Biosystems Design of *Corynebacterium glutamicum* for Bio-Production

Jian Zha[1]*, Zhen Zhao[1], Zhengyang Xiao[2], Thomas Eng[5], Aindrila Mukhopadhyay[5], Mattheos A.G. Koffas[3,4]*, Yinjie J. Tang[2]*

[1]School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, Shaanxi 710021, China

[2]Department of Energy, Environmental and Chemical Engineering, Washington University in Saint Louis, Missouri 63130, USA

[3]Department of Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, New York 12180, USA

[4]Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, New York 12180, USA

[5] Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

*__Correspondence__:

JZ: zhajian1985@sust.edu.cn

MK: koffam@rpi.edu

YJT: yinjie.tang@seas.wustl.edu

Abstract

Corynebacterium glutamicum, a natural glutamate-producing bacterium adopted for industrial production of amino acids, has been extensively explored recently for high-level biosynthesis of amino acid derivatives, bulk chemicals such as organic acids and short-chain alcohols, aromatics, and natural products including polyphenols and terpenoids. Here, we review the recent advances with a focus on biosystem design principles, metabolic characterization and modeling, omics analysis, utilization of non-model feedstock, emerging CRISPR tools for *Corynebacterium* strain engineering, biosensors, and novel strains of *C. glutamicum*. Future research directions for developing *C. glutamicum* cell factories are also discussed.

Keywords

biosystem design, CRISPR, metabolic models, polyphenol, terpenoid.

Introduction

The growing concerns on climate change and energy supply have driven fast development of microbial manufacturing of diverse bioproducts from renewable resources [1-3]. One of the most commonly used industrial microbes is *Corynebacterium glutamicum*, a gram-positive and nonpathogenic bacterium adopted industrially for the production of amino acids. *C. glutamicum* demonstrates several physiological properties advantageous to fermentative production, such as high rates of sugar consumption under either aerobic or anaerobic conditions regardless of cell density, high tolerance to osmotic pressure and various chemicals (including the final products), and capability of simultaneously utilizing mixtures of sugars without carbon catabolite repression [4]. Recently, the product portfolio of this host platform has been expanded substantially to cover organic acids, short-chain alcohols, phenolics, and plant natural products (**Figure 1**), attributed to the elucidation of more physiological information, the establishment of genome-scale models, and the development of sophisticated genetic manipulation tools. In this review, we summarize the latest progress on the engineering of *C. glutamicum*, with a focus on biomanufacturing, utilization of various substrates, emerging approaches of gene editing and metabolic regulation, metabolic modeling and omics analysis, and novel strains of *C. glutamicum*.

Production of primary metabolites, amino acids and amino acid derivatives

C. glutamicum has been applied industrially to produce 17 natural amino acids (except glycine, methionine and aspartate [5-8]) as well as amino acid derivatives such as 5-aminovalerate and polyglutamic acid (PGA) (**Table 1**) [9-11]. The general principles of strain engineering include: (1) introduction of the biosynthetic pathway consisting of

heterologous genes, (2) balancing of the amino acid biosynthetic pathway and the downstream pathway, and (3) deletion or suppression of competing pathways. For example, the heterologous pathway involving genes *davTBA* responsible for aminovaleramide formation from lysine was overexpressed in a lysine-producing *C. glutamicum* strain, followed by expression of various aldehyde reductase orthologues for the generation of 5-hydroxyvaleric acid. The resulting strain achieved a titer of 52 g/L in fed-batch fermentation [12]. Another example is the production of glutaric acid. The L-lysine catabolic pathway from *P. putida* was expressed in *C. glutamicum*, converting L-lysine to glutaric acid, with a titer of 105 g/L [13]. Moreover, *C. glutamicum* metabolism has been studied by ^{13}C-Metabolic Flux Analysis (MFA). The metabolic knowledge led to heterologous expression of transhydrogenase and site-directed mutagenesis of pentose phosphate pathway enzymes to promote co-factor balance and L-methionine production [14]. In addition to amino acids and their derivatives, *C. glutamicum* is an excellent host to synthesize various organic acids (i.e., lactate, succinate, pyruvate, and α-ketoglutarate) [15,16] and short-chain alcohols (**Table 1**) [17].

Biosynthesis of natural products

C. glutamicum is a GRAS (generally regarded as safe) microbe that can produce pharmaceuticals and nutraceuticals. It has a strong shikimate pathway for the synthesis of phenylalanine and tyrosine, which are primary building blocks for polyphenol biosynthesis. Polyphenols usually exhibit antimicrobial properties. *C. glutamicum* is naturally more resistant to polyphenols than *E. coli*, and can even metabolize polyphenols as carbon sources under certain conditions. As a consequence, *C. glutamicum* has been recently engineered to produce diverse subgroups of flavonoid compounds including

naringenin, kaempferol, eriodictyol, and cyanidin 3-*O*-glucoside [18,19]. Moreover, *C. glutamicum* has been employed to produce aromatics, such as indole, protocatechuate, 4-hydroxybenzoate and 4-aminobenzoate (**Figure 1**) [4]. *C. glutamicum* has also been used to synthesize various terpenoids, including astaxanthin, valencene, and lycopene [20]. However, its performance for the biosynthesis of natural products is generally lower than those obtained in *E. coli, S. cerevisiae*, or *Y. lipolytica* [21]. One possible reason is that enzyme expression in *C. glutamicum* leads to insoluble inclusion bodies. To improve the expression of heterologous proteins, the fusion of a soluble peptide tag has been shown to be an effective approach [18].

