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REVIEW

Updates on industrial production of amino acids using *Corynebacterium glutamicum*

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Abstract L-Amino acids find various applications in biotechnology. L-Glutamic acid and its salts are used as flavor enhancers. Other L-amino acids are used as food or feed additives, in parenteral nutrition or as building blocks for the chemical and pharmaceutical industries. L-amino acids are synthesized from precursors of central carbon metabolism. Based on the knowledge of the biochemical pathways microbial fermentation processes of food, feed and pharma amino acids have been developed. Production strains of *Corynebacterium glutamicum*, which has been used safely for more than 50 years in food biotechnology, and *Escherichia coli* are constantly improved using metabolic engineering approaches. Research towards new processes is ongoing. Fermentative production of L-amino acids in the million-ton-scale has shaped modern biotechnology and its markets continue to grow steadily. This review focusses on recent achievements in strain development for amino acid production including the use of CRISPRi/dCas9, genome-reduced strains, biosensors and synthetic pathways to enable utilization of alternative carbon sources.

Keywords L-Glutamate · L-Lysine · GABA · L-Ornithine · L-Proline · L-Arginine · L-Citrulline · CRISPRi-dCas9 · Genome-reduced strains · Biosensors · Flexible feedstock concept

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Introduction

This review summarizes recent technological achievements in *C. glutamicum* strain development for amino acid production (Fig. 1) and applications to broadening the substrate spectrum of this bacterium to improve production of glutamate-family amino acids. *C. glutamicum* strain development has benefited from target gene identification by CRISPR interference, from riboswitch-based control of biosynthetic genes, from transcription factor-based screening and from synthetic biology approaches such as genome reduction to generate chassis strains. Moreover, this review highlights recent developments of metabolic engineering efforts for production of L-ornithine, L-citrulline, L-arginine, L-proline and gamma-aminobutyrate (GABA) as well as for access to alternative carbon sources.

CRISPRi/dCas9-based engineering of L-lysine producing strains

C. glutamicum is used for the industrial production of L-glutamate, L-lysine and other amino acids. Strain development and improvement make use of classical mutagenesis and selection or screening (sometimes combined with omics analysis) and rational approaches such as metabolic engineering. Target identification often involves various parallel omics techniques, while target verification relies on more tedious methods such as deletion mutagenesis, which depends on rare homologous double-crossover events. Recently, CRISPR interference (CRISPRi) technology with the deactivated Cas9 protein (dCas9) was used to determine the effects of target gene repression (Fig. 1) on L-lysine and L-glutamate production (Cleto et al. 2016). Effects on amino acid production due to CRISPRi/dCas9-mediated

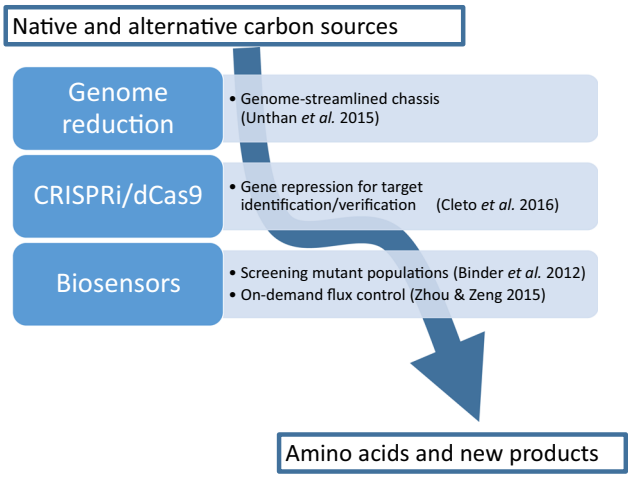

Fig. 1 Technologies recently applied in *C. glutamicum* strain development. Selected examples comprise genome reduction (Unthan et al. 2015b), riboswitch-based biosensors (Zhou and Zeng 2015a), transcriptional regulator-based biosensors (Binder et al. 2012) and CRISPRi/dCas9 gene repression (Cleto et al. 2016)

target gene repression (*pgi, pck,* and *pyk* encoding phosphoglucoisomerase, PEP carboxykinase, and pyruvate kinase, respectively) were comparable to levels achieved by target gene deletion (Cleto et al. 2016). This fast approach may potentially be operated in parallel for many target genes.

