Biotechnology Journal
www.biotechnology-journal.com

Biotechnol. J. 2015, 10, 1170–1184

DOI 10.1002/biot.201400590

Review

Engineering microbial cell factories: Metabolic engineering of *Corynebacterium glutamicum* with a focus on non-natural products

Sabine A. E. Heider and Volker F. Wendisch

Chair of Genetics or Prokaryotes, Faculty of Biology & CeBiTec, Bielefeld University, Germany

Corynebacterium glutamicum is the workhorse of biotechnological amino acid production. For more than 50 years amino acid producing strains of this actinomycete have been improved by classical breeding, metabolic engineering and systems and synthetic biology approaches. This review focusses mainly on recent developments on *C. glutamicum* strain development for non-natural products. Recently, metabolite sensors have accelerated classical strain breeding. Synthetic pathways for access to alternative carbon sources, such as pentoses, and to new products, such as α,ω-amino acids, α,ω-diamines, α-keto acids, isobutanol, carotenoids and terpenes, have been embedded in the central metabolism of *C. glutamicum*. Furthermore, *C. glutamicum* is a chassis for new and improved production processes that has been improved in two ways: by rendering it biotin prototrophic and by curing it from its prophage DNA followed by further genome reduction. The first combinations of this chassis approach with production will be highlighted. Although their transfer to industrial scale processes will have to be evaluated, these recent achievements indicate how synthetic biology helps realizing proof-of-principles. Moreover, current and future synthetic biology technology developments hold the promise to explore the full potential of *C. glutamicum* as production host for value-added chemicals.

Received 17 DEC 2014
Revised 26 MAY 2015
Accepted 02 JUL 2015

Keywords: Biosensor · Chassis organism · *Corynebacterium glutamicum* · Isoprenoids · Synthetic biology

1 Introduction

L-amino acids find various applications in food and feed biotechnology [1–4] and as synthons for the chemical industry [5]. Essential L-amino acids are used in human parenteral nutrition and as feed supplements, while L-glutamate and its salts are used as flavor enhancers [5, 6]. The growing world population and a higher demand for animal products drive the amino acid market growth, which is countered by increased production facilities, strain optimization and amino acid process intensification. Fermentative production of L-amino acids in the million-ton-scale can be considered a major achievement of biotechnology [6].

Amino acid producing strains of *Corynebacterium glutamicum*, which has been used safely for more than 50 years in food biotechnology [6–8], and of *Escherichia coli* are continuously improved using metabolic engineering approaches [5, 6]. This review focuses on metabolic engineering of *C. glutamicum* by sensor-based methods of classical strain breeding, synthetic pathway engineering for access to alternative carbon sources and for rendering this bacterium biotin-prototrophic. A large variety of compounds can be produced by *C. glutamicum* (Table 1) with a traditional focus on L-amino acids such as L-glutamate and L-lysine [9], but also L-arginine [10], L-alanine [11], L-isoleucine [4], L-methionine [12], L-serine [13] or L-valine [14] (Table 1). D-Amino acids including D-arginine and D-serine are also produced [15]. Decar-

Correspondence: Prof. Volker F. Wendisch, Chair of Genetics or Prokaryotes, Faculty of Biology & CeBiTec, Bielefeld University, Universitaetsstr. 25, 33615 Bielefeld, Germany
E-mail: volker.wendisch@uni-bielefeld.de

Abbreviations: DW, dry weight; **FPP**, farnesyl pyrophosphate; **GABA**, γ-aminobutyrate; **GPP**, geranyl pyrophosphate; **IPP**, isopentenyl pyrophosphate; **KIV**, 2-ketoisovalerate; **MEP**, methylerythritol phosphate

Table 1. Selected compounds produced by *Corynebacterium glutamicum*

Product	Application	Titer (g L^{-1})	Productivity (g L^{-1} h^{-1})	Reference
Amino acids				
L-Arginine	Building block in chemistry	93	1.3	[10]
L-Alanine	Component of infusion solutions, dietics	98	3.10	[186]
L-Citrulline		7.7	0.32	[27]
L-Glutamate	Flavor-enhancer	100	3.00	[9]
L-Histidine	Dietary supplementation	23	0.19	[187]
L-Isoleycine	Component of infusion solutions, dietics	21	0.21	[4]
L-Leucine	Building block for novel pharmaceutical	24	0.60	[188]
L-Lysine	Feed industry	120	4.00	[189]
L-Methionine	Feed industry, pharmaceutical applications	2.9	0.02	[12]
L-Ornithine	Dietary supplement in food and pharmaceutical industries	52	1.3	[90]
L-Phenylalanine	Precursor for peptide sweetener Aspartam	28	0.40	[4]
L-Proline	Animal feed and in chemical synthesis	13	0.52	[93]
L-Serine		43	0.44	[13]
L-Threonine	Feed industry	52	0.58	[52]
L-Tryptophane	Feed industry	58	0.73	[2]
L-Valine	Dietary products, pharmaceuticals, cosmetics, precursor for antibiotic and herbicide synthesis	150	0.27	[14]
D-Arginine	Building block in chemistry	0.5	0.01	[15]
D-Lysine	Building blocks in chemistry	4.2	0.11	[15]
D-Ornithine	Building blocks in chemistry	6.3	0.13	[15]
D-Serine	Building blocks in chemistry	8.5	0.12	[15]
GABA	Pharmaceuticals, functional foods or in polyamide 4	39	0.54	[82]
Diamines				
Cadaverine	Precursor of polyamide 5,5	88	2.2	[17]
Putrescine	Drop-in for production of polyamides such as PA4,10 or PA4,6	19	0.6	[16]
Alcohols				
Ethanol	Bulk chemical, alternative bio-fuel	6.9	2.3	[38]
Isobutanol	Bulk chemical, alternative bio-fuel	73	2.6	[104]
1,2-propandiol		1.8	0.18	[190]
Organic acids				
Glycolate	Cosmetic industry, rinsing agent, precursor to specialized poylmers	5.3	0.1	[191]
Ketoisovalerate	Therapeutic agent for chronic kidney disease	22	0.22	[19]
D-Lactate	Precursor to biodegradable polymer	120	40	[192]
Oleic acid	Pharmaceuticals, cosmetics, fuels	0.21		[41]
Palmitic acid	Pharmaceuticals, cosmetics, fuels	0.05		[41]
Panthothenate	Vitamine, food additive	1.8	0.04	[40]
Succinate	Precursor to specialized polyesters, acidity regulator in food applications	146	3.2	[23]
Biopolymers				
Poly-hydroxybutyrate	Biopolymers, bioplastics	0.7		[42]
Terpenoids				
Astaxanthin	Antioxidant, food coloring	8.4×10^{-3}	0.15×10^{-3}	[44]
β-Carotene	Provitamin A, food coloring	23×10^{-3}	0.9×10^{-3}	[43]
C.p.450	Antioxidant, light filter	16×10^{-3}		[43]
Decaprenoxanthin	Antioxidant, light filter	22×10^{-3}		[43]
Lycopene	Antioxidant, food coloring	2.3×10^{-3}		[43]
Pinene	Flavor, fragrence	0.2×10^{-3}	3.7×10^{-6}	[45]
Sarcinaxanthin	Antioxidant, light filter	21×10^{-3}		[43]
Valencene	Flavor, fragrance	0.2×10^{-3}	10×10^{-6}	[46]
Zeaxanthin	Food coloring	4.5×10^{-3}		[43]

boxylation leads to the diamines putrescine [16] and cadaverine [17]. A number of organic acids, mostly immediate precursors of the amino acids, can be produced, e.g. α-ketoglutarate [18], 2-ketoisovalerate [19], 2-ketoisocaproate [20] or pyruvate [21]. Importantly, succinate production under aerobic and oxygen-deprivation conditions has been widely studied [22–31] including succinate transport [32–35]. Alcohols such as isobutanol [36, 37] and in particular ethanol fermentation under oxygen-deprivation conditions have been developed [38, 39]. Other products include vitamins [40], fatty acids[41], polymers [42], carotenoids [43, 44] and terpenes [45, 46].

Recently the field has gained new impetus [47] since the genome of *C. glutamicum* has been cleared of its prophage DNA [48] and was further reduced by deletion of genes shown to have no negative effect on growth in glucose medium [49]. Such genome-stream lined strains were used for the production of L-citrulline [50] and astaxanthin [44]. In this review selected examples for the accomplishments of metabolic engineering of *C. glutamicum* are given; the reader is referred to reviews covering complementary examples [8, 18, 51–54].

2 Realizing a flexible feedstock concept: Access to alternative carbon sources

In industry, molasses, glucose from starch hydrolysis or thick juice find application [55]. Unlike many other microorganisms such as *E. coli*, *B. subtilis* or *S. cerevisiae*, *C. glutamicum* is able to simultaneously utilize a variety of carbon sources [6] and typically neither shows diauxic growth nor sequential utilization on mixed carbon sources, which is highly appreciated for industrial applications [56]. Furthermore, this advantageous characteristic prevails when exogenous carbon utilization pathways were introduced, as it was observed when cultivated on glucose with glycerol [57], xylose and arabinose [58], on cellobiose with xylose and glucose [59] or on hexuronates and glucose [60].

To meet the quest for more sustainable and less costly biotechnological processes *C. glutamicum* has been successfully engineered for the consumption of alternative feed stocks with particular attention to those attained from agro- and industry-wastes, which therefore do not have competing applications in human nutrition. Since biomass hydrolysates consist of complex mixtures of different carbon compounds *C. glutamicum* is favorable for processes based on agro-wastes due to its ability to co-utilize many different carbon sources. Beyond that, *C. glutamicum*, in contrast to many other microorganisms, shows relatively high resistance to various growth inhibitors, such as acetate, alcohols, furans, phenols, and acids, which form during lignocellulose pretreatment [61, 62] which is appreciated in fermentations based on plant hydrolysates.

Xylose utilization in *C. glutamicum* has been established via the isomerase [63] and the Weimberg pathway [64], the latter of which avoids carbon loss in xylose-based production of L-glutamate-family amino acids. Growth and production (e.g. of amino acids, diamines or carotenoids) has been described based on crude glycerol, the major byproduct in biodiesel processes [65], on pentoses like arabinose and xylose, which constitute the most abundant sugars in lignocellulosic hydrolysates [58], on hexuronic acids as present in pectin [60] and on the amino sugars glucosamine as well as the monomeric unit of chitin, *N*-acetyl glucosamine [66, 67]. Succinic and lactic acid production by *C. glutamicum* from renewable resources was achieved and comprehensively reviewed recently [25].

Apart from enabling access to alternative monomeric carbon sources that can be purified from agro- and industrial wastes, *C. glutamicum* has also been engineered for the utilization of polymers such as starch [68, 69] or glucans [70, 71]. Growth and synthesis of various bioproducts from rice straw and wheat bran hydrolysates [58, 72, 73] as well as grass juice [74] was shown.

3 Metabolic engineering of L-glutamate amino acid family products: GABA, L-arginine, L-ornithine, L-proline and putrescine

C. glutamicum can be considered a good source of L-glutamate-derived products since it is an exquisite producer of L-glutamate [53]. Various triggers of glutamate overproduction have been investigated in order to optimize production and excretion [75]: addition of ethambutol [76] or penicillin [77] as well as upward temperature shifts [78, 79] are only a few of the analyzed inducers of glutamate production.

3.1 Engineering γ-amino-butyrate (GABA) producing strains

L-glutamate can be deaminated to α-ketoglutarate [18] or decarboxylated to the α,ω-amino acid GABA. Efficient conversion of glutamate to GABA, which can be used in pharmaceuticals, functional foods or in polyamide 4, required expression of glutamate decarboxylase genes from *E. coli* or lactobacilli and deletion of the endogenous GABA uptake genes [80]. By deletion of *pknG*, encoding for serine/threonine protein kinase G, intracellular glutamate concentration in a fermentative process from glucose as carbon source could be increased and thereby GABA production enhanced further up to 31 g/L [81]. More recently Choi and co-workers investigated growth and production from glucose at different pH and achieved a maximal GABA concentration of about 39 g/L by fed-bath culture of a recombinant *C. glutamicum* [82].