Utilization of cellulosic sugars and non-model feedstock

C. glutamicum can use glucose, sucrose and fructose but not pentoses [22,23]. Recent research to expand the spectrum of *C. glutamicum* carbon sources targets methanol, chitin, pentoses (xylose and arabinose) from hemicellulosic hydrolysates, galactose and lactose that are abundant in whey-based fermentation media, and glycerol that is a major by-product from the biodiesel industry [24] (**Figure 1**). The relevant strategies for strain engineering toward sugar utilization contain adaptive evolution, introduction of sugar transporters from other microbes, activation of cryptic transporters, and expression of sugar pathway genes for subsequent catabolism [25]. *C. glutamicum* contains an endogenous yet silent glycerol-catabolizing pathway. Earlier attempts regarding glycerol utilization in this bacterium involved activation of the endogenous pathway or introduction of heterologous pathways; however, these methods only led to limited success [26]. A recent study optimized the expression of the heterologous genes involving *glpF* (encoding aquaglyceroporin), *dhaD* (encoding glycerol dehydrogenase),

and *dhaK* (encoding ATP-dependent dihydroxyacetone kinase). The best strain achieved a glycerol utilization rate of 1.34 g/g DCW/h and the maximum specific growth rate of 0.37 h^{-1} with glycerol as the sole carbon source [26].

A consolidated process using starch as the feedstock has been achieved in *C. glutamicum* that lacks hydrolases to decompose starch. Surface display of α-amylase from *Streptococcus bovis* enabled the engineered *C. glutamicum* to degrade starch into glucose, which is then metabolized to produce lysine [27,28]. On the other hand, a co-culture approach has been applied. Through the division of labor [29], the partner strain (α-amylase-producing *E. coli*) is designed to digest starch into glucose, whereas *C. glutamicum* uses glucose to produce value-added chemicals [30].

Recently, new methods have been developed to depolymerize lignin [31]. While a range of molecules can be released from lignin, aromatic molecules such as *para*-coumarate and ferulate are natively catabolized by *C. glutamicum* [32,33]. Therefore, lignocellulosic biomass could release both monomeric sugars and aromatics as feedstock for this organism.

New tools to engineer *C. glutamicum*

Traditional gene knockout or knockin in *C. glutamicum* uses allelic exchange plasmids, which is a multi-step and overall inefficient process. Better gene modifications can be achieved by CRISPR/Cas9 in conjunction with ssDNA-binding repair protein RecT from *E. coli* **(Figure 2)** [34]. Adapting these techniques to *C. glutamicum* has required some optimization; expressing *S. pyogenes* Cas9 alone can generate double-strand breaks that are highly toxic to the cell, thus leading to a low genome editing efficiency especially when Cas9 is expressed constitutively. In contrast, Cas12a

(Cpf1) from *Francisella novicida* is non-toxic and highly efficient in nucleotide modifications with the aid of single-stranded DNA. With Cas12a, a 5' PAM (Protospacer Adjacent Motif) sequence 5′-NYTV-3′ preceding a 21 bp targeting spacer sequence can introduce double-strand breaks [35]. Inspired by this, similar toolboxes have been developed for *C. glutamicum* genome editing through optimized expression of guide RNA and Cas9, and coexpression of recombinases [36]. Another newly developed tool is the adenine/cytosine base editor. In this system, the catalytically dead Cas9 is fused to a cytosine deaminase (CDA) or adenine deaminase (AID), which enables base pair transition from C:G to T:A or from A:T to G:C. Expression of the guide RNA and the fusion construct Cas9-CDA or Cas9-AID triggers precise base editing in either the genome or the plasmid [37]. By applying this tool to the sequences of ribosome-binding sites or promoter regions, the pathway genes can be regulated in parallel and their expression levels can be varied in a large range [37]. Moreover, the genome-targeting scope of such base editors has been expanded by loosening the 3' PAM sequence requirements from a 5'-NGG-3' to 5'-NG-3' using the Cas9 variants, thus providing 3.9-fold more target loci for *C. glutamicum* gene modifications [36].

The CRISPR system has been investigated in the interference of gene expression (CRISPRi) **(Figure 2)**. By employing a catalytically-dead Cas9 endonuclease that binds to one or several target sequences simultaneously with the aid of guide RNAs, the expression of the target gene(s) can therefore be repressed or, in some cases, activated [38]. For example, *C. glutamicum* was engineered for carotenoid production and CRISPRi tested 74 genes involved in its central metabolism, regulatory genes, and biosynthetic pathways. Such an effort led to the identification of new target genes for

increased carotenoid bioproduction [39]. On the other hand, a synthetic small regulatory RNA (sRNA)-based gene knockdown strategy has been developed in *C. glutamicum* (**Figure 2**). This system contains an RNA chaperone Hfq from *E. coli* and a rationally designed sRNA consisting of the *E. coli* MicC (mRNA-interfering complementary OmpC) scaffold and a target binding site. Upon expression in *C. glutamicum*, the sRNA binds to the mRNA of the target genes, represses translation and enzyme synthesis, and regulates the production of the target compounds [40].

Biosensors are useful in metabolic engineering. *C. glutamicum* contains many native transcription factors that respond to amino acids to trigger the expression of exporters. In addition, some endogenous regulatory proteins are responsive to native metabolites or natural products [41,42]. For example, MarR (multiple antibiotic resistance regulator)-type regulator CrtR, which represses the transcription of the promoter of the *crt* operon (PcrtE) and its own gene (PcrtR), can sense intracellular geranylgeranyl pyrophosphate (GGPP), and the CrtR/PcrtE switch can be used to screen GGPP-overproducing strains for the production of carotenoids [42]. Recently, other biosensors have been discovered in *C. glutamicum* such as ShiR, NCgl0581, and CgmR, in addition to previously identified biosensors such as Lrp, GlxR, LysG [43]. They can be applied in the screening of efficient producers or as a switch to modulate biosynthetic pathways in a dynamic manner. For instance, various dynamic pathway regulation tools have been reported, including quorum-sensing-based genetic circuits [44] and synthetic metabolic switches (responsive to cell growth [26] or effector molecules such as gluconate [45] and ferulic acid [46]).

