Metabolic pathway engineering for production of 1,2-propanediol and 1-propanol by Corynebacterium glutamicum

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Abstract

Background: Production of the versatile bulk chemical 1,2-propanediol and the potential biofuel 1-propanol is still dependent on petroleum, but some approaches to establish bio-based production from renewable feed stocks and to avoid toxic intermediates have been described. The biotechnological workhorse Corynebacterium glutamicum has also been shown to be able to overproduce 1,2-propanediol by metabolic engineering. Additionally, C. glutamicum has previously been engineered for production of the biofuels ethanol and isobutanol but not for 1-propanol.

Results: In this study, the improved production of 1,2-propanediol by C. glutamicum is presented. The product yield of a C. glutamicum strain expressing the heterologous genes gldA and mgsA from Escherichia coli that encode methylglyoxal synthase gene and glycerol dehydrogenase, respectively, was improved by additional expression of alcohol dehydrogenase gene yqhD from E. coli leading to a yield of 0.131 mol/mol glucose. Deletion of the endogenous genes hdpA and ldh encoding dihydroxyacetone phosphate phosphatase and lactate dehydrogenase, respectively, prevented formation of glycerol and lactate as by-products and improved the yield to 0.343 mol/mol glucose. To construct a 1-propanol producer, the operon ppdABC from Klebsiella oxytoca encoding diol dehydratase was expressed in the improved 1,2-propanediol producing strain ending up with 12 mM 1-propanol and up to 60 mM unconverted 1,2-propanediol. Thus, B12-dependent diol dehydratase activity may be limiting 1-propanol production.

Conclusions: Production of 1,2-propanediol by C. glutamicum was improved by metabolic engineering targeting endogenous enzymes. Furthermore, to the best of our knowledge, production of 1-propanol by recombinant C. glutamicum was demonstrated for the first time.

Keywords: Corynebacterium glutamicum, Metabolic engineering, 1-propanol, 1,2-propanediol
its genome [19] and creating a genome-streamlined chassis organism [20]. Metabolic engineering aimed at the production of not only many other amino acids [14, 21] but also for example at monomers of bioplastics (e.g., cadaverine [22, 23] and putrescine [23]), organic acids [24], carotenoids [25], and biofuels. C. glutamicum was engineered for isobutanol production and shown to exhibit less toxicity to isobutanol than E. coli [26, 27]. The isobutanol yield by recombinant C. glutamicum was competitive with E. coli [28]. In particular, overproduction of the biofuel ethanol under oxygen deprivation conditions is well-described for C. glutamicum and shown to be efficient [29–31]. Importantly, under these conditions C. glutamicum showed high tolerance to organic acid, furan, and phenolic inhibitors present in lignocellulose hydrolysates [30]. Thus, C. glutamicum is a promising alternative biofuel production host. To enable sustainable production from several alternative carbon sources, the substrate spectrum of C. glutamicum was widened by metabolic engineering [32]. Since 1,2-propanediol production by C. glutamicum has been shown [12] in principle, this study aimed at improving 1,2-propanediol production and at producing 1-propanol as derived compound. This primary alcohol, also named n-propanol, finds application in the solvent, cosmetic, and pharmaceutical industries, in antiseptic solutions, as precursor for diesel fuels and in the plastics industry and finally as biofuel [33–35]. C. glutamicum has previously been engineered for production of the biofuels ethanol [31] and isobutanol [26–28] but not for 1-propanol. Natural microorganisms are not known to secrete significant amounts of 1-propanol. However, Propionibacterium freudenreichii has been engineered for the direct conversion of propionyl-CoA to 1-propanol [34]. Engineered E. coli strains either convert 2-ketobutyrate to 1-propanol by variants of the threonine and citramate pathways [36, 37] or by extending succinate dissimilation [35]. Finally, 1,2-propanediol can be converted in a two-step conversion to 1-propanol by diol dehydratase from Klebsiella oxytoca [33]. The latter pathway was chosen in this study for construction of a C. glutamicum 1-propanol-producing strain.

Results

Co-overexpression of yqhD from E. coli increased 1,2-propanediol production

C. glutamicum has previously been engineered for 1,2-propanediol production by expressing the heterologous genes mgsA and gldA encoding methylglyoxal synthase gene and glycerol dehydrogenase from E. coli [12]. Expression of these genes as artificial operon from the plasmid pEKE3-mgsA-gldA in C. glutamicum WT yielded 19 ± 1 mM 1,2-propanediol within 51 h (Fig. 2) when using modified CGXII minimal medium with a decreased nitrogen content (5 g/L ammonium sulfate) and 184 ± 1 mM glucose as sole carbon source. Thus, the base strain produced 1,2-propanediol with a yield of 0.103 mol/mol glucose.

Methylglyoxal is a toxic intermediate of the conversion of dihydroxyacetone phosphate (DHAP) to 1,2-propanediol (Fig. 1), and in E. coli, additional overexpression of the alcohol dehydrogenase genes yqhD or fucO was shown to increase the yield of 1,2-propanediol from glycerol [10]. Heterologous expression of yqhD with mgsA and gldA from plasmid pEKE3-mgsA-yqhD-gldA in C. glutamicum WT improved 1,2-propanediol production by about 27 % as 24 ± 1 mM 1,2-propanediol accumulated after 51 h (Fig. 2b), which correlated to a product yield of 0.131 mol/mol. Both C. glutamicum WT(pEKE3-mgsA-gldA) and WT(pEKE3-mgsA-yqhD-gldA) grew and utilized glucose as growth substrate slightly slower than the empty vector carrying control strain C. glutamicum WT(pEKE3) (Fig. 2a). The addition of alcohol dehydrogenase gene fucO as fourth gene of the heterologously expressed operon on plasmid pEKE3-mgsA-yqhD-fucO-gldA did not further improve 1,2-propanediol production as compared to WT(pEKE3-mgsA-yqhD-gldA) (data not shown).

A comparison between strains WT(pEKE3-mgsA-gldA) and WT(pEKE3-mgsA-yqhD-gldA) with respect to byproduct formation revealed that acetol, the direct precursor of 1,2-propanediol (Fig. 1), accumulated to higher concentrations in supernatants of WT(pEKE3-mgsA-gldA) than of WT(pEKE3-mgsA-yqhD-gldA), i.e., 14 mM as compared 5 mM, after glucose was depleted (Fig. 2b). On the other hand, WT(pEKE3-mgsA-gldA) only produced 8 ± 1 mM glycerol as a by-product, whereas the additional expression of yqhD resulted in accumulation of 42 ± 1 mM (Fig. 2c). Interestingly, the empty vector control produced 32 ± 3 mM dihydroxyacetone (DHA), while C. glutamicum strains WT(pEKE3-mgsA-gldA) and WT(pEKE3-mgsA-yqhD-gldA) accumulated less than 5 mM DHA (Fig. 2c). Thus, preventing glycerol formation by the so far best producing strain WT(pEKE3-mgsA-yqhD-gldA) offers the potential to improve 1,2-propanediol production.

