Harnessing biocompatible chemistry for developing improved and novel microbial cell factories

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

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Organisations

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19/05/2015 → present
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Research outputs:

Systems Biology – A Guide for Understanding and Developing Improved Strains of Lactic Acid Bacteria

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

General information
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Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Colorado State University
Corresponding author: Solem, C.
Contributors: Liu, J., Chan, S. H. J., Chen, J., Solem, C., Jensen, P. R.
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Alterations in the transcription factors GntR1 and RamA enhance the growth and central metabolism of Corynebacterium glutamicum

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

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Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Hamburg University of Technology, Technical University of Denmark
Corresponding author: Solem, C.
Contributors: Wang, Z., Liu, J., Chen, L., Zeng, A., Solem, C., Jensen, P. R.
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Publication information
A bacterial cell factory for efficient production of ethanol from whey

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

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Contributors: Jensen, P. R., Liu, J., Solem, C., Dantoft, S. H.
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High-level production of diacetyl in a metabolically engineered lactic acid bacterium

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

General information
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Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining
Engineering Lactococcus lactis into a cell factory for production of butanol isomers

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

The non-dairy lactic acid bacterium Lactococcus lactis KF147 can utilize xylose as the sole energy source. To assess whether KF147 could serve as a platform organism for converting second generation sugars into useful chemicals, we characterized growth and product formation for KF147 when grown on xylose. In a defined medium KF147 was found to co-metabolize xylose and arginine, resulting in bi-phasic growth. Especially at low xylose concentrations, arginine significantly improved growth rate. To facilitate further studies of the xylose metabolism, we eliminated arginine catabolism by deleting the arcA gene encoding the arginine deiminase. The fermentation product profile suggested two routes for xylose degradation, the phosphoketolase pathway and the pentose phosphate pathway. Inactivation of the phosphoketolase pathway redirected the entire flux through the pentose phosphate pathway whereas over-expression of phosphoketolase increased the flux through the phosphoketolase pathway. In general, significant amounts of the mixed-acid products, including lactate, formate, acetate and ethanol, were formed irrespective of xylose concentrations. To demonstrate the potential of KF147 for converting xylose into useful chemicals we chose to redirect metabolism towards ethanol production. A synthetic promoter library was used to drive the expression of codon-optimized versions of the Zymomonas mobilis genes encoding pyruvate decarboxylase and alcohol dehydrogenase, and the outcome was a strain producing ethanol as the sole fermentation product with a high yield corresponding to 83% of the theoretical maximum. The results clearly indicate the great potential of using the more metabolically diverse non-dairy L. lactis strains for bio-production based on xylose containing feedstocks.
Micro-organism for the production of stereo-specific s, s-2,3-butanediol

The invention relates to a genetically modified lactic acid bacterium capable of producing (S,S)-2,3-butanediol stereo specifically from glucose under aerobic conditions. Additionally the invention relates to a method for producing (S,S)-2,3-butanediol and L-acetoin using the genetically modified lactic acid bacterium, under aerobic conditions in the presence of a source of iron-containing porphyrin or a source of metal ions (Fe3+/Fe2+). The lactic acid bacterium is genetically modified to express heterologous genes encoding enzymes catalysing the stereo-specific synthesis of (S,S)-2,3-butanediol; and additionally a number of genes are deleted in order to maximise the production of (S,S)-2,3-butanediol as compared to other products of oxidative fermentation.

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Organisations: Systems Biotechnology, National Food Institute, Research group for Microbial Biotechnology and Biorefining
Contributors: Solem, C., Jensen, P. R., Chen, J., Liu, J.
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Acetoin and 2,3 butanediol isomers synthesis in metabolically engineered Lactococcus lactis

Harnessing the biosynthetic machinery of living cells is a common approach used for producing a broad range of useful chemicals. Here, we divert inherent metabolic routes in L. lactis to produce (3R)-acetoin and the derived 2,3 butanediol isomers. Efficient production of (3R)-acetoin was accomplished using a strain where the competing lactate, acetate and ethanol forming pathways had been blocked. By introducing different alcohol dehydrogenases into this strain, either EcBdh from Enterobacter cloacae or SadB from Achromobacter xylosoxidans, it was possible to achieve high-yield production of m-BDO or R-BDO respectively. To achieve biosustainable production of these chemicals from dairy waste, we transformed the above strains with the lactose plasmid pLP712. This enabled efficient production of (3R)-acetoin, m-BDO and R-BDO from processed whey waste, with titers of 27, 51, and 32.1g/L respectively. The corresponding yields obtained were 0.42, 0.47 and 0.40 g/g lactose, which is 82%, 89%, and 76% of maximum theoretical yield respectively. These results clearly demonstrate that L. lactis is an excellent choice as a cell factory for transforming lactose containing dairy waste into value added chemicals.

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Contributors: Kandasamy, V., Liu, J., Dantoft, S. H., Solem, C., Jensen, P. R.
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A novel cell factory for efficient production of ethanol from dairy waste

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

General information
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Organisations: National Food Institute, Research group for Microbial Biotechnology and Biorefining, Arla Foods
Contributors: Liu, J., Dantoft, S. H., Würtz, A., Jensen, P. R., Solem, C.
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Web of Science (2016): Indexed yes
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Combining metabolic engineering and biocompatible chemistry for efficient production of food ingredients

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

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

Integrating biocompatible chemistry and manipulating cofactor partitioning in metabolically engineered Lactococcus lactis for fermentative production of (3S)-acetoin

Biocompatible chemistry (BC), i.e. non-enzymatic chemical reactions compatible with living organisms, is increasingly used in conjunction with metabolically engineered microorganisms for producing compounds that do not usually occur naturally. Here we report production of one such compound, (3S)-acetoin, a valuable precursor for chiral synthesis, using a metabolically engineered Lactococcus lactis strain growing under respiratory conditions with ferric iron serving as a BC component. The strain used has all competing product pathways inactivated, and an appropriate cofactor balance is achieved by fine-tuning the respiratory capacity indirectly via the hemin concentration. We achieve high-level (3S)-acetoin production...
production with a final titer of 66 mM (5.8 g/L) and a high yield (71% of the theoretical maximum). To the best of our knowledge, this is the first report describing production of (3S)-acetoin from sugar by microbial fermentation, and the results obtained confirm the potential that lies with BC for producing useful chemicals.

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

**General information**
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Contributors: Liu, J., Kandasamy, V., Würtz, A., Jensen, P. R., Solem, C.
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Scopus rating (2016): CiteScore 3.57 SJR 1.2 SNIP 1.194
Web of Science (2016): Impact factor 3.42
Synthesis of (3R)-acetoin and 2,3-butanediol isomers by metabolically engineered Lactococcus lactis

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

**Metabolic Engineering 11**
Jianming Liu (Participant)
National Food Institute
Research group for Microbial Biotechnology and Biorefining

**Description**
Metabolic Engineering 11 will be held from June 26-30, 2016 at the Awaji Yumebutai International Conference Center and The Westin Awaji Island in Kobe, Japan. The conference is chaired by Akihiko Kondo, Kobe University and Hiroshi Shimizu, Osaka University. Sessions integrate the recent achievements made in the fields of systems biology, synthetic biology, biochemical engineering, synthetic enzyme, evolutionary engineering, integrated omics, tools and methods, and emerging techniques, healthcare, biofuels, chemicals and materials, biologics, microbial and mammalian systems, and other disciplines and applications.

**Related event**

**Metabolic Engineering 11**
25/06/2016 → 30/06/2016
Kobe, Japan
Activity: Attending an event › Participating in or organising a conference