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Research Groups
31/07/2015 → present
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iLoop
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VIP

Bacterial Cell Factories
08/08/2014 → 19/12/2014 Former
VIP

CFB - Core Flow
14/12/2012 → 14/02/2014 Former
VIP

Publications:

**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.

**General information**
State: Published
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), Herrgård, M. (Intern), Sommer, M. O. A. (Intern), Feist, A. (Intern), Mohamed, E. T. T. (Intern)
Publication date: 16 Nov 2017

**Publication information**
IPC: C12P 7/ 52 A I
Patent number: WO2017194696
Date: 16/11/2017
Priority date: 09/06/2016
Priority number: EP20160173673
Original language: English
Main Research Area: Technical/natural sciences
Source: espacenet
Source-ID: WO2017194696
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**
State: Published
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
Generation of a platform strain for ionic liquid tolerance using adaptive laboratory evolution

There is a need to replace petroleum-derived with sustainable feedstocks for chemical production. Certain biomass feedstocks can meet this need as abundant, diverse, and renewable resources. Specific ionic liquids (ILs) can play a role in this process as promising candidates for chemical pretreatment and deconstruction of plant-based biomass feedstocks as they efficiently release carbohydrates which can be fermented. However, the most efficient pretreatment ILs are highly toxic to biological systems, such as microbial fermentations, and hinder subsequent bioprocessing of fermentative sugars obtained from IL-treated biomass. To generate strains capable of tolerating residual ILs present in treated feedstocks, a tolerance adaptive laboratory evolution (TALE) approach was developed and utilized to improve growth of two different Escherichia coli strains, DH1 and K-12 MG1655, in the presence of two different ionic liquids, 1-ethyl-3-methylimidazolium acetate ([C2C1Im][OAc]) and 1-butyl-3-methylimidazolium chloride ([C4C1Im]Cl). For multiple parallel replicate populations of E. coli, cells were repeatedly passed to select for improved fitness over the course of approximately 40 days. Clonal isolates were screened and the best performing isolates were subjected to whole genome sequencing. The most prevalent mutations in tolerant clones occurred in transport processes related to the functions of mdtJI, a multidrug efflux pump, and yhdP, an uncharacterized transporter. Additional mutations were enriched in processes such as transcriptional regulation and nucleotide biosynthesis. Finally, the best-performing strains were compared to previously characterized tolerant strains and showed superior performance in tolerance of different IL and media combinations (i.e., cross tolerance) with robust growth at 8.5% (w/v) and detectable growth up to 11.9% (w/v) [C2C1Im][OAc]. The generated strains thus represent the best performing platform strains available for bioproduction utilizing IL-treated renewable substrates, and the TALE method was highly successful in overcoming the general issue of substrate toxicity and has great promise for use in tolerance engineering.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, iLoop, ALE Technology & Software Development, Research Groups, Global Econometric Modeling, Network Reconstruction in Silico Biology, Joint Bioenergy Institute
Authors: Mohamed, E. T. (Intern), Wang, S. (Ekstern), Lennen, R. M. (Intern), Herrgård, M. J. (Intern), Simmons, B. A. (Ekstern), Singer, S. W. (Ekstern), Feist, A. M. (Intern)
Number of pages: 15
Publication date: 2017
Main Research Area: Technical/natural sciences

Publication information
Journal: Microbial Cell Factories
Volume: 16
Issue number: 1
Article number: 204
ISSN (Print): 1475-2859
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.92 SJR 1.446 SNIP 1.228
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.501 SNIP 1.24 CiteScore 4.08
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.
Systemic biology solutions for biochemical production challenges

There is an urgent need to significantly accelerate the development of microbial cell factories to produce fuels and chemicals from renewable feedstocks in order to facilitate the transition to a biobased society. Methods commonly used within the field of systems biology including omics characterization, genome-scale metabolic modeling, and adaptive laboratory evolution can be readily deployed in metabolic engineering projects. However, high performance strains usually carry tens of genetic modifications and need to operate in challenging environmental conditions. This additional complexity compared to basic science research requires pushing systems biology strategies to their limits and often spurs innovative developments that benefit fields outside metabolic engineering. Here we survey recent advanced applications of systems biology methods in engineering microbial production strains for biofuels and -chemicals.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, iLoop, Synthetic Biology Tools for Yeast, Research Groups, Global Econometric Modeling
Authors: Hansen, A. S. L. (Intern), Lennen, R. M. (Intern), Sonnenschein, N. (Intern), Herrgard, M. (Intern)
Pages: 85-91
Publication date: 2017
Main Research Area: Technical/natural sciences

