was demonstrated to increase serine tolerance from 1.6 g/L to 25 g/L. During fed batch fermentation, the resulting strain lead to the serine production titer of 11.7 g/L with yield of 0.43 g/g glucose, which is the highest yield reported so far for any organism.

General information
State: Published
Organisations: Bacterial Cell Factories, Novo Nordisk Foundation Center for Biosustainability, iLoop, National Food Institute
Authors: Mundhada, H. (Intern), Schneider, K. (Intern), Christensen, H. B. (Intern), Nielsen, A. T. (Intern)
Number of pages: 10
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Publication date: 2016
Main Research Area: Technical/natural sciences

Publication information
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Web of Science (2017): Indexed yes
Amino acids, E. coli, Fermentation, L-serine production, L-serine toxicity, Metabolic engineering

Scopus rating (2016): CiteScore 4.14 SJR 1.411 SNIP 1.163
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.613 SNIP 1.37 CiteScore 4.44
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.589 SNIP 1.401 CiteScore 4.16
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.621 SNIP 1.425 CiteScore 4.44
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.639 SNIP 1.366 CiteScore 4.04
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.668 SNIP 1.483 CiteScore 4.08
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.538 SNIP 1.357
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.491 SNIP 1.356
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.238 SNIP 1.288
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.368 SNIP 1.362
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.458 SNIP 1.43
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 1.123 SNIP 1.239
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 1.094 SNIP 1.249
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 1.041 SNIP 1.228
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 1.197 SNIP 1.278
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 1.07 SNIP 1.177
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 1.102 SNIP 1.541
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 1.511 SNIP 1.567
Original language: English
Amino acids, E. coli, Fermentation, L-serine production, L-serine toxicity, Metabolic engineering

10.1002/bit.25844
Source: FindIt
Source-ID: 2281899305
Publication: Research - peer-review › Journal article – Annual report year: 2015
Enhanced protein and biochemical production using CRISPRi-based growth switches

Production of proteins and biochemicals in microbial cell factories is often limited by carbon and energy spent on excess biomass formation. To address this issue, we developed several genetic growth switches based on CRISPR interference technology. We demonstrate that growth of *Escherichia coli* can be controlled by repressing the DNA replication machinery, by targeting *dnaA* and *oriC*, or by blocking nucleotide synthesis through *pyrF* or *thyA*. This way, total GFP-protein production could be increased by up to 2.2-fold. Single-cell dynamic tracking in microfluidic systems was used to confirm functionality of the growth switches. Decoupling of growth from production of biochemicals was demonstrated for mevalonate, a precursor for isoprenoid compounds. Mass yield of mevalonate was increased by 41%, and production was maintained for more than 45 h after activation of the *pyrF*-based growth switch. The developed methods represent a promising approach for increasing production yield and titer for proteins and biochemicals.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Research Groups, Research Centre Julich (FZJ)
Authors: Li, S. (Intern), Jendresen, C. B. (Intern), Grünberger, A. (Ekstern), Ronda, C. (Intern), Ingemann Jensen, S. (Intern), Noack, S. (Ekstern), Nielsen, A. T. (Intern)
Number of pages: 11
Pages: 274-284
Publication date: 2016
Main Research Area: Technical/natural sciences

Publication information
Journal: Metabolic Engineering
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ISSN (Print): 1096-7176
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.33 SJR 3.54 SNIP 1.864
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.611 SNIP 1.822 CiteScore 8.2
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.381 SNIP 2.034 CiteScore 7.23
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 4.004 SNIP 2.185 CiteScore 8.43
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.032 SNIP 1.858 CiteScore 6.72
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 3.124 SNIP 2.144 CiteScore 6.75
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 2.373 SNIP 1.802
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 2.575 SNIP 1.421
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.757 SNIP 1.028
Gas Fermentation using Thermophilic Moorella Species for production of Biochemicals

Gas fermentation is a promising technology which gained increasing attention over the last years. In this process, acetogenic bacteria convert gases rich in H₂, CO₂, and CO, into compounds of higher value. The gas can derive from industrial off-gas or from waste streams via gasification. In the gas fermentation processes that are nearly on commercial level, mesophilic acetogens are used to mainly produce ethanol and butanediol. However, thermophilic acetogens, such as Moorella thermoacetica would allow for easy downstream processing when producing volatile products such as acetone.

This thesis starts with a review of the feedstock potential for gas fermentation and how thermophilic production strains as well as unconventional fermentation processes such as mixotrophy can help to exploit this potential. I analyzed a process with respect to thermodynamic and economic considerations, in which acetone is produced from cornstover derived syngas with M. thermoacetica hypothetically overexpressing the respective heterologous pathway. To make such a process feasible, some challenges have to be overcome. A system for genetic manipulation has been published in 2013, and I describe my attempts to extent the published genetic toolbox. One crucial tool is a robust selection marker. We characterized a thermostable beta-galactosidase and its application as screening marker in thermophiles. In 1981, strain M, thermoautotrophica, reported to be closely related to M. thermoacetica, was isolated. Together with collaborators, I identified M. thermoautotrophica to be a mixed culture of M. thermoacetica strains. We de novo sequenced the genome of several M. thermoacetica strains to shed light on the taxonomic relations within the genus Moorella. Although well studied, some aspects of the physiology of M. thermoacetica are still unknown. I performed an RNA-seq study of M. thermoacetica grown on sugar and gaseous substrates (H₂/CO₂ and H₂/CO₂/CO) to obtain insights into the transcription profile. To facilitate the research with anaerobes and thermophiles such as Moorella species, I developed together with a colleague a device that enables the fully automated generation of growth curves in mid-sized cultures. In the respective chapter, we elaborate on the role of 3D printing in the construction of novel lab equipment and present the aforementioned solution for automated tracking of bacterial growth. In conclusion, this thesis describes several projects which help to pave the way for biochemical production with the thermophile M. thermoacetica in an economically competitive way.

General information
State: Published
Organisations: Bacterial Cell Factory Optimization, Novo Nordisk Foundation Center for Biosustainability, Bacterial Synthetic Biology, Research Groups
Authors: Redl, S. M. A. (Intern), Nielsen, A. T. (Intern)
Number of pages: 309
Publication date: 2016

Publication information
Publisher: Novo Nordisk Foundation Center for Biosustainability
Economically viable biobased production of bulk chemicals and biofuels typically requires high product titers. During microbial bioconversion this often leads to product toxicity, and tolerance is therefore a critical element in the engineering of production strains. Here, a systems biology approach was employed to understand the chemical stress response of *Escherichia coli*, including a genome-wide screen for mutants with increased fitness during chemical stress. Twelve chemicals with significant production potential were selected, consisting of organic solvent-like chemicals (butanol, hydroxy-γ-butyrolactone, 1,4-butanediol, furfural), organic acids (acetate, itaconic acid, levulinic acid, succinic acid), amino acids (serine, threonine) and membrane-intercalating chemicals (decanoic acid, geraniol). The transcriptional response towards these chemicals revealed large overlaps of transcription changes within and between chemical groups, with functions such as energy metabolism, stress response, membrane modification, transporters and iron metabolism being affected. Regulon enrichment analysis identified key regulators likely mediating the transcriptional response, including CRP, RpoS, OmpR, ArcA, Fur and GadX. These regulators, the genes within their regulons and the above mentioned cellular functions therefore constitute potential targets for increasing *E. coli* chemical tolerance. Fitness determination of genome-wide transposon mutants (Tn-seq) subjected to the same chemical stress identified 294 enriched and 336 depleted mutants and experimental validation revealed up to 60 % increase in mutant growth rates. Mutants enriched in several conditions contained, among others, insertions in genes of the Mar-Sox-Rob regulon as well as transcription and translation related gene functions. The combination of the transcriptional response and mutant screening provides general targets that can increase tolerance towards not only single, but multiple chemicals.
Biochemicals, Chemical stress, Tolerance, Systems biology, Transcription analysis, Tn-seq, E. coli

Electronic versions:
Genome_wide_Escherichia_coli_stress_response_and_improved_tolerance_towards_industrially_relevant_chemicals.pdf

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Relations
Projects:
Genome-wide Escherichia coli stress response and improved tolerance towards industrially relevant chemicals
Publication: Research - peer-review › Journal article – Annual report year: 2016

Increasing production yield of tyrosine and mevalonate through inhibition of biomass formation
Microbial cell factories have been engineered to produce a variety of biochemicals, ranging from biofuels, food additives to pharmaceuticals. However, for most compounds, the production yield is far from reaching economical targets. Accumulation of excess biomass contributes to decreasing production yields, and a method for limiting biomass formation while allowing for continued production of biochemicals is therefore desirable. In this study, we investigated eight different culturing setups aiming at inhibiting biomass formation of Escherichia coli, based on nutrient limitations or the addition of growth inhibitors. The ability to control cell growth and the production of biochemicals, exemplified by mevalonate and tyrosine, was characterized. An increased mass yield of both mevalonate and tyrosine was achieved by limiting phosphate, sulfate or magnesium in the media. Sulfate limitation, in particular, resulted in an increase in mass yield of mevalonate and tyrosine by 80% and 50%, respectively. By tracking production and biomass concentrations, it was observed that the production was maintained for more than 10 h after inhibition of cell growth, despite cell maintenance requirements. The outlined method serves as promising approach for increasing production yield of a range of different biochemicals.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
The extensive use of cell factories for production of biochemicals is still facing a number of challenges to become economically competitive. One of the problems that need to be overcome is the toxicity of certain chemical products, which prevents yields high enough for their implementation in industry. This thesis aims at contributing to developing and characterizing tools for the use of alternative hosts organisms with high tolerance towards toxic compounds, such as *Pseudomonas putida*. The thesis also focuses on identifying target compounds that may be relevant to produce in this strain such as p-coumaric acid, and the mechanisms responsible for such natural tolerance. Moreover, the tolerance towards the highly toxic compound p-hydroxystyrene was improved as an alternative strategy to deal with the effect on growth during the production of such compounds. Expanding the toolbox for alternative microorganisms as well as identifying mechanisms that can be applied in the design and construction of cell factories will assist in the advance of biosustainability, leading us to a future free of petroleum-based industry.
Predictable tuning of protein expression in bacteria

We comprehensively assessed the contribution of the Shine-Dalgarno sequence to protein expression and used the data to develop EMOPEC (Empirical Model and Oligos for Protein Expression Changes; http://emopec.biosustain.dtu.dk). EMOPEC is a free tool that makes it possible to modulate the expression level of any *Escherichia coli* gene by changing only a few bases. Measured protein levels for 91% of our designed sequences were within twofold of the desired target level.

