ACCESS TO *N*-ALKYLATED AMINO ACIDS BY MICROBIAL FERMENTATION

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N-methylated amino acids are found in many pharmaceutically active compounds and have been shown to improve pharmacokinetic properties as constituents of peptide drugs since *N*-methylation of amino acids may result in conformational changes, improved proteolytic stability and higher lipophilicity of the peptide drug.[1] *N*-methylated amino acids are mainly produced chemically or by biocatalysis, however with low yields or high costs for co-factor regeneration.

First, we established a fermentative route for production of *N*-mehtyl-L-glutamate by *Pseudomonas putida* from glucose and glycerol. Interception of the C1 assimilation pathway of *Methylobacterium extorquence* yielded *N*-methyl-L-glutamate titers of 17.9 g L^{-1} in fed-batch cultivation.[2] Due to high substrate specificity of this C1 assimilation pathway genes, we continued with an independent pathway for extension of the product range. Therefore, we focus on pathway-design for *N*-methylated amino acids by the industrially relevant production host *Corynebacterium glutamicum*. Metabolic engineering of *C. glutamicum* led to an expanded product range of proteinogenic amino acids like L-valine[2] but also ω-amino acids like γ-aminobutyrate and diamines like putrescine[3]. The rare imine reductase DpkA from *P. putida* KT2440 catalyzes the reductive methylamination of pyruvate as side activity. Implementation of DpkA into the central carbon metabolism of the pyruvate overproducing *C. glutamicum* strain ELB-P[4] yielded *N*-methyl-L-alanine production. Optimization of carbon- and nitrogen ratios of the minimal medium allowed production of up to 10.5 g L^{-1} when cultivated in shake flasks. *N*-methyl-L-alanine titers of 31.7 g L^{-1} with a yield of 0.71 g per g glucose were achieved in fed-batch cultivation[5]. Due to the somewhat relaxed substrate scope of DpkA, the product portfolio of *N*-methylated amino acids produced by fermentation could be successfully extended. Changing the base strain to a glyoxylate producing *C. glutamicum* strain[6] achieved production of 2.6 g L^{-1} sarcosine, the *N*-methylated glycine derivative, from glucose. Sarcosine production based on the second generation feedstocks xylose and arabinose led to higher product titers than glucose-based production and optimization of substrate composition led to a titer of 8.7 g L^{-1} sarcosine. This is the first example in which a *C. glutamicum* process using lignocellulosic pentoses is superior to glucose-based production.

By mutation of the active site of DpkA, a mutant with higher specific activity towards glyoxylate (30.3 ± 2.7 U mg^{-1}; wild type enzyme 25.7 ± 1.8 U mg^{-1}) was identified. Therefore, the mutant DpkA^{F117L} was incorporated into the production strain and enabled faster sarcosine production. Additionally, this mutation led to an increased activity towards reductive ethylamination of glyoxylate (31.2 ± 1.1 U mg^{-1}; wild type enzyme 25.3 ± 3.2 U mg^{-1}). As a result, the fermentative production of *N*-ethylglycine showed enhanced volumetric productivity compared to the strain harboring the wild type enzyme.

Fermentative access to *N*-methylated amino acids was achieved by two independent pathway designs. First, we enabled *N*-methyl-L-glutamate production by pathway interception in *P. putida.* Additionally, introduction of the imine reductase gene *dpkA* from *P. putida* into various 2-oxoacid producing *C. glutamicum* strains extended the product range. Optimization of medium composition, preferred substrate specificity of the strain or the enzyme itself resulted in excellent production yields.

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