On-demand flux engineering of L-lysine producing strains

An intracellular riboswitch-based L-lysine biosensor has been developed to control gene expression according to the metabolic demand (Fig. 1). Since *C. glutamicum* lacks a L-lysine riboswitch, the L-lysine riboswitches of *E. coli* and *B. subtilis* were used as L-lysine biosensors to control the citrate synthase gene *gltA* (Eikmanns et al. 1994) in L-lysine producing *C. glutamicum* strains (Zhou and Zeng 2015b). Previously, a low citrate synthase flux has been shown to improve L-lysine production (van Ooyen et al. 2012). At low intracellular L-lysine concentrations the riboswitch maintains a confirmation that allows free access of the ribosome binding site of *gltA* and citrate synthase is synthesized. At L-lysine concentrations exceeding 25–100 μM, the L-lysine riboswitch changes its conformation and in consequence the *gltA* ribosome binding site is masked and *gltA* translation ceases. Thus, a L-lysine-dependent translational OFF-switch was used for negative on-demand control of citrate synthase (Zhou and Zeng 2015b).

Positive on-demand control of L-lysine export was achieved by constructing a synthetic L-lysine ON-switch controlling translation of L-lysine export gene *lysE* (Zhou and Zeng 2015a). When the L-lysine concentration exceeded 0.1 mM, the *lysE* ribosome binding site was de-masked and *lysE* translation initiated. This increased the L-lysine yield on glucose by 89 % (Zhou and Zeng 2015a). Combining the OFF-switch to reduce *gltA* translation and the L-lysine ON-switch to increase L-lysine efflux further improved the L-lysine yield on glucose (Zhou and Zeng 2015a).

Repurposing natural genetic control circuits as shown for the lysine riboswitches may be extended to other genetic control mechanisms and in particular may include on-demand flux control by amino acid-responsive transcriptional regulators since these were also used as biosensors in strain screening.

Intracellular amino acid sensors in *C. glutamicum*

In *C. glutamicum*, two amino acid-responsive transcriptional regulators, Lrp (Mustafi et al. 2012) and the L-lysine regulator LysG (Binder et al. 2012), have been used to develop genetically-encoded biosensors (Fig. 1). Transcriptional fusions of one of their target promoters with a promoterless fluorescence reporter gene (Mustafi et al. 2014) is suitable to monitor intracellular amino acid concentrations of millions of single cells in fluorescence activated cell screening/sorting (FACS).

The transcriptional activator Lrp was used in a biosensor for development of strains overproducing branched-chain amino acids or L-methionine (Mustafi et al. 2012). At elevated intracellular concentrations of L-valine, L-leucine, L-isoleucine or L-methionine, Lrp activates the expression of the *brnFE* operon, which encodes an export system for these amino acids (Lange et al. 2012; Trotschel et al. 2005). The transcriptional fusion of the *brnF* promoter with promoterless *gfp* allowed intracellular detection and quantification of these amino acids (Mustafi et al. 2012) and led to improved L-valine producing strains (Mustafi et al. 2014).

The LysR-type transcriptional regulator LysG activates transcription of *lysE*, the gene for the L-lysine exporter (Vrljic et al. 1996), at elevated intracellular concentrations of L-lysine and basic amino acids in general (Bellmann et al. 2001). The L-lysine biosensor approach was used to screen populations of cells either expressing variants of *murE*-encoded UDP-*N*-acetylmuramoy-*L*-alanyl-D-glutamate:meso-diaminopimelate ligase (Binder et al. 2013) or *lysC*-encoded aspartate kinase (Schendzielorz et al. 2014). As the almost linear relationship between intracellular L-lysine concentration and biosensor response plateaus at about 25 mM (Binder et al. 2012), the dynamic range of the biosensor system needs to be expanded.