General information

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Synthetic Biology, Bacterial Cell Factory Optimization, iLoop, Research Groups
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Main Research Area: Technical/natural sciences

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Volume: 13
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 11.58 SJR 19.544 SNIP 5.24
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 20.9 SNIP 6.131 CiteScore 15.62
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 15.179 SNIP 4.815 CiteScore 13.54
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 10.093 SNIP 3.513 CiteScore 12.21
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 8.644 SNIP 2.969 CiteScore 10.1
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 9.535 SNIP 2.862 CiteScore 9.56
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 8.667 SNIP 2.474
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 7.497 SNIP 2.352
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 6.51 SNIP 2.017
Scopus rating (2007): SJR 5.618 SNIP 1.881
Scopus rating (2006): SJR 6.26 SNIP 2.144
Scopus rating (2005): SJR 2.724 SNIP 0.866
Original language: English
Bacteria, Gene expression, Genetic engineering
Production of Sulfated Organic Compounds in Cell Factories

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
Authors: Jendresen, C. B. (Intern), Nielsen, A. T. (Intern)
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Publication date: 2016
Event: Poster session presented at Metabolic Engineering 11, Kobe, Japan.
Main Research Area: Technical/natural sciences
Source: PublicationPreSubmission
Source-ID: 124926936
Publication: Research › Poster – Annual report year: 2016

Surface Enhanced Raman Spectroscopy detection of p-coumaric acid from cell supernatant using gold-capped silicon nanopillar substrates
A standard protocol for analysis of microbial factories requires the screening of several populations in order to find the best performing ones. This is done with standard analytical methods (e.g. HPLC) with an expensive and time-consuming process. Surface Enhanced Raman Spectroscopy (SERS) is a highly sensitive spectroscopic technique which only requires drying a small volume of solution on an active substrate, with an analysis time of few minutes. Here we demonstrate the use of SERS to discriminate between two different bacterial populations based on detection of p-coumaric acid (pHCA) in cell supernatant. pHCA is a valuable secondary metabolite of genetically modified E. coli[1]. It is produced through deamination of tyrosine, and it has strong Raman and SERS activity[2],[3]. Gold capped silicon nanopillars were used as sensing substrates[4]. At first, they were successfully used to detect pHCA spiked in culture medium, in the same concentration range (10^-4 – 10^-5 M) commonly found in cell supernatant. For supernatant analysis, triplicate cultures of F]TAL modified (P strains) and control (C strains) E.coli strains were carried out according to the methods described by[5] and shown in Fig.1. Samples of cell supernatant were extracted from each culture at 0, 3, 24 and 48 h post seeding and their pHCA content was measured with HPLC[5]. For SERS analysis, aliquots of supernatant were diluted 10-fold with MilliQ water, and 1 µL droplets were dried on the SERS substrates. A MatLab analysis was performed to extract the height of the significant peak at 1169 cm^-1, with the results shown in Fig.2. The amplitude of the peak shows a different trend for P and C strains. A similar trend is obtained from HPLC. These promising results open up new possibilities for the use of SERS for high-throughput and automated evaluation of bacterial factories, allowing parallel analysis and discrimination of different strains.

General information
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Organisations: Department of Micro- and Nanotechnology, Nanoprobes, Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
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Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2016

Surface Enhanced Raman Spectroscopy detection of p-coumaric acid from cell supernatant using gold-capped silicon nanopillar substrates
The purpose of the project is to use Surface Enhanced Raman Spectroscopy (SERS) to discriminate between two different bacterial populations, based on their p-coumaric acid (pHCA) production. The pHCA concentration is measured in a droplet of diluted supernatant dried on SERS substrates, using a Raman microscope. By analyzing the SERS signal of pHCA from the supernatant, considering the peak height at the characteristic frequency (1169 cm^-1) it is possible to distinguish between a producing and control strain, as also confirmed by HPLC analysis.

General information
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Thermophilic acetogens for the production of higher value compounds from (biomass derived) waste gas streams

General information
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Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
Authors: Redl, S. (Intern), Jensen, T. Ø. (Intern), Nielsen, A. T. (Intern)
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The Ssr protein (T1E_1405) from Pseudomonas putida DOT-T1E enables oligonucleotide-based recombineering in platform strain P. putida EM42

Some strains of the soil bacterium Pseudomonas putida have become in recent years platforms of choice for hosting biotransformations of industrial interest. Despite availability of many genetic tools for this microorganism, genomic editing of the cell factory P. putida EM42 (a derivative of reference strain KT2440) is still a time-consuming endeavor. In this work we have investigated the in vivo activity of the Ssr protein encoded by the open reading frame T1E_1405 from Pseudomonas putida DOT-T1E, a plausible functional homologue of the β protein of the Red recombination system of λ phage of Escherichia coli. A test based on the phenotypes of pyrF mutants of P. putida (the yeast’s URA3 ortholog) was developed for quantifying the ability of Ssr to promote invasion of the genomic DNA replication fork by synthetic oligonucleotides. The efficiency of the process was measured by monitoring the inheritance of the changes entered into pyrF by oligonucleotides bearing mutated sequences. Ssr fostered short and long genomic deletions/insertions at considerable frequencies as well as single-base swaps not affected by mismatch repair. These results not only demonstrate the feasibility of recombineering in P. putida, but they also enable a suite of multiplexed genomic manipulations in this biotechnologically important bacterium.

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups, CSIC
Authors: Aparicio, T. (Ekstern), Ingemann Jensen, S. (Intern), Nielsen, A. T. (Intern), Víctor de Lorenzo, V. D. (Ekstern), Martínez-García, E. (Ekstern)
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Acceleration of cell factories engineering using CRISPR-based technologies

The constant demand of oil-derived products in the market has pushed science to develop alternative ways to cope with this demand. Therefore the development of efficient cell factories as sustainable alternative is an expanding trend. These are envisioned as future workhorse manufacturers of pharmaceuticals, biofuels and biomaterials. The focus of this thesis is to develop new genome engineering methods to relieve one of the major bottlenecks in metabolic engineering, the strain design and optimization. The aim is to generate an engineering tool-box applicable to different model organisms, which can potentially be standardized in an automatable platform and, in the future be integrated with metabolic modeling tools. In particularly it describes the technologies developed in the three widely used organisms: E. coli, S. cerevisiae and CHO mammalian cells using the recent breakthrough CRISPR/ Cas9 system. These include CRMAGE, a MAGE improved recombineering platform using CRISPR negative selection, CrEdit, a system for multi-loci marker-free simultaneous gene and pathway integrations and CRISPy a platform to accelerate genome editing in CHO cells.

General information

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups, Infection Microbiology, Infection Microbiology
Authors: Ronda, C. (Intern), Nielsen, A. T. (Intern), Molin, S. (Intern)
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Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.189 SNIP 1.062 CiteScore 2.98
Web of Science (2014): Indexed yes
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ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.944 SNIP 0.957 CiteScore 2.4
ISI indexed (2012): ISI indexed no
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.785 SNIP 0.726 CiteScore 1.94
ISI indexed (2011): ISI indexed no
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.787 SNIP 0.798
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.695 SNIP 0.749
BFI (2008): BFI-level 1
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CasEMBLR: Cas9-Facilitated Multiloci Genomic Integration of in Vivo Assembled DNA Parts in *Saccharomyces cerevisiae*

Homologous recombination (HR) in *Saccharomyces cerevisiae* has been harnessed for both plasmid construction and chromosomal integration of foreign DNA. Still, native HR machinery is not efficient enough for complex and marker-free genome engineering required for modern metabolic engineering. Here, we present a method for marker-free multiloci integration of *in vivo* assembled DNA parts. By the use of CRISPR/Cas9-mediated one-step double-strand breaks at single, double and triple integration sites we report the successful *in vivo* assembly and chromosomal integration of DNA parts. We call our method CasEMBLR and validate its applicability for genome engineering and cell factory development in two ways: (i) introduction of the carotenoid pathway from 15 DNA parts into three targeted loci, and (ii) creation of a tyrosine production strain using ten parts into two loci, simultaneously knocking out two genes. This method complements and improves the current set of tools available for genome engineering in *S. cerevisiae*.
is often a preferred option when compared to expression from episomal vectors. Existing approaches for achieving stable simultaneous genome integrations of multiple DNA fragments often result in relatively low integration efficiencies and furthermore rely on the use of selection markers. Results: Here, we have developed a novel method, CrEdit (CRISPR/Cas9 mediated genome Editing), which utilizes targeted double strand breaks caused by CRISPR/Cas9 to significantly increase the efficiency of homologous integration in order to edit and manipulate genomic DNA. Using CrEdit, the efficiency and locus specificity of targeted genome integrations reach close to 100% for single gene integration using short homology arms down to 60 base pairs both with and without selection. This enables direct and cost efficient inclusion of homology arms in PCR primers. As a proof of concept, a non-native beta-carotene pathway was reconstructed in S. cerevisiae by simultaneous integration of three pathway genes into individual intergenic genomic sites. Using longer homology arms, we demonstrate highly efficient and locus-specific genome integration even without selection with up to 84% correct clones for simultaneous integration of three gene expression cassettes. Conclusions: The CrEdit approach enables fast and cost effective genome integration for engineering of S. cerevisiae. Since the choice of the targeting sites is flexible, CrEdit is a powerful tool for diverse genome engineering applications.

