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Analysis and Engineering of Metabolic Pathways in Lysine Producing *Corynebacterium glutamicum*

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Corynebacterium glutamicum is used for large scale production of amino acids, whereby glutamate and lysine with current volumes of 1.500.000 and 700.000 tons per year display the major industrial products. Due to high industrial relevance the optimization of producer strains is of central interest, whereby developments in genetic engineering and molecular biology nowadays allow defined genetic manipulation of *C. glutamicum*. Hereby a crucial prerequisite for successful rational strain engineering is the identification of promising targets, which relies on a fundamental understanding of the underlying metabolic network.

In this context we develop novel approaches that allow the quantitative analysis of in vivo carbon fluxes in metabolic networks, i. e. intracellular activities of enzymes and pathways. Quantification of in vivo carbon fluxes allows investigating the effects of genetic or environmental modifications and thus precisely provides a global perspective on the integrated genetic and metabolic regulation within the intact metabolic network. The developed approaches are based on a combination of ^{13}C tracer experiments, mass spectrometric labeling analysis, metabolite balancing and isotopomer modelling [1-4]. By recent developments in cultivation techniques and analytics miniaturized carbon flux analysis in 96 well microtiter plates could be achieved [5]. This is beneficial for the investigation of large numbers of mutants and for expensive tracer substrates.

In recent years we have applied metabolic pathway analysis to investigate lysine production by *C. glutamicum*. Comparative analysis of different mutants, displaying subsequent generations of a genealogy of classical strain improvement, clarifies the impact of strain optimization on intracellular fluxes [6]. The strong influence of environmental conditions on the metabolism becomes obvious from comparative flux studies with different carbon sources, where substrate-specific differences in production characteristics can be related to drastic flux differences in key pathways [7, 8]. The studies provide a detailed insight into the functioning and regulation of central pathways and shed light on the NADPH metabolism in C. *glutamicum*, which is of central importance for lysine production.

Of special importance for holistic understanding of the *C. glutamicum* metabolism is the combination of metabolic pathway analysis with other profiling tools. The auspicious perspectives of such in depth-profiling approaches are demonstrated by combined application of metabolic pathway analysis with transcription profiling and metabolome analysis to lysine-producing *C. glutamicum* [9].

The knowledge obtained from metabolic pathway analysis displays a useful basis for rational design and optimization of industrial production strains. This is exemplified by targeted improvement of lysine production by *C. glutamicum* via engineering of NADPH supplying pathways, which is currently under investigation.

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