3.2 Production of L-ornithine, L-arginine, and L-proline

Ikeda and colleagues have pioneered production of the L-glutamate family amino acid L-arginine and introduced key mutations into the wild-type genome [83]. This strategy has been followed widely [84–89] and classical mutagenesis paired with metabolic engineering led to high-producing strains [10]. L-ornithine can be considered a key intermediate of L-arginine biosynthesis and a number of L-ornithine producing strains have been obtained [85, 90–92].

L-ornithine can be converted to L-proline, a secondary proteinogenic amino acid used for animal feed and in chemical synthesis [5, 6], in a reaction catalyzed by ornithine cyclodeaminase. This enzyme occurs in plants, animals and bacteria such as *Pseudomonas putida*. Heterologous expression of the *P. putida* gene in a L-ornithine accumulating strain enabled production of about 13 g/L L-proline with a yield of 0.36 g L-proline per g glucose [93].

3.3 Establishing and optimizing production of the diamine putrescine

L-ornithine can be decarboxylated to the α,ω-diamine putrescine (1,4-diaminobutane) [94], which finds application as drop-in for production of polyamides such as PA4,10 or PA4,6. Heterologous expression of the ornithine decarboxylase gene *speC* from *E. coli* in a L-ornithine producing *C. glutamicum* strain led to putrescine production [95]. Leaky expression of ornithine transcarbamoylase gene *argF* from an addiction plasmid enabled the highest putrescine yield (about 0.26 g/g) reported in bacteria and made the addition of antibiotics to the fermentation broth dispensable [16]. Recently, it was found that the TetR-family transcriptional repressor CgmR responds to putrescine and other diamines in physiologically relevant concentrations, which function as inducers of *cgmRA* expression [96]. Overexpression of the putative transport gene *cgmA* increased putrescine production [96]. Since *N*-acetylputrescine was formed as significant by-product of putrescine production, a systematic screen of all genome-encoded (putative) *N*-acetyltransferase genes revealed that a single *N*-acetyltransferase, named SnaA, caused putrescine acetylation [96]. SnaA uses acetyl-CoA or propionyl-CoA to acetylate putrescine and other diamines, but in particular the triamine spermidine and the tetraamine spermine [96]. In the absence of SnaA neither cadaverine [97] nor putrescine [96] were acetylated. Deletion of *snaA* in the putrescine producing strain PUT21 prevented acetylputrescine formation and improved the putrescine yield by about 41%. Based on analysis of a genome-scale stoichiometric model of a *C. glutamicum* strain with reduced ornithine transcarbamoylase activity, derepressed arginine biosynthesis, and heterologous expression of *E. coli* ornithine decarboxylase gene *speC*, glycolysis and anaplerosis were enhanced by plasmid-borne overexpression of the genes for glyceraldehyde 3-phosphate dehydrogenase and pyruvate carboxylase. Moreover, 2-oxoglutarate dehydrogenase activity was reduced by changing the translational start codon of the chromosomal gene for 2-oxoglutarate dehydrogenase subunit E1o and changing threonine 15 of OdhI to alanine. The latter modifications improved specific productivity to 0.045 g g^{-1} h^{-1} [98]. Putrescine was also produced from alternative substrates such as pentoses, hemicellulosic hydrolysates [72], hexuronic acids [60], crude glycerol [99], glucosamine [66], *N*-acetylglucosamine [67] and by a biotin-prototrophic putrescine producing strain [89].

3.4 Strain engineering for production of L-citrulline

L-ornithine can be carbamoylated by L-ornithine carbamoyltransferase ArgF to yield L-citrulline. A *C. glutamicum* strain accumulating L-citrulline as major product has recently been developed [50]. The arginine repressor gene *argR* was deleted to derepress L-arginine biosynthesis and deletion of the argininosuccinate synthetase gene *argG* blocked conversion of L-citrulline to L-arginine. When in addition *argF*, encoding L-ornithine carbamoylphosphate transferase, and $argB^{fbr}$, encoding a feedback-resistant variant of *N*-acetyl L-glutamate kinase, were overexpressed, about 44 mM L-citrulline were produced from glucose with a yield of 0.38 ± 0.01 g g^{-1} and a volumetric productivity of 0.32 ± 0.01 g L^{-1} h^{-1}. In principle, starch, xylose and glucosamine also served as substrates for the production of L-citrulline by the respective recombinant strains [50].

4 Metabolic engineering of L-aspartate amino acid family products: 5-aminovalerate and cadaverine

The α,ω-amino acid 5-aminovalerate can be converted to valerolactam, which in turn can be used for ring-opening polycondensation to yield nylon 5. L-lysine serves as precursor for 5-aminovalerate. Heterologous expression of the *Pseudomonas putida davAB* encoding δ-aminovaleramidase and lysine 2-monooxygenase in *E. coli* enabled production of 5-aminovalerate [100, 101]. Additional heterologous expression of *P. putida gabTD* genes encoding 5-aminovalerate aminotransferase and glutarate semialdehyde dehydrogenase allowed for production of the α,ω-dicarboxylic acid glutaric acid, when α-ketoglutarate was added externally in stoichiometric concentrations. Polyamide 5,5 can be generated using glutaric acid and cadaverine.

The α,ω-diamine cadaverine (1,5-diaminopentane) is amenable to fermentation by coupling L-lysine fermentation to L-lysine decarboxylation as first described by

Mimitsuka and colleagues [102]. Cadaverine yields were improved by export engineering and elimination of the acetylated by-product [51]. Moreover, alternative carbon sources were used for cadaverine production [69, 73, 103].

5 Metabolic engineering of isobutanol production

2-Ketoisovalerate (KIV), the key-metabolite of L-leucine and L-valine biosynthesis, also serves as precursor for isobutanol production. Isobutanol is an attractive alternative bio-fuel due to its higher energy density and lower hygroscopy compared to ethanol, which makes it more suitable for currently existing pipelines and combustion-engines. Apart from that, isobutanol is used as educt in the isobutene synthesis needed for the production of several other chemicals. Microbial production of isobutanol is challenging due to its high cell toxicity. *C. glutamicum* is one of the more isobutanol-tolerant industrial microorganisms; therefore, it was successfully used for the conversion of KIV to isobutanol in aerobic and anaerobic processes [37, 104]

For fermentative production of the precursor KIV in *C. glutamicum* transaminase gene *ilvE* was deleted and *ilvBNCD* genes, responsible for the KIV biosynthesis from pyruvate, were overexpressed. Deletion of *aceE* and *pqo* encoding for the pyruvate dehydrogenase subunit AceE and pyruvate quinone oxidoreductase Pqo, respectively, entailed enhanced pyruvate supply and led to 188 mM KIV, which is about 22 g L^{-1}, and a productivity of about 0.5 g L^{-1} h^{-1} [19]. Based on this strain, *C. glutamicum* was engineered for isobutanol production from glucose [36]. Since isobutanol is a more reduced compound than KIV or L-valine its biosynthesis from glucose requires more reduction equivalents. Thus, anaerobic production was preferably pursued in order to provide sufficient NADH supply. Efficient conversion of KIV to isobutanol under oxygen deprivation was achieved by inactivation of L-lactate and malate dehydrogenases by directed gene deletion, heterologous expression of the ketoacid decarboxylase gene *kivD* from *Lactococcus lactis* and the *pntAB* transhydrogenase genes from *E. coli*, and overexpression of the native alcohol dehydrogenase gene (*adhA*). In a two-phase fed-batch fermentation with an aerobic growth phase and subsequent anaerobic production phase this strain produced 175 mM isobutanol and an overall yield of about 0.48 mol per mol glucose [36]. In a later approach, Yamamoto and co-workers addressed avoidance of product inhibition by continuous extraction of isobutanol from the reaction mixture using oleyl alcohol. Continuous extraction and tripling of the cell concentration in the reaction mixture increased the isobutanol titer to 981 mM [104].

6 Metabolic engineering of production of carotenoids and terpenes

Isoprenoids denote an extensive group of natural secondary metabolites with more than 55 000 structures; a number which is constantly rising due to new discoveries [105, 106]. They serve versatile biological functions, like e.g. hormones, volatile signals, pigments or plant regulatory molecules [107, 108] and are commercially employed for a wide range of applications in the food, cosmetic and pharmaceutical industry [109, 110]. Apart from that, isoprenoids have recently emerged as sustainable alternatives to petroleum-derived fuels [110, 111]

All isoprenoids are composed of a varying number of isoprene units and are derived from the universal five-carbon precursor isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) [112] (Fig. 1). The C5 building block IPP can be synthesized by two distinct pathways, the mevalonic acid (MVA) pathway, which occurs mainly in eukaryotes, archaea, and a limited number of bacteria, and the methylerythritol phosphate (MEP) pathway, present in most bacteria and plant plastids [113–118].

6.1 *C. glutamicum* as host for production of C40 and C50 carotenoids

Carotenoids are yellow-to-red colored pigments originating from the isoprenoid biosynthetic pathway. They are ubiquitous in nature and synthesized by plants, fungi, algae, and bacteria. Carotenoids are classified according to the lengths of their carbon backbone with the majority consisting of a C40 backbone, but also C30 and C50 carotenoids are known [119]. Although numerous microbial carotenoids are identifiedand and their microbial production is intensively investigated accompanied by a rising interest for their efficient and environmental-friendly production by microbial hosts, only a few are produced to industrial scale by biological systems as they are often not cost competitive compared to chemical synthesis [114, 120–122]

C. glutamicum belongs to a rare group of bacteria that produce long chain C50 carotenoids, that have been mainly isolated from extremely halophilic archaea [123, 124] and from Gram-positive bacteria of the order Actinomycetales [125]. The cyclic C50 carotenoid decaprenoxanthin and its glucosides give *C. glutamicum* its characteristic yellow phenotype. The biosynthetic pathway was elucidated in *C. glutamicum* by Sandmann and co-workers and the respective carotenogenic genes have been identified [126–128]. The genome exhibits two carotenogenic gene clusters: the major of the two *crt*-clusters comprises the genes *crtE*, cg0722, *crtB*, *crtI*, $crtY_e$, $crtY_f$, *crtEb*, encoding enzymes for the full conversion of IPP and DMAPP to decaprenoxanthin, and one gene of unknown function (cg0722). A second small *crt* cluster includes the