Stopping glycerol formation by deleting the gene hdpA resulted in higher yields of 1,2-propanediol

Typically, glycerol is hardly secreted by C. glutamicum WT, although two enzymes involved in glycerol formation have been found, namely gpp-encoded glycerol-3-phosphatase [38] and butA-encoded (S,S)-butanediol dehydrogenase [39]. In the experiments described above, glycerol was produced by the recombinant strains WT(pEKE3-mgsA-gldA) and WT(pEKE3-mgsA-yqhD-gldA) but nearly not by the parent strain WT(pEKE3). This indicated that the heterologous enzymes present in these recombinants may be involved in glycerol formation. Since it is known that the gldA-encoded glycerol
dehydratase from *E. coli* accepts also dihydroxyacetone, acetol, and methylglyoxal as substrates [40] (Fig. 1), it was tested if dihydroxyacetone formation can be prevented. Secretion of dihydroxyacetone by *C. glutamicum* WT occurs under certain conditions, e.g., acidic conditions [41], and was observed for WT(pEKEx3) under the conditions of 1,2-propanediol production described above. Two enzymes may be involved in DHA production, namely DHAP phosphatase encoded by *hdpA* [42] and a predicted kinase related to dihydroxyacetone kinase encoded by cg1497 [43]. To test if these enzymes are relevant for glycerol formation from DHA by the 1,2-propanediol-producing strain WT(pEKEx3-*mgsA*-yqhD-*gldA*), both genes were deleted by homologous recombination individually and in combination. The resulting strains *C. glutamicum Δcg1497(pEKEx3-*mgsA*-yqhD-*gldA*)*, Δ*hdpA*(pEKEx3-*mgsA*-yqhD-*gldA*) and Δcg1497Δ*hdpA*(pEKEx3-*mgsA*-yqhD-*gldA*) were grown as described above for WT(pEKEx3-*mgsA*-yqhD-*gldA*). The deletion of the gene cg1497 had no impact on the 1,2-propanediol formation (data not shown). Upon deletion of *hdpA*, 1,2-propanediol production increased by about 90 % (Fig. 3b), while the double deletion mutant showed no further increase (data not shown). After 51 h of cultivation, *C. glutamicum Δcg1497Δ*hdpA*(pEKEx3-*mgsA*-yqhD-*gldA*) accumulated 46 ± 4 mM 1,2-propanediol, which corresponds to a product yield of 0.249 mol/mol. *C. glutamicum* WT (pEKEx3-*mgsA*-yqhD-*gldA*) and Δ*hdpA*(pEKEx3-*mgsA*-yqhD-*gldA*) grew with comparable growth rates, utilized glucose comparably fast (Fig. 3a), and accumulated comparable concentrations (5 and 7 mM, respectively). However, glycerol was not a significant by-product (<5 mM) of

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**Fig. 1** Scheme of the engineered metabolic pathway for the production of 1,2-propanediol and 1-propanol in *C. glutamicum*. Reactions are represented by arrows (preferred direction and cofactors), while dashed lines indicate multiple reaction steps. Genes coding for relevant enzymes are depicted next to the arrows: cg1497, predicted kinase related to dihydroxyacetone kinase; *hdpA*, dihydroxyacetone phosphate phosphatase (*HdpA*); *fucO*, propanediol oxidoreductase/lactaldehyde reductase (*FucO*); *gldA*, glycerol dehydrogenase (*GldA*); *ldhA*, L-lactate dehydrogenase (*LdhA*); *mgsA*, methylglyoxal synthase (*MgsA*); *ppdABC*, diol dehydratase (*PpdABC*); *yqhD*, aldehyde reductase (*YqhD*). Abbreviations: ADP adenose diphosphate, ATP adenose triphosphate, DHAP dihydroxyacetone phosphate, GAP glyceraldehyde 3-phosphate, PPP pentose phosphate pathway, TCA citric acid cycle, Vit. B12 vitamin B12.
the \textit{hdpA} deletion strain, while the parental strain accumulated more than 40 mM glycerol (Fig. 3c). Thus, preventing DHA formation from DHAP by deletion of \textit{hdpA} prevented subsequent formation of glycerol from DHA and improved 1,2-propanediol production.

Deleting \textit{ldh} prevented transient L-lactate accumulation and led to faster and higher 1,2-propanediol production

The deletion of \textit{hdpA} prevented formation of about 40 mM glycerol but increased 1,2-propanediol accumulation by about 22 mM only (Fig. 3). Since 1,2-propanediol
is more reduced than glycerol and since it is known that C. glutamicum utilizes excess NADH to reduce pyruvate to L-lactate, lactate formation may compete with 1,2-propanediol formation for NADH. In C. glutamicum, L-lactate is formed by fermentative, NADH-dependent lactate dehydrogenase LdhA under oxygen deprivation conditions [44] but transiently also during aerobic cultivation [45]. Re-uptake and re-utilization of lactate does not generate NADH but menaquinol, because both L- and D-lactate dehydrogenases LldD and Dld oxidize lactate to pyruvate in menaquinone-dependent reactions [45, 46]. Thus, ldh was deleted and the resulting strain C. glutamicum ΔhdpAΔldh(pEKEx3-mpgA-yqhD-gldA) was compared to strain ΔhdpA(pEKEx3-mpgA-yqhD-gldA) in batch cultivations. As consequence of introducing the ldh deletion, 1,2-propanediol production increased by about 38 %. C. glutamicum strain ΔhdpAΔldh(pEKEx3-mpgA-yqhD-gldA) accumulated 63 ± 4 mM 1,2-propanediol (Fig. 4b), which corresponds to a product yield of 0.343 mol/mol. Moreover, the ldh deletion strain utilized glucose faster and accumulated 1,2-propanediol faster than the parental strain, while the growth rates of both strains were comparable (Fig. 4a). Neither DHA nor glycerol accumulated to significant concentrations (<5 mM), but more acetol (15 mM as compared to 7 mM) was produced by the ldh deletion strain (Fig. 4b). Lactate formation by the ldh deletion strain was not detectable (<1 mM), while the parental strains and all other strains mentioned in Figs. 2, 3, and 4 accumulated lactate to low concentrations (between 1 and 4 mM) over the whole fermentation process. Taken together, ldh deletion improved 1,2-propanediol production considerably.