Multi-scale models and omics analysis to assist *C. glutamicum* engineering

A DBTL (design-build-test-learn) cycle for *C. glutamicum* engineering involves: 1) *design* pathways, 2) *build* genetic constructs, 3) *test* strains for desired traits, and 4) *learn* new strategies for the next cycle of DBTL. In the design stage, metabolic modeling predicts strain metabolism and identify biosynthesis bottlenecks. Several computational design tools, including models and algorithms, have been developed to greatly accelerate such a process. The recently updated genome-scale metabolic model of *C. glutamicum*, i.e., model *i*CW773 established for strain ATCC 13032, consists of 773 genes, 950 metabolites, and 1207 reactions [47]. This model coupled with flux balance analysis and computational strain design could suggest the genetic interventions leading to hyaluronic acid overproduction. Engineering efforts following such predictions led to 28.7 g/L of hyaluronic acid (0.21-0.97 MDa) in fed-batch fermentation [48]. In another example, model-guided metabolic engineering reconstructed the TCA cycle, blocked product degradation, enhanced transport system, and improved gamma-aminobutyric acid (GABA) production (achieving 23 g/L) [49]. Similarly, a pool influx kinetics approach integrated dynamic ^{13}C labeling with model-based analysis, leading to the identification of key genes for improving L-histidine production in *C. glutamicum* [50]. Recently, an enzyme-constrained metabolic model was developed [51]. This model improved the prediction of *C. glutamicum* phenotypes and revealed the trade-off between biomass yield and enzyme usage efficiency, which could guide strain engineering for L-lysine production. In parallel to mechanistic models, data driven approaches (such as AI) have been reported to facilitate successful DBTL cycles in other model organisms such as *E. coli* [52] and *S. cerevisiae* [53]. Moreover, the Automated Recommendation Tool (ART) for machine learning applications has been built to design synthetic biology components

196 (such as promoters) [54]. The same machine learning approaches may enhance *C.*
197 *glutamicum* strain development and biomanufacturing [55].

198 Omics analyses are important tools to facilitate DBTL strain development. In a
199 putrescine-producing *C. glutamicum* strain obtained via adaptive evolution, key
200 engineering loci were identified at the genetic level using whole genome sequencing and
201 at the protein level using comparative proteomics analysis. Subsequent engineering
202 efforts guided by the omics studies further increased the titer of putrescine by 30% [56].
203 In another study, transcriptomic and metabolomic data were analyzed to uncover the
204 association between cellular metabolism and the amino acid-producing phenotype,
205 suggesting that active pentose phosphate pathway and glyoxylate cycle are correlated
206 with efficient production of branched-chain amino acids [57]. On the other hand,
207 bio-production scale-up from laboratory flasks to industrial fermenters requires
208 multi-scale process analyses and optimizations. Thereby, various process models
209 have been built to predict *C. glutamicum* fermentations [58], to gain insights
210 into cell metabolism under bioreactor conditions [59], and to quantify bioreactor
211 mass transfer, hydromechanics, and power input [60]. Moreover, the integration of
212 process models with intracellular omics analysis under scale-down conditions provide
213 valuable perspectives on *C. glutamicum* physiologies inside inhomogeneous industrial
214 fermenters [61].

215 **Novel *C. glutamicum* strains for metabolic engineering applications**

216 While genomic tools and computational model development have reached maturity
217 for the ATCC 13032 type strain, differences between the type strain and other *C.*
218 *glutamicum* isolates remain an untapped reservoir of potential metabolic capacity.
A phylogenetic analysis of the 26 most common *C. glutamicum* isolates described in
the

219 literature identified 9 distinct groups with unique genomic islands and complex
220 polymorphisms that may be related to their specific amino acid secretion phenotypes [62].
221 These *C. glutamicum* isolates can have differing potentials to produce desirable
222 heterologous bioproducts. *N*-Acetylglucosamine (GlcNAc) is a monosaccharide with
223 potential applications in human health. Deng and coworkers introduced the
224 *Caenorhabditis elegans GNA1* gene (encoding glucosamine-6-phosphate
225 acetyltransferase) into different *C. glutamicum* isolates and detected GlcNAc titers at 3.0
226 g/L in the S9114 isolate. In contrast, ATCC 13032 produced 0.5 g/L GlcNAc. The
227 authors were able to adapt standard *C. glutamicum* gene modification tools in the S9114
228 isolate to further boost titers in batch mode to 6.9 g/L in rich media [63]. Similarly,
229 Banerjee and coworkers tested the production of a 5 gene isoprenol production pathway
230 in a transformation-improved Δ*mrr* ATCC 13032 strain as well as in isolate BRC-JBEI
231 1.1.2, and found that isoprenol titers were at the lower detection limit (15 mg/L) in the
232 type strain but was twenty-fold higher in BRC-JBEI 1.1.2 [64]. Many (>500) genes in
233 these *C. glutamicum* isolates lack any functional characterization and have no known
234 homologs in other species, and this trend will likely hold as more genomes from related
235 *Corynebacteria* are identified from diverse microbiomes using high quality metagenomic
236 assembly approaches. Functional genomics approaches using parallel transposon
237 mutagenized mutant libraries that have been applied in other bacterial hosts will enable
238 the comparison of gene function across these isolates, providing insights into the
239 unknown genes harbored in these strains [65].
240

241 **Conclusions and Outlook for the Industry**

C. glutamicum has superior capability in the biosynthesis of diverse amino acids, organic acids, short-chain alcohols, and their derivatives, many of which are bulk chemicals. The fermentation facilities and bio-separation techniques for *C. glutamicum* factories have been established, facilitating the commercialization of other compounds beyond amino acids. Meanwhile, the development of omics analyses and high-throughput cultivate/screen [66] is momentously speeding strain characterization and development. Additionally, the existence of a natural aromatic-degrading pathway and the strong resistance to aromatic inhibitors in hemicellulosic hydrolysates suggest promising potentials of *C. glutamicum* for the utilization of lignocellulose to produce diverse chemicals [64]. On the other hand, it should be noted that *C. glutamicum* is not the best chassis organism for all compounds. For example, natural products are synthesized in this bacterium at low yields. To improve the functions of the plant-derived pathways in *C. glutamicum*, several approaches can be employed, including transporter engineering or cell wall remodeling to increase efflux of the final products, enzyme modifications to enhance catalytic performances, and modular pathway engineering [67,68]. In addition, advanced metabolic modeling and emerging AI technologies may accelerate *C. glutamicum* engineering to synthesize various high value products.