Publication information
Journal: Current Opinion in Biotechnology
Volume: 45
ISSN (Print): 0958-1669
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.55 SJR 3.331 SNIP 2.1
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 3.113 SNIP 2.143 CiteScore 7.99
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 3.271 SNIP 2.068 CiteScore 7.45
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.322 SNIP 2.198 CiteScore 7.93
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
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
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.
Benefits of selective feeding

Industrial processes using microbial cells allow the conversion of renewable-carbon feedstocks into a complex range of chemical products at comparatively low temperatures and pressures (1). In contrast, traditional chemical manufacturing relies mainly on energy-intensive conversions of petroleum-derived carbon feedstocks. However, record-low oil prices are making it difficult for biotechnology processes to compete with traditional manufacturing, particularly for low-cost bulk products such as biofuels and commodity chemicals. On page 583 of this issue, Shaw et al. (2), report a cost-effective technology to control contamination in nonsterilized process equipment (see the figure). This technology has the potential to greatly lower the cost of producing fermentation-derived chemicals with microbial processes.

Genome-wide Escherichia coli stress response and improved tolerance towards industrially relevant chemicals

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.
Transient overexpression of DNA adenine methylase enables efficient and mobile genome engineering with reduced off-target effects

Homologous recombination of single-stranded oligonucleotides is a highly efficient process for introducing precise mutations into the genome of _E. coli_ and other organisms when mismatch repair (MMR) is disabled. This can result in the rapid accumulation of off-target mutations that can mask desired phenotypes, especially when selections need to be employed following the generation of combinatorial libraries. While the use of inducible mutator phenotypes or other MMR evasion tactics have proven useful, reported methods either require non-mobile genetic modifications or costly oligonucleotides that also result in reduced efficiencies of replacement. Therefore a new system was developed, Transient Mutator Multiplex Automated Genome Engineering (TM-MAGE), that solves problems encountered in other methods for oligonucleotide-mediated recombination. TM-MAGE enables nearly equivalent efficiencies of allelic replacement to the use of strains with fully disabled MMR and with an approximately 12-to 33-fold lower off-target mutation rate. Furthermore, growth temperatures are not restricted and a version of the plasmid can be readily removed by sucrose counterselection. TM-MAGE was used to combinatorially reconstruct mutations found in evolved salt-tolerant strains, enabling the identification of causative mutations and isolation of strains with up to 75% increases in growth rate and greatly reduced lag times in 0.6 M NaCl.
Transient overexpression of DNA adenine methylase enables efficient and mobile genome engineering with reduced off-target effects.pdf

10.1093/nar/gkv1090

Analysis of gene essentiality in Escherichia coli across strains and growth conditions

Different types of knock-out studies have for years been applied in addressing the question of gene essentiality in various organisms. The development within the field of next generation sequencing has paved the way for more extensive studies due to the high throughput. One of these fairly resent methods is transposon insertion sequencing (Tn-Seq), in which a mutant library is constructed by randomly inserting transposons into the genome, the position of which is determined by sequencing. By knowing the number of inserts in each gene in the initial library it is possible to determine if genes are either essential or detrimental for growth in the test condition in question. In this study the TN-Seq method was used to
investigate the differences in gene essentiality between four laboratory strains of E. coli subjected to four different growth conditions to investigate the reason for the differences in osmotic and chemical stress tolerance that exists between the strains as well as to assess the commonalities. Based on the sequencing data we identified genes that were essential for growth under the different conditions, some of which are essential in all conditions across strains and others that are specifically essential under certain growth conditions and/or in certain strains. This knowledge is important in the effort to engineer more stress tolerant strains, which are highly relevant for industrial purposes. Here is presented the bioinformatic analysis of the data, which includes one to one comparisons for each strain in each condition to the control condition and a multivariate analysis including all strains across conditions.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, iLoop, Research Groups, Department of Systems Biology
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Number of pages: 1
Publication date: 2015
Event: Abstract from First Annual Danish Bioinformatics Conference, Odense, Denmark.
Main Research Area: Technical/natural sciences
Publication: Research - peer-review › Conference abstract for conference – Annual report year: 2015

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
State: Published
Organisations: Bacterial Cell Factories, Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factory Optimization, Research Groups
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Number of pages: 10
Publication date: 2015
Main Research Area: Technical/natural sciences

Publication information
Journal: Scientific Reports
Volume: 5
Article number: 17874
ISSN (Print): 2045-2322
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
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
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.103 SNIP 1.544 CiteScore 4.75
Web of Science (2014): Indexed yes
Combinatorial Strategies for Improving Multiple-Stress Resistance in Industrially Relevant Escherichia coli Strains.