**Production of 1-propanol by recombinant C. glutamicum**

A 1,2-propanediol-producing E. coli strain produced 1-propanol when the ppdABC operon from K. oxytoca, which encodes a vitamin B$_{12}$-dependent 1,2-propanediol dehydratase, was expressed [33, 47]. After vitamin B$_{12}$-dependent 1,2-propanediol dehydratase has converted 1,2-propanediol to 1-propanal, the latter is reduced to 1-propanol by alcohol dehydrogenases such as YqhD [48]. Thus, the operon ppdABC of K. oxytoca was cloned into the expression vector pVWEx1, which is compatible with expression vector pEKEx3, and used to transform 1,2-propanediol-producing strains. Cultivated in minimal medium with 217 ± 1 mM glucose and 10 μM vitamin B$_{12}$, C. glutamicum strain ΔhdpAΔldh(pEKEx3-mpgA-yqhD-gldA)(pVWEx1-ppdABC) accumulated 1-propanol to the highest concentration (12 ± 1 mM) after 70 h (Fig. 5a). This strain did not accumulate significant concentrations of glycerol, DHA, and acetol (data not shown). However, 1,2-propanediol was still the main product (62 ± 2 mM).

As expected from the 1,2-propanediol production experiments, deletions of genes hdpA and ldh were beneficial for 1-propanol production since strain WT(pEKEx3-mpgA-yqhD-gldA)(pVWEx1-ppdABC) accumulated almost two-fold less 1-propanol (7 ± 1 mM) and 1,2-propanediol (30 ± 1 mM; Fig. 5b).

Strain WT(pEKEx3-mpgA-yqhD-gldA)(pVWEx1-ppdABC) that did not overexpress yqhD from E. coli, which presumably is involved in reduction of 1-propanol to 1-propanol, only accumulated 2 ± 1 mM 1-propanol and utilized glucose incompletely (Fig. 5a). Accordingly, this strain only produced 9 ± 2 mM 1,2-propanediol and 43 ± 4 mM glycerol (Fig. 5c).

Taken together, 1-propanol was produced for the first time by recombinant C. glutamicum and strain ΔhdpAΔldh (pEKEx3-mpgA-yqhD-gldA)(pVWEx1-ppdABC) accumulated 1-propanol up to a concentration of 12 mM. Besides vitamin B$_{12}$-dependent 1,2-propanediol dehydratase, also
Alcohol dehydrogenase YqhD appeared to be involved in converting 1,2-propanediol to 1-propanol.

**Discussion**

In this study, production of 1,2-propanediol by *C. glutamicum* was improved and production of the biofuel molecule 1-propanol by *C. glutamicum* was shown for the first time. It has been shown previously that expression of the heterologous methylglyoxal synthase gene *mgsA* from *E. coli* was required for 1,2-propanediol and had to be coupled with glycerol dehydrogenase either encoded by heterologous gene *gldA* from *E. coli* or endogenous cgR_2242 [12]. Within 96 h, up to 25 mM 1,2-propanediol and 44 mM acetol were produced from 333 mM glucose as a sole carbon source [12]. Using a comparable strain but the cultivation setup employed in this study, it was possible to produce 19 mM 1,2-propanediol in 51 h from 184 mM glucose by overexpression of *mgsA* and *gldA* from *E. coli* in *C. glutamicum* WT (Fig. 2). Notably, accumulation of 1,2-propanediol and side products started after the cells entered the stationary phase, thus, production was not coupled to growth (Fig. 2).

Alcohol dehydrogenase YqhD proved beneficial for 1,2-propanediol production (increased by 27 % to a yield of 0.131 mol/mol glucose, Fig. 2), presumably because conversion of methylglyoxal to acetol and 1,2-propanediol was improved by YqhD. This enzyme has the following characteristics: a reductase activity for at least 12 aldehydes and thus increasing tolerance to aldehydes as aldehyde scavenger; preferring aldehydes over alcohols as substrates; a better conversion of alcohols longer than three carbon atoms; dependence of NADPH/NADP and divalent cations (e.g., zinc) as cofactors [48]. Notably, YqhD is NADPH-dependent [48] as compared to the NADH-dependent GldA, thus, YqhD is coupled to anabolic metabolism, which is driven by NADPH. Overexpression of *yqhD* proved beneficial for production of, e.g., 3-hydroxypropionic acid by *E. coli* [49], poly(3-hydroxypropiolate) from glycerol using engineered *Klebsiella pneumoniae* [50], short-chain alcohols by *E. coli* [51], or acetol by *E. coli* [52].

Heterologous expression of *gldA* and *yqhD* from *E. coli* resulted in production of the side-product glycerol since these aldehyde reductases reduced DHA to glycerol [40]. Two possible enzymes were considered to be involved in the reduction of DHA metabolism, namely cg1497 and *hdpA* [42, 43]. Only the deletion of *hdpA* prevented glycerol formation and improved 1,2-propanediol production increasing the yield by about 90 % up to 0.249 mol/mol glucose (Fig. 3). The strain lacking endogenous *hdpA* showed improved 1,2-propanediol production due to two possible advantages. First of all, DHAP is not converted to DHA and, thus, supply of DHAP for the MgsA reaction to methylglyoxal was improved. Secondly, preventing reduction of DHA to glycerol increased provision of the redox cofactor NADH for the reactions converting methylglyoxal to 1,2-propanediol. Formation of glycerol as side-product of *C. glutamicum* strains expressing heterologous *gldA* and/or *yqhD* is distinct from glycerol production of *C. glutamicum* WT. In *C. glutamicum* WT, glycerol is formed from glycerol 3-phosphate by glycerol 3-phosphate...
phosphatase Gpp [38]. Since C. glutamicum WT secretes DHA under certain condition [41, 42], it is devoid of an enzyme catalyzing reduction of DHA to glycerol as efficient as observed in recombinants expressing heterologous gldA and/or yqhD from E. coli.

With the additional deletion of the gene ldh, it was possible to further increase the 1,2-propanediol production by about 38% resulting in a yield of 0.343 mol/mol (Fig. 4). Deletion of ldh is a common strategy to improve production of organic acids under oxygen deprivation conditions [53, 54] since L-lactate is secreted by C. glutamicum under conditions of excess NADH. Two factors may have led to improved 1,2-propanediol production as result of ldh deletion. Firstly, provision of NADH for reduction of methylglyoxal to acetol and 1,2-propanediol is increased since pyruvate is not reduced to L-lactate. Secondly, pyruvate and possibly also other intermediates of glycolysis may accumulate as consequence of ldh deletion. This accumulation is plausible since deletion of pyruvate kinase Pyk led to accumulation of pyruvate and other glycolytic intermediates [55, 56]. In E. coli, methylglyoxal reacts spontaneously with glutathione to form a hemithioacetal, followed by detoxification by the glyoxalase system leading to the production of D-lactate [57]. C. glutamicum lacks glutathione but possesses mycothiol as its primary low molecular weight thiol [58]. A number of mycothiol-dependent reactions have been described for C. glutamicum including formaldehyde oxidation to formate [59, 60]. Although the reaction between mycothiol and methylglyoxal is currently not known in C. glutamicum, the overexpression of msbA-encoding mycothiol glycosyltransferase led to an increased robustness towards methylglyoxal [61].