Author contributions

Writing – original draft preparation: JZ, ZZ; Writing – review & editing: ZX, TE, AM, MK, YT.

Conflict of interest statement

The authors declare that they have no known competing financial interestsor personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

JZ acknowledges startup funds from Shaanxi University of Science and Technology. TE and AM acknowledge funding from the DOE Joint BioEnergy Institute (http://www.jbei.org) supported by the US Department of Energy, Office of Science, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the US Department of Energy. YT acknowledges funding from the NSF (grant number 2225809).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- ● of special interest
- ●● of outstanding interest

1. Rischer H, Szilvay GR, Oksman-Caldentey KM: **Cellular agriculture - industrial biotechnology for food and materials**. *Curr. Opin. Biotechnol.* 2020, **61**:128-134.
2. Fröhling M, Hiete M: **Sustainability and life cycle assessment in industrial biotechnology: a review of current approaches and future needs**. *Adv. Biochem. Eng. Biotechnol.* 2020, **173**:143-203.
3. Ko YS, Kim JW, Lee JA, Han T, Kim GB, Park JE, Lee SY: **Tools and strategies of systems metabolic engineering for the development of microbial cell factories for chemical production**. *Chem. Soc. Rev.* 2020, **49**:4615-4636.
4. Kogure T, Inui M: **Recent advances in metabolic engineering of *Corynebacterium glutamicum* for bioproduction of value-added aromatic chemicals and natural products**. *Appl. Microbiol. Biotechnol.* 2018, **102**:8685-8705.
5. Yu S, Zheng B, Chen Z, Huo YX: **Metabolic engineering of *Corynebacterium glutamicum* for producing branched chain amino acids**. *Microb. Cell Fact.* 2021, **20**:230.
6. Wendisch VF, Jorge JMP, Pérez-García F, Sgobba E: **Updates on industrial production of amino acids using *Corynebacterium glutamicum***. *World J. Microbiol. Biotechnol.* 2016, **32**:105.
7. Zhang X, Gao Y, Chen Z, Xu G, Zhang X, Li H, Shi J, Koffas MA, Xu ZJMcf: **High-yield production of L-serine through a novel identified exporter combined with synthetic pathway in Corynebacterium glutamicum**. 2020, **19**:1-14.
8. Zhang X, Lai L, Xu G, Zhang X, Shi J, Koffas MAG, Xu Z: **Rewiring the Central Metabolic Pathway for High-Yield l-Serine Production in Corynebacterium glutamicum by Using Glucose**. 2019, **14**:1800497.
9. Tsuge Y, Matsuzawa H: **Recent progress in production of amino acid-derived chemicals using *Corynebacterium glutamicum***. *World J. Microbiol. Biotechnol.* 2021, **37**:49.
10. Rohles C, Pauli S, Gießelmann G, Kohlstedt M, Becker J, Wittmann C: **Systems metabolic engineering of *Corynebacterium glutamicum* eliminates all by-products for selective and high-yield production of the platform chemical 5-aminovalerate**. *Metab. Eng.* 2022, **73**:168-181.
11. Xu G, Zha J, Cheng H, Ibrahim MHA, Yang F, Dalton H, Cao R, Zhu Y, Fang J, Chi K, et al.: **Engineering Corynebacterium glutamicum for the de novo biosynthesis of tailored poly-γ-glutamic acid**. *Metabolic Engineering* 2019, **56**:39-49.
12. Sohn YJ, Kang M, Baritugo K-A, Son J, Kang KH, Ryu M-H, Lee S, Sohn M, Jung YJ, Park K, et al.: **Fermentative high-level production of 5-hydroxyvaleric acid**

by metabolically engineered *Corynebacterium glutamicum*. *ACS Sustainable Chem. Eng.* 2021, **9**:2523-2533.

● This study shows high-level production of 5-hydroxyvaleric acid based on lysine-producing *C. glutamicum*. Via selection of optimal pathway enzymes in the artificial synthesis pathway of 5-hydroxyvaleric acid, and by decreasing the degradation of key intermediate, a hyperproducing strain was developed with a product titer of 52 g/L in fed-batch fermentation.

13. Han T, Kim GB, Lee SY: **Glutaric acid production by systems metabolic engineering of an l-lysine–overproducing Corynebacterium glutamicum**. *Proceedings of the National Academy of Sciences* 2020, **117**:30328-30334.
14. Liu B, Sun X, Liu Y, Yang M, Wang L, Li Y, Wang J: **Increased NADPH supply enhances glycolysis metabolic flux and L-methionine production in *Corynebacterium glutamicum***. *Foods* 2022, **11**:1031.

●● *C. glutamicum* was engineered for L-methionine production and ^{13}C-MFA revealed the glycolysis flux changes and NADPH supplies in the engineered strain, leading to new strategies for strain imporvement.

15. Briki A, Kaboré K, Olmos E, Bosselaar S, Blanchard F, Fick M, Guedon E, Fournier F, Delaunay S: ***Corynebacterium glutamicum*, a natural overproducer of succinic acid?** *Eng. Life Sci.* 2020, **20**:205-215.
16. Becker J, Rohles CM, Wittmann C: **Metabolically engineered *Corynebacterium glutamicum* for bio-based production of chemicals, fuels, materials, and healthcare products**. *Metab. Eng.* 2018, **50**:122-141.

● This review summarizes recent trends in metabolic engineering of *C. glutamicum* for the bioproduction of diverse chemicals and basic tools used thereof.