High-cell-density fermentation for industrial production of chemicals can impose numerous stresses on cells due to high substrate, product, and by-product concentrations; high osmolarity; reactive oxygen species; and elevated temperatures. There is a need to develop platform strains of industrial microorganisms that are more tolerant toward these typical processing conditions. In this study, the growth of six industrially relevant strains of Escherichia coli was characterized under eight stress conditions representative of fed-batch fermentation, and strains W and BL21(DE3) were selected as platforms for transposon (Tn) mutagenesis due to favorable resistance characteristics. Selection experiments, followed by either targeted or genome-wide next-generation-sequencing-based Tn insertion site determination, were performed to identify mutants with improved growth properties under a subset of three stress conditions and two combinations of individual stresses. A subset of the identified loss-of-function mutants were selected for a combinatorial approach, where strains with combinations of two and three gene deletions were systematically constructed and tested for single and multistress resistance. These approaches allowed identification of (i) strain-background-specific stress resistance phenotypes, (ii) novel gene deletion mutants in E. coli that confer single and multistress resistance in a strain-background-dependent manner, and (iii) synergistic effects of multiple gene deletions that confer improved resistance over single deletions. The results of this study underscore the suboptimality and strain-specific variability of the genetic network regulating growth under stressful conditions and suggest that further exploration of the combinatorial gene deletion space in multiple strain backgrounds is needed for optimizing strains for microbial bioprocessing applications.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, Bacterial Cell Factories, iLoop, Research Groups
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Pages: 6223-6242
Publication date: 2014
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied and Environmental Microbiology
Volume: 80
Issue number: 19
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
Library sequencing strategies for comparative analysis of stress resistance mechanisms in Escherichia coli strains

Transposon insertion sequencing (Tn-Seq) has recently emerged as a powerful next-generation sequencing method that enables querying the contributions of all genes in a bacterial genome toward the fitness of a growing organism. In this method, transposon insertion mutant libraries are constructed and subjected to growth selections. Following selection, the locations of all insertions in the population are counted and can be compared between a control and a target condition, enabling the identification of genes that are both conditionally essential and conditionally detrimental. We have exploited
Tn-Seq to probe the basis for the large variations in osmotic and acetate stress tolerance of different laboratory strains of Escherichia coli (K-12 MG1655, BL21(DE3), W, and Crooks). Little is currently known to explain the source of this variation and to enable rational engineering to impart stress tolerance. Tn-Seq revealed many differences and similarities in resistance mechanisms at the genetic level across strains, allowing correlations to be made with growth phenotypes. Cross-strain comparisons of conditionally essential genes and their relative essentiality also suggest a large degree of variation in metabolic flux distributions and regulation of gene expression between strains. A number of direct targets for metabolic engineering of stress resistance via loss-of-function mutations were also discovered, and we show that deletion of a selection of these genes results in improved growth under the original selection condition.
Identification of Transport Proteins Involved in Free Fatty Acid Efflux in Escherichia coli