 Provision of NAD(P)H for reduction of acetol to 1,2-propanediol may still be limiting since even strain C. glutamicum ΔhdpAxldh produced up to 15 mM acetol (Fig. 4). Notably, the accumulation of acetol increased after glucose was depleted while the 1,2-propanediol concentration decreased. Thus, 1,2-propanediol may be taken up again and oxidized to acetol to generate NADH, which may provide the cells with ATP in oxidative phosphorylation. Currently, it is not known whether oxidation of 1,2-propanediol occurs via the heterologous GldA from E. coli or by an endogenous enzyme. Interestingly, in a recombinant cyanobacterium producing 1,2-propanediol, alternative NADPH-alcohol dehydrogenases led to higher 1,2-propanediol titers, while acetol was not produced as side-product [11].

Additionally, the production of 1-propanol by C. glutamicum is reported for the first time in this study. Heterologous expression of the operon ppdABC from K. oxytoca encoding diol dehydratase in a 1,2-propanediol producing C. glutamicum strain was required for 1-propanol production of up to 12 mM (Fig. 5). Diol dehydratase PpdABC has the following characteristics: consisting of three subunits (α, β, and γ) with two units of a heterotrimer building the quaternary structure; indicated that the α- and γ-subunit promote the correct folding of each subunit; substrates are 1,2-propanediol, glycerol and 1,2-ethanediol with Km values of 0.08 μM, 0.73 mM, and 0.56 mM, respectively; lack of stereospecificity accepting (R)- and (S)-1,2-propanediol; dependent of adenosylcobalamin and divalent cations (e.g., potassium) as cofactors [62–64]. The observation that 1,2-propanediol was still the major product (up to 62 mM; Fig. 5) indicated that 1,2-propanediol to is not converted efficiently to 1-propanol by B12-dependent diol dehydratase PpdABC and YqhD. However, vitamin B12 may be limiting since it is not known if C. glutamicum can synthesize vitamin B12. In addition, provision of the cofactor NADPH may be a bottleneck.

There is potential for improving 1-propanol production with C. glutamicum as exemplified for E. coli [33, 47]. Overexpression of ppdABC in E. coli BW25113 for conversion of DHAP to 1,2-propanediol yielded 0.036 mol/mol 1-propanol from glucose [33], which is comparable to the yield of 0.032 mol/mol reported here (Fig. 5). The yield with C. glutamicum doubled as consequence of deleting ldh and hdpA (Fig. 5). Jain et al. (2014) optimized 1-propanol production by E. coli further [47]. The improvements included co-cultivation of one strain converting glucose to 1,2-propanediol and a second strain converting 1,2-propanediol to 1-propanol [47]. The first strain was improved by overexpressing an optimized gene set for conversion of DHAP to 1,2-propanediol and by deleting four genes to improve NADH provision [47]. Furthermore, heterologous expression of a gene coding for formate dehydrogenase and feeding the additional carbon source sodium formate and yeast extract improved the redox balance [47]. The second strain harbored a synthetic diol dehydratase gene cluster with optimized gene order (ppdA-C-B) and separation by linker sequences [47]. These metabolic engineering and medium optimization approaches may be helpful for improving 1-propanol production by the C. glutamicum strains described in this study. A number of engineering strategies to improve NADPH provision in C. glutamicum have been developed and include, e.g., transmembrane transhydrogenase PntAB [65], phosphoglucone isomerase mutants [66], NADPH-dependent glyceraldehyde-3-phosphate dehydrogenase [67], or NAD kinase [68]. Thus, production of 1-propanol may be increased further over the proof-of-concept established in this study.

**Conclusions**

Metabolic engineering improved 1,2-propanediol production by C. glutamicum. Deletion of the endogenous genes hdpA and ldh combined with overexpression of the E. coli genes mgsA, gldA, and yqhD resulted in strain producing
1,2-propanediol from glucose in mineral salt medium with a product yield of 0.343 mol/mol. Further strain engineering led to strain capable of producing 1-propanol. This is the first report of 1-propanol production by recombinant C. glutamicum.

Materials and methods

Microorganisms, media, and cultivation conditions

In Table 1, all C. glutamicum strains and plasmids which were used for this study are presented. The E. coli strain DH5α [69] was used for the plasmid construction and was cultured in lysogeny broth complex medium (LB) [70]. Precultivation of C. glutamicum was performed in LB with 2 % glucose by inoculation from LB plates. For the main cultures of C. glutamicum, the cells of an overnight preculture were harvested by centrifugation (10 min; 3220 × g) and transferring the appropriate volume for an optical density (λ = 600 nm) (OD<sub>600</sub>) of 1 in 50-mL cultures. These cells were washed with CGXII minimal medium [71] without carbon source and without urea and ammonium sulfate. The cells were again centrifuged and resuspended with the same CGXII. As sole nitrogen source 5 g/L ammonium sulfate were added and as sole carbon source, glucose was used in the measured concentration given in the results. All cultivations of C. glutamicum were carried out in a volume of 50 mL in 500-mL baffled flasks at 30 °C and 120 rpm. The gene expression was induced by adding 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at inoculation of the main culture. When appropriate, the medium was supplemented with 25 μg/mL kanamycin and 100 μg/mL spectinomycin. For 1-propanol production, it was necessary to add 10 μM of vitamin B<sub>12</sub> to the medium. Growth was observed by measuring the OD<sub>600</sub> using the V-1200 spectrophotometer (VWR International, Darmstadt, Germany) by diluting the samples into an OD<sub>600</sub> range of 0.05–0.25. Additionally, 1-mL samples were taken at the time points given in the results and centrifuged (10 min; 16,000 × g), and the resulting supernatants were stored at −20 °C until further analysis.