17. Hasegawa S, Jojima T, Suda M, Inui M: **Isobutanol production in *Corynebacterium glutamicum*: suppressed succinate by-production by pckA inactivation and enhanced productivity via the Entner-Doudoroff pathway**. *Metab. Eng.* 2020, **59**:24-35.
18. Zha J, Zang Y, Mattozzi M, Plassmeier J, Gupta M, Wu X, Clarkson S, Koffas MAG: **Metabolic engineering of *Corynebacterium glutamicum* for anthocyanin production**. *Microb. Cell Fact.* 2018, **17**:143.
19. Kallscheuer N, Vogt M, Bott M, Marienhagen J: **Functional expression of plant-derived *O*-methyltransferase, flavanone 3-hydroxylase, and flavonol synthase in *Corynebacterium glutamicum* for production of pterostilbene, kaempferol, and quercetin**. *J. Biotechnol.* 2017, **258**:190-196.
20. Wolf S, Becker J, Tsuge Y, Kawaguchi H, Kondo A, Marienhagen J, Bott M, Wendisch VF, Wittmann C: **Advances in metabolic engineering of *Corynebacterium glutamicum* to produce high-value active ingredients for food, feed, human health, and well-being**. *Essays Biochem.* 2021, **65**:197-212.
21. Mai J, Li W, Ledesma-Amaro R, Ji XJ: **Engineering plant sesquiterpene synthesis into yeasts: a review**. *J. Agric. Food Chem.* 2021, **69**:9498-9510.

22. Choi JW, Jeon EJ, Jeong KJ: **Recent advances in engineering *Corynebacterium glutamicum* for utilization of hemicellulosic biomass**. *Curr. Opin. Biotechnol.* 2019, **57**:17-24.
23. Zhang B, Jiang Y, Li Z, Wang F, Wu XY: **Recent progress on chemical production from non-food renewable feedstocks using *Corynebacterium glutamicum***. *Front. Bioeng. Biotechnol.* 2020, **8**:606047.
24. Wendisch VF, Nampoothiri KM, Lee J-H: **Metabolic engineering for valorization of agri- and aqua-culture sidestreams for production of nitrogenous compounds by *Corynebacterium glutamicum***. *Front. Microbiol.* 2022, **13**:835131.
25. Stella RG, Wiechert J, Noack S, Frunzke J: **Evolutionary engineering of *Corynebacterium glutamicum***. *Biotechnol. J.* 2019, **14**:e1800444.
26. Wei L, Zhao J, wang Y, Gao J, Du M, zhang Y, Xu N, Du H, Ju J, Liu Q, et al.: **Engineering of *Corynebacterium glutamicum* for high-level γ-aminobutyric acid production from glycerol by dynamic metabolic control**. *Metab. Eng.* 2022, **69**:134-146.
27. Tateno T, Fukuda H, Kondo A: **Production of L-lysine from starch by *Corynebacterium glutamicum* displaying alpha-amylase on its cell surface**. *Appl. Microbiol. Biotechnol.* 2007, **74**:1213-1220.
28. Tateno T, Okada Y, Tsuchidate T, Tanaka T, Fukuda H, Kondo A: **Direct production of cadaverine from soluble starch using *Corynebacterium glutamicum* coexpressing alpha-amylase and lysine decarboxylase**. *Appl. Microbiol. Biotechnol.* 2009, **82**:115-121.
29. Roell GW, Zha J, Carr RR, Koffas MA, Fong SS, Tang YJ: **Engineering microbial consortia by division of labor**. *Microb. Cell Fact.* 2019, **18**:35.
30. Sgobba E, Stumpf AK, Vortmann M, Jagmann N, Krehenbrink M, Dirks-Hofmeister ME, Moerschbacher B, Philipp B, Wendisch VF: **Synthetic *Escherichia coli-Corynebacterium glutamicum* consortia for L-lysine production from starch and sucrose**. *Bioresour. Technol.* 2018, **260**:302-310.
● This work shows the construction of a mutualistic *E. coli-C. glutamicum* consortium to achieve lysine production from starch.
31. Zhang J, Zou D, Singh S, Cheng G: **Recent developments in ionic liquid pretreatment of lignocellulosic biomass for enhanced bioconversion**. *Sustainable Energy Fuels* 2021, **5**:1655-1667.
32. Kallscheuer N, Vogt M, Kappelmann J, Krumbach K, Noack S, Bott M, Marienhagen J: **Identification of the *phd* gene cluster responsible for phenylpropanoid utilization in *Corynebacterium glutamicum***. *Appl. Microbiol. Biotechnol.* 2016, **100**:1871-1881.
33. Mhatre A, Shinde S, Jha AK, Rodriguez A, Wardak Z, Jansen A, Gladden JM, George A, Davis RW, Varman AM: ***Corynebacterium glutamicum* as an efficient omnivorous microbial host for the bioconversion of lignocellulosic biomass**. *Front. Bioeng. Biotechnol.* 2022, **10**:827386.