Escherichia coli has been used as a platform host for studying the production of free fatty acids (FFA) and other energy-dense compounds useful in biofuel applications. Most of the FFA produced by E. coli are found extracellularly. This finding suggests that a mechanism for transport across the cell envelope exists, yet knowledge of proteins that may be responsible for export remains incomplete. Production of FFA has been shown to cause cell lysis, induce stress responses, and impair basic physiological processes. These phenotypes could potentially be diminished if efflux rates were increased. Here, a total of 15 genes and operons were deleted and screened for their impact on cell viability and titer in FFA-producing E. coli. Deletions of acrAB and rob and, to a lower degree of statistical confidence, emrAB, mdtEF, and mdtABCD reduced multiple measures of viability, while deletion of tolC nearly abolished FFA production. An acrAB emrAB deletion strain exhibited greatly reduced FFA titers approaching the tolC deletion phenotype. Expression of efflux pumps on multicopy plasmids did not improve endogenous FFA production in an acrAB+ strain, but plasmid-based expression of acrAB, mdtEF, and an mdtEF-tolC artificial operon improved the MIC of exogenously added decanoate for an acrAB mutant strain. The findings suggest that AcrAB-TolC is responsible for most of the FFA efflux in E. coli, with residual activity provided by other resistance-nodulation-cell division superfamily-type efflux pumps, including EmrAB-TolC and MdtEF-TolC. While the expression of these proteins on multicopy plasmids did not improve production over the basal level, their identification enables future engineering efforts.
Fatty acid metabolism is an attractive route to produce liquid transportation fuels and commodity oleochemicals from renewable feedstocks. Recently, genes and enzymes, which comprise metabolic pathways for producing fatty acid-derived compounds (e.g. esters, alkanes, olefins, ketones, alcohols, polyesters) have been elucidated and used in engineered microbial hosts. The resulting strains often generate products at low percentages of maximum theoretical yields, leaving significant room for metabolic engineering. Economically viable processes will require strains to approach theoretical yields, particularly for replacement of petroleum-derived fuels. This review will describe recent progress toward this goal, highlighting the scientific discoveries of each pathway, ongoing biochemical studies to understand each enzyme, and metabolic engineering strategies that are being used to improve strain performance.
Modulating Membrane Composition Alters Free Fatty Acid Tolerance in Escherichia coli

Microbial synthesis of free fatty acids (FFA) is a promising strategy for converting renewable sugars to advanced biofuels and oleochemicals. Unfortunately, FFA production negatively impacts membrane integrity and cell viability in Escherichia coli, the dominant host in which FFA production has been studied. These negative effects provide a selective pressure against FFA production that could lead to genetic instability at industrial scale. In prior work, an engineered E. coli strain harboring an expression plasmid for the Umbellularia californica acyl-acyl carrier protein (ACP) thioesterase was shown to have highly elevated levels of unsaturated fatty acids in the cell membrane. The change in membrane content was hypothesized to be one underlying cause of the negative physiological effects associated with FFA production. In this work, a connection between the regulator of unsaturated fatty acid biosynthesis in E. coli, FabR, thioesterase expression, and unsaturated membrane content was established. A strategy for restoring normal membrane saturation levels and increasing tolerance towards endogenous production of FFAs was implemented by modulating acyl-ACP pools with a second thioesterase (from Geobacillus sp. Y412MC10) that primarily targets medium chain length, unsaturated acyl-ACPs. The strategy succeeded in restoring membrane content and improving viability in FFA producing E. coli while maintaining FFA titers. However, the restored fitness did not increase FFA productivity, indicating the existence of additional metabolic or regulatory barriers.
Engineering Escherichia coli to synthesize free fatty acids

Fatty acid metabolism has received significant attention as a route for producing high-energy density, liquid transportation fuels and high-value oleochemicals from renewable feedstocks. If microbes can be engineered to produce these compounds at yields that approach the theoretical limits of 0.3–0.4 g/g glucose, then processes can be developed to replace current petrochemical technologies. Here, we review recent metabolic engineering efforts to maximize production of free fatty acids (FFA) in Escherichia coli, the first step towards production of downstream products. To date, metabolic engineers have succeeded in achieving higher yields of FFA than any downstream products. Regulation of fatty acid metabolism and the physiological effects of fatty acid production will also be reviewed from the perspective of identifying future engineering targets.

General information
State: Published
Organisations: University of Wisconsin-Madison
Authors: Lennen, R. (Intern), Pfleger, B. F. (Ekstern)
Pages: 659-667
Publication date: 2012
Main Research Area: Technical/natural sciences

Publication information
Journal: Trends in Biotechnology
Volume: 30
Issue number: 12
ISSN (Print): 0167-7799
Ratings:
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 9.97 SJR 4.037 SNIP 3.143
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 4.091 SNIP 3.391 CiteScore 9.72
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 4.344 SNIP 3.35 CiteScore 10.31
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 3.763 SNIP 3.151 CiteScore 10.5
ISI indexed (2013): ISI indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 3.353 SNIP 3.083 CiteScore 9.77
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 3.321 SNIP 3.05 CiteScore 9.82
Isolation of improved free fatty acid overproducing strains of Escherichia coli via Nile red based high-throughput screening