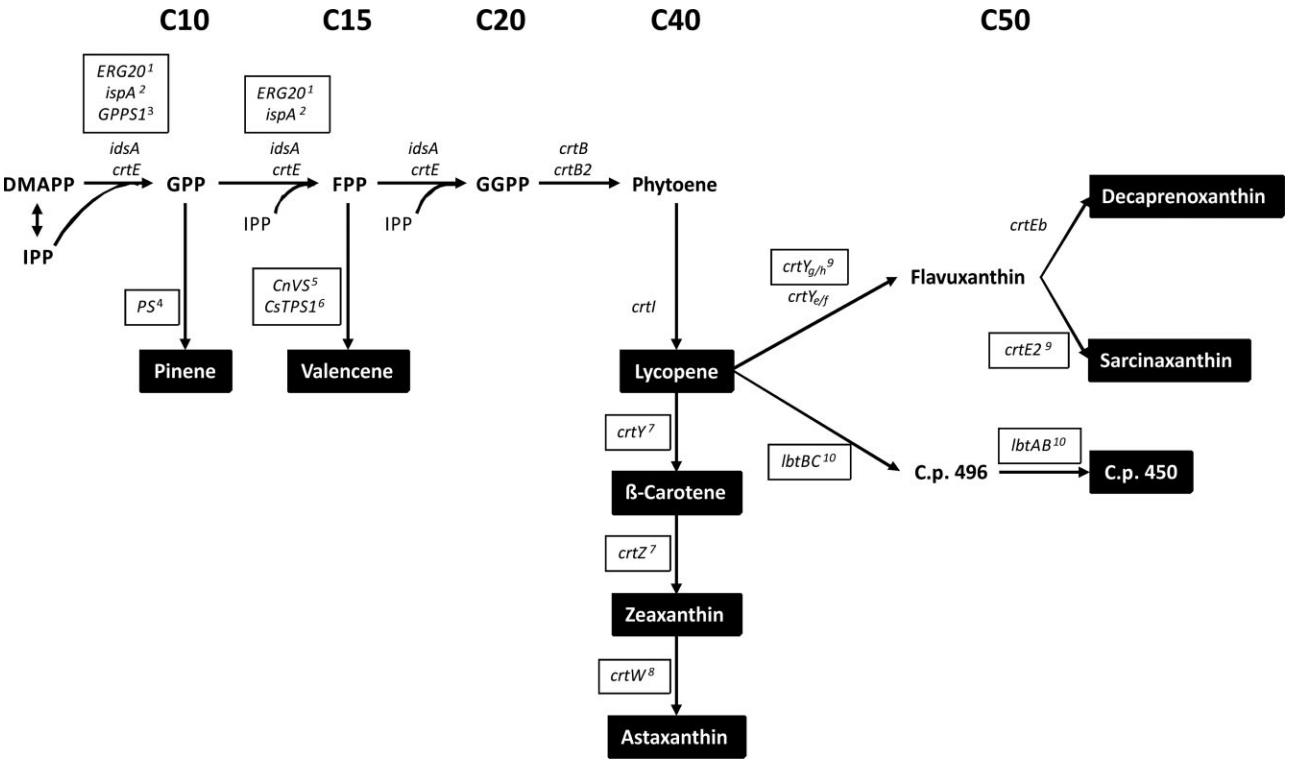

Figure 1. Biosynthesis of endogenous and heterologous terpenoids by recombinant *C. glutamicum*. Production of the monoterpene pinene, the sesquiterpene valencene, C40 carotenoids and C50 carotenoids are depicted (black boxes). Gene names are indicated next to the reactions catalyzed by the respective gene products. Heterologously expressed genes for pinene production ([3]*Pinus taeda*, [4]*Abies grandis*), for valencene production ([1]*S. cerevisiae*, [2]*E. coli*, [5]*Citrus sinensis*, [6]*Callitropsis nootkatensis*) and for carotenoid production ([7]*P. ananatis*, [8]*B. aurantiaca*, [9]*M. luteus*, [10]*Dietzia* sp. CQ4) are illustrated in frames. (GAP, Glyceraldehyde 3-phosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; DMAPP, dimethylallyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate)

gene *crtB2* (cg2672), which codes for a second functional phytoene synthase [128]. The carotenoid glucosyltransferase of *C. glutamicum*, which was demonstrated to glucosylate decaprenoxanthin as well as other cyclic C50 carotenoids, is encoded by gene *crtX* (cg0730), located about 6 kb downstream the major *crt*-cluster [129].

The heterologous expression of *C. glutamicum* carotenogenic genes has been conducted in several studies [125, 126, 130, 131], but also the potential of this Gram-positive organism for carotenoid overproduction has been investigated [43, 128] (Fig. 1). A platform strain has been engineered for elevated production of a variety of different C40 and C50 carotenoids up to 3–4 mg g^{-1} DW [43]. In that work the strain design focused on the terminal carotenoid pathway. Therefore, genes for conversion of lycopene to decaprenoxanthin were deleted, yielding the lycopene accumulating platform strain (LYC1). Flux towards lycopene was enhanced by plasmid-borne overexpression of an artificial operon comprising the native genes *crtE*, *crtB* and *crtI*. Heterologous expression of lycopene processing genes from various organisms, *Pantoea ananatis*, *Micrococcus luteus* or *Dietzia* sp. resulted in the production of the respective carotenoid. Furthermore, the presence or absence of an appropriate glucoslyltranferase allowed the production of carotenoids in both, their diglucosylated and in their non-glucosylated form [43].

6.2 Short chain isoprenoids

Apart from carotenoids, several other isoprenoid compounds are synthesized by *C. glutamicum*, involved in the menaquinone and cytochrome biosynthesis or the composition of the cell wall [132, 133]. Isoprenoid precursors in *C. glutamicum* derive from the MEP-pathway [44, 134, 135]. Recently, the optimization of the IPP precursor supply for carotenoid production was investigated by chromosomal overexpression of genes of the MEP pathway [44].

Moreover, the production of short chain isoprenoids has been facilitated in *C. glutamicum*: production of the sesquiterpene valencene was achieved by heterologous expression of the (+)-valencene synthase gene from the sweet orange *Citrus sinensis*. Increased titers were obtained upon expression of valencene synthase from Nootka cypress with up to 0.25 ± 0.03 mg g^{-1} DW [46]. However, expression of the valencene synthase gene alone was not sufficient to enable valencene production, most likely due to a deficit of the immediate precursor farnesyl pyrophosphate (FPP). On that account the deletion

of the two native geranylgeranyl pyrophosphate (GGPP) synthases genes *idsA* and *crtE* and the heterologous production of FPP synthase either from *Escherichia coli* or from *Saccharomyces cerevisiae* was necessary to establish the sesquiterpene production [46, 136]. In addition to that, production of the monoterpenoid pinene, deriving from geranyl pyrophosphate (GPP) could be achieved in metabolically-engineered *C. glutamicum* [45]. GPP synthase and pinene synthase from *Pinus taeda* and *Abies grandis*, respectively, were co-expressed with two native MEP-pathway genes, encoding 1-deoxy-d-xylulose-5-phosphate synthase (Dxs) and isopentenyl diphosphate isomerase (Idi), which resulted in an α-pinene yield of about 0.027 ± 0.007 mg g^{-1} DW [45]. Thus, the yield for this monoterpene was about ten-fold lower than the yield for the sesquiterpene (+)-valencene [46]. An overview of synthetic pathways towards endogenous and heterologous terpenoids by recombinant *C. glutamicum* is given in Fig. 1.

7 Chassis for metabolic engineering: Biotin prototrophy and genome reduction

Apart from high product formation in terms of yields, titers and productivities metabolic engineering also aims at reduction of production costs. Both aspects are important for making microbial production processes economically viable. Hence, the use of inexpensive media components for fermentation and the eschewal of cost-intense supplements are desirable.

7.1 Engineering biotin prototrophic *C. glutamicum*

C. glutamicum is auxotrophic for biotin. While biotin limitation elicits L-glutamate production, sufficient biotin is required for L-lysine production [137, 138]. *C. glutamicum* possesses only two biotin-containing proteins: pyruvate carboxylase and acetyl-CoA carboxylase. Both proteins are biotinylated at a specific lysyl residue by biotin-protein ligase BirA [139].

Unexpectedly, most of the genes for biotin biosynthesis and even its regulation are present in *C. glutamicum*: it possesses the biotin uptake system BioYMN, the transcriptional regulator BioQ and functional enzymes for the last three reactions of biotin synthesis starting from pimeloyl-CoA, namely, BioA, BioD, and BioB [89]. While initially biotin auxotrophy of *C. glutamicum* was suggested to be due to the lack of BioF [140], heterologous expression of *bioF* from *E. coli*, encoding 7-keto-8-aminopelargonic acid synthase, enabled growth of *C. glutamicum* without added biotin, but required pimelic acid supplementation instead. Two pathways of pimeloyl-CoA/ACP biosynthesis exist: the *E. coli* BioC-BioH pathway [141] and the *Bacillus subtilis* BioI-BioW pathway [142]. In *E. coli*, BioC methylates malonyl-CoA to yield a methyl ester that enters fatty acid biosynthesis and after two elongation cycles is demethylated to pimeloyl-ACP by BioH [141]. However, combined expression of *bioF*, *bioC* and *bioH* from *E. coli* did not render *C. glutamicum* biotin prototrophic. Possibly, the type-I fatty acid synthase complex (FAS-I) of *C. glutamicum*, unlike type-II fatty acid synthase (FAS-II) from *E. coli*, may not accept methyl-malonyl-CoA as substrate and/or BioH may not gain access to methyl-pimeloyl-ACP in FAS-I [89]. By contrast, plasmid-borne expression of the *bioWAFDBI* gene cluster from *B. subtilis* resulted in biotin-independent growth of *C. glutamicum* for at least eight serial transfers to biotin-depleted medium and production of L-lysine, L-arginine, putrescine and lycopene could be demonstrated [89]. Thus, the P450 enzyme BioI catalysing oxidative cleavage of fatty acyl chains appears compatible with FAS-I of *C. glutamicum* [143]. While it can be postulated from two studies on engineering biotin prototrophic *C. glutamicum* strains [89, 143] that heterologous expression of *bioI* combined with *bioF* would be sufficient to endow *C. glutamicum* with biotin-prototrophy, this still needs to be demonstrated experimentally.

7.2 Genome stream-lined *C. glutamicum* for use as chassis organism

Although metabolic engineering has advanced during the last decades, incomplete understanding of the metabolic and regulatory complexity of the microbial cell remains a big challenge in strain development. Systematic genome reduction follows the aim to reduce this complexity by creating a simplified and defined chassis that can be engineered more efficiently [144, 145]. Theoretically, a minimal genome should only possess the essential parts that define a living cell. Importantly, the genetic repertoire to maintain homeostasis with respect to pH, osmolality, salts etc. in response to ever changing environmental conditions in nature is dispensable if such an organism is kept under stable physiological conditions. Biotechnology rather targets chassis organisms with reduced genome sizes that, however, retain robust growth and production characteristics under defined cultivation conditions.

There are two basic approaches towards a minimal genome following either a top-down, in which the genome is gradually reduced in either a targeted or untargeted manner, or a bottom-up strategy, where the entire genome is created from scratch [47]. Since the former strategy can be implemented more easily, several studies on rational genome reduction of biotechnologically relevant bacteria have been performed trimming the genome in a range from 7 to 22% [146–148]. Also in *C. glutamicum* extensive genome engineering has been conducted in the last years.