34. Wang Y, Liu Y, Zheng P, Sun J, Wang M: **Microbial base editing: a powerful emerging technology for microbial genome engineering**. *Trends Biotechnol.* 2021, **39**:165-180.
35. Zhang J, Yang F, Yang Y, Jiang Y, Huo YX: **Optimizing a CRISPR-Cpf1-based genome engineering system for *Corynebacterium glutamicum***. *Microb. Cell Fact.* 2019, **18**:60.
36. Wang Y, Liu Y, Li J, Yang Y, Ni X, Cheng H, Huang T, Guo Y, Ma H, Zheng P, et al.: **Expanding targeting scope, editing window, and base transition capability of base editing in *Corynebacterium glutamicum***. *Biotechnol. Bioeng.* 2019, **116**:3016-3029.
37. Wang Y, Cheng H, Liu Y, Liu Y, Wen X, Zhang K, Ni X, Gao N, Fan L, Zhang Z, et al.: **In-situ generation of large numbers of genetic combinations for metabolic reprogramming via CRISPR-guided base editing**. *Nat. Commun.* 2021, **12**:678.
●● This study shows the application of Cas/nucleotide deaminase base editors to modify *in situ* the sequences of ribosome binding sites, 5' untranslated regions, or promoters of genes in target pathways simultaneously, generating thousands of mutants with varied performances in the production of target compounds. This work provides a useful method for large-scale fine-tuning of multigene expression.
38. Cress BF, Toparlak ÖD, Guleria S, Lebovich M, Stieglitz JT, Englaender JA, Jones JA, Linhardt RJ, Koffas MAG: **CRISPathBrick: modular combinatorial assembly of type II-A CRISPR arrays for dCas9-mediated multiplex transcriptional repression in *E. coli***. *ACS Synth. Biol.* 2015, **4**:987-1000.
39. Göttl VL, Schmitt I, Braun K, Peters-Wendisch P, Wendisch VF, Henke NA: **CRISPRi-library-guided target identification for engineering carotenoid production by *Corynebacterium glutamicum***. *Microorganisms* 2021, **9**:670.
40. Sun D, Chen J, Wang Y, Li M, Rao D, Guo Y, Chen N, Zheng P, Sun J, Ma Y: **Metabolic engineering of *Corynebacterium glutamicum* by synthetic small regulatory RNAs**. *J. Ind. Microbiol. Biotechnol.* 2019, **46**:203-208.
41. Zhao N, Song J, Zhang H, Lin Y, Han S, Huang Y, Zheng S: **Development of a transcription factor-based diamine biosensor in *Corynebacterium glutamicum***. *ACS Synth. Biol.* 2021, **10**:3074-3083.
42. Henke NA, Austermeier S, Grothaus IL, Götker S, Persicke M, Peters-Wendisch P, Wendisch VF: ***Corynebacterium glutamicum* CrtR and its orthologs in Actinobacteria: conserved function and application as genetically encoded biosensor for detection of geranylgeranyl pyrophosphate**. *Int. J. Mol. Sci.* 2020, **21**.
43. Wang Y, Zheng P, Sun J: **Recent advances in developing enabling technologies for *Corynebacterium glutamicum* metabolic engineering**. *Chin. J. Biotechnol.* 2021, **37**:1603-1618.
44. Liu H, Shi F, Tan S, Yu X, Lai W, Li Y: **Engineering a bifunctional ComQXPA-PsrfA quorum-sensing circuit for dynamic control of gene**

expression in *Corynebacterium glutamicum*. *ACS Synth. Biol.* 2021, **10**:1761-1774.

45. Wiechert J, Gätgens C, Wirtz A, Frunzke J: **Inducible expression systems based on xenogeneic silencing and counter-silencing and design of a metabolic toggle switch**. *ACS Synth. Biol.* 2020, **9**:2023-2038.
46. Siebert D, Altenbuchner J, Blombach B: **A timed off-switch for dynamic control of gene expression in *Corynebacterium glutamicum***. *Front. Bioeng. Biotechnol.* 2021, **9**:704681.
47. Zhang Y, Cai J, Shang X, Wang B, Liu S, Chai X, Tan T, Zhang Y, Wen T: **A new genome-scale metabolic model of *Corynebacterium glutamicum* and its application**. *Biotechnol. Biofuels* 2017, **10**:169.

●This study reports a new and accurate genome-scale metabolic model of ATCC 13032, i.e., *i*CW773, for the prediction of potential targets for L-proline production. This model provides a high-quality platform for strain design to conduct efficient bio-production by *C. glutamicum*.

48. Cheng F, Yu H, Stephanopoulos G: **Engineering *Corynebacterium glutamicum* for high-titer biosynthesis of hyaluronic acid**. *Metab. Eng.* 2019, **55**:276-289.

●● Model *i*CW773 was applied coupled with flux balance analysis and algorithm OptForce$_{MUST}$ for hyaluronic acid biosynthesis to predict genetic interventions in *C. glutamicum* leading to the overproducing phenotype. By following the prediction, the production titer reached 28.7 g/L with 50% less production of the byproduct.

49. Zhang Y, Zhao J, Wang X, Tang Y, Liu S, Wen T: **Model-Guided Metabolic Rewiring for Gamma-Aminobutyric Acid and Butyrolactam Biosynthesis in Corynebacterium glutamicum ATCC13032**. 2022, **11**:846.
50. Feith A, Schwentner A, Teleki A, Favilli L, Blombach B, Takors R: **Streamlining the analysis of dynamic ^{13}C-labeling patterns for the metabolic engineering of *Corynebacterium glutamicum* as L-histidine production host**. *Metabolites* 2020, **10**:458.

● The pool influx kinetics (PIK) approach was built to assist DBTL cycles for identifying promising metabolic engineering targets to imporve L-histidine production.

51. Niu J, Mao Z, Mao Y, Wu K, Shi Z, Yuan Q, Cai J, Ma H: **Construction and Analysis of an Enzyme-Constrained Metabolic Model of Corynebacterium glutamicum**. 2022, **12**:1499.
52. Opgenorth P, Costello Z, Okada T, Goyal G, Chen Y, Gin J, Benites V, de Raad M, Northen TR, Deng K, et al.: **Lessons from two design-build-test-learn cycles of dodecanol production in *Escherichia coli* aided by machine learning**. *ACS Synth. Biol.* 2019, **8**:1337-1351.

● Two cycles of DBTL were performed with machine learning algorithms integrated in between for the engineering of *E. coli* to produce dodecanol

53. Zhang J, Petersen SD, Radivojevic T, Ramirez A, Pérez-Manríquez A, Abeliuk E, Sánchez BJ, Costello Z, Chen Y, Fero MJ, et al.: **Combining mechanistic and**

machine learning models for predictive engineering and optimization of tryptophan metabolism. *Nature Communications* 2020, **11**:4880.

54. Radivojević T, Costello Z, Workman K, Garcia Martin H: **A machine learning Automated Recommendation Tool for synthetic biology**. *Nature Communications* 2020, **11**:4879.
55. Liao X, Ma H, Tang YJ: **Artificial intelligence: a solution to involution of design-build-test-learn cycle**. *Curr. Opin. Biotechnol.* 2022, **75**:102712.

●● This review article describes the applications of machine learning and data mining for metabolic engineering and strain development by following the DBTL cycle.