Biological production of hydrocarbons is an attractive strategy to produce drop-in replacement transportation fuels. Several methods for converting microbially produced fatty acids into reduced compounds compatible with petrodiesel have been reported. For these processes to become economically viable, microorganisms must be engineered to approach the theoretical yield of fatty acid products from renewable feedstocks such as glucose. Strains with increased titers can be obtained through both rational and random approaches. While powerful, random approaches require a genetic selection or facile screen that is amenable to high throughput platforms. Here, we present the use of a high throughput screen for fatty acids based on the hydrophobic dye Nile red. The method was applied to screening a transposon library of a free fatty acid overproducing strain of Escherichia coli in search of high producing mutants. Ten gene targets were identified via primary and secondary screening. A strain comprising a clean knockout of one of the identified genes led to a 20% increase in titer over the baseline strain. A selection strategy that combines these findings and can act in an iterative fashion has been developed and can be used for future strain optimization in hydrocarbon producing strains. © 2011 American Institute of Chemical Engineers Environ Prog, 2012
Kinetic modeling of free fatty acid production in Escherichia coli based on continuous cultivation of a plasmid free strain

The microbial production of free fatty acids (FFAs) and reduced derivatives is an attractive process for the renewable production of diesel fuels. Toward this goal, a plasmid-free strain of Escherichia coli was engineered to produce FFAs by integrating three copies of a thioesterase gene from Umbellularia californica (BTE) under the control of an inducible promoter onto the chromosome. In batch culture, the resulting strain produced identical titers to a previously reported strain that expressed the thioesterase from a plasmid. The growth rate, glucose consumption rate, and FFA production rate of this strain were studied in continuous cultivation under carbon limitation. The highest yield of FFA on glucose was observed at a dilution rate of 0.05 h⁻¹ with the highest specific productivity observed at a dilution rate of 0.2 h⁻¹. The observed yields under the lowest dilution rate were 15% higher than that observed in batch cultures. An increase in both productivity and yield (~40%) was observed when the composition of the nutrients was altered to shift the culture toward non-carbon limitation. A deterministic model of the production strain has been proposed and indicates that maintenance requirements for this strain are significantly higher than wild-type E. coli. Biotechnol. Bioeng. 2012; 109:1518–1527. © 2012 Wiley Periodicals, Inc.
Bacterial production of free fatty acids from freshwater macroalgal cellulose

The predominant strategy for using algae to produce biofuels relies on the overproduction of lipids in microalgae with subsequent conversion to biodiesel (methyl-esters) or green diesel (alkanes). Conditions that both optimize algal growth and lipid accumulation rarely overlap, and differences in growth rates can lead to wild species outcompeting the desired lipid-rich strains. Here, we demonstrate an alternative strategy in which cellulose contained in the cell walls of multicellular algae is used as a feedstock for cultivating biofuel-producing microorganisms. Cellulose was extracted from an environmental sample of Cladophora glomerata-dominated periphyton that was collected from Lake Mendota, WI, USA. The resulting cellulose cake was hydrolyzed by commercial enzymes to release fermentable glucose. The hydrolysis mixture was used to formulate an undefined medium that was able to support the growth, without supplementation, of a free fatty acid (FFA)-overproducing strain of Escherichia coli (Lennen et. al 2010). To maximize free fatty acid production from glucose, an isopropyl β-d-1-thiogalactopyranoside (IPTG)-inducible vector was constructed to express the Umbellularia californica acyl–acyl carrier protein (ACP) thioesterase. Thioesterase expression was optimized by inducing cultures with 50 μM IPTG. Cell density and FFA titers from cultures grown on algae-based media reached 50% of those (~90 μg/mL FFA) cultures grown on rich Luria–Bertani broth supplemented with 0.2% glucose. In comparison, cultures grown in two media based on AFEX-pretreated corn stover generated tenfold less FFA than cultures grown in algae-based media. This study demonstrates that macroalgal cellulose is a potential carbon source for the production of biofuels or other microbially synthesized compounds.