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Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.501 SNIP 1.24 CiteScore 4.08
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.672 SNIP 1.471 CiteScore 4.25
Web of Science (2014): Indexed yes
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Scopus rating (2013): SJR 1.686 SNIP 1.43 CiteScore 4.22
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.392 SNIP 1.312 CiteScore 3.69
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.417 SNIP 1.38 CiteScore 3.91
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.609 SNIP 1.463
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.276 SNIP 1.206
Web of Science (2009): Indexed yes
Development of aptamers for in vivo and in vitro biosensor applications

The nylon in the clothes we wear; the paracetamol in the painkillers we eat; and LEGO® blocks we may buy for our children – they all are manufactured from chemical material based on petroleum. Most of us are blissfully unaware of how our dependency on oil, transcends the use of fuel-based transportation. Nylon is made from adipic acid made from petroleum. Paracetamol is made from phenol, which is made from petroleum and LEGO® bricks are made up of acrylonitrile, butadiene, and styrene all petroleum based monomers. To reduce our dependency on oil many pharmaceuticals, nutraceuticals and building block chemicals are now being sustainably produced in bacterial cell-factories. The development of new bacterial cell-factories is a difficult and expensive process, in part due to time required to screen for and optimize productions strains. A new promising way of reducing the development time is generating new and faster ways of screening and optimizing using biosensors.

In this thesis we develop new functional biological recognition modules for biosensors. These DNA- and RNA-based recognition modules are called aptamers and are developed to interact with targets of choice. Aptamers are developed through a laborious process; which suffers from high error-rates and, therefore, the process has undergone significant improvements. Here we present two new versions of aptamer development schemes that have been used to identify aptamers against snake venom toxin (with a possible pharmaceutical application) and small molecule food additives (for optimization production in cell factories). Additionally, the characterization an all-polymer physicochemical biosensor is presented for the detection of antibiotics in food products.

These results have lead to the ongoing development of a high-throughput allpolymeric biosensor device at DTU Nanotech and also resulted in extended funding of 3M DKK from the Danish National Innovation Foundation, Biosyntia and The Technical University of Denmark to advance the use of aptamers and biosensors in cell-factory development.
Differential expression of small RNAs under chemical stress and fed-batch fermentation in *E. coli*

Bacterial small RNAs (sRNAs) are recognized as posttranscriptional regulators involved in the control of bacterial lifestyle and adaptation to stressful conditions. Although chemical stress due to the toxicity of precursor and product compounds is frequently encountered in microbial bioprocessing applications, the involvement of sRNAs in this process is not well understood. We have used RNA sequencing to map sRNA expression in *E. coli* under chemical stress and high cell density fermentation conditions with the aim of identifying sRNAs involved in the transcriptional response and those with potential roles in stress tolerance.

**General information**

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Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factories, Research Groups, Bacterial Cell Factory Optimization  
Authors: Rau, M. H. (Intern), Nielsen, A. T. (Intern), Long, K. (Intern)  
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Scopus rating (2016): CiteScore 4.05 SJR 2.065 SNIP 1.122  
Web of Science (2016): Indexed yes  
BFI (2015): BFI-level 1  
Scopus rating (2015): SJR 2.287 SNIP 1.172 CiteScore 4.3  
Web of Science (2015): Indexed yes  
BFI (2014): BFI-level 1  
Scopus rating (2014): SJR 2.297 SNIP 1.205 CiteScore 4.18  
Web of Science (2014): Indexed yes  
BFI (2013): BFI-level 1  
Scopus rating (2013): SJR 2.141 SNIP 1.174 CiteScore 4.39  
ISI indexed (2013): ISI indexed yes  
Web of Science (2013): Indexed yes  
BFI (2012): BFI-level 1  
Scopus rating (2012): SJR 2.181 SNIP 1.225 CiteScore 4.61  
ISI indexed (2012): ISI indexed yes  
Web of Science (2012): Indexed yes  
BFI (2011): BFI-level 1  
Scopus rating (2011): SJR 2.271 SNIP 1.197 CiteScore 4.38  
ISI indexed (2011): ISI indexed yes  
Web of Science (2011): Indexed yes  
BFI (2010): BFI-level 1  
Scopus rating (2010): SJR 2.109 SNIP 1.038  
Web of Science (2010): Indexed yes  
BFI (2009): BFI-level 1  
Scopus rating (2009): SJR 2.181 SNIP 1.015  
Web of Science (2009): Indexed yes  
BFI (2008): BFI-level 1  
Scopus rating (2008): SJR 2.067 SNIP 1.005  
Web of Science (2008): Indexed yes  
Scopus rating (2007): SJR 1.846 SNIP 1.04
Introduction: Bacterial small RNAs (sRNAs) are often expressed in response to changing environmental conditions and function to modulate gene expression. Although chemical stress is routinely encountered in microbial processing applications, the cellular response and the involvement of sRNAs in this process is poorly understood. We have used RNA sequencing to map the *Escherichia coli* sRNome during chemical stress and high cell density fermentations with the aim of identifying sRNAs involved in the stress response and those with potential roles in stress tolerance.

Methods: RNA sequencing libraries were prepared from RNA isolated from *E. coli* MG1655 cells subjected to chemical stress with twelve compounds. The strain was also grown under high cell density fermentation conditions, where cells were harvested in four growth phases.

Results: We have discovered over 250 novel intergenic transcripts, adding to the roughly 200 previously reported sRNAs in *E. coli*. There are 84 and 139 differentially expressed sRNAs under fermentation and chemical stress conditions, respectively. In the latter case, approximately 30 exhibit significant expression changes in multiple conditions, suggesting their involvement in a more general chemical stress response.

Conclusions: This study has revealed a wealth of hitherto undescribed sRNAs and an atlas of expression under 17 growth conditions. A significant fraction of the sRNAs exhibit specific expression patterns during fermentation, and a group of them are differentially expressed in the presence of multiple chemicals, suggesting they may play regulatory roles during these stress conditions. These are candidates for
improving stress tolerance and our understanding of the regulatory network during fermentation.

General information
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Authors: Rau, M. H. (Intern), Bojanovic, K. (Intern), Nielsen, A. T. (Intern), Long, K. (Intern)
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Editorial overview: Chemical biotechnology: Interdisciplinary concepts for modern biotechnological production of biochemicals and biofuels

General information
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Authors: Bornscheuer, U. T. (Ekstern), Nielsen, A. T. (Intern)
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Web of Science (2017): Indexed yes
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Scopus rating (2016): CiteScore 8.55 SJR 3.331 SNIP 2.1
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.113 SNIP 2.143 CiteScore 7.99
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.271 SNIP 2.068 CiteScore 7.45
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.322 SNIP 2.198 CiteScore 7.93
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.508 SNIP 2.327 CiteScore 7.93
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 3.313 SNIP 2.089 CiteScore 7.76
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 3.56 SNIP 2.223
Highly Active and Specific Tyrosine Ammonia-Lyases from Diverse Origins Enable Enhanced Production of Aromatic Compounds in Bacteria and Saccharomyces cerevisiae

Phenylalanine and tyrosine ammonia-lyases form cinnamic acid and p-coumaric acid, which are precursors of a wide range of aromatic compounds of biotechnological interest. Lack of highly active and specific tyrosine ammonia-lyases has previously been a limitation in metabolic engineering approaches. We therefore identified 22 sequences in silico using synteny information and aiming for sequence divergence. We performed a comparative in vivo study, expressing the genes intracellularly in bacteria and yeast. When produced heterologously, some enzymes resulted in significantly higher production of p-coumaric acid in several different industrially important production organisms. Three novel enzymes were found to have activity exclusively for phenylalanine, including an enzyme from the low-GC Gram-positive bacterium Brevibacillus laterosporus, a bacterial-type enzyme from the amoeba Dictyostelium discoideum, and a phenylalanine ammonia-lyase from the moss Physcomitrella patens (producing 230 μM cinnamic acid per unit of optical density at 600 nm [OD600]) in the medium using Escherichia coli as the heterologous host). Novel tyrosine ammonia-lyases having higher reported substrate specificity than previously characterized enzymes were also identified. Enzymes from Herpetosiphon aurantiacus and Flavobacterium johnsoniae resulted in high production of p-coumaric acid in Escherichia coli (producing 440 μM p-coumaric acid OD600 unit−1 in the medium) and in Lactococcus lactis. The enzymes were also efficient in Saccharomyces cerevisiae, where p-coumaric acid accumulation was improved 5-fold over that in strains expressing previously characterized tyrosine ammonia-lyases.
Moorella Strains for the Production of Biochemicals from Syngas