56. Li Z, Shen YP, Jiang XL, Feng LS, Liu JZ: **Metabolic evolution and a comparative omics analysis of *Corynebacterium glutamicum* for putrescine production**. *J. Ind. Microbiol. Biotechnol.* 2018, **45**:123-139.
57. Ma Y, Chen N, Cui Y, Du L, Ma Q, Xie X: **Transcriptomic and metabolomics analyses reveal metabolic characteristics of L-leucine- and L-valine-producing *Corynebacterium glutamicum* mutants**. *Ann. Microbiol.* 2019, **69**:457-468.
58. Lira-Parada PA, Pettersen E, Pérez-García F, Bar N: **The development of a fed-batch *Corynebacterium glutamicum* fermentation model**. *IFAC-PapersOnLine* 2019, **52**:231-237.
59. Lira-Parada PA, Sinner P, Kohlstedt M, Kager J, Wittmann C, Herwig C, Bar N: **Linking process and metabolic modelling for the estimation of carbon flux distribution in *Corynebacterium glutamicum* growth in spent sulfite liquor**. *IFAC-PapersOnLine* 2022, **55**:228-233.
60. Seletzky JM, Noak U, Fricke J, Welk E, Eberhard W, Knocke C, Büchs J: **Scale-up from shake flasks to fermenters in batch and continuous mode with *Corynebacterium glutamicum* on lactic acid based on oxygen transfer and pH**. *Biotechnol. Bioeng.* 2007, **98**:800-811.
61. Limberg MH, Schulte J, Aryani T, Mahr R, Baumgart M, Bott M, Wiechert W, Oldiges M: **Metabolic profile of 1,5-diaminopentane producing *Corynebacterium glutamicum* under scale-down conditions: blueprint for robustness to bioreactor inhomogeneities**. *Biotechnol. Bioeng.* 2017, **114**:560-575.
62. Yang J, Yang S: **Comparative analysis of *Corynebacterium glutamicum* genomes: a new perspective for the industrial production of amino acids**. *BMC Genomics* 2017, **18**:940.
63. Deng C, Lv X, Liu Y, Li J, Lu W, Du G, Liu L: **Metabolic engineering of *Corynebacterium glutamicum* S9114 based on whole-genome sequencing for efficient *N*-acetylglucosamine synthesis**. *Synth. Syst. Biotechnol.* 2019, **4**:120-129.

● This study reports a new *C. glutamicum* strain for efficient *N*-acetylglucosamine synthesis.

64. Banerjee D, Eng T, Sasaki Y, Srinivasan A, Oka A, Herbert RA, Trinh J, Singan VR, Sun N, Putnam D, et al.: **Genomics characterization of an engineered**

Corynebacterium glutamicum **in bioreactor cultivation under ionic liquid stress**. *Front. Bioeng. Biotechnol.* 2021, **9**:766674.

65. Swaney MH, Sandstrom S, Kalan LR: **Cobamide sharing is predicted in the human skin microbiome**. *mSystems* 2022, **7**:e0067722.
66. Täuber S, Blöbaum L, Steier V, Oldiges M, Grünberger A: **Microfluidic single-cell scale-down bioreactors: a proof-of-concept for the growth of *Corynebacterium glutamicum* at oscillating pH values**. *Biotechnol. Bioeng.* 2022, **119**:3194-3209.
67. Banerjee D, Eng T, Lau AK, Sasaki Y, Wang B, Chen Y, Prahl J-P, Singan VR, Herbert RA, Liu Y, et al.: **Genome-scale metabolic rewiring improves titers rates and yields of the non-native product indigoidine at scale**. *Nature Communications* 2020, **11**:5385.
68. Keasling J, Garcia Martin H, Lee TS, Mukhopadhyay A, Singer SW, Sundstrom E: **Microbial production of advanced biofuels**. *Nature Reviews Microbiology* 2021, **19**:701-715.
69. Wang Y, Xu J, Jin Z, Xia X, Zhang W: **Improvement of acetyl-CoA supply and glucose utilization increases L-leucine production in *Corynebacterium glutamicum***. *Biotechnol. J.* 2022, **17**:e2100349.
70. Chen J, Wang Y, Guo X, Rao D, Zhou W, Zheng P, Sun J, Ma Y: **Efficient bioproduction of 5-aminolevulinic acid, a promising biostimulant and nutrient, from renewable bioresources by engineered *Corynebacterium glutamicum***. *Biotechnol. Biofuels.* 2020, **13**:41.
71. Xu G, Zha J, Cheng H, Ibrahim MHA, Yang F, Dalton H, Cao R, Zhu Y, Fang J, Chi K, et al.: **Engineering *Corynebacterium glutamicum* for the *de novo* biosynthesis of tailored poly-γ-glutamic acid**. *Metab. Eng.* 2019, **56**:39-49.
72. Gießelmann G, Dietrich D, Jungmann L, Kohlstedt M, Jeon EJ, Yim SS, Sommer F, Zimmer D, Mühlhaus T, Schroda M, et al.: **Metabolic engineering of *Corynebacterium glutamicum* for high-level ectoine production: design, combinatorial assembly, and implementation of a transcriptionally balanced heterologous ectoine pathway**. *Biotechnol. J.* 2019, **14**:e1800417.
73. Ghiffary MR, Prabowo CPS, Sharma K, Yan Y, Lee SY, Kim HU: **High-level production of the natural blue pigment indigoidine from metabolically engineered *Corynebacterium glutamicum* for sustainable fabric dyes**. *ACS Sustainable Chem. Eng.* 2021, **9**:6613-6622.

● The blue pigment indigoidine was synthesized by *C. glutamate* with the highest titer. Strain engineering involved systems metabolic engineering of the biosynthesis pathway and expression of indigoidine synthetase.

74. Jin Q, Pan F, Hu C-F, Lee SY, Xia X-X, Qian Z-G: **Secretory production of spider silk proteins in metabolically engineered Corynebacterium glutamicum for spinning into tough fibers**. *Metabolic Engineering* 2022, **70**:102-114.
75. Schwardmann LS, Dransfeld AK, Schäffer T, Wendisch VF: **Metabolic engineering of *Corynebacterium glutamicum* for sustainable production of the aromatic**

cicarboxylic acid dipicolinic acid. *Microorganisms* 2022, **10**:730.