Genome-reduction of *C. glutamicum* wild type and a L-lysine producing strain

Bacteria have evolved in Nature to cope with changing conditions in their habitats (e.g. temperature, pH, etc.). By contrast, bacteria used as production hosts in biotechnology face more stable environments under production conditions and, thus, may benefit if freed from the metabolic burden of enzymes and genes not relevant under these conditions (e.g. for maintaining intracellular pH). Moreover, reduced metabolic and regulatory complexity may be beneficial for engineering bacterial hosts (Feher et al. 2007; Mampel et al. 2013).

For biotechnological applications, achieving a minimal genome, i.e. the smallest genome required for survival under optimal conditions, is not helpful since multiple auxotrophies (e.g. for amino acids and vitamins) preclude cost-efficient fermentation processes. Biotechnologically relevant genome-reduced chassis organisms have to retain robust growth and production characteristics under defined cultivation conditions. These conditions and characteristics have to be defined prior to initiating a genome reduction project.

Genome reduction by a bottom-up strategy, i.e. creating the entire genome from scratch (de Lorenzo 2015), has not been applied for biotechnologically relevant microorganisms. By contrast, genome reduction by a top-down strategy (Fig. 1), i.e. by gradually reducing genome size in either a targeted or untargeted manner, has been used and the genomes of biotechnologically relevant bacteria have been reduced by 7–22 % (Hashimoto et al. 2005; Leprince et al. 2012; Manabe et al. 2011). The relatively small genome of *C. glutamicum* strain R has been reduced by 5.7 % by deletion of 188 open reading frames in eight regions not shared with the wild-type *C. glutamicum* strain ATCC 13032 (Suzuki et al. 2005). The genome of the *C. glutamicum* wild-type strain ATCC 13032 was reduced by 6 % by deletion of prophage sequences (Baumgart et al. 2013). The resulting strain, MB001, did not show unfavorable properties under the previously defined growth conditions. Moreover, MB001 showed no defects under stress conditions and exhibited increased transformation efficiency and plasmid copy number. Subsequently, the genome-reduced *C. glutamicum* strain MB001 was engineered for the production of the carotenoids lycopene, decaprenoxanthin and astaxanthin (Heider et al. 2014), and the amino acids L-citrulline with a yield on glucose of 0.38 ± 0.01 g g^{-1} (Eberhardt et al. 2014). Similarly, MB001-based strains for the production of the amino acids L-ornithine, L-arginine, and L-proline and the diamine putrescine were developed (Jensen et al. 2015). High-level and uniform protein overproduction was achieved by introducing the DE3 region of *E. coli* BL21(DE3) harboring the T7 RNA polymerase gene under control of the lacUV5 promoter into the chromosome of *C. glutamicum* MB001 (Kortmann et al. 2015).

Corynebacterium glutamicum MB001 was also the starting point for further genome reduction. Several *omics* data sets, evolutionary considerations, and prior knowledge on the function of genes were used to classify all genes of *C. glutamicum* with respect to their essentiality and importance for maintaining fast growth in a previously defined medium. 36 of 41 gene clusters with a high content of non-essential or unclassifiable genes could be deleted (Unthan et al. 2015b). Genome reduction was not only reported for *C. glutamicum* wild-type strain ATCC 13032, but also for a L-lysine-producing model strain (Unthan et al. 2015a, b). The genome-reduced strains were phenotyped in multi-well plates in a mini pilot plant allowing for automatic separation of supernatants from biomass in a robotic workflow (Rohe et al. 2012; Unthan et al. 2015b). After at-line clarification, amino acids were quantified using the ninhydrin assay and the genome-reduced L-lysine producer strains were categorized with respect to growth rate and amino acid titer (Unthan et al. 2015b).