C. glutamicum represents a promising target for creating a minimal organism as its comparably small genome

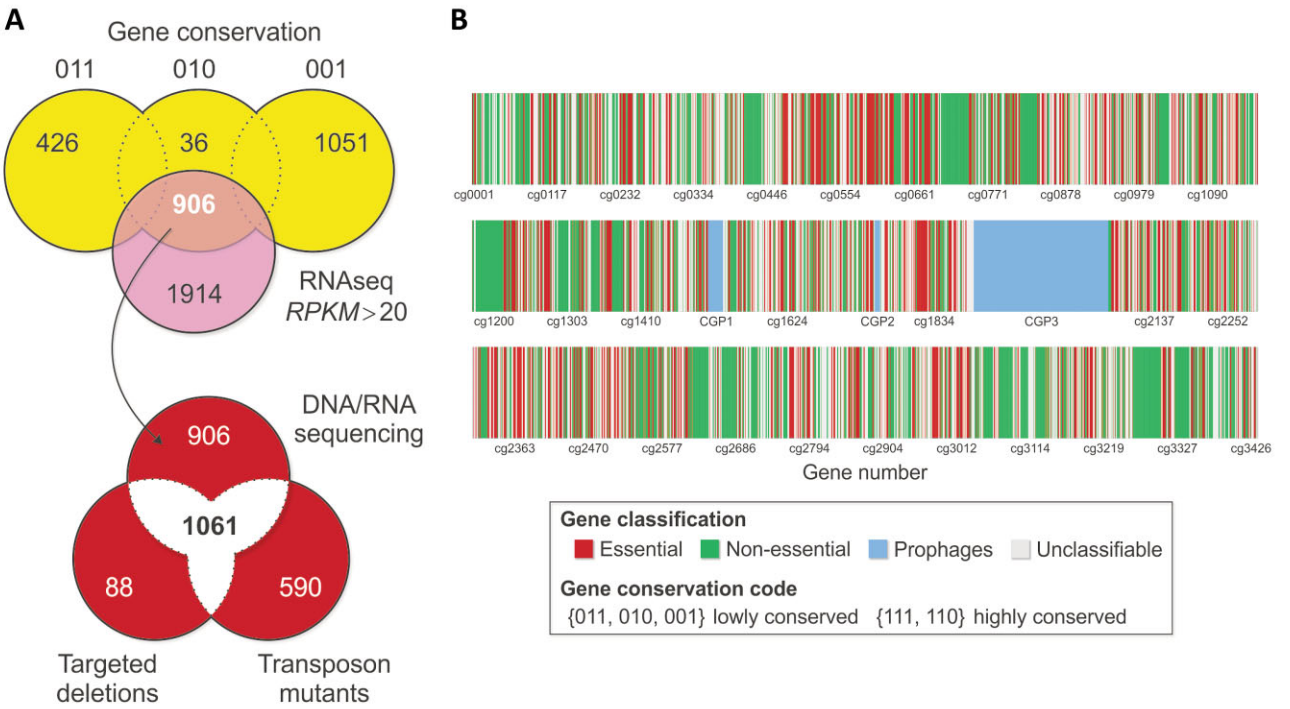

Figure 2. Classification of gene essentiality for *C. glutamicum* ATCC 13032 by (**A**) combining data from genome and RNA sequencing as well as targeted and untargeted knockout studies and (**B**) genome map with classification results of essential, non-essential, and unclassifiable genes (modified from [49]). A *C. glutamicum* strain devoid of prophages (CGP123) has also been described [48]. Reprinted with permission from Wiley [49].

was sequenced and made available in 2003 [134, 149], a wide range of genetic engineering tools are established and *omics* data are constantly expanding [150–152]. In 2005, Suzuki et al. undertook several investigations on targeted genome reduction in *C. glutamicum* R [153–155]. By comparison of the genomes of the two *C. glutamicum* strains, R and ATCC 13032, they identified eight distinct regions that were targeted for deletion in *C. glutamicum* R [154]. A combined deletion of 188 open reading frames, including transposable elements, prophages, genes for phenylacetic acid degradation as well as genes of unknown function, yielded a genome reduction of 5.7% [155]. Genome reduction efforts have recently been described also for *C. glutamicum* ATCC 13032. In a first attempt the prophage-cured strain MB001 was constructed with a 6% reduced genome. This strain did not show any unfavorable properties under the standard and stress conditions tested; it rather exhibited increased transformation efficiency and plasmid copy number [48]. Subsequently, an elaborate analysis of the *C. glutamicum* genome and a classification of genes with essentiality for maintaining fast growth on defined medium identified 41 clusters with a high content of non-essential or unclassifiable genes and sizes ranging from 3.7 to 49.7 kbp as potential targets for deletion (Fig. 2). Out of those 41 clusters 36 were successfully deleted and thereby the prediction for non-essentiality was confirmed [49]. Experiments for step-wise reduction of the native genome are still ongoing and first combinatory deletions have been conducted. A deletion of all irrelevant gene clusters identified in this study would decrease the genome by 22%, which would be comparable to the highest genome reduction achieved for *E. coli* [156] and *B. subtilis* [146]. However, due to different sizes of the wild-type genomes, the reduced genomes of *E. coli* and *B. subtilis* are still larger than the genome-reduced *C. glutamicum* strain MB001.

As first applications, *C. glutamicum* MB001 was already used for metabolic engineering of carotenoid, protein and amino acid producing strains. *C. glutamicum* MB001 was engineered for improved precursor supply for carotenoid production by chromosomal overexpression of the MEP-pathway genes [44]. This concept was applied for the production of lycopene, decaprenoxanthin and astaxanthin [44]. To enable high-level and uniform protein overproduction, part of the DE3 region of *E. coli* BL21(DE3) including the T7 RNA polymerase gene 1 under control of the lacUV5 promoter was integrated into the chromosome of *C. glutamicum* MB001 [157]. As test cases, *eyfp* and the endogenous pyruvate kinase gene *pyk* were expressed in MB001(DE3) and single-cell analysis revealed that 99% of the cells were fluorescent due to *eyfp* expression from the T7 promoter, while *pyk* overexpression led to a specific activity of 135 U mg^{-1} for pyruvate kinase [157]. *C. glutamicum* MB001 also served as start-

ing point to develop an L-citrulline overproduction strain and a yield of 0.38 ± 0.01 g · g^{-1} was achieved [50].

8 Intracellular metabolite sensors and classical mutagenesis

Amino acid excreting bacteria such as *C. glutamicum* and acidophilic *Pantoea ananatis* have been isolated based on auxanographic plate assays, i.e. in overlays with L-glutamate auxotrophs as indicator strains [6, 158, 159]. Directed engineering towards the overproduction of metabolites can be supported by the use of genetically-encoded biosensors that detect the level of a specific compound and translate this information into an optical signal [160–162]. So for example, intracellular amino acid concentrations can be visualized based on transcriptional regulatory proteins and their metabolite binding capacities in combination with fluorescent reporter proteins. High intracellular concentrations of either L-leucine, L-isoleucine, L-valine or L-methionine are sensed by the transcriptional regulator Lrp in *C. glutamicum* [163], which in response activates expression of a promoter-less gene encoding a fluorescent protein fused to the Lrp-regulated promoter of the *brnFE* operon [164]. The construction and proof of principle of the biosensor based on the Lrp-BrnFE was described in detail by Mustafi et al. [165]. Among the fluorescent mutants also mutants excreting branched-chain amino acid could be isolated [165]. It has to be noted that in this setup transport deficient mutants unable to excrete amino acids give rise to the same fluorescent phenotype. This biosensor strategy has been followed for various amino acids including L-lysine, *O*-acetyl serine and *O*-acetyl homoserine, of L-serine and of L-arginine [166]. As an extension, activation of *E. coli* SoxR by an increased cellular NADPH demand e.g. for biocatalysis was employed to isolate variants of NADPH-dependent enzymes [167, 168].

9 Concluding remarks and outlook

C. glutamicum biotechnology is constantly evolving and strain development embraces more and more approaches. Classical mutagenesis and selection [169], overcoming allosteric feedback inhibition of key enzymes [170, 171], and metabolic engineering, like e.g. engineering of cofactor availability [172], have been combined with systems biology [173–177], omics analyses such as transcriptome sequencing [178] and quantitative proteomics [179] and the respective high-throughput screening [180]. As reviewed here, synthetic biology approaches have been applied to *C. glutamicum* strain development with two aims: (i) embedding synthetic pathways into the host metabolism to generate new function, e.g. access to alternative carbon sources or enabling formation of new products, and (ii) developing the host into a versatile chassis, e.g. by rendering *C. glutamicum* biotin prototrophic or by reducing its genome size without compromising its fast growth in defined glucose-based medium. First proof-of-concept applications of genome-stream lined *C. glutamicum* strains equipped with synthetic pathways leading to formation of non-native products have been reported already. Besides the strategies to engineer *C. glutamicum* mentioned here further strategies are followed, including protein or riboswitch engineering [172, 181] and microscale cultivation at single-cell level [182]. Moreover, general understanding of basic cell functions and its adaption to environmental changes on the genetic level contribute to rational strain design and conception of cultivation conditions, respectively [183–185]. We foresee rapid and continuous technical improvements that help to gain deeper insights into *C. glutamicum* physiology and genetics and that will drive strain engineering and process intensification.

Prof. Dr. **Volker F. Wendisch** is Chair of Genetics of Prokaryotes at the Faculty of Biology at Bielefeld University. Since 2010 he is member of the board of Center for Biotechnology CeBiTec and since 2014 member of the senate of Bielefeld University. He studied biology in Köln University. After having completed his PhD at the Research Center Jülich in 1997, he worked as postdoctoral researcher at the University of California, Berkeley, CA, USA. From 2006 to 2009 he was Professor for Metabolic Engineering at Münster University. Prof. Wendisch's research interests concern genome-based metabolic engineering of industrially relevant microorganisms, systems and synthetic microbiology.

Dr. **Sabine A. E. Heider** is a postdoctoral researcher at the Chair of Genetics of Prokaryotes at the Faculty of Biology at Bielefeld University. Besides, she is the local coordinator of the CLIB Graduate Cluster Industrial Biotechnology at Bielefeld University. She studied her Bachelor of Science Chemistry und Biotechnology at the Niederrhein University of Applied Sciences, Krefeld, Germany and her Master of Science Biotechnology at the WestfälischeWilhelms University, Münster, Germany. She received her PhD in July 2014 from Bielefeld University for her investigation of the carotenoid biosynthesis in *Corynebacterium glutamicum* for terpenoid production.

We acknowledge support by EU-FP7 project PROMYSE (289540), by ZIM project (KF2969003SB2), and by BMBF project Genome reduction (0316017A).

The authors declare no financial or commercial conflict of interest.

