Industrial Use of Corynebacterium

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Corynebacterium species, in particular *C. glutamicum* and mutants derived from the latter are used in industry for the production of a number of amino acids. Among the products L-glutamate with more than 1.800.000 t/a and L-lysine with 850.000 t/a are the most impressive products with respect to production volume, but other amino acids, like L-valine, L-isoleucine, L-proline, L-arginine, for instance, are also produced with *C. glutamicum*. Furthermore, organisms closely related to *C. glutamicum* are known and used, like *Corynebacterium ammoniagenes*, or those more recently isolated, like *Corynebacterium efficiens*, and *Corynebacterium glutamicum* R. This shows the vital industrial interest in this group of bacteria, apparently particularily suited for production purposes.

Besides the interest in "classical" amino acid production, *C. glutamicum* has attracted further interest. A first reason is that the high metabolic capacity of *C. glutamicum* has aroused interest to use this and to make other compounds like for instance bulk chemicals with engineered strains. Indeed, impressive success is obtained in this field, demonstrating that formation of ethanol or succcinic acid is possible with *C. glutamicum*. A second reason is the unique cell wall structure of C. glutamicum which represents the core structure of the cell wall in Corynebacterianeae to which also Mycobacterium tuberculosis belongs. The study of the cell wall in *C. glutamicum* is instrumental to understand this significantly more complex structure in *M. tuberculosis*. Indeed, studies on enzymes like glysosyltransferases and membrane-bound glysosyltransferases in *C. glutamicum* has helped to understand the function of the ortologuos enzymes in M. tuberculosis. Thus studies with *C. glutamicum* are of highest interest both from an applicative and a scientific view, and a wealth of approaches and methodologies is used to understand this fascinating organism.

In the talk I will illustrate that export is essential when considering production of metabolites like amino acids, but I will mainly focus on our recent development of a superior L-serine producer of *C. glutamicum*. Interestingly, such strain was notoriously difficult if not impossible to obtain by classical approaches. Using externally added L-serine, we found that L-serine is utilised by *C. glutamicum* up to rates of 20 nmol min^{-1} $mg(dry\ weight)^{-1}$ and tracing of 13-C-labelled L-serine identified that the L-serine carbon is present in a number of metabolites encluding pyruvate as well as glycine. We identified a serine dehydratase and deletion of its structural gene, *sdaA*, led to reduced L-serine degradation. To prevent the additional degradation to glycine by serinehydroxymethyl transferase activity and regulation of the *glyA* gene was studied. Unfortunately this gene is essential in *C. glutamicum*. We therefore also studied synthesis of its cofactor 5,6,7,8-tetrahydrofolate, THF,

and deleted the biosynthesis genes *pabABC* to make the strain THF dependend thereby reducing serinehydroxymethyl transferase activity. With a strain deleted of *pabABC* and *sdaA* together with overexpression of the L-serine biosynthesis pathway genes, encluding a *serA* gene where we deleted the regulatory domain of 3-phosphoglycerate dehydrogenase, more than 60 g l^{-1} L-serine were easily obtained under industrial relevant conditions. This demonstrates that the consequent application of metabolic engineering can profitably make use of the high intracellular metabolite availability of *C. glutamicum* for a number of purposes as required in white biotechnology.