Production of the L-glutamate-derived amino acids L-ornithine, L-citrulline, L-arginine, L-proline and gamma-aminobutyrate (GABA)

Corynebacterium glutamicum, a bacterium discovered in 1956 for its natural capacity to accumulate L-glutamate extracellularly (Kinoshita et al. 2004), is currently used for the industrial production of L-glutamate (about 3 million tons in 2014) and, thus, is considered an excellent host for the production of glutamate-derived amino acids (Wendisch 2014) such as L-ornithine, L-citrulline, L-arginine, L-proline and GABA (Table 1).

L-Arginine biosynthesis (Fig. 2) initiates with L-glutamate and proceeds via the intermediates L-ornithine and L-citrulline. L-Ornithine is a non-essential amino acid that can be used in the food industry as a dietary supplement (Kim et al. 2015c) or in the pharmaceutical industry e.g. in the treatment of liver disease (Salvatore et al. 1964) or wound healing (Shi et al. 2002). A number of groups engineered *C. glutamicum* for L-ornithine production (Hwang et al. 2008; Jensen et al. 2015; Kim et al. 2015a, c; Matsui and Oikawa 2010; Schneider et al. 2011). L-ornithine is secreted as result of interrupting L-arginine biosynthesis by deletion of the ornithine carbamoyltransferase gene *argF*. Deletion of the arginine repressor gene *argR* leads to de-repression of the L-arginine biosynthesis genes. The pacemaker enzyme *N*-acetylglutamate kinase is alleviated from feedback inhibition by L-arginine by introducing point mutations into its

Table 1 Titer, yields and volumetric productivities for recently engineered *C. glutamicum* strains overproducing selected amino acids

Amino acid	Titer (g L^{-1})	Yield (g g^{-1})	Volumetric productivity (g L^{-1} h^{-1})	References
L-Ornithine	51.5	0.24	1.29	Kim et al. (2015c)
	20.8	0.52	0.46	Jensen et al. (2015)
L-Citrulline	7.7	0.38	0.32	Eberhardt et al. (2014)
L-Arginine	92.5	0.40	1.28	Park et al. (2014)
L-Proline	12.7	0.36	0.42	Jensen and Wendisch (2013)
GABA	38.6	0.10	0.54	Choi et al. (2015)