Recombinant DNA work

All oligonucleotides used in this study were obtained from Eurofins MWG Operon (Ebersberg, Germany) or metabion international AG (Planegg, Germany) (Table 2). The plasmid construction was carried out with PCR fragments (KOD, Novagen, Darmstadt, Germany) generated with genomic DNA of C. glutamicum WT, E. coli DH5α (DNA preparation described by [72]), or K. oxytoca DSM4798 (DSMZ, Braunschweig, Germany) as template DNA. These...
### Table 2 Oligonucleotides used in this study

| Oligonucleotide name | Sequence (5′ → 3′) | Purpose |
|----------------------|---------------------|---------|
| cg1497_upstrm_fw_pK19 | GACTCTAGAGGATCCCTTAACGGCCGGGCTC | pK19mobsa8-Δcg1497 |
| cg1497_upstrm_rv     | GGGTAGCTTGGATTTGCTTATTTGCAAAGGGAGCGAATCTCAGACATAC | pK19mobsa8-Δcg1497 |
| cg1497_downstrm_fw   | ACAAAATCTACCACCCCGGATGACCTTCACTCTACGATGACATGGGGA | pK19mobsa8-Δcg1497 |
| cg1497_downstrm_rv_pK19 | CGAGCTCGTACCCCCGAGCCGTTGATTTGAGGCTGATGATGCTGTT | pK19mobsa8-Δcg1497 |
| Dcg1497_upstrm_v3    | CCAGCTCCAGACGAGCC | Verification of cg1497 deletion by PCR |
| Dcg1497_rv_v3        | AAGGAAGTGGCGATCCCGTGATTC | Verification of cg1497 deletion by PCR |
| nagD_upstrm_fw_pK19  | GACTCTAGAGGATCCCTTAACGGCCGGGCTC | pK19mobsa8-ΔhdpaA |
| nagD_upstrm_rv       | GGGTAGCTTGGATTTGCTTATTTGCAAAGGGAGCGAATCTCAGACATAC | pK19mobsa8-ΔhdpaA |
| nagD_downstrm_fw     | ACAAAATCTACCACCCCGGATGACCTTCACTCTACGATGACATGGGGA | pK19mobsa8-ΔhdpaA |
| nagD_downstrm_rv_pK19 | CGAGCTCGTACCCCCGAGCCGTTGATTTGAGGCTGATGATGCTGTT | pK19mobsa8-ΔhdpaA |
| DnagD_fw             | GATGAAACAGCAGCTGTCCG | Verification of hdpa deletion by PCR |
| DnagD_rv             | GGGTAGCTTGGATTTGCTTATTTGCAAAGGGAGCGAATCTCAGACATAC | Verification of hdpa deletion by PCR |
| ldhfw                | TGATGGCCACAGGATGCGAT | Verification of ldh deletion by PCR |
| ldhrev               | CCAATGATCGAGGATGATGA | Verification of ldh deletion by PCR |
| mgsA_fw_x3           | GACTCTAGAGGATCCCTTAACGGCCGGGCTCAGATGGAAAGCAGC | pEEx3-mgsA-ΔgldA, pEEx3-mgsA-yqhD-ΔgldA, pEEx3-mgsA-ΔgldA, pEEx3-mgsA-ΔfucO-ΔgldA |
| mgsA_rv_gld_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-ΔgldA |
| gldA_fw_mgs_DS       | GAGAGCTCGTACCCCCGAGCCGTTGATTTGAGGCTGATGATGCTGTT | pEEx3-mgsA-ΔgldA, pEEx3-mgsA-yqhD-ΔgldA, pEEx3-mgsA-ΔfucO-ΔgldA |
| mgsA_rv              | TTACTTCAGACCGGATCGGCA | pEEx3-mgsA-ΔgldA |
| yqhD_fw_mgs          | CGCCCGGATGCTGCAGAGAAGAGGAGGCGCTCCAGATGAAGAACATTTATTCCGCA | pEEx3-mgsA-yqhD-ΔgldA |
| yqhD_rv              | TTACTTCAGACCGGATCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| gldA_rv_yqh_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| gldA_rv_gld_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| yqhD-fw_mgs_DS       | GAGAGCTCGTACGCCGCAATCTTCAGACGGAAGAGGAGGCGCTCCAGATGAAGAACATTTATTCCGCA | pEEx3-mgsA-yqhD-ΔgldA |
| yqhD_rv_gld_DS       | GAGAGCTCGTACGCCGCAATCTTCAGACGGAAGAGGAGGCGCTCCAGATGAAGAACATTTATTCCGCA | pEEx3-mgsA-yqhD-ΔgldA |
| fucO-fw_yqh_DS       | GAGAGCTCGTACGCCGCAATCTTCAGACGGAAGAGGAGGCGCTCCAGATGAAGAACATTTATTCCGCA | pEEx3-mgsA-yqhD-ΔgldA |
| fucO_rv_gld_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| fucO_rv_gld_DS       | GAGAGCTCGTACGCCGCAATCTTCAGACGGAAGAGGAGGCGCTCCAGATGAAGAACATTTATTCCGCA | pEEx3-mgsA-yqhD-ΔgldA |
| gldA_fw_fuc_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| gldA_fw_gld_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| gldA_rv               | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| gldA_rv_gld_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| gldA_rv_fuc_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| gldA_rv_gld_DS       | TATCTCAATAAGTACCTACGACGGCTCCGGCA | pEEx3-mgsA-yqhD-ΔgldA |
| ppdABC_ko_fw_x1      | CTGCAAGCTCGTACGCCGCAATCTTCAGACGGAAGAGGAGGCGCTCCAGATGAAGAACATTTATTCCGCA | pWEx1-ppdABC |
| ppdABC_ko_rv_x1      | CGGAGCCGCGCTTGACATCTTCAGACGGAAGAGGAGGCGCTCCAGATGAAGAACATTTATTCCGCA | pWEx1-ppdABC |
| gldA_seq             | GAACTTGGTGCTAACAACCCCTG | Sequencing primer for gldA |
| yqhD_seq             | GTATTTGCCCGTCGTCGATC | Sequencing primer for yqhD |
| fucO_seq             | GACCAATAAACCCCGTGATAC | Sequencing primer for fucO |
| ppdABC_seq1          | CGAACGAGCAGCCGCGGTGTT | Sequencing primer for ppdABC |
| ppdABC_seq2          | ACAGACAGACCGCCATCCAC | Sequencing primer for ppdABC |
| ppdABC_seq3          | TACCTCCAGCAGCTGCGGCTT | Sequencing primer for ppdABC |
| ppdABC_seq4          | AATCCCTCCGACGCTGCGGCTT | Sequencing primer for ppdABC |
| ppdABC_seq5          | CGAACGAGCAGCCGCGGTGTT | Sequencing primer for ppdABC |

Purpose: Verification of deletion by PCR

Δ: Deletion
fragments were cloned via Gibson Assembly [73] (enzymes provided by NEB, Frankfurt am Main, Germany) into the linearized vectors, and the resulting reaction was used for the transformation of \emph{E. coli} DH5α cells using the calcium chloride method [70]. Therefore, pEKEx3 and pK19mobsacB were digested with the restriction enzyme \emph{SmaI} and pVWEx1 with \emph{BamHI} (Fermentas/Thermo Scientific, St. Leon-Rot, Germany). For the purification of the PCR fragments and the digested plasmids, the PCR purification kit or MinElute PCR purification kit (QIAGEN, Hilden, Germany) were applied. The plasmids were isolated from \emph{E. coli} by using the QIAprep spin miniprep kit (QIAGEN, Hilden, Germany). All resulting vectors were sequenced to confirm the correctness of the cloned DNA fragments (SCF, CeBiTec, Bielefeld, Germany). The transformation of \emph{C. glutamicum} was performed with electrocompetent cells [74] by electroporation [71] in a GenePulser Xcell™ plus PC Module (BioRad, München, Germany) but using LB with 2 % glucose in all stages of cultivation. All enzymes and kit systems were used like recommended in the manufacturer’s manuals.