10 References

[1] Hermann, T., Industrial production of amino acids by coryneform bacteria. *J. Biotechnol.* 2003, *104*, 155–172.
[2] Leuchtenberger, W., Huthmacher, K., Drauz, K., Biotechnological production of amino acids and derivatives: Current status and prospects. *Appl. Microbiol. Biotechnol.* 2005, *69*, 1–8.
[3] Eggeling, L., Bott, M., A giant market and a powerful metabolism: L-lysine provided by *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2015, *99*, 3387–3394.
[4] Ikeda, M., Amino acid production processes. *Adv. Biochem. Eng. Biotechnol.* 2003, *79*, 1–35.
[5] Wendisch, V. F., Steinbüchel, A., Preface, in: Volker, F. W. (Ed.), *Amino Acid Biosynthesis – Pathways, Regulation and Metabolic Engineering*, Springer 2007, pp. V-VII.
[6] Bott, M., Eggeling, L. (Eds.), *Handbook of Corynebacterium glutamicum*, CRC Press LLC 2005.
[7] Becker, J., Wittmann, C., Systems and synthetic metabolic engineering for amino acid production – the heartbeat of industrial strain development. *Curr. Opin. Biotechnol.* 2012, *23*, 718–726.
[8] Mitsuhashi, S., Current topics in the biotechnological production of essential amino acids, functional amino acids, and dipeptides. *Curr. Opin. Biotechnol.* 2014, *26*, 38–44.
[9] Ault, A., The monosodium glutamate story: The commercial production of MSG and other amino acids. *J. Chem. Educ.* 2004, *81*, 347–355.
[10] Park, S. H., Kim, H. U., Kim, T. Y., Park, J. S. et al., Metabolic engineering of *Corynebacterium glutamicum* for L-arginine production. *Nat. Commun.* 2014, *5*, 4618.
[11] Jojima, T., Fujii, M., Mori, E., Inui, M., Yukawa, H., Engineering of sugar metabolism of *Corynebacterium glutamicum* for production of amino acid L-alanine under oxygen deprivation. *Appl. Microbiol. Biotechnol.* 2010, *87*, 159–165.
[12] Park, S. D., Lee, J. Y., Sim, S. Y., Kim, Y., Lee, H. S., Characteristics of methionine production by an engineered *Corynebacterium glutamicum* strain. *Metab. Eng.* 2007, *9*, 327–336.
[13] Zhu, Q., Zhang, X., Luo, Y., Guo, W. et al., L-Serine overproduction with minimization of by-product synthesis by engineered *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2015, *99*, 1665–1673.
[14] Hasegawa, S., Suda, M., Uematsu, K., Natsuma, Y. et al., Engineering of *Corynebacterium glutamicum* for high-yield L-valine production under oxygen deprivation conditions. *Appl. Environ. Microbiol.* 2013, *79*, 1250–1257.
[15] Stabler, N., Oikawa, T., Bott, M., Eggeling, L., *Corynebacterium glutamicum* as a host for synthesis and export of D-amino acids. *J. Bacteriol.* 2011, *193*, 1702–1709.
[16] Schneider, J., Eberhardt, D., Wendisch, V. F., Improving putrescine production by *Corynebacterium glutamicum* by fine-tuning ornithine transcarbamoylase activity using a plasmid addiction system. *Appl. Microbiol. Biotechnol.* 2012, *95*, 169–178.
[17] Kind, S., Neubauer, S., Becker, J., Yamamoto, M. et al., From zero to hero – production of bio-based nylon from renewable resources using engineered *Corynebacterium glutamicum*. *Metab. Eng.* 2014, *25*, 113–123.
[18] Wieschalka, S., Blombach, B., Bott, M., Eikmanns, B. J., Bio-based production of organic acids with *Corynebacterium glutamicum*. *Microb. Biotechnol.* 2013, *6*, 87–102.
[19] Krause, F. S., Blombach, B., Eikmanns, B. J., Metabolic engineering of *Corynebacterium glutamicum* for 2-ketoisovalerate production. *Appl. Environ. Microbiol.* 2010, *76*, 8053–8061.
[20] Buckle-Vallant, V., Krause, F. S., Messerschmidt, S., Eikmanns, B. J., Metabolic engineering of *Corynebacterium glutamicum* for 2-ketoisocaproate production. *Appl. Microbiol. Biotechnol.* 2014, *98*, 297–311.
[21] Wieschalka, S., Blombach, B., Eikmanns, B. J., Engineering *Corynebacterium glutamicum* for the production of pyruvate. *Appl. Microbiol. Biotechnol.* 2012, *94*, 449–459.
[22] Litsanov, B., Kabus, A., Brocker, M., Bott, M., Efficient aerobic succinate production from glucose in minimal medium with *Corynebacterium glutamicum*. *Microb. Biotechnol.* 2012, *5*, 116–128.
[23] Okino, S., Noburyu, R., Suda, M., Jojima, T. et al., An efficient succinic acid production process in a metabolically engineered *Corynebacterium glutamicum* strain. *Appl. Microbiol. Biotechnol.* 2008, *81*, 459–464.
[24] Litsanov, B., Brocker, M., Bott, M., Toward homosuccinate fermentation: Metabolic engineering of *Corynebacterium glutamicum* for anaerobic production of succinate from glucose and formate. *Appl. Environ. Microbiol.* 2012, *78*, 3325–3337.
[25] Tsuge, Y., Hasunuma, T., Kondo, A., Recent advances in the metabolic engineering of *Corynebacterium glutamicum* for the production of lactate and succinate from renewable resources. *J. Ind. Microbiol. Biotechnol.* 2015, *42*, 375–389.
[26] Rados, D., Turner, D. L., Fonseca, L. L., Carvalho, A. L. et al., Carbon flux analysis by ^{13}C nuclear magnetic resonance to determine the effect of CO_2 on anaerobic succinate production by *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 2014, *80*, 3015–3024.
[27] Yamauchi, Y., Hirasawa, T., Nishii, M., Furusawa, C., Shimizu, H., Enhanced acetic acid and succinic acid production under microaerobic conditions by *Corynebacterium glutamicum* harboring *Escherichia coli* transhydrogenase gene *pntAB*. *J. General Applied Microbiol.* 2014, *60*, 112–118.
[28] Ma, X., Zhang, X., Wang, B., Mao, Y. et al., Engineering microorganisms based on molecular evolutionary analysis: A succinate production case study. *Evol. Appl.* 2014, *7*, 913–920.
[29] Zhou, Z., Wang, C., Chen, Y., Zhang, K. et al., Increasing available NADH supply during succinic acid production by *Corynebacterium glutamicum*. *Biotechnol. Prog.* 2015, *31*, 12–19.
[30] Okino, S., Inui, M., Yukawa, H., Production of organic acids by *Corynebacterium glutamicum* under oxygen deprivation. *Appl. Microbiol. Biotechnol.* 2005, *68*, 475–480.
[31] Inui, M., Murakami, S., Okino, S., Kawaguchi, H. et al., Metabolic analysis of *Corynebacterium glutamicum* during lactate and succinate productions under oxygen deprivation conditions. *J. Mol. Microbiol. Biotechnol.* 2004, *7*, 182–196.
[32] Fukui, K., Koseki, C., Yamamoto, Y., Nakamura, J. et al., Identification of succinate exporter in *Corynebacterium glutamicum* and its physiological roles under anaerobic conditions. *J. Biotechnol.* 2011, *154*, 25–34.
[33] Huhn, S., Jolkver, E., Kramer, R., Marin, K., Identification of the membrane protein SucE and its role in succinate transport in *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2011, *89*, 327–335.
[34] Youn, J. W., Jolkver, E., Kramer, R., Marin, K., Wendisch, V. F., Identification and characterization of the dicarboxylate uptake system DccT in *Corynebacterium glutamicum*. *J. Bacteriol.* 2008, *190*, 6458–6466.
[35] Youn, J. W., Jolkver, E., Kramer, R., Marin, K., Wendisch, V. F., Characterization of the dicarboxylate transporter DctA in *Corynebacterium glutamicum*. *J. Bacteriol.* 2009, *191*, 5480–5488.

[36] Blombach, B., Riester, T., Wieschalka, S., Ziert, C. et al., *Corynebacterium glutamicum* tailored for efficient isobutanol production. *Appl. Environ. Microbiol.* 2011, *77*, 3300–3310.
[37] Smith, K. M., Cho, K. M., Liao, J. C., Engineering *Corynebacterium glutamicum* for isobutanol production. *Appl. Microbiol. Biotechnol.* 2010, *87*, 1045–1055.
[38] Inui, M., Kawaguchi, H., Murakami, S., Vertes, A. A., Yukawa, H., Metabolic Engineering of *Corynebacterium glutamicum* for Fuel Ethanol Production under Oxygen-Deprivation Conditions. *J. Mol. Microbiol. Biotechnol.* 2004, *8*, 243–254.
[39] Jojima, T., Noburyu, R., Sasaki, M., Tajima, T. et al., Metabolic engineering for improved production of ethanol by *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2015, *99*, 1165–1172.
[40] Hüser, A. T., Chassagnole, C., Lindley, N. D., Merkamm, M. et al., Rational design of a *Corynebacterium glutamicum* pantothenate production strain and its characterization by metabolic flux analysis and genome-wide transcriptional profiling. *Appl. Environ. Microbiol.* 2005, *71*, 3255–3268.
[41] Takeno, S., Takasaki, M., Urabayashi, A., Mimura, A. et al., Development of fatty acid-producing *Corynebacterium glutamicum* strains. *Appl. Environ. Microbiol.* 2013, *79*, 6776–6783.
[42] Liu, Q., Ouyang, S. P., Kim, J., Chen, G. Q., The impact of PHB accumulation on L-glutamate production by recombinant *Corynebacterium glutamicum*. *J. Biotechnol.* 2007, *132*, 273–279.
[43] Heider, S. A., Peters-Wendisch, P., Netzer, R., Stafnes, M. et al., Production and glucosylation of C50 and C40 carotenoids by metabolically engineered *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2014, *98*, 1223–1235.
[44] Heider, S. A., Wolf, N., Hofemeier, A., Peters-Wendisch, P., Wendisch, V. F., Optimization of the IPP precursor supply for the production of lycopene, decaprenoxanthin and astaxanthin by *Corynebacterium glutamicum*. *Front. Bioeng. Biotechnol.* 2014, *2*, 28.
[45] Kang, M. K., Eom, J. H., Kim, Y., Um, Y., Woo, H. M., Biosynthesis of pinene from glucose using metabolically-engineered *Corynebacterium glutamicum*. *Biotechnol. Lett.* 2014, *36*, 2069–2077.
[46] Frohwitter, J., Heider, S. A., Peters-Wendisch, P., Beekwilder, J., Wendisch, V. F., Production of the sesquiterpene (+)-valencene by metabolically engineered *Corynebacterium glutamicum*. *J. Biotechnol.* 2014, *191*, 205–213.
[47] de Lorenzo, V., Chassis organism from *Corynebacterium glutamicum*: The way towards biotechnological domestication of Corynebacteria. *Biotechnol. J.* 2015, *10*, 244–245.
[48] Baumgart, M., Unthan, S., Ruckert, C., Sivalingam, J. et al., Construction of a prophage-free variant of *Corynebacterium glutamicum* ATCC 13032 for use as a platform strain for basic research and industrial biotechnology. *Appl. Environ. Microbiol.* 2013, *79*, 6006–6015.
[49] Unthan, S., Baumgart, M., Radek, A., Herbst, M. et al., Chassis organism from *Corynebacterium glutamicum* – a top-down approach to identify and delete irrelevant gene clusters. *Biotechnol. J.* 2015, *10*, 290–301.
[50] Eberhardt, D., Jensen, J. V. K., Wendisch, V. F., L-citrulline production by metabolically engineered *Corynebacterium glutamicum* from glucose and alternative carbon sources. *AMB Express* 2014, *4*, 85.
[51] Becker, J., Wittmann, C., Bio-based production of chemicals, materials and fuels – *Corynebacterium glutamicum* as versatile cell factory. *Curr. Opin. Biotechnol.* 2012, *23*, 631–640.
[52] Becker, J., Wittmann, C., Advanced biotechnology: Metabolically engineered cells for the bio-based production of chemicals and fuels, materials, and health-care products. *Angew. Chem. Int. Ed.* 2015, *54*, 3328–3350.
[53] Wendisch, V. F., Microbial production of amino acids and derived chemicals: Synthetic biology approaches to strain development. *Curr. Opin. Biotechnol.* 2014, *30*, 51–58.
[54] Burkovski, A., *Corynebacterium glutamicum – From Systems Biology to Biotechnological Applications*, Caister Academic Press, Norfolk, UK 2015.
[55] Meiswinkel, T., Lindner, S., Wendisch, V. F., Thick juice-based production of amino acids and putrescine by *Corynebacterium glutamicum*. *J. Biotechnol. Biomater.* 2014, *4*, 167.
[56] Wendisch, V. F., de Graaf, A. A., Sahm, H., Eikmanns, B. J., Quantitative determination of metabolic fluxes during coutilization of two carbon sources: Comparative analyses with *Corynebacterium glutamicum* during growth on acetate and/or glucose. *J. Bacteriol.* 2000, *182*, 3088–3096.
[57] Rittmann, D., Lindner, S. N., Wendisch, V. F., Engineering of a glycerol utilization pathway for amino acid production by *Corynebacterium glutamicum*. *Appl Environ Microbiol* 2008, *74*, 6216–6222.
[58] Gopinath, V., Meiswinkel, T. M., Wendisch, V. F., Nampoothiri, K. M., Amino acid production from rice straw and wheat bran hydrolysates by recombinant pentose-utilizing *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2011, *92*, 985–996.
[59] Sasaki, M., Jojima, T., Inui, M., Yukawa, H., Simultaneous utilization of D-cellobiose, D-glucose, and D-xylose by recombinant *Corynebacterium glutamicum* under oxygen-deprived conditions. *Appl. Microbiol. Biotechnol.* 2008, *81*, 691–699.
[60] Hadiati, A., Krahn, I., Lindner, S., Wendisch, V. F., Engineering of *Corynebacterium glutamicum* for growth and production of L-ornithine, L-lysine, and lycopene from hexuronic acids. *Bioresour. Bioprocess.* 2014, *1*, 25.
[61] Matano, C., Meiswinkel, T. M., Wendisch, V. F., Amino acid production from rice straw hydrolysate, in: Watson, R. R., Preedy, V. R., Zibadi, S. (Eds.), *Wheat and Rice in Disease Prevention and Health*, Elsevier/Academic Press 2014, pp. 493–505.
[62] Sakai, S., Tsuchida, Y., Okino, S., Ichihashi, O. et al., Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested *Corynebacterium glutamicum* R. *Appl. Environ. Microbiol.* 2007, *73*, 2349–2353.
[63] Kawaguchi, H., Vertes, A. A., Okino, S., Inui, M., Yukawa, H., Engineering of a xylose metabolic pathway in *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 2006, *72*, 3418–3428.
[64] Radek, A., Krumbach, K., Gatgens, J., Wendisch, V. F. et al., Engineering of *Corynebacterium glutamicum* for minimized carbon loss during utilization of d-xylose containing substrates. *J. Biotechnol.* 2014, *192*, 156–160.
[65] Meiswinkel, T. M., Rittmann, D., Lindner, S. N., Wendisch, V. F., Crude glycerol-based production of amino acids and putrescine by *Corynebacterium glutamicum*. *Bioresour. Technol.* 2013, *145*, 254–258.
[66] Uhde, A., Youn, J. W., Maeda, T., Clermont, L. et al., Glucosamine as carbon source for amino acid-producing *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2013, *97*, 1679–1687.
[67] Matano, C., Uhde, A., Youn, J. W., Maeda, T. et al., Engineering of *Corynebacterium glutamicum* for growth and L-lysine and lycopene production from *N*-acetyl-glucosamine. *Appl. Microbiol. Biotechnol.* 2014, *98*, 5633–5643.
[68] Seibold, G., Auchter, M., Berens, S., Kalinowski, J., Eikmanns, B. J., Utilization of soluble starch by a recombinant *Corynebacterium glutamicum* strain: Growth and lysine production. *J. Biotechnol.* 2006, *124*, 381–391.
[69] Tateno, T., Okada, Y., Tsuchidate, T., Tanaka, T. et al., Direct production of cadaverine from soluble starch using *Corynebacterium glutamicum* coexpressing alpha-amylase and lysine decarboxylase. *Appl. Microbiol. Biotechnol.* 2009, *82*, 115–121.
[70] Tsuchidate, T., Tateno, T., Okai, N., Tanaka, T. et al., Glutamate production from beta-glucan using endoglucanase-secreting *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2011, *90*, 895–901.