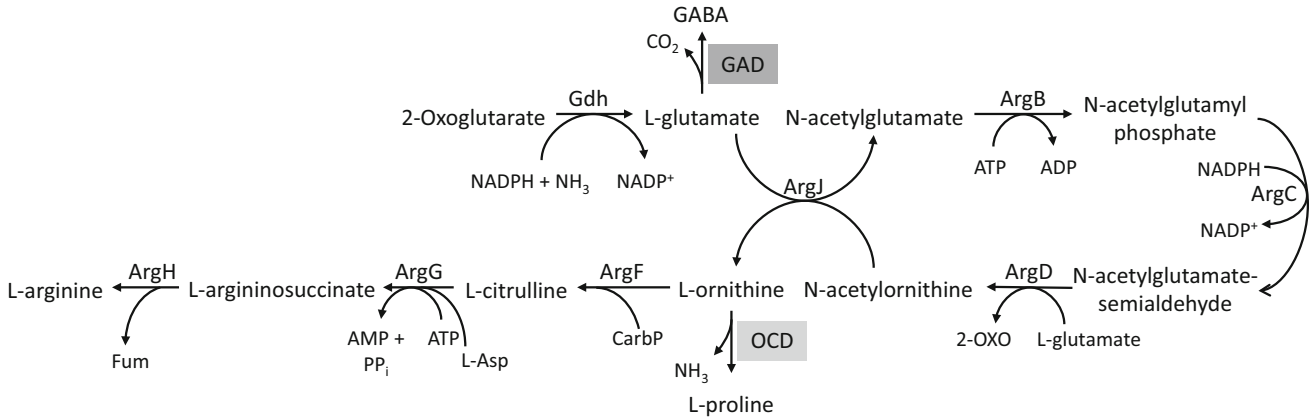

Fig. 2 Biosynthetic pathways of the L-glutamate derived amino acids L-ornithine, L-citrulline, L-arginine, L-proline and gamma-aminobutyrate. Heterologous reactions are highlighted in *boxes*. Abbreviations: Gdh, glutamate dehydrogenase; ArgB, *N*-acetylglutamate kinase; ArgC, *N*-acetyl-gamma-glutamyl-phosphate reductase; ArgD, *N*-acetylornithine aminotransferase; ArgJ, ornithine acetyltransferase; ArgF, ornithine carbamoyltransferase; ArgG, argininosuccinate synthetase; ArgH, argininosuccinate lyase; GAD, glutamate decarboxylase (*Escherichia coli*); OCD, ornithine cyclodeaminase (*Pseudomonas putida*); 2-OXO, 2-oxoglutaric acid; L-glut, L-glutamic acid; CarP, carbamoyl phosphate; L-Asp, L-aspartic acid; Fum, fumaric acid; GABA, gamma-aminobutyrate

gene, *argB*. Supply of L-glutamate and of NADPH for L-ornithine production was improved by increased promoter activity of L-glutamate dehydrogenase gene *gdh* and by decreased expression of *pgi*, which encodes the key enzyme phosphoglucoisomerase from the oxidative pentose phosphate pathway. After combining these changes with introduction of a second copy of the arginine biosynthesis operon in the genome-streamlined strain MB001 L-ornithine yields of about 0.52 g ornithine g^{-1} glucose were achieved (Jensen et al. 2015). Kim et al. 2015c reported an L-ornithine titer of 51.5 g L^{-1} and a volumetric productivity of 1.29 g L^{-1} h^{-1} (Kim et al. 2015c).

L-Citrulline is an intermediate of arginine biosynthesis (Fig. 2) derived from L-ornithine by ornithine carbamoyltransferase. L-Citrulline plays an important role in human health and nutrition (Eberhardt et al. 2014; Hao et al. 2015; Ikeda et al. 2009). L-citrulline is excreted as consequence of deletion of *argG,* which codes for argininosuccinate synthetase, the enzyme converting L-citrulline to *N*-argininosuccinate (Hao et al. 2015). Engineering an L-ornithine producing strain to an efficient L-citrulline producing strain required the overexpression of *argF* as well as deletion of *argG*. An L-citrulline titer of about 44 mM L-citrulline and a yield of 0.38 g L-citrulline g^{-1} glucose were achieved (Eberhardt et al. 2014).

L-Arginine is a semi-essential amino acid, i.e. there is no dietary requirement for healthy adults, but for infants and growing children or adults under catabolic stress or with dysfunction of the small intestine or kidney (Flynn et al. 2002), and it has applications in food, cosmetics and pharmaceutical industries (Ikeda et al. 2009; Park et al. 2014). Rationally engineered L-arginine producing strains were constructed in a similar manner as L-citrulline and L-ornithine producing strains, however, L-citrulline was a side-product of L-arginine production (Ikeda et al. 2009; Schneider et al. 2011; Jensen et al. 2015). This limitation was overcome by random mutagenesis and screening (Park et al. 2014). L-Arginine titers of about 90 g L^{-1} were achieved.

L-Proline is a secondary proteinogenic amino acid which finds applications in the feed, pharmaceutical or cosmetic industries (Jensen and Wendisch 2013). In a widespread biosynthetic pathway leading to L-proline, L-glutamate is converted to L-proline in three enzyme-catalysed and one spontaneous reaction (Eggeling and Bott 2005; Jensen and

Wendisch 2013). Heterologous expression of the ornithine cyclodeaminase gene *ocd* from *Pseudomonas putida* in an L-ornithine production strain (Fig. 2) enabled production of about 13 g L^{-1} of proline with a yield of 0.36 g proline g^{-1} glucose (Jensen and Wendisch 2013).

