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Publications:

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

General information

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Organisations: National Food Institute, Research Group for Microbial Biotechnology and Biorefining
Authors: Jensen, P. R. (Intern), Liu, J. (Intern), Solem, C. (Intern), Dantoft, S. H. (Intern)
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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.

**General information**

State: Published
Organisations: National Food Institute, Research Group for Microbial Biotechnology and Biorefining, Systems Biotechnology
Authors: Kandasamy, V. (Intern), Liu, J. (Intern), Dantoft, S. H. (Intern), Solem, C. (Intern), Jensen, P. R. (Intern)
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**Bibliographical note**

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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 Ingredients Group P/S
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**Publication Information**

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The potential that lies in harnessing the chemical synthesis capabilities inherent in living organisms is immense. Here we demonstrate how the biosynthetic machinery of Lactococcus lactis can be diverted to make (3R)-acetoin and the derived 2,3-butanediol isomers meso-(2,3)-butanediol (m-BDO) and (2R,3R)-butanediol (R-BDO). Efficient production of (3R)-acetoin was accomplished using a strain where the competing lactate, acetate and ethanol forming pathways had been blocked. By introducing different alcohol dehydrogenases into this strain, either EcBDH from Enterobacter cloacae or SadB from Achromobacter xylosoxidans, it was possible to achieve high-yield production of m-BDO or R-BDO respectively. To achieve biosustainable production of these chemicals from dairy waste, we transformed the above strains with the lactose plasmid pLP712. This enabled efficient production of (3R)-acetoin, m-BDO and R-BDO from processed whey waste, with titers of 27, 51, and 32 g/L respectively. The corresponding yields obtained were 0.42, 0.47 and 0.40 g/g lactose, which is 82%, 89%, and 76% of maximum theoretical yield respectively. These results clearly demonstrate that L. lactis is an excellent choice as a cell factory for transforming lactose containing dairy waste into value added chemicals.
The modern global society faces great challenges in supply of energy, feed, food, and other products in a sustainable way. One way to mitigate the negative effects of providing these local eco-services is to convert biomass – instead of petroleum or natural gas – into a variety of food, feed, biomaterials, energy and fertilizer, maximizing the value of the biomass and minimizing the waste. This integrated approach corresponds to the biorefinery concept and is gaining attention in many parts of the world (Kam & Kam 2004). Energy, food and feed production is the driver for development in this area, but as biorefineries become more and more sophisticated with time, other products will be developed. Today, almost all organic chemicals - and also fertilizer - are produced from crude oil and petroleum and technologies with are driven by fossil energy, thus referred to as petro-chemicals and fossil fertilizer. It is generally anticipated that white biotechnology, the use of fermentation and enzymatic processes will play a key role for future cleaner production of bulk chemicals, energy carriers as well as fertilizer from biomass sources by saving resources and reduce negative environmental impacts from the chemical production. In order to replace fossil based energy carriers, chemicals and fertilizer, cost is the critical challenge for success. Thus, easily accessible and low costs biomass feedstock is a prerequisite for making bio-based production economically feasible. Industrial, agriculture and municipal biowastes have the potential to be that resource. However, it is of great importance to be aware of how to utilize the different sources of biowaste and for which purpose. In October 2012, a new EU project, funded under the FP7 programme was launched with partners from the EU, Africa and Malaysia. The objective of the proposed project is to show and demonstrate the technical roadmap - a strategy - for efficient technological utilization of selected significant biowaste in five African countries - Morocco, Egypt, Ghana, South Africa, and Kenya- derived from both the industrial and agricultural sector, thus, turning biowaste into a new resource for sustainable products. Our group is involved in developing strains and microbial fermentation processes for these bioconversions.
Complete Genome Sequence of Pediococcus pentosaceus Strain SL4

Pediococcus pentosaceus SL4 was isolated from a Korean fermented vegetable product, kimchi. We report here the whole-genome sequence (WGS) of P. pentosaceus SL4. The genome consists of a 1.79-Mb circular chromosome (G+C content of 37.3%) and seven distinct plasmids ranging in size from 4 kb to 50 kb.
Source: Internal funding (public)
Name of research programme: Stipendie fra udlandet
Project: PhD