[71] Adachi, N., Takahashi, C., Ono-Murota, N., Yamaguchi, R. et al., Direct L-lysine production from cellobiose by *Corynebacterium glutamicum* displaying beta-glucosidase on its cell surface. *Appl. Microbiol. Biotechnol.* 2013, *97*, 7165–7172.
[72] Meiswinkel, T. M., Gopinath, V., Lindner, S. N., Nampoothiri, K. M., Wendisch, V. F., Accelerated pentose utilization by *Corynebacterium glutamicum* for accelerated production of lysine, glutamate, ornithine and putrescine. *Microb. Biotechnol.* 2013, *6*, 131–140.
[73] Buschke, N., Becker, J., Schafer, R., Kiefer, P. et al., Systems metabolic engineering of xylose-utilizing *Corynebacterium glutamicum* for production of 1,5-diaminopentane. *Biotechnol. J.* 2013, *8*, 557–570.
[74] Neuner, A., Wagner, I., Sieker, T., Ulber, R. et al., Production of L-lysine on different silage juices using genetically engineered *Corynebacterium glutamicum*. *J. Biotechnol.* 2013, *163*, 217–224.
[75] Shimizu, H., Hirasawa, T., Production of glutamate and glutamate-related amino acids: Molecular mechanism analysis and metabolic engineering, in: Wendisch, V. F. (Ed.), *Amino Acid Biosynthesis – Pathways, Regulation and Metabolic Engineering*, Springer, Heidelberg 2007, pp. 1–38.
[76] Radmacher, E., Stansen, K. C., Besra, G. S., Alderwick, L. J. et al., Ethambutol, a cell wall inhibitor of *Mycobacterium tuberculosis*, elicits L-glutamate efflux of *Corynebacterium glutamicum*. *Microbiology* 2005, *151*, 1359–1368.
[77] Kim, J., Fukuda, H., Hirasawa, T., Nagahisa, K. et al., Requirement of de novo synthesis of the OdhI protein in penicillin-induced glutamate production by *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2010, *86*, 911–920.
[78] Lapujade, P., Goergen, J. L., Engasser, J. M., Glutamate excretion as a major kinetic bottleneck for the thermally triggered production of glutamic acid by *Corynebacterium glutamicum*. *Metab. Eng.* 1999, *1*, 255–261.
[79] Delaunay, S., Gourdon, P., Lapujade, P., Mailly, E. et al., An improved temperature triggered process for glutamate production with *Corynebacterium glutamicum*. *Enz. Microb. Technol.* 1999, *25*, 762–768.
[80] Zhao, Z., Ding, J. Y., Ma, W. H., Zhou, N. Y., Liu, S. J., Identification and characterization of gamma-aminobutyric acid uptake system GabPCg (NCgl0464) in *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 2012, *78*, 2596–2601.
[81] Okai, N., Takahashi, C., Hatada, K., Ogino, C., Kondo, A., Disruption of *pknG* enhances production of gamma-aminobutyric acid by *Corynebacterium glutamicum* expressing glutamate decarboxylase. *AMB Express* 2014, *4*, 20.
[82] Choi, J. W., Yim, S. S., Lee, S. H., Kang, T. J. et al., Enhanced production of gamma-aminobutyrate (GABA) in recombinant *Corynebacterium glutamicum* by expressing glutamate decarboxylase active in expanded pH range. *Microb. Cell Fact.* 2015, *14*, 21.
[83] Ikeda, M., Mitsuhashi, S., Tanaka, K., Hayashi, M., Reengineering of a *Corynebacterium glutamicum* L-arginine and L-citrulline producer. *Appl. Environ. Microbiol.* 2009, *75*, 1635–1641.
[84] Reddy, G. K., Wendisch, V. F., Characterization of 3-phosphoglycerate kinase from *Corynebacterium glutamicum* and its impact on amino acid production. *BMC Microbiol.* 2014, *14*, 54.
[85] Schneider, J., Niermann, K., Wendisch, V. F., Production of the amino acids l-glutamate, L-lysine, L-ornithine and L-arginine from arabinose by recombinant *Corynebacterium glutamicum*. *J. Biotechnol.* 2011, *154*, 191–198.
[86] Petri, K., Walter, F., Persicke, M., Ruckert, C., Kalinowski, J., A novel type of *N*-acetylglutamate synthase is involved in the first step of arginine biosynthesis in *Corynebacterium glutamicum*. *BMC Genomics* 2013, *14*, 713.
[87] Schendzielorz, G., Dippong, M., Grunberger, A., Kohlheyer, D. et al., Taking control over control: Use of product sensing in single cells to remove flux control at key enzymes in biosynthesis pathways. *ACS. Synth. Biol.* 2014, *3*, 21–29.
[88] Kessler, N., Walter, F., Persicke, M., Albaum, S. P. et al., ALLocator: An interactive web platform for the analysis of metabolomic LC-ESI-MS datasets, enabling semi-automated, user-revised compound annotation and mass isotopomer ratio analysis. *PLoS One* 2014, *9*, e113909.
[89] Peters-Wendisch, P., Gotker, S., Heider, S. A., Komati Reddy, G. et al., Engineering biotin prototrophic *Corynebacterium glutamicum* strains for amino acid, diamine and carotenoid production. *J. Biotechnol.* 2014, *192*, 346–354.
[90] Kim, S. Y., Lee, J., Lee, S. Y., Metabolic engineering of *Corynebacterium glutamicum* for the production of L-ornithine. *Biotechnol. Bioeng.* 2015, *112*, 416–421.
[91] Hwang, J. H., Hwang, G. H., Cho, J. Y., Effect of increased glutamate availability on L-ornithine production in *Corynebacterium glutamicum*. *J. Microbiol. Biotechnol.* 2008, *18*, 704–710.
[92] Matsui, D., Oikawa, T., Detection of D-ornithine extracellularly produced by *Corynebacterium glutamicum* ATCC 13032::*argF*. *Biosci. Biotechnol. Biochem.* 2010, *74*, 2507–2510.
[93] Jensen, J. V., Wendisch, V. F., Ornithine cyclodeaminase-based proline production by *Corynebacterium glutamicum*. *Microb. Cell Fact.* 2013, *12*, 63.
[94] Schneider, J., Wendisch, V. F., Biotechnological production of polyamines by bacteria: Recent achievements and future perspectives. *Appl. Microbiol. Biotechnol.* 2011, *91*, 17–30.
[95] Schneider, J., Wendisch, V. F., Putrescine production by engineered *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2010, *88*, 859–868.
[96] Nguyen, A. Q., Schneider, J., Wendisch, V. F., Elimination of polyamine *N*-acetylation and regulatory engineering improved putrescine production by *Corynebacterium glutamicum*. *J. Biotechnol.* 2015, *201*, 75–85.
[97] Kind, S., Jeong, W. K., Schroder, H., Zelder, O., Wittmann, C., Identification and elimination of the competing *N*-acetyldiaminopentane pathway for improved production of diaminopentane by *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 2010, *76*, 5175–5180.
[98] Nguyen, A. Q., Schneider, J., Wendisch, V. F., Elimination of polyamine *N*-acetylation and regulatory engineering improved putrescine production by *Corynebacterium glutamicum*. *J. Biotechnol.* 2015, *201*, 75–85.
[99] Meiswinkel, T. M., Rittmann, D., Lindner, S. N., Wendisch, V. F., Crude glycerol-based production of amino acids and putrescine by *Corynebacterium glutamicum*. *Bioresour. Technol.* 2013, *145*, 254–258.
[100] Park, S. J., Kim, E. Y., Noh, W., Park, H. M. et al., Metabolic engineering of *Escherichia coli* for the production of 5-aminovalerate and glutarate as C5 platform chemicals. *Metab. Eng.* 2013, *16*, 42–47.
[101] Adkins, J., Jordan, J., Nielsen, D. R., Engineering *Escherichia coli* for renewable production of the 5-carbon polyamide building-blocks 5-aminovalerate and glutarate. *Biotechnol. Bioeng.* 2013, *110*, 1726–1734.
[102] Mimitsuka, T., Sawai, H., Hatsu, M., Yamada, K., Metabolic engineering of *Corynebacterium glutamicum* for cadaverine fermentation. *Biosci. Biotechnol. Biochem.* 2007, *71*, 2130–2135.
[103] Ikeda, N., Miyamoto, M., Adachi, N., Nakano, M. et al., Direct cadaverine production from cellobiose using beta-glucosidase displaying *Escherichia coli*. *AMB Express* 2013, *3*, 67.
[104] Yamamoto, 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*, 2938–2948.