GABA is a non-protein amino acid that can be used in the pharmaceutical and food industries. Moreover, the lactamization of GABA yields 2-pyrrolidone, which can be converted chemically to the bio-plastic polyamide 4 (Kawasaki et al. 2005; Park et al. 2013). L-Glutamate can be decarboxylated to GABA by glutamate decarboxylase (Fig. 2) and heterologous expression of glutamate decarboxylase genes usually from *Escherichia coli* or *Lactobacillus brevis* resulted in GABA production under conditions triggering L-glutamate production (Choi et al. 2015; Okai et al. 2014; Shi et al. 2013; Takahashi et al. 2012). GABA titers of about 39 g L^{-1} were achieved in fed-batch cultures (Choi et al. 2015).

Utilization of alternative carbon source derived from complex polymers

Corynebacterium glutamicum utilizes a relative broad spectrum of carbon sources for its growth and energy supply, including e.g. the monosaccharides glucose, fructose and ribose as well as the disaccharides sucrose and maltose (Blombach and Seibold 2010). However, *C. glutamicum* wild type is neither able to catabolise a number of polymeric carbon sources (e.g. starch, cellulose, hemicellulose, lignocellulose or chitin) nor their monomeric constituents such as xylose, arabinose, or *N*-acetylglucosamine. To realize a flexible feedstock concept (Table 2), *C. glutamicum* was engineered to catabolize e.g. starch, xylose, arabinose, glycerol or levoglucosan by heterologous gene expression (Kim et al. 2015b; Zahoor et al. 2012). Notably, when present in blends *C. glutamicum* utilizes many carbon sources simultaneously (Blombach and Seibold 2010; Gerstmeir et al. 2003), an ideal characteristic when considering hydrolysates of complex carbon sources such as lignocellulose. Substrate co-utilization is typically also observed for *C. glutamicum* strains engineered to catabolize non-native carbon sources (Gopinath et al. 2011; Meiswinkel et al. 2013; Rittmann et al. 2008; Seibold et al. 2006).

Starch is a major feedstock for industrial amino acid production, however, fermentations are based on glucose derived from enzymatic starch hydrolysis. Heterologous production and secretion of α-amylase from *Streptomyces griseus* in *C. glutamicum* enabled growth with starch as sole or combined carbon source (Seibold et al. 2006). Surface display of α-amylase from *Streptococcus bovis* anchored via *pgsA* from *Bacillus subtilis* enabled starch catabolism as well (Tateno et al. 2007). Nevertheless, complete utilization of starch was not observed since some starch degradation products still remained in the medium (Seibold et al. 2006). Pullulanase (EC 3.2.1.41), a debranching enzyme, enables a complete and efficient conversion of the branched polysaccharides into small fermentable sugars during saccharification process (Hii et al. 2012). Heterologous expression pullulanases has been reported in *E. coli* with expression of *pul3YH5* from *Exiguobacterium acetylicum* YH5 (Qiao et al. 2015) and in *Bacillus subtilis* with expression of *apuA* from *Delsulforococcus muscosus*, whereas heterologous expression of pullulanases in *C. glutamicum* has not yet been reported.

Lignocellulose hydrolysates, which can be obtained from residual plant biomass, support growth of *C. glutamicum* since they contain natural carbon substrates of this bacterium such as cellulose-derived glucose or acetate (Gopinath et al. 2011; Meiswinkel et al. 2013). Efficient conversion of residual plant biomass to value-added products including biofuels or chemicals requires their complete utilization. Lignocellulose contains cellulose (40–50 %), hemicellulose (25–30 %) and lignin (10–20 %) (Anwar et al. 2014; Wyman 1999), and cannot be utilized directly by most microorganisms including *C. glutamicum*. Pre-treatment of lignocellulosic biomass e.g. by alkali/acidic or enzymatic hydrolysis into its monomeric components (Anwar et al. 2014; Rumbold et al. 2010) yields glucose, xylose, arabinose, acetate and galactose as major products of hydrolysis (Aristidou and Penttila 2000). Endoglucanases and exoglucanases depolymerize cellulose incompletely to the disaccharide cellobiose which is subsequently cleaved by β-glucosidase (Schwarz 2001). For an industrially scalable process, heterologous cellulases need to be produced and secreted by the host itself (Adham et al. 2001; Paradis et al. 1987) and synthetic microcellulosomes based on genes from *Clostridium cellulovorans* may increase efficiency (Hyeon et al. 2011; Lambertz et al. 2014). *C. glutamicum* R is able to use cellobiose as a result of a mutation in phosphotransferase permease subunit BglF which imports methyl β-glucoside, arbutin and salicin (Kotrba et al. 2003).