In the process of sugar fermentation, a significant portion of lignocellulosic biomass is left unused. An alternative is the gasification into syngas, a carbon-rich gas mixture. Syngas serves as energy and carbon source for acetogenic bacteria, which can then produce biochemicals. In the syngas fermentation process even the recalcitrant lignin portion can be fully converted into higher value compounds. Still the cost-effectiveness of this process requires better understanding of the metabolism and modification of the acetogenic strains. In my PhD project I am laying the basis for production of higher value bio-chemicals (acetone) from syngas using Moorella strains as cell factories. Moorella has outstanding abilities that make it especially suitable for the syngas fermentation process (thermophily, carbon source utilization). Firstly, the project focuses on understanding the primary metabolism in acetogenic bacteria. The main research aspect is to determine acceptance of, and the exact growth rates on different carbon sources (C1, C6, gaseous substrates) in different Moorella strains. Genome analysis on pathway level is performed to link the genotype to the phenotype. Differential expression analysis between heterotrophic and autotrophic growth (RNA-seq) serves to elucidate the regulatory mechanisms underlying carbon source utilization. In the second part of my project I am developing tools for genetic manipulation of Moorella strains. For example, a pyrF deletion strain, which allows heterologous gene expression was constructed. These tools developed in my project will be applied to engineer bacterial cell factories for production of higher value biochemicals like acetone.

Moorella Strains for the Production of Biochemicals from Syngas

In the process of sugar fermentation, a significant portion of lignocellulosic biomass is left unused. An alternative is the gasification into syngas, a carbon-rich gas mixture. Syngas serves as energy and carbon source for acetogenic bacteria, which can then produce biochemicals. In the syngas fermentation process even the recalcitrant lignin portion can be fully converted into higher value compounds. However, cost-effectiveness of this process requires better understanding of the metabolism and modification of the acetogenic strains. The present project lays the basis for production of higher value biochemicals (acetone) from syngas using Moorella strains. Moorella has outstanding abilities like thermophily and carbon source conversion yields that make it especially suitable for the syngas fermentation process. The present project focuses on understanding the primary metabolism in acetogenic bacteria. The main research aspect is hereby to determine the acceptance of, and the exact growth rates on different carbon sources (C1 and C6 compounds and gaseous substrates) in different Moorella strains. Genome analysis on pathway level is performed to link the genotype to the phenotype. Differential expression analysis (RNA-seq) between heterotrophic and autotrophic growth serves to elucidate the regulatory mechanisms underlying carbon source utilization. In the second part of the project tools for genetic manipulation of Moorella strains are developed. For example, a pyrF deletion strain, which allows heterologous gene expression was constructed. These tools and knowledge will be applied to engineer strains with outstanding abilities for the production of higher value biochemicals which are currently derived from fossil sources.
Seven gene deletions in seven days: fast generation of Escherichia coli strains tolerant to acetate and osmotic stress

Generation of multiple genomic alterations is currently a time consuming process. Here, a method was established that enables highly efficient and simultaneous deletion of multiple genes in Escherichia coli. A temperature sensitive plasmid containing arabinose inducible lambda Red recombineering genes and a rhamnose inducible flippase recombinase was constructed to facilitate fast marker-free deletions. To further speed up the procedure, we integrated the arabinose inducible lambda Red recombineering genes and the rhamnose inducible FLP into the genome of E. coli K-12 MG1655. This system enables growth at 37°C, thereby facilitating removal of integrated antibiotic cassettes and deletion of additional genes in the same day. Phosphorothioated primers were demonstrated to enable simultaneous deletions during one round of electroporation. Utilizing these methods, we constructed strains in which four to seven genes were deleted in E. coli W and E. coli K-12. The growth rate of an E. coli K-12 quintuple deletion strain was significantly improved in the presence of high concentrations of acetate and NaCl. In conclusion, we have generated a method that enables efficient and simultaneous deletion of multiple genes in several E. coli variants. The method enables deletion of up to seven genes in as little as seven days.

General information
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Organisations: Bacterial Cell Factories, Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
Authors: Ingemann Jensen, S. (Intern), Lennen, R. (Intern), Herrgard, M. (Intern), Nielsen, A. T. (Intern)
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Scopus rating (2016): CiteScore 4.63 SJR 1.625 SNIP 1.401
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Scopus rating (2015): SJR 2.057 SNIP 1.684 CiteScore 5.3
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.103 SNIP 1.544 CiteScore 4.75
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.886 SNIP 1.51 CiteScore 4.06
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.458 SNIP 0.896 CiteScore 2.44
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
ISI indexed (2011): ISI indexed no
Original language: English
Electronic versions:
Seven_gene_deletions_in_seven_days.pdf
DOIs:
Accelerating Genome Editing in CHO Cells Using CRISPR Cas9 and CRISPy, a Web-Based Target Finding Tool

Chinese hamster ovary (CHO) cells are widely used in the biopharmaceutical industry as a host for the production of complex pharmaceutical proteins. Thus, genome engineering of CHO cells for improved product quality and yield is of great interest. Here, we demonstrate for the first time the efficacy of the CRISPR Cas9 technology in CHO cells by generating site-specific gene disruptions in COSMC and FUT8, both of which encode proteins involved in glycosylation. The tested single guide RNAs (sgRNAs) created an indel frequency up to 47.3% in COSMC, while an indel frequency up to 99.7% in FUT8 was achieved by applying lectin selection. All eight sgRNAs examined in this study resulted in relatively high indel frequencies, demonstrating that the Cas9 system is a robust and efficient genome editing methodology in CHO cells. Deep sequencing revealed that 85% of the indels created by Cas9 resulted in frameshift mutations at the target sites, with a strong preference for single base indels. Finally, we have developed a user-friendly bioinformatics tool, named “CRISPy” for rapid identification of sgRNA target sequences in the CHO-K1 genome. The CRISPy tool identified 1,970,449 CRISPR targets divided into 27,553 genes and lists the number of off-target sites in the genome. In conclusion, the proven functionality of Cas9 to edit CHO genomes combined with our CRISPy database have the potential to accelerate genome editing and synthetic biology efforts in CHO cells.
Increased tolerance towards serine obtained by adaptive laboratory evolution

The amino acid serine has previously been identified as one of the top 30 candidates of value added chemicals, making the production of serine from glucose attractive. Production of serine have previously been attempted in E. coli and C. glutamicum, however, titers sufficient for commercial applications have not yet been achieved. This is partly due to the fact that the key serine degradation pathway (serine to glycine), encoded by glyA, has not yet been successfully deleted in E. coli or C. glutamicum. So far, the most successful attempts of serine production have been achieved using a C. glutamicum auxotroph for the cofactor of glyA, however, this requires the use of rich fermentation media or the addition of folic acid. Here, we demonstrate that the two major pathways for degradation of serine can be deleted in E. coli MG1655. In addition to the conversion of serine to glycine (encoded by glyA), the conversion of serine to pyruvate (encoded by sdaA, sdaB and tdcG) was also deleted. As expected, the resulting strain turned out to be susceptible to even low concentrations of serine in the media. In order to improve the tolerance of the strain towards serine, adaptive laboratory evolution was implemented using a state-of-the-art robotics platform. The strain was grown under inhibiting concentrations of serine in minimal media and was periodically transferred to new media during mid log phase. After achieving a desired increase in growth rate, the concentration was serine was gradually increased. During the evolution experiment, the serine tolerance was increased substantially. Genome re-sequencing was subsequently used to analyze the genotype of a number of selected strains. These results reveal insights towards the adaptation process as well as the mechanism of serine tolerance.

General information
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Organisations: Novo Nordisk Foundation Center for Biosustainability, Research Groups, Applied Metabolic Engineering, Bacterial Cell Factory Optimization, iLoop, University of California
Trash to treasure: Production of biofuels and commodity chemicals via syngas fermenting microorganisms

Fermentation of syngas is a means through which unutilized organic waste streams can be converted biologically into biofuels and commodity chemicals. Despite recent advances, several issues remain which limit implementation of industrial-scale syngas fermentation processes. At the cellular level, the energy conservation mechanism of syngas fermenting microorganisms has not yet been entirely elucidated. Furthermore, there was a lack of genetic tools to study and ultimately enhance their metabolic capabilities. Recently, substantial progress has been made in understanding the intricate energy conservation mechanisms of these microorganisms. Given the complex relationship between energy conservation and metabolism, strain design greatly benefits from systems-level approaches. Numerous genetic manipulation tools have also been developed, paving the way for the use of metabolic engineering and systems biology approaches. Rational strain designs can now be deployed resulting in desirable phenotypic traits for large-scale production. © 2013 Elsevier Ltd.