[105] Thulasiram, H. V., Erickson, H. K., Poulter, C. D., Chimeras of two isoprenoid synthases catalyze all four coupling reactions in isoprenoid biosynthesis. *Science* 2007, *316*, 73–76.
[106] Liang, P. H., Reaction kinetics, catalytic mechanisms, conformational changes, and inhibitor design for prenyltransferases. *Biochemistry* 2009, *48*, 6562–6570.
[107] Ajikumar, P. K., Tyo, K., Carlsen, S., Mucha, O. et al., Terpenoids: Opportunities for biosynthesis of natural product drugs using engineered microorganisms. *Mol. Pharm.* 2008, *5*, 167–190.
[108] Heider, S. A., Peters-Wendisch, P., Wendisch, V. F., Beekwilder, J., Brautaset, T., Metabolic engineering for the microbial production of carotenoids and related products with a focus on the rare C50 carotenoids. *Appl. Microbiol. Biotechnol.* 2014, *98*, 4355–4368.
[109] Marienhagen, J., Bott, M., Metabolic engineering of microorganisms for the synthesis of plant natural products. *J. Biotechnol.* 2013, *163*, 166–178.
[110] Tippmann, S., Chen, Y., Siewers, V., Nielsen, J., From flavors and pharmaceuticals to advanced biofuels: Production of isoprenoids in *Saccharomyces cerevisiae*. *Biotechnol. J.* 2013, *8*, 1435–1444.
[111] Rude, M. A., Schirmer, A., New microbial fuels: A biotech perspective. *Curr. Opin. Microbiol.* 2009, *12*, 274–281.
[112] Ruzicka, L., The isoprene rule and the biogenesis of terpenic compounds. *Experientia* 1953, *9*, 357–367.
[113] Daum, M., Herrmann, S., Wilkinson, B., Bechthold, A., Genes and enzymes involved in bacterial isoprenoid biosynthesis. *Curr. Opin. Chem. Biol.* 2009, *13*, 180–188.
[114] Lee, P. C., Schmidt-Dannert, C., Metabolic engineering towards biotechnological production of carotenoids in microorganisms. *Appl. Microbiol. Biotechnol.* 2002, *60*, 1–11.
[115] Rohmer, M., The discovery of a mevalonate-independent pathway for isoprenoid biosynthesis in bacteria, algae and higher plants. *Nat. Prod. Rep.* 1999, *16*, 565–574.
[116] Lange, B. M., Rujan, T., Martin, W., Croteau, R., Isoprenoid biosynthesis: The evolution of two ancient and distinct pathways across genomes. *Proc. Natl. Acad. Sci. U.S.A.* 2000, *97*, 13172–13177.
[117] Kirby, J., Keasling, J. D., Biosynthesis of plant isoprenoids: Perspectives for microbial engineering. *Annu. Rev. Plant Biol.* 2009, *60*, 335–355.
[118] Rohmer, M., Knani, M., Simonin, P., Sutter, B., Sahm, H., Isoprenoid biosynthesis in bacteria: A novel pathway for the early steps leading to isopentenyl diphosphate. *Biochem. J.* 1993, *295*, 517–524.
[119] Heider, S. A., Peters-Wendisch, P., Wendisch, V. F., Beekwilder, J., Brautaset, T., Metabolic engineering for the microbial production of carotenoids and related products with a focus on the rare C50 carotenoids. *Appl. Microbiol. Biotechnol.* 2014, *98*, 4355–4368.
[120] Cutzu, R., Coi, A., Rosso, F., Bardi, L. et al., From crude glycerol to carotenoids by using a *Rhodotorula glutinis* mutant. *World J. Microbiol. Biotechnol.* 2013, *29*, 1009–1017.
[121] Das, A., Yoon, S. H., Lee, S. H., Kim, J. Y. et al., An update on microbial carotenoid production: Application of recent metabolic engineering tools. *Appl. Microbiol. Biotechnol.* 2007, *77*, 505–512.
[122] Harada, H., Misawa, N., Novel approaches and achievements in biosynthesis of functional isoprenoids in *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 2009, *84*, 1021–1031.
[123] Pfander, H., C45-carotenoids and C50-carotenoids. *Pure Appl. Chem.* 1994, *66*, 2369–2374.
[124] Kelly, M., Jensen, S. L., Bacterial carotenoids .26. C50-carotenoids .2. Bacterioruberin. *Acta Chem. Scand.* 1967, *21*, 2578–2580.
[125] Netzer, R., Stafsnes, M. H., Andreassen, T., Goksoyr, A. et al., Biosynthetic pathway for gamma-cyclic sarcinaxanthin in *Micrococcus luteus*: Heterologous expression and evidence for diverse and multiple catalytic functions of C(50) carotenoid cyclases. *J. Bacteriol.* 2010, *192*, 5688–5699.
[126] Krubasik, P., Kobayashi, M., Sandmann, G., Expression and functional analysis of a gene cluster involved in the synthesis of decaprenoxanthin reveals the mechanisms for C50 carotenoid formation. *Eur. J. Biochem.* 2001, *268*, 3702–3708.
[127] Krubasik, P., Takaichi, S., Maoka, T., Kobayashi, M. et al., Detailed biosynthetic pathway to decaprenoxanthin diglucoside in *Corynebacterium glutamicum* and identification of novel intermediates. *Arch. Microbiol.* 2001, *176*, 217–223.
[128] Heider, S. A., Peters-Wendisch, P., Wendisch, V. F., Carotenoid biosynthesis and overproduction in *Corynebacterium glutamicum*. *BMC Microbiol.* 2012, *12*, 198.
[129] Heider, S. A., Peters-Wendisch, P., Netzer, R., Stafnes, M. et al., Production and glucosylation of C_{40} and C_{50} carotenoids by metabolically engineered *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2014, *98*, 1223–1235.
[130] Tao, L., Yao, H., Cheng, Q., Genes from a *Dietzia* sp. for synthesis of C40 and C50 beta-cyclic carotenoids. *Gene* 2007, *386*, 90–97.
[131] Song, G. H., Kim, S. H., Choi, B. H., Han, S. J., Lee, P. C., Heterologous carotenoid-biosynthetic enzymes: Functional complementation and effects on carotenoid profiles in *Escherichia coli*. *Appl. Environ. Microbiol.* 2013, *79*, 610–618.
[132] Bott, M., Niebisch, A., Respiratory energy metabolism, in: Eggeling, L., Bott, M., (Eds.), *Handbook of Corynebacterium glutamicum*, CRC Press, Boca Raton, USA 2005, pp. 305–332.
[133] Daffé, M., The cell envelope of Corynebacteria, in: Eggeling, L., Bott, M. (Eds.), *Handbook of Corynebacterium glutamicum* CRC Press, Boca Raton, USA 2005, pp. 9–34.
[134] Kalinowski, J., Bathe, B., Bartels, D., Bischoff, N. et al., The complete *Corynebacterium glutamicum* ATCC 13032 genome sequence and its impact on the production of L-aspartate-derived amino acids and vitamins. *J. Biotechnol.* 2003, *104*, 5–25.
[135] Sandmann, G., Yukawa, H., Vitamin synthesis: Carotenoids, biotin and pantothenate, in: Eggeling, L., Bott, M. (Eds.), *Handbook of Corynebacterium glutamicum*, , CRC Press, Boca Raton, USA 2005, pp. 399–417.
[136] Heider, S. A., Peters-Wendisch, P., Beekwilder, J., Wendisch, V. F., IdsA is the major geranylgeranyl pyrophosphate synthase involved in carotenogenesis in *Corynebacterium glutamicum*. *FEBS J.* 2014.
[137] Udaka, S., Screening method for microorganisms accumulating metabolites and its use in the isolation of *Micrococcus glutamicus*. *J. Bacteriol.* 1960, *79*, 745–755.
[138] Peters-Wendisch, P. G., Schiel, B., Wendisch, V. F., Katsoulidis, E. et al., Pyruvate carboxylase is a major bottleneck for glutamate and lysine production by *Corynebacterium glutamicum*. *J. Mol. Microbiol. Biotechnol.* 2001, *3*, 295–300.
[139] Peters-Wendisch, P., Stansen, K. C., Gotker, S., Wendisch, V. F., Biotin protein ligase from *Corynebacterium glutamicum*: Role for growth and L-lysine production. *Appl. Microbiol. Biotechnol.* 2012, *93*, 2493–2502.
[140] Hatakeyama, K., Kobayashi, M., Yukawa, H., Analysis of biotin biosynthesis pathway in coryneform bacteria: *Brevibacterium flavum*. *Methods Enzymol.* 1997, *279*, 339–348.
[141] Agarwal, V., Lin, S., Lukk, T., Nair, S. K., Cronan, J. E., Structure of the enzyme-acyl carrier protein (ACP) substrate gatekeeper complex required for biotin synthesis. *Proc Natl. Acad. Sci. U.S.A.* 2012, *109*, 17406–17411.
[142] Lin, S., Cronan, J. E., Closing in on complete pathways of biotin biosynthesis. *Mol. Biosyst.* 2011, *7*, 1811–1821.
[143] Ikeda, M., Miyamoto, A., Mutoh, S., Kitano, Y. et al., Development of biotin-prototrophic and -hyperauxotrophic *Corynebacterium glutamicum* strains. *Appl. Environ. Microbiol.* 2013, *79*, 4586–4594.
[144] Feher, T., Papp, B., Pal, C., Posfai, G., Systematic genome reductions: Theoretical and experimental approaches. *Chem. Rev.* 2007, *107*, 3498–3513.
[145] Mampel, J., Buescher, J. M., Meurer, G., Eck, J., Coping with complexity in metabolic engineering. *Trends Biotechnol.* 2013, *31*, 52–60.

[146] Manabe, K., Kageyama, Y., Morimoto, T., Ozawa, T. et al., Combined effect of improved cell yield and increased specific productivity enhances recombinant enzyme production in genome-reduced *Bacillus subtilis* strain MGB874. *Appl. Environ. Microbiol.* 2011, *77*, 8370–8381.

[147] Leprince, A., de Lorenzo, V., Voller, P., van Passel, M. W., Martins dos Santos, V. A., Random and cyclical deletion of large DNA segments in the genome of *Pseudomonas putida*. *Environ. Microbiol.* 2012, *14*, 1444–1453.

[148] Hashimoto, M., Ichimura, T., Mizoguchi, H., Tanaka, K. et al., Cell size and nucleoid organization of engineered *Escherichia coli* cells with a reduced genome. *Mol. Microbiol.* 2005, *55*, 137–149.

[149] Ikeda, M., Nakagawa, S., The *Corynebacterium glutamicum* genome: Features and impacts on biotechnological processes. *Appl Microbiol Biotechnol* 2003, *62*, 99–109.

[150] Wendisch, V. F., Genome-wide expression analysis in *Corynebacterium glutamicum* using DNA microarrays. *J. Biotechnol.* 2003, *104*, 273–285.

[151] Pfeifer-Sancar, K., Mentz, A., Ruckert, C., Kalinowski, J., Comprehensive analysis of the *Corynebacterium glutamicum* transcriptome using an improved RNAseq technique. *BMC Genomics* 2013, *14*, 888.

[152] Schaffer, S., Burkovski, A., Proteomics, in: Eggeling, L., Bott, M. (Eds.), *Handbook of Corynebacterium glutamicum*, CRC Press, Boca Raton, Florida, USA 2005, pp. 99–118.

[153] Suzuki, N., Okayama, S., Nonaka, H., Tsuge, Y. et al., Large-scale engineering of the *Corynebacterium glutamicum* genome. *Appl. Environ. Microbiol.* 2005, *71*, 3369–3372.

[154] Suzuki, N., Tsuge, Y., Inui, M., Yukawa, H., Cre/loxP-mediated deletion system for large genome rearrangements in *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2005, *67*, 225–233.

[155] Suzuki, N., Nonaka, H., Tsuge, Y., Inui, M., Yukawa, H., New multiple-deletion method for the *Corynebacterium glutamicum* genome, using a mutant lox sequence. *Appl. Environ. Microbiol.* 2005, *71*, 8472–8480.

[156] Posfai, G., Plunkett, G., 3rd, Feher, T., Frisch, D. et al., Emergent properties of reduced-genome *Escherichia coli*. *Science* 2006, *312*, 1044–1046.

[157] Kortmann, M., Kuhl, V., Klaffl, S., Bott, M., A chromosomally encoded T7 RNA polymerase-dependent gene expression system for *Corynebacterium glutamicum*: Construction and comparative evaluation at the single-cell level. *Microb. Biotechnol.* 2015, *8*, 253–265.

[158] Hara, Y., Kadotani, N., Izui, H., Katashkina, J. I. et al., The complete genome sequence of *Pantoea ananatis* AJ13355, an organism with great biotechnological potential. *Appl. Microbiol. Biotechnol.* 2012, *93*, 331–341.

[159] Udaka, S., Screening method for microorganisms accumulating metabolites and its use in the isolation of *Micrococcus glutamicus*. *J. Bacteriol.* 1960, *79*, 754–755.

[160] Dietrich, J. A., McKee, A. E., Keasling, J. D., High-throughput metabolic engineering: Advances in small-molecule screening and selection. *Annu. Rev. Biochem.* 2010, *79*, 563–590.