Construction of \emph{C. glutamicum} deletion strains
To delete the genes cg1497 and \emph{hdpA} new plasmids were constructed by using the suicide vector pK19mobsacB [75]. For the deletion of cg1497, genomic regions flanking this gene were amplified via PCR from genomic DNA of \emph{C. glutamicum} using the primer pairs cg1497\_upstrm\_fw\_pK19/cg1497\_upstrm\_rv and cg1497\_dwnstrm\_fw/cg1497\_dwnstrm\_rv\_pK19 (Table 2). The resulting PCR fragments were purified and cloned via Gibson-Assembly into the linearized vector pK19mobsacB resulting in the plasmid pK19mobsacB\_Δcg1497 (Table 1). The deletion of the gene cg1497 was carried out with this plasmid by a two-step homologous recombination procedure described before [71]. For the verification of the correct in-frame deletion of the gene cg1497, a PCR (\emph{Taq} DNA polymerase with ThermoPol® Buffer, NEB, Frankfurt am Main, Germany) was performed using the primer pair Dcg1497\_fw\_v3/Dcg1497\_rv\_v3 (Table 2). Accordingly the deletion of \emph{hdpA} (cg2474) was realized, using the primer pairs nagD\_upstrm\_fw\_pK19/nagD\_upstrm\_rv and nagD\_dwnstrm\_fw/nagD\_dwnstrm\_rv\_pK19 (Table 2) for the cloning procedure of the plasmid pK19mobsacB\_ΔhdpA (Table 1) and the primer pair DnagD\_fw\_DnagD\_rv (Table 2) for the verification of the in-frame deletion via PCR. The plasmid pK19mobsacB\_Δldh (Table 1) was already available [28]. Thus, the primer pair ldh\_fw\_ldh\_rev (Table 2) was used to verify the successful in-frame deletion of \emph{ldh} after the two-step homologous recombination.

GC-MS measurements
The supernatants of the samples taken in the cultivation were analyzed using a TRACE GC ULTRA connected to an AS 3000 Auto-sampler and to an SQ Single Quadrupole Mass Spectrometer using a TG-WAXMS (length: 30 m; I.D.: 0.25 mm; film: 0.25 µm) (Thermo Scientific, Dreieich, Germany). The thawed supernatants were directly diluted 1:10 with methanol (HPLC gradient grade; VWR Chemicals, Fontenay-sous-Bois, France) or after an additional 1:10 dilution step with water (Milli-Q grade). Prior to injection, the diluted samples were centrifuged (10 min; 16,000 × g) and the resulting supernatant was used for analysis. The operating setup was the following: the temperature of the MS transfer line and the ion source were held at 230 °C; the injector temperature was set to 220 °C and a gradient was used for the oven (holding 40 °C for 1 min, increasing the temperature with a rate of 12 °C/min up to 230 °C and holding this for 5 min); in the constant flow mode, the flow rate of the carrier gas helium was 1 mL/min using the splitless mode of the injector (split flow: 10 mL/min; splitless time: 1.5 min; focus liner: 5 × 8 × 105 mm, splitless for 50-mm needle with glass wool); the electron impact ionization energy was 70 eV. The compounds 1,2-propanediol and acetol were measured with this method by creating a calibration curve with an external standard. The peaks were identified by retention time and were quantified using the intensity of one specific m/z value (1,2-propanediol: m/z = 45; acetol: m/z = 43). For the computational quantification, the program Xcalibur 2.1 (2.1.0 SP1.1160, Thermo Scientific, Dreieich, Germany) was employed.

HPLC measurements
The compounds glucose, glycerol, DHA, lactate, propanal, and 1-propanol were quantified with a HPLC system
(1200 series, Agilent Technologies, Böblingen, Germany). As a immmobile phase, an organic acid resin column (300 × 8 mm) with the appropriate pre-column (40 × 8 mm) (Chromatographie-Service GmbH, Langerwehe, Germany) was installed and heated up to 60 °C while the mobile phase was 5 mM sulfuric acid in water (Milli-Q grade) with a flow rate of 0.8 mL/min or 1 mL/min. The signals were acquired with a refractive index detector (glucose, glycerol, propanol, and 1-propanol) and a diode array detector at a signal wavelength of 210 nm and a reference wavelength of 360 nm (DHA, lactate). For the calibration curve, external standards for every component were prepared and the supernatants of the samples from the cultivations were measured undiluted after thawing.

**Abbreviations**

Δ: deletion; ADP-adenosine diphosphate; ATP-adenosine triphosphate; butA: gene coding for 3,4-DHB dehydratase (BuTA); CellBiTec: Center for Biotechnology; cg1497: gene coding for predicted kinase related to dihydroxyacetone kinase; _C. glutamicum_: Corynebacterium glutamicum; CoA: Coenzyme A; crgA, crgB: gene coding for putative aldo-keto reductase (AKR); DHAP: dihydroxyacetone (phosphate); DNA: deoxyribonucleic acid; DSMZ: German Collection of Microorganisms and Cell Cultures; E. coli: Escherichia coli; fucO: gene coding for propanediol oxidoreductase/lactaldehyde reductase (FucO); GC: MS: gas chromatography–mass spectrometry; gdA: gene coding for glyceral dehydrogenase (GdA); gpd: gene coding for glyceral-3-phosphate dehydrogenase (Gpd); HdpA: gene coding for dihydroxyacetone phosphate phosphatase (HdpA); HPLC: High-performance liquid chromatography; IPTG: isopropyl-β-D-thiogalactopyranoside; LD: oxygen; L-lactate dehydrogenase (LdhA); mgsA: gene coding for glycerol-3-phosphatase (Gpp); mValA: gene encoding _myxothecin_ glycosyltransferase (MhA); NADH and NAD: reduced or oxidized form of nicotinamide adenine dinucleotide, respectively; NADPH and NADP: reduced and oxidized form of nicotinamide adenine dinucleotide phosphate, respectively; NEN: New England Biolabs; OD_600_: optical density at wavelength (in) 600 nm; PCR: polymerase chain reaction; PntA/B: transmembrane transhydrogenase; pppAEC: operon coding for diol dehydratase (PpddABC); PPP: pentose phosphate pathway; Pyk: pyruvate kinase; rpm: revolutions per minutes; SciF: Sequencing Core Facility; TCA: citric acid cycle; _V._ _b._ _b._ vitamin B_2_; WT: wild type; yqH: gene coding for aldehyde reductase (YqH).