General information
State: Published
Organisations: Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Bioenergy and Environment Inc.
Pages: 435-446
Publication date: 2011
Main Research Area: Technical/natural sciences

Publication information
Journal: Applied Microbiology and Biotechnology
Volume: 91
Issue number: 2
ISSN (Print): 0175-7598
Ratings:
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.57 SJR 1.177 SNIP 1.173
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.254 SNIP 1.217 CiteScore 3.43
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.327 SNIP 1.458 CiteScore 3.71
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.533 SNIP 1.432 CiteScore 4.3
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.507 SNIP 1.286 CiteScore 4
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Membrane stresses induced by overproduction of free fatty acids in Escherichia coli

Microbiologically produced fatty acids are potential precursors to high-energy-density biofuels, including alkanes and alkyl ethyl esters, by either catalytic conversion of free fatty acids (FFAs) or enzymatic conversion of acyl-acyl carrier protein or acyl-coenzyme A intermediates. Metabolic engineering efforts aimed at overproducing FFAs in Escherichia coli have achieved less than 30% of the maximum theoretical yield on the supplied carbon source. In this work, the viability, morphology, transcript levels, and protein levels of a strain of E. coli that overproduces medium-chain-length FFAs was compared to an engineered control strain. By early stationary phase, an 85% reduction in viable cell counts and exacerbated loss of inner membrane integrity were observed in the FFA-overproducing strain. These effects were enhanced in strains endogenously producing FFAs compared to strains exposed to exogenously fed FFAs. Under two sets of cultivation conditions, long-chain unsaturated fatty acid content greatly increased, and the expression of genes and proteins required for unsaturated fatty acid biosynthesis were significantly decreased. Membrane stresses were further implicated by increased expression of genes and proteins of the phage shock response, the MarA/Rob/SoxS regulon, and the nuo and cyo operons of aerobic respiration. Gene deletion studies confirmed the importance of the phage shock proteins and Rob for maintaining cell viability; however, little to no change in FFA titer was observed after 24 h of cultivation. The results of this study serve as a baseline for future targeted attempts to improve FFA yields and titers in E. coli. © 2011, American Society for Microbiology.
A process for microbial hydrocarbon synthesis: Overproduction of fatty acids in Escherichia coli and catalytic conversion to alkanes

The development of renewable alternatives to diesel and jet fuels is highly desirable for the heavy transportation sector, and would offer benefits over the production and use of short-chain alcohols for personal transportation. Here, we report the development of a metabolically engineered strain of Escherichia coli that overproduces medium-chain length fatty acids via three basic modifications: elimination of β-oxidation, overexpression of the four subunits of acetyl-CoA carboxylase, and expression of a plant acyl–acyl carrier protein (ACP) thioesterase from Umbellularia californica (BTE). The expression level of BTE was optimized by comparing fatty acid production from strains harboring BTE on plasmids with four different copy numbers. Expression of BTE from low copy number plasmids resulted in the highest fatty acid production. Up to a seven-fold increase in total fatty acid production was observed in engineered strains over a negative control strain (lacking β-oxidation), with a composition dominated by C12 and C14 saturated and unsaturated fatty acids. Next, a strategy for producing undecane via a combination of biotechnology and heterogeneous catalysis is demonstrated. Fatty acids were extracted from a culture of an overproducing strain into an alkane phase and fed to a Pd/C plug flow reactor, where the extracted fatty acids were decarboxylated into saturated alkanes. The result is an enriched alkane stream that can be recycled for continuous extractions. Complete conversion of C12 fatty acids extracted from culture to undecane has been demonstrated yielding a concentration of 0.44 g L−1 (culture volume) undecane. Biotechnol. Bioeng. 2010;106:193–202. © 2010 Wiley Periodicals, Inc.
Projects:

Functional investigations of cell wall alterations in chemical-evolved E. coli strains

One of the largest barriers to achieving economical bio-based production of bulk chemicals such as biofuels and polymer precursors is poor tolerance of microbial production hosts toward high concentrations of excreted product. These concentrations are often in excess of 100 g/L in order to minimize capital and downstream purification costs. However virtually all chemicals at these levels result in stresses and poor growth in the majority of microbial hosts, which can decrease product yields and productivities. To help address this issue, we utilized a robotic platform to evolve parallel populations of Escherichia coli K-12 MG1655 for enhanced growth in the presence of toxic concentrations of 11 chemicals representing diverse functional classes that are of interest as biofuels or their precursors, polymer precursors, and other bulk chemicals and intermediates. Resequencing of over 200 strains and subsequent reconstruction of sets of mutations has provided unparalleled insight on the genomic basis of tolerance. In addition to more specific mechanisms for individual chemicals or classes of chemicals, many broader mechanisms of tolerance have been putatively identified that recur in strains evolved on different chemicals.