General information
State: Published
Organisations: Bacterial Cell Factories, Novo Nordisk Foundation Center for Biosustainability, Research Groups, University of California
Authors: Latif, H. (Ekstern), Zeidan, A. (Intern), Nielsen, A. T. (Intern), Zengler, K. (Intern)
Pages: 79-87
Publication date: 2014
Main Research Area: Technical/natural sciences

Publication information
Journal: Current Opinion in Biotechnology
Volume: 27
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BFI (2017): BFI-level 2
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BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.55 SJR 3.331 SNIP 2.1
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.113 SNIP 2.143 CiteScore 7.99
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.271 SNIP 2.068 CiteScore 7.45
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.322 SNIP 2.198 CiteScore 7.93
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.508 SNIP 2.327 CiteScore 7.93
Comparative study on aptamers as recognition elements for antibiotics in a label-free all-polymer biosensor

We present an all-polymer electrochemical microfluidic biosensor using Topas® as substrate and a conductive polymer bilayer as electrode material. The conductive bilayer consists of tosylate doped poly(3,4-ethylenedioxythiophene) (PEDOT:TsO) and the hydroxymethyl derivative PEDOT-OH:TsO, which was covalently functionalized with two aptamer probes with affinity to ampicillin or kanamycin A, respectively. Using electrochemical impedance spectroscopy (EIS) we were able to detect ampicillin in a concentration range from 100pM to 1 μM and kanamycin A from 10nM to 1mM. The obtained EIS spectra were fitted with an equivalent circuit model successfully explaining the impedance signal. Real samples from regular ultra-high temperature treated low-fat milk spiked with ampicillin were successfully tested to assess the functionality of the sensor with real samples. In conclusion, we have demonstrated the applicability of the newly developed platform for real time, label-free and selective impedimetric detection of commonly used antibiotics. Additionally it was possible to detect ampicillin in a milk sample at a concentration below the allowed maximum residue limit (MRL) in the European Union. © 2013 Elsevier B.V.

General information
State: Published
Organisations: Department of Micro- and Nanotechnology, Polymer Microsystems for Medical Diagnostics, Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factories, CFB - Core Flow
Authors: Dapra, J. (Intern), Lauridsen, L. H. (Intern), Nielsen, A. T. (Intern), Rozlosnik, N. (Intern)
Pages: 315-320
Publication date: 2013
Characterization of a Feedback-Resistant Mevalonate Kinase from the Archaeon Methanosarcina mazei

The mevalonate pathway is utilized for the biosynthesis of isoprenoids in many bacterial, eukaryotic, and archaeal organisms. Based on previous reports of its feedback inhibition, mevalonate kinase (MVK) may play an important regulatory role in the biosynthesis of mevalonate pathway-derived compounds. Here we report the purification, kinetic characterization, and inhibition analysis of the MVK from the archaeon Methanosarcina mazei. The inhibition of the M. mazei MVK by the following metabolites derived from the mevalonate pathway was explored: dimethylallyl diphosphate (DMAPP), geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), isopentenyl monophosphate (IP), and diphosphomevalonate. M. mazei MVK was not inhibited by DMAPP, GPP, FPP, diphosphomevalonate, or IP, a proposed intermediate in an alternative isoprenoid pathway present in archaea. Our findings suggest that the M. mazei MVK represents a distinct class of mevalonate kinases that can be differentiated from previously characterized MVKs based on its inhibition profile.

General information
State: Published
Organisations: Genencor, Inc.
Number of pages: 7
Pages: 7772-7778
Publication date: 2011
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 77
Issue number: 21
ISSN (Print): 0099-2240
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 4.08
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.891 SNIP 1.308 CiteScore 4.14
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.857 SNIP 1.384 CiteScore 4.02
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.899 SNIP 1.414 CiteScore 4.25
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.975 SNIP 1.429 CiteScore 4.29
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.914 SNIP 1.455 CiteScore 4.12
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.887 SNIP 1.436
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
A Bistable Switch and Anatomical Site Control Vibrio cholerae Virulence Gene Expression in the Intestine

A fundamental, but unanswered question in host-pathogen interactions is the timing, localization and population distribution of virulence gene expression during infection. Here, microarray and in situ single cell expression methods were used to study Vibrio cholerae growth and virulence gene expression during infection of the rabbit ligated ileal loop model of cholera. Genes encoding the toxin-coregulated pilus (TCP) and cholera toxin (CT) were powerfully expressed early in the infectious process in bacteria adjacent to epithelial surfaces. Increased growth was found to co-localize with virulence gene expression. Significant heterogeneity in the expression of tcpA, the repeating subunit of TCP, was observed late in the infectious process. The expression of tcpA, studied in single cells in a homogeneous medium, demonstrated unimodal induction of tcpA after addition of bicarbonate, a chemical inducer of virulence gene expression. Striking bifurcation of the population occurred during entry into stationary phase: one subpopulation continued to express tcpA, whereas the expression declined in the other subpopulation. ctxA, encoding the A subunit of CT, and toxT, encoding the proximal master regulator of virulence gene expression also exhibited the bifurcation phenotype. The bifurcation phenotype was found to be reversible, epigenetic and to persist after removal of bicarbonate, features consistent with bistable switches. The bistable switch requires the positive-feedback circuit controlling ToxT expression and formation of the CRP-cAMP complex during entry into stationary phase. Key features of this bistable switch also were demonstrated in vivo, where striking heterogeneity in tcpA expression was observed in luminal fluid in later stages of the infection. When this fluid was diluted into artificial seawater, bacterial aggregates continued to express tcpA for prolonged periods of time. The bistable control of virulence gene expression points to a mechanism that could generate a subpopulation of V. cholerae that continues to produce TCP and CT in the rice water stools of cholera patients.

General information
State: Published
Organisations: Department of Systems Biology, Stanford University School of Medicine
Authors: Nielsen, A. T. (Intern), Dolganov, N. A. (Ekstern), Rasmussen, T. (Intern), Otto, G. (Ekstern), Miller, M. (Ekstern), Felt, S. (Ekstern), Torreilles, S. (Ekstern), Schoolnik, G. (Ekstern)
Number of pages: 23
Publication date: 2010
Main Research Area: Technical/natural sciences

Publication information
Journal: P L o S Pathogens
vpsA- and luxO-independent biofilms of *Vibrio cholerae*

The natural life cycle of *Vibrio cholerae* involves the transitioning of cells between different environmental surfaces such as the chitinous shell of Crustaceae and the epithelial layer of the human intestine. Previous studies using static biofilm systems showed a strict dependence of biofilm formation on the vps and lux genes, which are essential for exopolysaccharide formation and cell-cell signaling, respectively. The authors' report here that in biofilms grown under hydrodynamic conditions, Delta vpsA and Delta luxO mutants of *V. cholerae* do form pronounced, three-dimensional biofilms that resemble all aspects of wild-type biofilms. By genetic experiments, it was shown that in hydrodynamically grown biofilms this independence of vpsA is due to the expression of rpoS, which is a negative regulator of vpsA.
expression. Biofilms also underwent substantial dissolution after 96 h that could be induced by a simple stop of medium flow. The studies indicate that metabolic conditions control the reversible attachment of cells to the biofilm matrix and are key in regulating biofilm cell physiology via RpoS. Furthermore, the results redefine the roles of vps and quorum-sensing in V. cholerae biofilms.
RpoS controls the Vibrio cholerae mucosal escape response

*Vibrio cholerae* causes a severe diarrhoeal disease by secreting a toxin during colonization of the epithelium in the small intestine. Whereas the initial steps of the infectious process have been intensively studied, the last phases have received little attention. Confocal microscopy of *V. cholerae* O1-infected rabbit ileal loops captured a distinctive stage in the infectious process: 12 h post-inoculation, bacteria detach from the epithelial surface and move into the fluid-filled lumen. Designated the "mucosal escape response," this phenomenon requires RpoS, the stationary phase alternative sigma factor. Quantitative in vivo localization assays corroborated the rpoS phenotype and showed that it also requires HapR. Expression profiling of bacteria isolated from ileal loop fluid and mucus demonstrated a significant RpoS-dependent upregulation of many chemotaxis and motility genes coincident with the emigration of bacteria from the epithelial surface. In stationary phase cultures, RpoS was also required for upregulation of chemotaxis and motility genes, for production of flagella, and for movement of bacteria across low nutrient swarm plates. The hapR mutant produced near-normal numbers of flagellated cells, but was significantly less motile than the wild-type parent. During in vitro growth under virulence-inducing conditions, the rpoS mutant produced 10- to 100-fold more cholera toxin than the wild-type parent. Although the rpoS mutant caused only a small over-expression of the genes encoding cholera toxin in the ileal loop, it resulted in a 30% increase in fluid accumulation compared to the wild-type. Together, these results show that the mucosal escape response is orchestrated by an RpoS-dependent genetic program that activates chemotaxis and motility functions. This may furthermore coincide with reduced virulence gene expression, thus preparing the organism for the next stage in its life cycle.