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

DS carried out all experimental work and data analysis, as well as drafted and revised the manuscript. VFW conceived, supervised, and managed the study, as well as revised and finalized the manuscript. Both authors read and approved the manuscript.

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**References**

1.  Soxen, RA, Anand, P, Saran, S, Iasar, J, Agarwal, L. Microbial production and applications of 1,2-propanediol. Indian J Microbiol. 2010;50–11. doi:10.1007/s12088-010-0017-x.

2.  Chauvel, A, Lefebvre, G. Petrochemical processes: volume 2: major oxygenated, chlorinated and nitrated derivatives. Paris: Editions Technip; 1989.

3.  Unthan, S, Baumgart, M, Radek, A, Herbst, M, Siebert, D, Brühl, N, et al. Chassis organism from _Corynebacterium glutamicum_ - a top-down approach to identify and delete relevant gene clusters. Biotechnol J. 2014. doi:10.1002/biot.201400041.

4.  Schneider, J, Wendisch, V, Putrécine production by engineered _Corynebacterium glutamicum_. Appl Microbiol Biotechnol. 2010;88:859–68. doi:10.1007/s00253-010-1279-x.

5.  Heider, S, Wolf, N, Hofmeier, A, Peters-Wendisch, P, Wendisch, VF. Optimization of the IPP precursor supply for the production of lycopene, decaprenoxanthin and astaxanthin by _Corynebacterium glutamicum_. Front Bioeng Biotechnol. 2014;2:28. doi:10.3389/fbioe.2014.00028.

6.  Smith, KM, Cho, K, Liao, JC. Engineering _Corynebacterium glutamicum_ for isobutanol production. Appl Microbiol Biotechnol. 2010;84:1045–55. doi:10.1007/s00253-010-1292-6.

7.  Yamaoto, S, Suda, M, Niimi, S, Inui, M, Yukawa, H. Strain optimization for efficient isobutanol production using _Corynebacterium glutamicum_ under oxygen deprivation. Biotechnol Bioeng. 2013;110:2936–48. doi:10.1002/bit.24961.
28. Blombach B, Riefer T, Wieschalker S, Zierl C, Youn J, Wendisch V, et al. Corynebacterium glutamicum tailored for efficient isobutanol production. Appl Environ Microbiol. 2011;77:3300–10. doi:10.1128/AEM.02972-10.

29. Inui M, Kawaguchi H, Murakami S, Vértés AA, Yukawa H. Metabolic engineering of Corynebacterium glutamicum for fuel ethanol production under oxygen-deprivation conditions. J Mol Microbiol Biotechnol. 2009;18:243–54. doi:10.1515/JMMB.2009.087.

30. Sakai S, Tsuchiya Y, Nakamoto H, Okino S, Ichihashi O, Kawaguchi H, et al. Effect of lipoxygenase-derived inhibitors on growth of and ethanol production by growth-arrested Corynebacterium glutamicum. Appl Environ Microbiol. 2007;73:2349–54. doi:10.1128/AEM.02880-06.

31. Jojima T, Nobuyuki R, Sasaki M, Tajima T, Suda M, Yukawa H, et al. Metabolic engineering for improved production of ethanol by Corynebacterium glutamicum. Appl Microbiol Biotechnol. 2015;99:1165–72. doi:10.1007/s00253-014-6223-4.

32. Zahoor A, Lindner SN, Wendisch VF. Metabolic engineering of Corynebacterium glutamicum aimed at alternative carbon sources and new products. Comput Struct Biotechnol J. 2012;3:210004. doi:10.5936/csbj.20120004.

33. Jain R, Yan Y. Dehydratase mediated 1-propanol production in metabolically engineered Escherichia coli. Microbiol Cell Fact. 2011;10:97. doi:10.1186/1475-286X-10-97.

34. Ammar EM, Wang Z, Yang S. Metabolic engineering of Propionibacterium freudenreichii for n-propanol production. Appl Microbiol Biotechnol. 2013;97:4677–80. doi:10.1007/s00253-013-4861-6.

35. Srinagan K, Liu X, Westbrook A, Akawi L, Pyne ME, Moe-Young M, et al. Biochemical, genetic, and metabolic engineering strategies to enhance coproduction of 1-propanol and ethanol in engineered Escherichia coli. Appl Microbiol Biotechnol. 2014;98:6499–515. doi:10.1007/s00253-014-6093-9.

36. Shen CR, Liao JC. Synergy as design principle for metabolic engineering of 1-propanol production in Escherichia coli. Metab Eng. 2013;17:4–12. doi:10.1016/j.ymben.2012.07.006.

37. Choi YJ, Park JH, Kim TY, Lee SY. Metabolic engineering of Escherichia coli for the production of 1-propanol. Metab Eng. 2012;14:477–86. doi:10.1016/j.ymben.2012.07.006.

38. Lindner SN, Meiswinkel TM, Panhorst M, Youn J, Wiegel L, Wendisch VF. Glycerol-3-phosphate of Corynebacterium glutamicum. J Biotechnol. 2012;159:26–24. doi:10.1016/j.jbiotec.2012.02.003.

39. Jojima T, Igarashi T, Moteki Y, Suda M, Yukawa H, Inui M. Promiscuous activity of (S)-butanediol dehydrogenase is responsible for glycerol production from 1,3-dihydroxyacetone in Corynebacterium glutamicum under oxygen-deprived conditions. Appl Microbiol Biotechnol. 2014;98:6499–515. doi:10.1007/s00253-014-6093-9.

40. Subedi KP, Kim I, Kim J, Min B, Park C. Role of GldA in diacetyl lactonization and methyglyoxal metabolism of Escherichia coli K12. FEMS Microbiol Lett. 2008;279:180–8. doi:10.1111/j.1574-6968.2007.01082.x.

41. Thomas DR, Haas L, Dr. Dl, Achim DR, Maruz Obuch, Volker F. Prof. Dr. Wendisch, inventors. Preparing dihydroxyacetone, useful e.g. in cosmetic composition, comprises culturing microorganisms in microgromm, adjusting pH of the medium, contacting cells with a base and culturing the microorganism in presence of carbohydrates. 2008.

42. Jojima T, Igarashi T, Gunji W, Suda M, Inui M, Yukawa H. Identification of a HAD superfamily phosphatase, HdpA, involved in 1,3-dihydroxyacetone production during sugar catabolism in Corynebacterium glutamicum. J Biotech. 2008;125:486–97. doi:10.1016/j.jbiotec.2008.03.003.

43. Okino S, Nobuyuki R, Suda M, Tajima T, Inui M, Yukawa H. A proficient succinic acid production process in a metabolically engineered Corynebacterium glutamicum strain. Appl Microbiol Biotechnol. 2008;81:459–65. doi:10.1007/s00253-008-0938-y.