One class of common mutations across chemical conditions are coding mutations in genes related to cell wall biogenesis, maintenance, and recycling. It is suspected that many of the strains harboring these mutations feature altered cell morphologies and altered membrane protein and lipid compositions. In order to understand the connection between genotype and phenotype for cell wall mutations, it is proposed to conduct work at EMSL to further characterize the phenotype of a subset of evolved strains with confirmed morphological changes. The proposed tests include using cryogenic transmission electron microscopy and helium ion microscopy to observe cross-sectional and surface modifications of single cells, and performing differential membrane proteomics and lipidomics analyses.
The data obtained from this study will be used to develop further targeted tests on strains with cell wall mutations, and will ultimately be integrated together with other datasets concerning other types of mutations to develop predictive models of chemical and stress tolerance. The direct effect of cell wall mutations on endogenous production and excretion of relevant chemicals will also be tested by employing them directly in engineered production host strains.

Novo Nordisk Foundation Center for Biosustainability

Research Groups

iLoop

Pacific Northwest National Laboratory
Period: 01/10/2015 → 30/09/2016
Number of participants: 2
Project Manager, academic:
Lennen, Rebecca (Intern)
Herrgard, Markus (Intern)

Project

Generation of Raw Substrate Utilizing Platform Strains

Novo Nordisk Foundation Center for Biosustainability

Research Groups

iLoop

Network Reconstruction in Silico Biology
Period: 01/06/2015 → 30/04/2019
Number of participants: 4
Phd Student:
Tharwat Tolba Mohamed, Elsayed (Intern)
Supervisor:
Lennen, Rebecca (Intern)
Feist, Adam (Intern)
Main Supervisor:
Herrgard, Markus (Intern)

Project

Generation of Raw Substrate Utilizing Platform Strains

Technical University of Denmark
Period: 01/06/2015 → 31/05/2018
Number of participants: 4
Phd Student:
Tharwat Tolba Mohamed, Elsayed (Intern)
Supervisor:
Feist, Adam (Intern)
Lennen, Rebecca (Intern)
Main Supervisor:
Herrgard, Markus (Intern)

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

Small Regulatory RNAs in Bacterial Production Organisms

Department of Systems Biology
Period: 01/11/2013 → 17/11/2016
Number of participants: 6
Phd Student:
Bojanovic, Klara (Intern)
Supervisor:
Molin, Søren (Intern)
Main Supervisor:
Long, Katherine (Intern)
Examiner:
Lennen, Rebecca (Intern)
Kallipolitis, Birgitte Haahr (Ekstern)
Valverde, Claudio (Ekstern)

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

Relations
Publications:
Small RNA-Controlled Gene Regulatory Networks in *Pseudomonas putida*
Project: PhD

Activities:

*Adaptive laboratory evolution of product-tolerant hosts for biobased chemical production*
Period: 26 Jul 2016
Rebecca Lennen (Invited speaker)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
iLoop

Related event

*2016 Society for Industrial Microbiology Annual Meeting*
24/07/2016 → 29/07/2016
New Orleans, United States
Activity: Talks and presentations › Conference presentations

*Metabolic Engineering XI*
Period: 28 Jun 2016
Rebecca Lennen (Participant)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
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Description
Adaptive laboratory evolution of product-tolerant hosts for enhanced production of biobased chemicals

Related event

*Metabolic Engineering XI*
26/06/2016 → 30/06/2016
Awaji, Japan
Activity: Attending an event › Participating in or organising a conference

Unraveling the functional basis of chemical tolerance in evolved strains of *Escherichia coli*
Period: 14 Oct 2015
Rebecca Lennen (Speaker)
Novo Nordisk Foundation Center for Biosustainability
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**Description**
Oral presentation

**Related event**

**EMBL Symposium: New Approaches and Concepts in Microbiology**
11/10/2015 → 14/10/2015
Heidelberg, Germany
Activity: Talks and presentations › Conference presentations

**2015 Synthetic Biology: Engineering, Evolution & Design (SEED)**
Period: 11 Jun 2015
Rebecca Lennen (Participant)
Novo Nordisk Foundation Center for Biosustainability
Research Groups

**Description**
Large-scale determination of causative mutations for enhanced chemical tolerance in evolved strains of Escherichia coli

**Related event**

**2015 Synthetic Biology: Engineering, Evolution & Design (SEED)**
10/06/2015 → 13/06/2015
Boston, United States
Activity: Attending an event › Participating in or organising a conference

**Large-scale determination of causative mutations for enhanced chemical tolerance in evolved strains of Escherichia coli**
Period: 20 May 2015
Rebecca Lennen (Speaker)
Novo Nordisk Foundation Center for Biosustainability
Research Groups