Recently, a combined approach of metabolic engineering and adaptive evolution of *C. glutamicum* ATCC 13032 has been reported with respect to cellobiose utilization as C-source. Genes for a cellodextrin transporter and β-glucosidase were expressed and the resulting strain was evolved. Secreted and cell-displayed forms of β-glucosidase led to complete, but slow consumption of 20 g L^{-1} cellobiose in 4 days (Lee et al. 2016). In a similar approach, 1 g L^{-1} of L-lysine was produced from 20 g L^{-1} of cellobiose after 4 days based on a strain harboring β-glucosidase Sde1394 from *Saccharophagus degradans* (Adachi et al. 2013).

The hemicellulose-derived pentose sugars xylose and arabinose are not natural C-sources for *C. glutamicum*.

Table 2 *C. glutamicum* strains engineered for utilization of selected nonnative carbon sources

Carbon source	Heterologous/endogenous genes (over) expressed	References
Starch	*amyA* (α-amylase) from *Streptomyces griseus*	Seibold et al. (2006)
Cellobiose	Sde1394 (β-glucosidase) from *Saccharophagus degradans*	Eberhardt et al. (2014)
Levoglucosan	EU751287.1 (levoglucosan kinase) from *Lipomyces starkeyi*	Kim et al. (2015b)
Xylose	*xylA* (xylose isomerase) from *Xanthomonas campestris* and endogenous *xylB* (xylulokinase)	Kawaguchi et al. (2006), Meiswinkel et al. (2013)
Arabinose	*araA* (arabinose isomerase), *araB* (ribulokinase), and *araD* (ribulose 5-phosphate 4-epimerase) from *Escherichia coli*	Kawaguchi et al. (2008), Schneider et al. (2011)
Galactose	*galK* (galactokinase), *galT* (UDP-glucose-1-*P*-uridylyltransferase), *galE* (UDP-galactose-4-epimerase) and *galM* (aldose-1-epimerase) from *Lactococcus cremonis*	Barrett et al. (2004)
Glucosamine	Endogenous *nagB* (glucosamine-6-phosphate deaminase)	Uhde et al. (2013)
N-Acetyl-glucosamine	Endogenous *nagB* and *nagA* (*N*-acetylglucosamine-6-phosphate deacetylase) and *nagE* (*N*-acetyl-glucosamine-specific PTS) from *Corynebacterium glycinophilum*	Matano et al. (2014)

Since *C. glutamicum* possesses xylulokinase XylB, heterologous expression of xylose isomerase gene *xylA* from *E. coli* was sufficient to enable growth with xylose as sole carbon source (Kawaguchi et al. 2006). Faster growth was observed when endogenous *xylB* was overexpressed in combination with heterologous expression of *xylA* from *Xanthomonas campestris* (Meiswinkel et al. 2013). Xylose-based production has been described e.g. for L-glutamate and L-lysine (Gopinath et al. 2011; Meiswinkel et al. 2013), cadaverine (Buschke et al. 2011), and putrescine (Meiswinkel et al. 2013). Arabinose utilization was achieved by heterologous expression of the *araBAD* operon of *E. coli*, which encodes arabinose isomerase AraA, ribulokinase AraB, and ribulose 5-phosphate 4-epimerase AraD (Kawaguchi et al. 2008; Schneider et al. 2011). The arabinose uptake system from *C. glutamicum* strain ATCC 31831 was not only useful to accelerate import and catabolism of arabinose, but also utilization of xylose (Sasaki et al. 2009).