**General information**

**State:** Published

**Organisations:** Stanford University School of Medicine, Stanford University

**Authors:** Nielsen, A. T. (Intern), Dolganov, N. A. (Ekstern), Otto, G. (Ekstern), Miller, M. C. (Ekstern), Wu, C. Y. (Ekstern), Schoolnik, G. K. (Ekstern)

**Number of pages:** 16

**Pages:** 933-948

**Publication date:** 2006

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

**Publication information**

**Journal:** PLoS Pathogens

**Volume:** 2

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**Ratings:**

- BFI (2017): BFI-level 2
- Web of Science (2017): Indexed Yes
- BFI (2016): BFI-level 2
- Scopus rating (2016): SJR 4.466 SNIP 1.635 CiteScore 6.46
- BFI (2015): BFI-level 2
- Scopus rating (2015): SJR 5.019 SNIP 1.783 CiteScore 7.14
- Web of Science (2015): Indexed yes
Chitin, an insoluble polymer of GlcNAc, is an abundant source of carbon, nitrogen, and energy for marine microorganisms. Microarray expression profiling and mutational studies of Vibrio cholerae growing on a natural chitin surface, or with the soluble chitin oligosaccharides (GlcNAc)\(_{2-6}\), GlcNAc, or the glucosamine dimer (GlcN)\(_2\) identified three sets of differentially regulated genes. We show that (i) ChiS, a sensor histidine kinase, regulates expression of the (GlcNAc)\(_{2-6}\) gene set, including a (GlcNAc)\(_2\) catabolic operon, two extracellular chitinases, a chitoporin, and a PilA-containing type IV pilus, designated ChiRP (chitin-regulated pilus) that confers a significant growth advantage to V. cholerae on a chitin surface; (ii) GlcNAc causes the coordinate expression of genes involved with chitin chemotaxis and adherence and with the transport and assimilation of GlcNAc; (iii) (GlcN)\(_2\) induces genes required for the transport and catabolism of nonacetylated chitin residues; and (iv) the constitutively expressed MSHA pilus facilitates adhesion to the chitin surface independent of surface chemistry. Collectively, these results provide a global portrait of a complex, multistage V. cholerae program for the efficient utilization of chitin.

**General information**

**State:** Published  
**Organisations:** Stanford University School of Medicine, Johns Hopkins University  
**Authors:** Meibom, K. L. (Ekstern), Li, X. B. (Ekstern), Nielsen, A. T. (Intern), Wu, C. (Ekstern), Roseman, S. (Ekstern), Schoolnik, G. K. (Ekstern)  
**Number of pages:** 6  
**Pages:** 2524-2529
Single nucleotide polymorphism genotyping using locked nucleic acid (LNA)

Locked nucleic acid (LNA®) is a new class of bicyclic high affinity DNA analogs. LNA-containing oligonucleotides confer significantly increased affinity against their complementary DNA targets, increased mismatch discrimination (ΔTm) and allow full control of the melting point of the hybridization reaction. LNA chemistry is completely compatible with the traditional DNA phosphoramidite chemistry and therefore LNA-DNA mixmer oligonucleotides can be designed with complete freedom for optimal performance. These properties render LNA oligonucleotides very well suited for SNP genotyping and have enabled several approaches for enzyme-independent SNP genotyping based on allele-specific hybridization. In addition, allele-specific PCR assays relying on enzymatically-enhanced discrimination can be improved using LNA-modified oligonucleotides. The use of LNA transforms enzyme-independent genotyping approaches into experimentally simple, robust and cost-effective assays, which are highly suited for genotyping in clinical and industrial settings.
In situ examination of microbial populations in a model drinking water distribution system
A flow cell set-up was used as a model drinking water distribution system to analyze the in situ microbial population. Biofilm growth was followed by transmission light microscopy for 81 days and showed a biofilm consisting of microcolonies separated by a monolayer of cells. Protozoans (ciliates and flagellates) were often seen attached to the microcolonies. The biofilm was hybridized with oligonucleotide probes specific for all bacteria and the α- and β-subclass of Proteobacteria and visualized with a scanning confocal laser microscope. Hybridization showed that the microcolonies primarily consisted of a mixed population of α- and β-Proteobacteria. 65 strains from the inlet water and 20 from the biofilm were isolated on R2A agar plates and sorted into groups with amplified rDNA restriction analysis. The 16S rDNA gene was sequenced for representatives of the abundant groups. A phylogenetic analysis revealed that the majority of the isolated strains from the bulk water and biofilm were affiliated to the family of Comamonadaceae in the β-lineage of Proteobacteria. The majority of the strains from the α-lineage were affiliated to the family of Sphingomonadaceae. We were unable to detect any strains from the Pseudomonas genus and found a low abundance of bacteria affiliated to the γ-subclass of Proteobacteria where Pseudomonas and E. coli are positioned. The analysis revealed a high bacterial diversity in the water phase as well as the biofilm, but no strains were found in both environments.

General information
State: Published
Organisations: Department of Systems Biology, Department of Environmental Engineering, Exiqon A/S
Pages: 283-288
Publication date: 2002
Main Research Area: Technical/natural sciences

Publication information
Journal: Water Science and Technology: Water Supply
Volume: 2
Issue number: 3
ISSN (Print): 1606-9749
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): SJR 0.26 SNIP 0.438 CiteScore 0.65
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.28 SNIP 0.415 CiteScore 0.64
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 0.271 SNIP 0.46 CiteScore 0.47
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 0.397 SNIP 0.387 CiteScore 0.57
ISI indexed (2013): ISI indexed no
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 0.317 SNIP 0.414 CiteScore 0.51
ISI indexed (2012): ISI indexed no
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 0.37 SNIP 0.357 CiteScore 0.53
ISI indexed (2011): ISI indexed no
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.293 SNIP 0.378
In situ identification of polyphosphate- and polyhydroxyalkanoate-accumulating traits for microbial populations in a biological phosphorus removal process

Polyphosphate- and polyhydroxyalkanoate (PHA)-accumulating traits of predominant microorganisms in an efficient enhanced biological phosphorus removal (EBPR) process were investigated systematically using a suite of non-culture-dependent methods. Results of 16S rDNA clone library and fluorescence in situ hybridization (FISH) with rRNA-targeted, group-specific oligonucleotide probes indicated that the microbial community consisted mostly of the alpha- (9.5% of total cells), beta- (41.3%) and gamma- (6.8%) subclasses of the class Proteobacteria, Flexibacter-Cytophaga (4.5%) and the Gram-positive high G+C (HGC) group (17.9%). With individual phylogenetic groups or subgroups, members of Candidatus Accumulibacter phosphatis in the beta-2 subclass, a novel HGC group closely related to Tetrasphaera spp., and a novel gamma-proteobacterial group were the predominant populations. Furthermore, electron microscopy with energy-dispersive X-ray analysis was used to validate the staining specificity of 4,6-diamino-2-phenylindole (DAPI) for intracellular polyphosphate and revealed the composition of polyphosphate granules accumulated in predominant bacteria as mostly P, Ca and Na. As a result, DAPI and PHA staining procedures could be combined with FISH to identify directly the polyphosphate- and PHA-accumulating traits of different phylogenetic groups. Members of Accumulibacter phosphatis and the novel gamma-proteobacterial group were observed to accumulate both polyphosphate and PHA. In addition, one novel rod-shaped group, closely related to coccus-shaped Tetrasphaera, and one filamentous group resembling Candidatus Nostocoidia limicola in the HGC group were found to accumulate polyphosphate but not PHA. No cellular inclusions were detected in most members of the alpha-Proteobacteria and the Cytophaga-Flavobacterium group. The diversified functional traits observed suggested that different substrate metabolisms were used by predominant phylogenetic groups in EBPR processes.

General information
State: Published
Organisations: Department of Microbiology, National Central University, National Cheng Kung University, Chuo University
Authors: Liu, W. (Ekstern), Nielsen, A. T. (Intern), Wu, J. (Ekstern), Tsai, C. (Ekstern), Matsuo, Y. (Ekstern), Molin, S. (Intern)
Number of pages: 13
Pages: 110-122
Publication date: 2001
Main Research Area: Technical/natural sciences

Publication information
Journal: Environmental Microbiology
Volume: 3
Issue number: 2
ISSN (Print): 1462-2912
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 5.02 SJR 2.221 SNIP 1.406
Locked Nucleic Acid’s or LNA are a new class of bicyclic DNA analogues that have a high affinity and specificity towards complementary nucleic acids. LNA containing oligonucleotides were used to develop a multiplex SNP genotyping assay based entirely on hybridization between capture probe and target. The approach incorporates a polymer microarray platform, photochemistry for immobilization of oligonucleotides onto microarrays, and a dedicated software tool to aid primer and capture probe design for highly multiplex genotyping. Furthermore, these technologies are combined in an integrated microfluidics platform for simple, highly multiplex and robust SNP genotyping.
Quantification of biofilm structures by the novel computer program COMSTAT

General information
State: Published
Organisations: Department of Microbiology, Department of Systems Biology, Center for Biomedical Microbiology, Department of Informatics and Mathematical Modeling, Image Analysis and Computer Graphics
Pages: 2395-2407
Publication date: 2000
Main Research Area: Technical/natural sciences

Publication information
Journal: Microbiology-Uk
Volume: 146
ISSN (Print): 0026-2617
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 0.82
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 0.79
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 0.6
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 0.72
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 0.63
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 0.58
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 1
BFI (2009): BFI-level 1
BFI (2008): BFI-level 1
Web of Science (2007): Indexed yes
Web of Science (2000): Indexed yes
Original language: English
Source: orbit
Source-ID: 177515
Publication: Research - peer-review › Journal article – Annual report year: 2000

Role of commensal relationships on the spatial structure of a surface-attached microbial consortium

General information
State: Published
Distribution of bacterial growth activity in flow-chamber biofilms