[161] Michener, J. K., Thodey, K., Liang, J. C., Smolke, C. D., Applications of genetically-encoded biosensors for the construction and control of biosynthetic pathways. *Metab. Eng.* 2012, *14*, 212–222.

[162] Zhang, F., Keasling, J., Biosensors and their applications in microbial metabolic engineering. *Trends Microbiol.* 2011, *19*, 323–329.

[163] Lange, C., Mustafi, N., Frunzke, J., Kennerknecht, N. et al., Lrp of *Corynebacterium glutamicum* controls expression of the *brnFE* operon encoding the export system for L-methionine and branched-chain amino acids. *J. Biotechnol.* 2012, *158*, 231–241.

[164] Kennerknecht, N., Sahm, H., Yen, M. R., Patek, M. et al., Export of L-isoleucine from *Corynebacterium glutamicum*: A two-gene-encoded member of a new translocator family. *J. Bacteriol.* 2002, *184*, 3947–3956.

[165] Mustafi, N., Grunberger, A., Kohlheyer, D., Bott, M., Frunzke, J., The development and application of a single-cell biosensor for the detection of L-methionine and branched-chain amino acids. *Metab. Eng.* 2012, *14*, 449–457.

[166] Binder, S., Schendzielorz, G., Stabler, N., Krumbach, K. et al., A high-throughput approach to identify genomic variants of bacterial metabolite producers at the single-cell level. *Genome Biol.* 2012, *13*, R40.

[167] Siedler, S., Schendzielorz, G., Binder, S., Eggeling, L. et al., SoxR as a single-cell biosensor for NADPH-consuming enzymes in *Escherichia coli*. *ACS Synth. Biol.* 2014, *3*, 41–47.

[168] Siedler, S., Lindner, S. N., Bringer, S., Wendisch, V. F., Bott, M., Reductive whole-cell biotransformation with *Corynebacterium glutamicum*: Improvement of NADPH generation from glucose by a cyclized pentose phosphate pathway using *pfkA* and *gapA* deletion mutants. *Appl. Microbiol. Biotechnol.* 2013, *97*, 143–152.

[169] Schrumpf, B., Eggeling, L., Sahm, H., Isolation and prominent characteristics of an L-lysine hyperproducing strain of *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 1992, *37*, 566–571.

[170] Chen, Z., Bommareddy, R. R., Frank, D., Rappert, S., Zeng, A. P., Deregulation of feedback inhibition of phosphoenolpyruvate carboxylase for improved lysine production in *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 2014, *80*, 1388–1393.

[171] Chen, Z., Meyer, W., Rappert, S., Sun, J., Zeng, A. P., Coevolutionary analysis enabled rational deregulation of allosteric enzyme inhibition in *Corynebacterium glutamicum* for lysine production. *Appl. Environ. Microbiol.* 2011, *77*, 4352–4360.

[172] Bommareddy, R. R., Chen, Z., Rappert, S., Zeng, A. P., 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*, 30–37.

[173] Wendisch, V. F., Bott, M., Kalinowski, J., Oldiges, M., Wiechert, W., Emerging *Corynebacterium glutamicum* systems biology. *J. Biotechnol.* 2006, *124*, 74.

[174] Takors, R., Bathe, B., Rieping, M., Hans, S. et al., Systems biology for industrial strains and fermentation processes-example: Amino acids. *J. Biotechnol.* 2007, *129*, 181–190.

[175] Woo, H. M., Park, J. B., Recent progress in development of synthetic biology platforms and metabolic engineering of *Corynebacterium glutamicum*. *J. Biotechnol.* 2014, *180*, 43–51.

[176] Park, J. H., Lee, S. Y., Towards systems metabolic engineering of microorganisms for amino acid production. *Curr. Opin. Biotechnol.* 2008, *19*, 454–460.

[177] Lee, S. Y., Lee, D. Y., Kim, T. Y., Systems biotechnology for strain improvement. *Trends Biotechnol.* 2005, *23*, 349–358.

[178] Neshat, A., Mentz, A., Ruckert, C., Kalinowski, J., Transcriptome sequencing revealed the transcriptional organization at ribosome-mediated attenuation sites in *Corynebacterium glutamicum* and identified a novel attenuator involved in aromatic amino acid biosynthesis. *J. Biotechnol.* 2014, *190*, 55–63.

[179] Voges, R., Corsten, S., Wiechert, W., Noack, S., Absolute quantification of *Corynebacterium glutamicum* glycolytic and anaplerotic enzymes by QconCAT. *J Proteomics* 2015, *113*, 366–377.

[180] Reimer, L. C., Spura, J., Schmidt-Hohagen, K., Schomburg, D., High-throughput screening of a *Corynebacterium glutamicum* mutant library on genomic and metabolic level. *PLoS One* 2014, *9*, e86799.

[181] Zhou, L. B., Zeng, A. P., Exploring lysine riboswitch for metabolic flux control and improvement of L-lysine synthesis in *Corynebacterium glutamicum*. *ACS Synth Biol* 2015, *4*, 729–734.

[182] Dusny, C., Grunberger, A., Probst, C., Wiechert, W. et al., Technical bias of microcultivation environments on single-cell physiology. *Lab Chip* 2015, *15*, 1822–1834.

[183] Barriuso-Iglesias, M., Barreiro, C., Flechoso, F., Martin, J. F., Transcriptional analysis of the F_0F_1 ATPase operon of *Corynebacterium glutamicum* ATCC 13032 reveals strong induction by alkaline pH. *Microbiology* 2006, *152*, 11–21.

[184] Barreiro, C., Nakunst, D., Huser, A. T., de Paz, H. D. et al., Microarray studies reveal a 'differential response' to moderate or severe heat shock of the HrcA- and HspR-dependent systems in *Corynebacterium glutamicum*. *Microbiology* 2009, *155*, 359–372.

[185] Patek, M., Holatko, J., Busche, T., Kalinowski, J., Nesvera, J., *Corynebacterium glutamicum* promoters: A practical approach. *Microb. Biotechnol.* 2013, *6*, 103–117.

[186] Jojima, T., Fujii, M., Mori, E., Inui, M., Yukawa, H., Engineering of sugar metabolism of *Corynebacterium glutamicum* for production of amino acid l-alanine under oxygen deprivation. *Appl. Microbiol. Biot.* 2010, *87*, 159–165.

[187] Mizukami, T., Hamu, A., Ikeda, M., Oka, T., Katsumata, R., Cloning of the ATP phosphoribosyl transferase gene of *Corynebacterium glutamicum* and application of the gene to L-histidine production. *Biosci. Biotechnol. Biochem.* 1994, *58*, 635–638.

[188] Vogt, M., Haas, S., Klaffl, S., Polen, T. et al., Pushing product formation to its limit: Metabolic engineering of *Corynebacterium glutamicum* for L-leucine overproduction. *Metab. Eng.* 2014, *22*, 40–52.

[189] Becker, J., Zelder, O., Hafner, S., Schroder, H., Wittmann, C., From zero to hero-design-based systems metabolic engineering of *Corynebacterium glutamicum* for L-lysine production. *Metab. Eng.* 2011, *13*, 159–168.

[190] Niimi, S., Suzuki, N., Inui, M., Yukawa, H., Metabolic engineering of 1,2-propanediol pathways in *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 2011, *90*, 1721–1729.

[191] Zahoor, A., Lindner, S. N., Wendisch, V. F., Metabolic engineering of *Corynebacterium glutamicum* aimed at alternative carbon sources and new products. *Comput. Struct. Biotechnol. J.* 2012, *3*, e201210004.

[192] Okino, S., Suda, M., Fujikura, K., Inui, M., Yukawa, H., Production of D-lactic acid by *Corynebacterium glutamicum* under oxygen deprivation. *Appl. Microbiol. Biotechnol.* 2008, *78*, 449–454.

Cover illustration

Special Issue: ESBES. This issue of BTJ highlights a selection of articles presented at the 10th European Symposium on Biochemical Engineering Sciences (ESBES), which was held in Lille, France, September 7–10, 2014. The issue is edited by Guilherme Ferreira and Philippe Jacques and includes articles on metabolic engineering, protein expression and bioprocess development. The cover shows the Grande Place in Lille. © bbsferrari – Fotolia.com

Biotechnology Journal – list of articles published in the August 2015 issue.

Research Article
Fermentation broth components influence droplet coalescence and hinder advanced biofuel recovery during fermentation
Arjan S. Heeres, Karin Schroën, Joseph J. Heijnen, Luuk A. M. van der Wielen and Maria C. Cuellar
http://dx.doi.org/10.1002/biot.201400570

Research Article
Modeling leucine's metabolic pathway and knockout prediction improving the production of surfactin, a biosurfactant from *Bacillus subtilis*
François Coutte, Joachim Niehren, Debarun Dhali, Mathias John, Cristian Versari and Philippe Jacques
http://dx.doi.org/10.1002/biot.201400541

Research Article
A xeno-free microcarrier-based stirred culture system for the scalable expansion of human mesenchymal stem/stromal cells isolated from bone marrow and adipose tissue
Joana G. Carmelo, Ana Fernandes-Platzgummer, Maria Margarida Diogo, Cláudia Lobato da Silva and Joaquim M. S. Cabral
http://dx.doi.org/10.1002/biot.201400586

Research Article
Dynamic flux balancing elucidates NAD(P)H production as limiting response to furfural inhibition in *Saccharomyces cerevisiae*
Pornkamol Unrean and Carl J. Franzen
http://dx.doi.org/10.1002/biot.201400833

Research Article
Newly designed and validated impedance spectroscopy setup in microtiter plates successfully monitors viable biomass online
Bettina Luchterhand, Jannis Nolten, Sadik Hafizovic, Tino Schlepütz, Sandra Janine Wewetzer, Elke Pach, Kristina Meier, Georg Wandrey and Jochen Büchs
http://dx.doi.org/10.1002/biot.201400534

Research Article
A versatile, non genetically modified organism (GMO)-based strategy for controlling low-producer mutants in *Bordetella pertussis* cultures using antigenic modulation
Philippe Goffin, Thomas Slock, Vincent Smessaert, Philippe De Rop and Philippe Dehottay
http://dx.doi.org/10.1002/biot.201400539

Research Article
Maximizing the utilization of *Laminaria japonica* as biomass via improvement of alginate lyase activity in a two-phase fermentation system
Yuri Oh, Xu Xu, Ji Young Kim and Jong Moon Park
http://dx.doi.org/10.1002/biot.201400860

Research Article
Improving recombinant protein production in the *Chlamydomonas reinhardtii* chloroplast using vivid Verde Fluorescent Protein as a reporter
Stephanie Braun-Galleani, Frank Baganz and Saul Purton
http://dx.doi.org/10.1002/biot.201400566

Research Article
Regulation of the NADH pool and NADH/NADPH ratio redistributes acetoin and 2,3-butanediol proportion in *Bacillus subtilis*
Teng Bao, Xian Zhang, Xiaojing Zhao, Zhiming Rao, Taowei Yang and Shangtian Yang
http://dx.doi.org/10.1002/biot.201400577

Research Article
Engineering a branch of the UDP-precursor biosynthesis pathway enhances the production of capsular polysaccharide in *Escherichia coli* O5:K4:H4
Donatella Cimini, Elisabetta Carlino, Alfonso Giovane, Ottavia Argenzio, Ileana Dello Iacono, Mario De Rosa and Chiara Schiraldi
http://dx.doi.org/10.1002/biot.201400602

Research Article
Phenotypic variability in bioprocessing conditions can be tracked on the basis of on-line flow cytometry and fits to a scaling law
Jonathan Baert, Romain Kinet, Alison Brognaux, Anissa Delepierre, Samuel Telek, Søren J. Sørensen, Leise Riber, Patrick Fickers and Frank Delvigne
http://dx.doi.org/10.1002/biot.201400537