
ERACoBioTech

CELL4CHEM

Expanding the substrate range for the production of medium-chain carboxylates by anaerobic fermentation with bacterial consortia

In the Cell4Chem project, tools and strategies will be developed to unlock the full potential of microbial communities facilitating bioconversion processes that result in the production of medium-chain carboxylates from lignocellulosic feedstock.

Medium-chain carboxylates (MCC) such as caproate and caprylate are speciality chemicals with broad application range, which can be produced by anaerobic fermentation of complex biomass with bacterial communities. Up to now, the utilisation of sustainable feedstocks is mostly limited to biomass with high ethanol or lactate content, as such electron donors are crucial for reaching efficient MCC production processes. The exploitation of more abundant lignocellulosic biomass has the potential of greatly expanding the application of this new anaerobic fermentation technology; however, it harbours two major bottlenecks, i.e. the poor hydrolysis of cellulose and low internal production of lactate. Cell4Chem tackles these issues on three engineering levels. On the first level, different bacterial strains will be genetically modified to create metabolic specialists for cellulose hydrolysis and lactate production. On the second level, these specialised bacterial strains will be combined in *de novo* constructed consortia with various wildtype microorganisms that can convert lactate to MCC. On the third engineering level, anaerobic bioreactors will be operated with microbiota, aiming at the development of process strategies for steering anaerobic fermentation towards MCC formation. The communities will be monitored over time using next-generation amplicon sequencing and meta-omics methods in order to follow community dynamics and process performance. Species-specific metabolic models will be constructed and combined to quantitative, mechanistic microbial community models, which both are parameterised towards the experimental data in order to elucidate determinants of observed dynamics, and to screen for optimal community compositions. The broad method spectrum of Cell4Chem will be applied in a concerted manner to ensure the optimal fit of microbial consortia in these engineered anaerobic fermentation ecosystems.
The metabolic potential of genetically modified bacteria will be assessed during cultivation on artificial substrates and real biomass, in constructed consortia together with wildtype strains and enrichment cultures including chain elongation bacteria. *Lactococcus lactis* will be genetically engineered to provide it with the capability to degrade and to utilize cellulose, in order to increase the production of lactate. Additionally, *Ruminiclostridium cellulolyticum* will be engineered to support the lactate production by *L. lactis*. We will prepare genetic elements (promotors, enzyme-encoding genes, secretion signals, surface anchors, immune tags etc.) in line with Synthetic Biology standards, which will greatly facilitate cloning and selection of optimal plasmid constructs, as well as exchange between laboratories. Exploiting experience from process engineering for steering anaerobic fermentation, the cultivation of selected consortia will be eventually scaled up and key process parameters for stable and efficient production will be exposed. Bioinformatic tools will be used to analyse meta-omics data revealing the structure and activity of microbial consortia. Consortia structure will be analysed by amplicon sequencing of the 16S rRNA genes. Genomes of consortia members will be reconstructed from metagenomes. Long- and short-read sequencing data will be mapped and re-assembled to obtain metagenome-assembled genomes (MAGs). Activity analysis of consortia will be based on metaproteomics. We will use a Systems Biology approach using state-of-the-art methods to model microbial consortia combining genome-scale metabolic modelling and meta-omics data integration. The metabolic phenotypes of the constructed consortia will be simulated. Integration of omics data will be used to elucidate metabolic cross-feeding interactions and to identify potential bottlenecks. Thus, the design of optimal microbial communities that reach highest product yields can be guided.
Lignocellulosic biomass, which is an abundant renewable resource, will be converted to valuable chemicals, specifically targeting caproate and caprylate. Currently, these compounds are recovered from palm and coconut oil, which has significantly detrimental environmental and socioeconomic impacts, in particular related to deforestation caused by palm cultivation. Hence, an unsustainable feedstock will be replaced by a widespread and renewable one issued from lignocellulosic material. Carboxylate production by anaerobic fermentation is indicated as the carboxylate platform, which can be part of the circular economy. The exploitation of lignocellulosic substrates for MCC production will greatly promote this technology and enhance its operative range.

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