In microbial communities such as those found in biofilms, individual organisms most often display heterogeneous behavior with respect to their metabolic activity, growth status, gene expression pattern, etc. In that context, a novel reporter system for monitoring of cellular growth activity has been designed. It comprises a transposon cassette carrying fusions between the growth rate-regulated Escherichia coli rrnBP1 promoter and different variant gfp genes. It is shown that the pi promoter is regulated in the same way in E. coli and Pseudomonas putida, making it useful for monitoring of growth activity in organisms outside the group of enteric bacteria. Construction of fusions to genes encoding unstable Gfp proteins opened up the possibility of the monitoring of rates of rRNA synthesis and, in this way, allowing on-line determination of the distribution of growth activity in a complex community. With the use of these reporter tools, it is demonstrated that individual cells of a toluene-degrading P. putida strain growing in a benzyl alcohol-supplemented biofilm have different levels of growth activity which develop as the biofilm gets older. Cells that eventually grow very slowly or not at all may be stimulated to restart growth if provided with a more easily metabolizable carbon source. Thus, the dynamics of biofilm growth activity has been tracked to the level of individual cells, cell clusters, and microcolonies.
Identification of a novel group of bacteria in sludge from a deteriorated biological phosphorus removal reactor

The microbial diversity of a deteriorated biological phosphorus removal reactor was investigated by methods not requiring direct cultivation. The reactor was fed with media containing acetate and high levels of phosphate (P/C weight ratio, 8:100) but failed to completely remove phosphate in the effluent and showed very limited biological phosphorus removal activity. Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S ribosomal DNA was used to investigate the bacterial diversity. Up to 11 DGGE bands representing at least 11 different sequence types were observed; DNA from the 6 most dominant of these bands was further isolated and sequenced. Comparative phylogenetic analysis of the partial 16S rRNA sequences suggested that one sequence type was affiliated with the alpha subclass of the Proteobacteria, one was associated with the Legionella group of the gamma subclass of the Proteobacteria, and the remaining four formed a novel group of the gamma subclass of the Proteobacteria with no close relationship to any previously described species. The novel group represented approximately 75% of the PCR-amplified DNA, based on the DGGE band intensities. Two oligonucleotide rRNA probes for this novel group were designed and used in a whole-cell hybridization analysis to investigate the abundance of this novel group in situ. The bacteria were coccoid and 3 to 4 μm in diameter and represented approximately 35% of the total population, suggesting a relatively close agreement with the results obtained by the PCR-based DGGE method. Further, based on electron microscopy and standard staining microscopic analysis, this novel group was able to accumulate granule inclusions, possibly consisting of polyhydroxyalkanoate, inside the cells.

General information

State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, National Central University, Clemson University, Northwestern University
Authors: Nielsen, A. T. (Intern), Liu, W. (Ekstern), Filipe, C. (Ekstern), Grady, L. (Ekstern), Molin, S. (Intern), Stahl, D. A. (Ekstern)
Pages: 1251-1258
Publication date: 1999
Main Research Area: Technical/natural sciences
Molecular tools for study of biofilm physiology

General information
State: Published
Organisations: Department of Systems Biology, Center for Systems Microbiology, National Food Institute, Division of Microbiology and Risk Assessment, Fødevaredirektoratet, University of Tennessee
Authors: Christensen, B. B. (Ekstern), Sternberg, C. (Intern), Andersen, J. B. (Intern), Palmer, R. J. (Ekstern), Nielsen, A. T. (Intern), Givskov, M. C. (Intern), Molin, S. (Intern)
Pages: 20-42
Publication date: 1999

Host publication information
Title of host publication: Methods in Enzymology
Publisher: Academic Press
Main Research Area: Technical/natural sciences
Source: orbit
Source-ID: 174055
Publication: Research - peer-review › Article in proceedings – Annual report year: 1999

Projects:

Inducible growth decoupling systems for improved production og biochemicals
Technical University of Denmark
Period: 01/09/2017 → 31/08/2020
Number of participants: 3
Phd Student:
Landberg, Jenny Marie (Intern)
Supervisor:
Nørholm, Morten (Intern)
Main Supervisor:
Nielsen, Alex Toftgaard (Intern)

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

Synthetic Biology tool Development for Protein engineering and study of adaptive evolution in Bacteria
Technical University of Denmark
Period: 01/11/2016 → 31/10/2019
Number of participants: 3
Phd Student:
Lauritsen, Ida (Intern)
Supervisor:
Nielsen, Alex Toftgaard (Intern)
Main Supervisor:
Nørholm, Morten (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU)
Project: PhD
**Synthetic biology solutions to phenotypic instability in cell factory engineering**

Technical University of Denmark  
Period: 01/02/2016 → 31/01/2019  
Number of participants: 3  
Phd Student:  
Sarup-Lytzen, Kira (Intern)  
Supervisor:  
Nielsen, Alex Toftgaard (Intern)  
Main Supervisor:  
Sommer, Morten Otto Alexander (Intern)  

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

**Microbial electrosynthesis for acetate production from carbon dioxide: innovative biocatalysts leading to enhanced performance**

Technical University of Denmark  
Period: 15/02/2014 → 25/08/2017  
Number of participants: 6  
Phd Student:  
Aryal, Nabin (Intern)  
Supervisor:  
Tremblay, Pier-Luc (Intern)  
Main Supervisor:  
Zhang, Tian (Intern)  
Examiner:  
Nielsen, Alex Toftgaard (Intern)  
Aulenta, Federico (Ekstern)  
Rotaru, Amelia-Elena (Ekstern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Samfinansierede - Virksomhed

**Relations**  
Publications:  
Microbial electrosynthesis for acetate production from carbon dioxide: innovative biocatalysts leading to enhanced performance  
Project: PhD

**Generate Prototype Expression Platforms for Membrane Integrated Enzymes in E. coli**

Department of Systems Biology  
Period: 01/09/2013 → 29/09/2016  
Number of participants: 6  
Phd Student:  
Vazquez Albacete, Dario (Intern)  
Main Supervisor:  
Nørholm, Morten (Intern)  
Examiner:  
Nielsen, Alex Toftgaard (Intern)  
Nielsen, Alex Toftgaard (Intern)  
Takos, Adam (Ekstern)  
Takos, Adam (Ekstern)  

**Financing sources**  
Source: Internal funding (public)  
Name of research programme: Marie Curie (EU-stipendium)
Relations
Publications:
Protein and DNA technologies for functional expression of membrane-associated cytochromes P450 in bacterial cell factories
Project: PhD

Bioactive compounds in mixed marine bacterial communities
Department of Systems Biology
Period: 01/05/2013 → 30/06/2016
Number of participants: 6
Phd Student:
Giubergia, Sonia (Intern)
Supervisor:
Nielsen, Kristian Fog (Intern)
Main Supervisor:
Gram, Lone (Intern)
Examiner:
Nielsen, Alex Toftgaard (Intern)
Ingham, Colin John (Ekstern)
Niedermeyer, Timo (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)
Project: PhD

Biosensors for Fermentation
Technical University of Denmark
Period: 01/05/2013 → 21/06/2017
Number of participants: 6
Phd Student:
Lehning, Christina Eva (Intern)
Supervisor:
Nielsen, Alex Toftgaard (Intern)
Main Supervisor:
Sommer, Morten Otto Alexander (Intern)
Examiner:
Molin, Søren (Intern)
Draheim, Roger R. (Ekstern)
Heinemann, Matthias (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)
Project: PhD

Decouple Growth from Central Metabolism in order to Control Growth during Production of Biochemicals
Department of Systems Biology
Period: 01/05/2013 → 01/09/2016
Number of participants: 6
Phd Student:
Li, Songyuan (Intern)
Main Supervisor:
Nielsen, Alex Toftgaard (Intern)
Examiner:
Nørholm, Morten (Intern)
Nørholm, Morten (Intern)
Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)

Relations
Publications:
Decoupling of Growth from Production of Biochemicals and Proteins
Project: PhD

Engineering of Thermophillic bacteria for the production of industrially relevant chemicals
Department of Systems Biology
Period: 01/05/2013 → 15/03/2017
Number of participants: 6
Phd Student:
Pogrebnyakov, Ivan (Intern)
Supervisor:
Sommer, Morten Otto Alexander (Intern)
Main Supervisor:
Nielsen, Alex Toftgaard (Intern)
Examiner:
Gram, Lone (Intern)
Hreggvidsson, Gudmundur Oli (Ekstern)
Kranenburg, Richard Van (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)
Project: PhD

Mining Bacteria Genomes for Novel Bioactive Compounds
Department of Systems Biology
Period: 01/05/2013 → 29/09/2016
Number of participants: 5
Phd Student:
Machado, Henrique (Intern)
Main Supervisor:
Gram, Lone (Intern)
Examiner:
Nielsen, Alex Toftgaard (Intern)
Schramm, Andreas (Ekstern)
Ziemert, Nadine (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)

Relations
Publications:
Marine Bacterial Genomics
Project: PhD

Production of biochemicals using P.putida as a host
Department of Systems Biology
Period: 01/05/2013 → 30/06/2016
Number of participants: 6
Phd Student:
Calero Valdayo, Patricia Maria (Intern)
Supervisor:
Molin, Søren (Intern)
Main Supervisor:
Nielsen, Alex Toftgaard (Intern)
Examiner:
Long, Katherine (Intern)
Ramos, Juan L. (Ekstern)
Tolker-Nielsen, Tim (Intern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)
Project: PhD