Growth with galactose, a component of lignocellulosic hydrolysates, became possible for *C. glutamicum* only via heterologous expression of genes from *Lactococcus lactis subsp. cremoris* MG1363 encoding for galactokinase (*galK*), UDP-glucose-1-*P*-uridylyltransferase (*galT*), UDP-galactose-4-epimerase (*galE*) and aldose-1-epimerase (*galM*) (Barrett et al. 2004).

Lignin is synthesized via radical reactions and is recalcitrant towards degradation. Its constituents are the aromatic coniferyl-, sinapyl- and *p*-coumaryl-alcohols. *C. glutamicum* is able to catabolize a wide spectrum of aromatic compounds, e.g. benzoate, phenol (Shen et al. 2005a), 3-hydroxybenzoate, gentisate (Shen et al. 2005b), and naphthalene (Lee et al. 2010; Shen et al. 2005a). Catabolism of various aromatic compounds via peripheral pathways involving ring modification reactions converges in the formation of the dihydroxylated benzene derivatives gentisate (from naphthalene and 3-hydrobenzoate; (Lee et al. 2010; Shen et al. 2005b), catechol (from benzyl alcohol, benzoate and phenol; (Shen et al. 2004), and protocatechuate (from ferulic acid, vanillin, vanillate, 4-hydroxybenzoate, caffeate, 4-cresol, *p*-coumarate, quinate, shikimate; (Merkens et al. 2005; Qi et al. 2007; Shen et al. 2005b). These key aromatic intermediates are then converted to pyruvate, fumarate or succinyl-CoA after aromatic ring cleavage by the dioxygenases catechol 1,2-dioxygenase, protocatechuate 3,4-dioxygenase, gentisate 1,2-dioxygenase, and hydroxyquinol 1,2-dioxygenase. This metabolic potential of *C. glutamicum* may be used in bioremediation, for bioconversion of aromatic pollutants or for production of amino acids from aromatic feedstocks (Shen et al. 2012).

The amino sugars glucosamine and *N*-acetyl glucosamine, which have the potential to serve as combined carbon and nitrogen sources, are the monomeric components of chitin and chitosan. Chitin, which can be found in exoskeletons of arthropods, in the cell walls of fungi, in the radula of gastropods, is an underutilized feedstock for fermentation. Consequently, *C. glutamicum* strains have been constructed to enable access to glucosamine and to *N*-acetylglucosamine (Matano et al. 2014; Uhde et al. 2013). Glucosamine uptake is mediated by the glucose specific permease PtsG, but fast growth on glucosamine as sole source of carbon and nitrogen required that the endogenous glucosamine-6-phosphate deaminase NagB was overproduced (Uhde et al. 2013). To enable growth of *C. glutamicum* with *N*-acetyl-glucosamine, the *N*-acetyl-glucosamine-specific PTS gene *nagE* from the related *C. glycinophilum* had to be expressed in addition to overexpression of endogenous *nagB* and endogenous *nagA* which encodes the cytoplasmic *N*-acetylglucosamine-6-phosphate deacetylase (Matano et al. 2014). Glucosamine-based as well as *N*-acetyl-glucosamine-based L-lysine production was reported (Matano et al. 2014; Uhde et al. 2013).

Outlook

Since its discovery more than 50 years ago, *C. glutamicum* has been optimized for amino acid production. New technologies and concepts ranging from metabolic engineering, genomics, transcriptomics, metabolomics, fluxomics to systems and synthetic biology have been applied to *C. glutamicum* strain development and developed further. This has also boosted engineering *C. glutamicum* for the production of other value-added products such as diamines, organic acids, carotenoids, proteins and biopolymers. New technologies like the CRISPR/Cas9 system are expected to contribute to improving production of amino acids (Cleto et al. 2016) and other value-added chemicals by the workhorse of industrial biotechnology *C. glutamicum*.

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Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no conflict of interest.

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