BioProChemBB

Bio-based production of chemical building blocks – Corynebacterium glutamicum as a platform for new and efficient bioprocesses

Corynebacterium glutamicum is firmly established at industrial level as a producer of the bulk amino acids L-glutamate or L-lysine. This project aimed at developing C. glutamicum as a designer bug, serving as a robust platform organism for new and efficient bioprocesses, such as the production of the chemical building blocks succinate, fumarate, malate, asparate and itaconate. Further aims were to engineer producer strains to tolerate dicarboxylic acid stress and to show the feasibility of a bio-based production process with newly constructed producer strains of C. glutamicum, including scale-up and downstream processing. The figure gives an overview of the general project approach.

Based on previous knowledge of C. glutamicum and on a newly developed stoichiometric network model, several first generation producer strains were generated for anaerobic succinate and malate production, and for aerobic succinate, aspartate and itaconate production. These first generation producer strains were analyzed in shake flasks and in bioreactors for their growth and production characteristics. By 13C-labelling experiments and in vivo NMR techniques, the intracellular C-fluxes and metabolites were determined in a succinate producer. In parallel, the minimal inhibitory resistance levels for succinic, fumaric, malic, aspartic and itaconic acids were also determined. By evaluating the dicarboxylic acid stress stimulon of C. glutamicum on the basis of transcriptome and proteome analyses, several genes/proteins were found to be differentially expressed under dicarboxylic acid stress conditions.

These genes comprise a number of encoding proteins involved in protein and amino acid biosynthesis, as well as some encoding factors involved in oxidative stress and some encoding regulatory proteins. In fact, inactivation of two of the regulators led to a positive effect on the survival of C. glutamicum cells in the presence of dicarboxylic acids. Further candidate genes are still under investigation.

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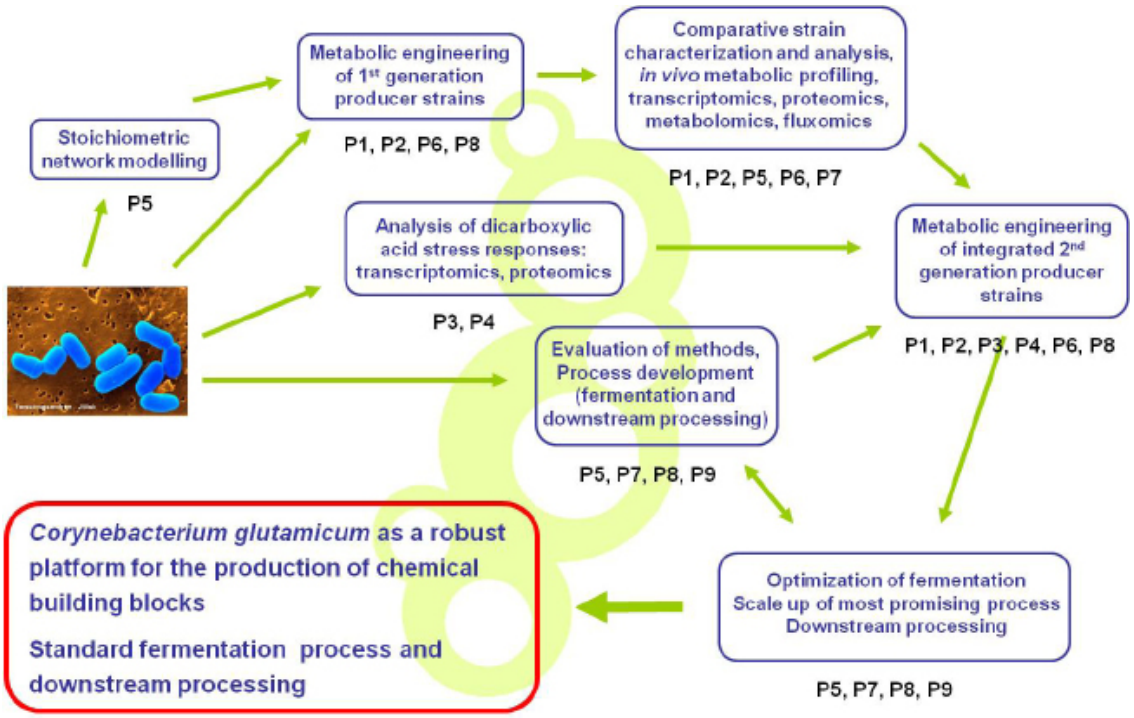
Based on the extensive analysis of the first generation producer strains, second generation producer strains of C. glutamicum were subsequently generated for succinate production under anaerobic conditions, and for pyruvate, succinate and itaconate production under aerobic conditions. These second generation producer strains showed improved substrate-specific yields, higher productivities and accumulated higher titers than the respective progenitors. In particular, the strains developed for succinate production from glucose and from glucose plus formate under anaerobic conditions, and those for succinate production from glycerol, as well as for itaconate production from glucose under aerobic conditions, are highly competitive when compared to industrially relevant microorganisms used for the production of the respective compounds.

Focusing on succinic acid production, a process has been developed, which is presently being adapted to the second generation producer strains. For recovery of the dicarboxylic acids in the culture broth, generic process concepts involving ion-exchange (ad)sorption and reactive extraction steps have been designed and experimentally applied for all organic acid products. The relevant characteristics were duly identified. Recovery processes have been implemented for low pH and for neutral pH conditions.

The results obtained will allow further metabolic engineering and the optimization of integrated second generation producer strains, as well as the implementation of robust fermentation processes with integrated downstream processing solutions.

General approach of the BioProChemBB project



P1, P2, … represent the partners involved in the respective elements of the work package. The products of interest, i.e., the chemical building blocks, are succinic, fumaric, malic, aspartic and itaconic acids. The final aim of the project is boxed in red.