The structure, dynamics and complexity of the bacterial transcriptome
Department of Systems Biology
Period: 01/05/2013 → 30/06/2016
Number of participants: 6
Phd Student:
D'Arrigo, Isotta (Intern)
Supervisor:
Sommer, Morten Otto Alexander (Intern)
Main Supervisor:
Long, Katherine (Intern)
Examiner:
Nielsen, Alex Toftgaard (Intern)
Ramos, Juan L. (Ekstern)
Vinther, Jeppe (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)
Project: PhD

Utilize Gasified Biomass and Waste Products (Synthesis Gas) for the Production of Biochemicals
Department of Systems Biology
Period: 01/05/2013 → 29/09/2016
Number of participants: 6
Phd Student:
Redl, Stephanie Maria Anna (Intern)
Supervisor:
Förster, Jochen (Intern)
Main Supervisor:
Nielsen, Alex Toftgaard (Intern)
Examiner:
Herrgard, Markus (Intern)
Simpson, Sean D. (Ekstern)
Soucaille, Philippe (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)

Relations
Publications:
Gas Fermentation using Thermophilic Moorella Species for production of Biochemicals
Standardization of DNA Vector Design-Processes

Department of Systems Biology
Period: 01/03/2013 → 21/04/2016
Number of participants: 6
Phd Student: Cavaleiro, Mafalda (Intern)
Supervisor: Nielsen, Alex Toftgaard (Intern)
Main Supervisor: Nørholm, Morten (Intern)
Examiner: Jensen, Michael Krogh (Intern)
Hillson, Nathan J. (Ekstern)
Nour-Eldin, Hussam Hassan (Ekstern)

Financing sources
Source: Internal funding (public)
Name of research programme: Marie Curie (EU-stipendium)

Microbial platform for expression of membrane integrated enzymes and sustainable production of high value chemicals

Department of Systems Biology
Period: 01/12/2012 → 27/01/2016
Number of participants: 6
Phd Student: Søgaard, Karina Marie (Intern)
Supervisor: Nielsen, Alex Toftgaard (Intern)
Main Supervisor: Nørholm, Morten (Intern)
Examiner: Kildegaard, Helene Faustrup (Intern)
Daley, Daniel Oliver (Ekstern)
Pedersen, Per Amstrup (Ekstern)

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

Relations
Publications:
Improved premises for cell factory development
Project: PhD

A riboswitch based method for in vivo selection of biocatalysts from large libraries

Department of Systems Biology
Period: 01/07/2012 → 02/09/2015
Number of participants: 5
Phd Student: Genee, Hans Jasper (Intern)
Main Supervisor: Sommer, Morten Otto Alexander (Intern)
Examiner: Nielsen, Alex Toftgaard (Intern)
Lorenzo, Victor de (Ekstern)
Financing sources
Source: Internal funding (public)
Name of research programme: Institut stipendie (DTU) Samf.
Project: PhD

Development of new metabolic engineering technologies for the production of biochemicals
Department of Systems Biology
Period: 01/06/2012 → 25/11/2015
Number of participants: 6
PhD Student:
Ronda, Carlotta (Intern)
Supervisor:
Molin, Søren (Intern)
Main Supervisor:
Nielsen, Alex Toftgaard (Intern)
Examiner:
Förster, Jochen (Intern)
Ingmer, Hanne (Ekstern)
de Gier, Jan-Willem (Ekstern)

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

Relations
Publications:
Acceleration of cell factories engineering using CRISPR-based technologies
Project: PhD

Aptameric Biosensor Arrays for Metabolic Engineering
Department of Systems Biology
Period: 01/12/2011 → 29/04/2015
Number of participants: 6
PhD Student:
Lauridsen, Lasse Holm (Intern)
Supervisor:
Sommer, Morten Otto Alexander (Intern)
Main Supervisor:
Nielsen, Alex Toftgaard (Intern)
Examiner:
Nørholm, Morten (Intern)
Gallivan, Justin (Ekstern)
Sloth Andersen, Ebbe (Ekstern)

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

Fysiologisk karakterisering af mikrobielle overfladesamfund
Department of Systems Biology
Period: 01/09/1997 → 23/05/2001
Number of participants: 4
PhD Student:
Nielsen, Alex Toftgaard (Intern)
Main Supervisor:
Bacterial physiology in biofilms
We have studied a microbial community capable of degrading toluene and derivative compounds through the use of quantitative in situ rRNA hybridization, gene expression using fluorescent reporters and gene transfer. The community is composed of 7 members of which 3 organisms are capable of degrading toluene to carbon dioxide and water. The community is grown as continuous surface cultures in flow chambers with benzyl alcohol as the only carbon source. Population structure (relative proportions of the 7 species as well as their positions in three dimensions) are determined using in situ RNA hybridization and confocal microscopy. Physiological activity is determined through quantitative rRNA hybridizations in single cells, from which growth rates are estimated. Specific gene expression is monitored through the use of green fluorescent protein as a reporter (allows single cell detection). Transfer of conjugative plasmids is followed as zygotic induction of GFP in transconjugant cells in the community. All methods used have been developed for applications in single cells for inspection in the fluorescence or confocal microscope. The goal is to build up an understanding of the way bacteria organise their activities in complex communities, and eventually to understand the coordinative aspects of this type of 'social life'. This first phase of the project was terminated at the end of 1999, due to the run-out of the Biotech framework grant. A new phase of this project financed through the anchoring of the Biotech grant will be initiated in 2000.

Department of Systems Biology
Spanish National Research Council
Period: 01/01/1996 → 31/12/1999
Number of participants: 10
Project participant:
Sternberg, Claus (Intern)
Andersen, Jens Bo (Intern)
Christensen, Bjarke Bak (Intern)
Givskov, Michael Christian (Intern)
Johansen, Tove (Intern)
Nielsen, Alex Toftgaard (Intern)
Heydorn, Arne (Intern)
de Lorenzo, Victor (Ekstern)
Ramos, Juan Luis (Ekstern)
Project Manager, organisational:
Molin, Søren (Intern)

Financing sources
Source: Unknown
Name of research programme: Ukendt
Amount: 6,000,000.00 Danish Kroner
Project

Activities:

University of Nottingham, Synthetic Biology Research Centre, Strategic Advisory Board (External organisation)
Period: 2015 → …
Alex Toftgaard Nielsen (Participant)
Novo Nordisk Foundation Center for Biosustainability

Research Groups
Bacterial Cell Factory Optimization

Description
Member of Scientific Advisory Board
Degree of recognition: International

Related external organisation

University of Nottingham, Synthetic Biology Research Centre, Strategic Advisory Board
Activity: Membership › Membership of committees, commissions, boards, councils, associations, organisations, or similar

Current Opinion in Biotechnology (Journal)
Period: Dec 2015
Alex Toftgaard Nielsen (Editor)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
Bacterial Cell Factory Optimization

Description
Section Editor, Chemical Biotechnology

Related journal

Current Opinion in Biotechnology
0958-1669
Central database
Activity: Research › Journal editor

Novo Nordisk Foundation Cluster Days
Period: 15 Nov 2015
Alex Toftgaard Nielsen (Lecturer)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
Bacterial Cell Factory Optimization

Description
A dual reporter system for monitoring protein folding and translation

Related external organisation

Unknown external organisation
Activity: Talks and presentations › Conference presentations

DuPont Research Seminar Series
Period: 5 Nov 2015
Alex Toftgaard Nielsen (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
Bacterial Cell Factory Optimization

Description
Optimizing production of biochemicals and proteins in E. coli

Related event

DuPont Research Seminar Series
05/11/2015 → …
Palo Alto, United States
Activity: Talks and presentations › Conference presentations
Technical University of Denmark (External organisation)
Period: 7 Aug 2015
Alex Toftgaard Nielsen (Chairman)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
Bacterial Cell Factory Optimization

Description
Chairman

Related external organisation
Technical University of Denmark
Kgs. Lyngby, Denmark
Activity: Membership › Membership in review committee

Opponent at PhD defence (External organisation)
Period: 12 Jun 2015
Alex Toftgaard Nielsen (Participant)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
Bacterial Cell Factory Optimization

Description
Opponent at Denis Selnihhin PhD defence at Aarhus University

Related external organisation
Activity: Membership › Membership in review committee

The 7th Copenhagen Bioscience Conference
Alex Toftgaard Nielsen (Organizer)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
Bacterial Cell Factory Optimization

Related event
The 7th Copenhagen Bioscience Conference: Cell Factories and Biosustainability – technologies for cell factory construction
17/05/2015 → 21/11/2015
Hillerød, Denmark
Activity: Attending an event › Participating in or organising a conference

Stuttgart University, Institut für Technische Biochemie, Seminar Series
Period: 22 Jan 2015
Alex Toftgaard Nielsen (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
Bacterial Cell Factory Optimization
Description
Production of Biochemicals in Bacteria

Related event
Stuttgart University, Institut für Technische Biochemie, Seminar Series
22/01/2015 → …
University of Stuttgart, Denmark
Activity: Talks and presentations › Conference presentations

C1NET – CHEMICALS FROM C1 GAS CONFERENCE
Period: 15 Jan 2015
Alex Toftgaard Nielsen (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
Bacterial Cell Factory Optimization

Description
Production of Biochemicals in Bacteria

Related event
C1NET – CHEMICALS FROM C1 GAS CONFERENCE
14/01/2015 → 16/01/2015
Nottingham, United Kingdom
Activity: Talks and presentations › Conference presentations