**Description**
Oral presentation

**Related event**

**Cell Factories and Biosustainability 2015**
17/05/2015 → 21/05/2015
Hillerød, Denmark
Activity: Talks and presentations › Conference presentations

**Advanced Genome Engineering Techniques**
Period: 9 Feb 2015 → 13 Feb 2015
Rebecca Lennen (Guest lecturer)
Novo Nordisk Foundation Center for Biosustainability
Research Groups

**Description**
Advanced Genome Engineering Techniques
Related event

**Advanced Genome Engineering Techniques**
09/02/2015 → 13/02/2015
San Diego, United States
Activity: Talks and presentations › Guest lectures, external teaching and course activities at other universities

**Project Management for Researchers**
Period: 2 Feb 2015 → 22 May 2015
Rebecca Lennen (Participant)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
iLoop

**Description**
Project Management for Researchers

Related event

**Project Management for Researchers**
02/02/2015 → 22/05/2015
Lyngby, Denmark
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.

**The Danish Microbiological Society Annual Congress 2014**
Period: 10 Nov 2014
Rebecca Lennen (Participant)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
iLoop

**Description**
Understanding and engineering stress tolerance in E. coli

Related event

**The Danish Microbiological Society Annual Congress 2014**
10/11/2014 → …
Copenhagen, Denmark
Activity: Attending an event › Participating in or organising a conference

**16th European Congress on Biotechnology**
Period: 14 Jul 2014
Rebecca Lennen (Participant)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
iLoop

**Description**
Comparative analysis of stress resistance mechanisms in laboratory Escherichia coli strains revealed by transposon insertion sequencing

Related event

**16th European Congress on Biotechnology**
13/07/2014 → 16/07/2014
Edinburgh, United Kingdom
Activity: Attending an event › Participating in or organising a conference
**Comparative cross-strain analysis of stress resistance mechanisms revealed by transposon insertion sequencing**

**Period:** 16 Jun 2014  
**Rebecca Lennen (Speaker)**  
**Novo Nordisk Foundation Center for Biosustainability**  
**Research Groups**  
**iLoop**

**Description**  
Oral presentation

**Related event**

**Metabolic Engineering X: Biological Design and Synthesis**  
15/06/2014 → 19/06/2014  
Vancouver, Canada  
**Activity:** Talks and presentations › Conference presentations

**14th International Conference on Systems Biology**  
**Period:** 30 Aug 2013  
**Rebecca Lennen (Participant)**  
**Novo Nordisk Foundation Center for Biosustainability**  
**Research Groups**  
**iLoop**

**Description**  
Systems biology approaches for the development of stress-tolerant industrial strains of E. coli

**Related event**

**14th International Conference on Systems Biology**  
30/08/2013 → 03/09/2013  
Copenhagen, Denmark  
**Activity:** Attending an event › Participating in or organising a conference

**The Sixth International Meeting on Synthetic Biology**  
**Period:** 10 Jul 2013  
**Rebecca Lennen (Participant)**  
**Novo Nordisk Foundation Center for Biosustainability**  
**Research Groups**  
**iLoop**

**Description**  
Mining the diversity of wild-type Escherichia coli strains for stress tolerance phenotypes

**Related event**

**The Sixth International Meeting on Synthetic Biology**  
09/07/2013 → 11/07/2013  
London, United Kingdom  
**Activity:** Attending an event › Participating in or organising a conference

**Copenhagen Bioscience Conference: Cell Factories and Biosustainability**  
**Period:** 6 May 2013  
**Rebecca Lennen (Participant)**  
**Novo Nordisk Foundation Center for Biosustainability**
Research Groups

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Description
Mining the diversity of wild-type Escherichia coli strains for stress tolerance phenotypes

Related event

Copenhagen Bioscience Conference: Cell Factories and Biosustainability
03/11/2013 → 06/11/2013
Denmark
Activity: Attending an event › Participating in or organising a conference

2nd advanced course on 13C-based metabolic flux analysis
Period: 22 Nov 2012 → 30 Nov 2012
Rebecca Lennen (Participant)
Novo Nordisk Foundation Center for Biosustainability
Research Groups
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Related event

2nd advanced course on 13C-based metabolic flux analysis
22/11/2012 → 30/11/2015
Jülich, Germany
Activity: Attending an event › Participating in or organising workshops, courses, seminars etc.