44. Okino S, Suda M, Fujiyuki K, Inui M, Yukawa H. Production of D-lactic acid by Corynebacterium glutamicum under oxygen deprivation. Appl Microbiol Biotechnol. 2008;81:459–65. doi:10.1007/s00253-008-0938-y.

45. Netzer R, Krause M, Rittmann D, Peters-Wendisch PG, Eggeling L, Wendisch VF, et al. Roles of pyruvate kinase and malic enzyme in Corynebacterium glutamicum for growth on carbon sources requiring gluconeoegenesis. Arch Microbiol. 2004;182:354–63. doi:10.1007/s00203-004-0170-4.

46. Gubler M, Metten JH, Shinskey JS. Cloning of the pyruvate kinase gene (pyk) of Corynebacterium glutamicum and site-specific inactivation of pyk in a lysine-producing Corynebacterium lactofermentum strain. Appl Environ Microbiol. 1994;60:2494–500.

47. Grabar TB, Zhou S, Shumagumi KT, Yomoan LP, Ingram LO. Methylglyoxal bypass identified as source of chiral contamination in (L+ and D–)–lactate fermentations by recombinant Escherichia coli. Biotechnol Lett. 2006;28:1257–35. doi:10.1007/s10529-006-9122-7.

48. Jarboe LR. YqhD: a broad-substrate range aldehyde reductase with various activities. J Biol Chem. 2003;278:22717–24. doi:10.1074/jbc.M801017200.

49. Lessmeier L, Hoefener M, Wendisch VF. Formaldehyde degradation in Corynebacterium glutamicum involves acetaldehyde dehydrogenase and mycothiol-dependent formaldehyde dehydrogenase. Microbiology. 2013;159:2651–61. doi:10.1099/mic.0.072414-3.

50. Witthoff S, Mühlroth A, Marienhagen J, Bott M. C1 metabolism in Corynebacterium glutamicum: an endogenous pathway for oxidation of methanol to carbon dioxide. Appl Environ Microbiol. 2013;79:6974–83. doi:10.1128/AEM.02705-13.

51. Liu Y, Chen C, Chaudhry MT, Si M, Zhang L, Wang Y, et al. Enhancing Corynebacterium glutamicum robustness by over-expressing a gene, mshA, for mycothiol glycosyltransferase. Biotechnol Lett. 2014;36:1453–9. doi:10.1007/s10529-014-1501-x.

52. Shibata N, Masuda I, Morimoto Y, Yasukawa N, Toraya T. Substrate-induced conformational change of a coenzyme B12-dependent enzyme: crystal structure of the substrate-free form of diol dehydratase. Biochemistry. 2002;41:12607–17. doi:10.1021/bi026106z.

53. Tobimasu T, Sakai T, Hashida Y, Mizoguchi N, Miyoshi S, Toraya T. Heterologous expression, purification, and properties of diol dehydratase, an adenosylcobalamin-dependent enzyme of Klebsiella oxytoca. Arch Biochem Biophys. 1997;347:132–40. doi:10.1016/S0003-9863(97)80204-8.

54. Shibata N, Nakayashi Y, Fukukawa M, Yamanishi M, Yasuoka N, Toraya T. Structural rationalization for the lack of stereospecificity in coenzyme B12-dependent diol dehydratase. J Biol Chem. 2002;277:21771–5. doi:10.1074/jbc.M3013200.

55. Kabus A, Georgi T, Wendisch VF, Bott M. Expression of the Escherichia coli ptaP genes encoding a membrane-bound transhydroxanase in Corynebacterium glutamicum improves L-lysine formation. Appl Microbiol Biotechnol. 2007;75:547–53. doi:10.1007/s00253-006-0804-9.

56. Marx A, Hans S, Möckel B, Bathe D, Ge G, Albert A. Metabolic phenotype of phosphoglucone isomerase mutants of Corynebacterium glutamicum. J Biotechnol. 2003;104:185–97. doi:10.1016/S0168-1656(03)00153-6.
67. Bommar Reddy RR, Chen Z, Rappert S, Zeng A. A de novo NADPH generation pathway for improving lysine production of Corynebacterium glutamicum by rational design of the coenzyme specificity of glyceraldehyde 3-phosphate dehydrogenase. Metab Eng. 2014;25:30–7. doi:10.1016/j.ymben.2014.06.005.

68. Shi F, Huan X, Wang X, Ning J. Overexpression of NAD kinases improves the L-isoleucine biosynthesis in Corynebacterium glutamicum sp. lactofermentum. Enzyme Microb Technol. 2012;51:73–80. doi:10.1016/j.enzmictec.2012.04.003.

69. Hanahan D. Studies on transformation of Escherichia coli with plasmids. J Mol Biol. 1983;166:557–80. doi:10.1016/S0022-2836(83)80028-8.

70. Sambrook J, Russell DW. Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 2001.

71. Eggeling L, Reyes O. Experiments. In: Eggeling L, Bott M, editors. Handbook of Corynebacterium glutamicum. Boca Raton, Fla: Taylor & Francis; 2005. p. 535–68.

72. Eikmanns BJ, Thum-Schmitz N, Eggeling L, Ludtke K, Sahm H. Nucleotide sequence, expression and transcriptional analysis of the Corynebacterium glutamicum gltA gene encoding citrate synthase. Microbiology. 1994;140:1817–26. doi:10.1099/13500872-140-8-1817.

73. Gibson DG, Young L, Chuang R, Venter JC, Hutchison CA, Smith HO. Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat Methods. 2009;6:343–5. doi:10.1038/NMETH.1318.

74. Follettie MT, Peoples OP, Ageropoulou C, Sinskey AJ. Gene structure and expression of the Corynebacterium flavum N13 ask-ask operon. J Bacteriol. 1993;175:4096–103.

75. Schäfer A, Tauch A, Jäger W, Kalinowski J, Thierbach G, Pühler A. Small mobilizable multi-purpose cloning vectors derived from the Escherichia coli plasmids pK18 and pK19: selection of defined deletions in the chromosome of Corynebacterium glutamicum. Gene. 1994;145:295–300. doi:10.1016/0378-1119(94)90324-7.

76. Abe S, Takayama K, Kinoshita S. Taxanomical studies on glutamic acid-producing bacteria. J Gen Appl Microbiol. 1967;3:295–300. doi:10.2323/jgam.3.295.

77. Peters-Wendisch PG, Schiel B, Wendisch VF, Katsoulidis E, Möckel B, Sahm H, et al. Pyruvate carboxylase is a major bottleneck for glutamate and lysine production by Corynebacterium glutamicum. J Mol Microbiol Biotechnol. 2001;3:295–300.