76. Labib M, Görtz J, Brüsseler C, Kallscheuer N, Gätgens J, Jupke A, Marienhagen J, Noack S: **Metabolic and process engineering for microbial production of protocatechuate with *Corynebacterium glutamicum***. *Biotechnol. Bioeng.* 2021, **118**:4414-4427.
77. Kim HS, Choi JA, Kim BY, Ferrer L, Choi JM, Wendisch VF, Lee JH: **Engineered *Corynebacterium glutamicum* as the platform for the production of aromatic aldehydes**. *Front. Bioeng. Biotechnol.* 2022, **10**:880277.
78. Li Z, Dong Y, Liu Y, Cen X, Liu D, Chen Z: **Systems metabolic engineering of *Corynebacterium glutamicum* for high-level production of 1,3-propanediol from glucose and xylose**. *Metab. Eng.* 2022, **70**:79-88.
79. Prabowo CPS, Shin JH, Cho JS, Chae TU, Lee SY: **Microbial production of 4-amino-1-butanol, a four-carbon amino alcohol**. *Biotechnol. Bioeng.* 2020, **117**:2771-2780.
80. Sasaki Y, Eng T, Herbert RA, Trinh J, Chen Y, Rodriguez A, Gladden J, Simmons BA, Petzold CJ, Mukhopadhyay A: **Engineering *Corynebacterium glutamicum* to produce the biogasoline isopentenol from plant biomass hydrolysates**. *Biotechnol. Biofuels* 2019, **12**:41.
81. Hasegawa S, Jojima T, Suda M, Inui M: **Isobutanol production in Corynebacterium glutamicum: Suppressed succinate by-production by pckA inactivation and enhanced productivity via the Entner–Doudoroff pathway**. *Metabolic Engineering* 2020, **59**:24-35.
82. Becker J, Kuhl M, Kohlstedt M, Starck S, Wittmann C: **Metabolic engineering of *Corynebacterium glutamicum* for the production of *cis*, *cis*-muconic acid from lignin**. *Microb. Cell Fact.* 2018, **17**:115.
83. Shin JH, Andersen AJC, Achterberg P, Olsson L: **Exploring functionality of the reverse β-oxidation pathway in *Corynebacterium glutamicum* for production of adipic acid**. *Microb. Cell Fact.* 2021, **20**:155.
84. Henke NA, Wendisch VF: **Improved astaxanthin production with *Corynebacterium glutamicum* by application of a membrane fusion protein**. *Mar. Drugs* 2019, **17**:621.
85. Burgardt A, Moustafa A, Persicke M, Sproß J, Patschkowski T, Risse JM, Peters-Wendisch P, Lee JH, Wendisch VF: **Coenzyme Q(10) biosynthesis established in the non-ubiquinone containing *Corynebacterium glutamicum* by metabolic engineering**. *Front. Bioeng. Biotechnol.* 2021, **9**:650961.
86. Kallscheuer N, Vogt M, Stenzel A, Gätgens J, Bott M, Marienhagen J: **Construction of a *Corynebacterium glutamicum* platform strain for the production of stilbenes and (2*S*)-flavanones**. *Metab. Eng.* 2016, **38**:47-55.

●● This is the first report on flavonoid or stilbene production in *C. glutamicum*. This work reports the natural aromatic degradation pathway and its effect on the production of flavonoids. The *de novo* biosynthesis of naringenin and resveratrol

was achieved through metabolic engineering modifications of the tyrosine biosynthetic pathway.

87. Kallscheuer N, Menezes R, Foito A, da Silva MH, Braga A, Dekker W, Sevillano DM, Rosado-Ramos R, Jardim C, Oliveira J, et al.: **Identification and microbial production of the raspberry phenol salidroside that is active against Huntington's Disease**. *Plant Physiol.* 2019, **179**:969-985.

Table 1. Recent achievements in *C. glutamicum*-based biosynthesis of compounds

Classification	Chemicals	Titer	Culture conditions	Reference
Amino acids	L-Leucine	40 g/L	Fermenter	[69]
and derivatives	5-Hydroxyvaleric acid	52 g/L	Fermenter	[12]
	5-Aminolevulinic acid	16.3 g/L	Fermenter	[70]
	Poly-γ-glutamic acid	21.3 g/L	Fermenter	[71]
	Ectoine	65.3 g/L	Fermenter	[72]
	Putrescine Indigoidine	12.5 g/L	Fermenter	[56]
	Spider silk protein	49.3 g/L	Fermenter	[73]
		0.56 g/L	Fermenter	[74]
Aromatics	Dipicolinic acid	2.5 g/L	Shake flask	[75]
	Protocatechuate	16 g/L	Fermenter	[76]
	Vanillin	0.31 g/L	Shake flask	[77]
Alcohols	1,3-Propanediol	98 g/L	Fermenter	[78]
	4-Amino-1-butanol	24 g/L	Fermenter	[79]
	Isoprenol	1.25 g/L	Shake flask	[80]
	(3-methyl-3-buten-1-ol)			
	Isobutanol	20.75 g/L	Shake flask	[81]
Organic Acids	Succinate	94 g/L	Fermenter	[15]
	Muconic acid	85 g/L	Fermenter	[82]
	Adipic acid	35 µg/L	Shake flask	[83]
Terpenoids	Astaxanthin	22 mg/L	Shake flask	[84]
	CoQ10	0.4 mg/L	Shake flask	[85]
Polyphenols	Cyanidin 3-*O*-glucoside	40 mg/L	Shake flask	[18]
	Naringenin	37 mg/L	Shake flask	[86]
	Resveratrol	158 mg/L	Shake flask	[86]
	Salidroside	9.7 g/L	Fermenter	[87]

Figure Legends

Figure 1. The portfolio of typical chemicals produced by engineered *C. glutamicum*. The chemicals include amino acids, their derivatives, organic acids, short-chain alcohols, fatty acids, aromatics, terpenoids, and polyphenols. The carbon sources for *C. glutamicum* include molasses and starch (common industrial fermentation media), hemicellulosic hydrolysates, xylose, methanol, glycerol, aromatics, etc.

Figure 2. The new genetic tools and models developed for metabolic engineering of *C. glutamicum*.

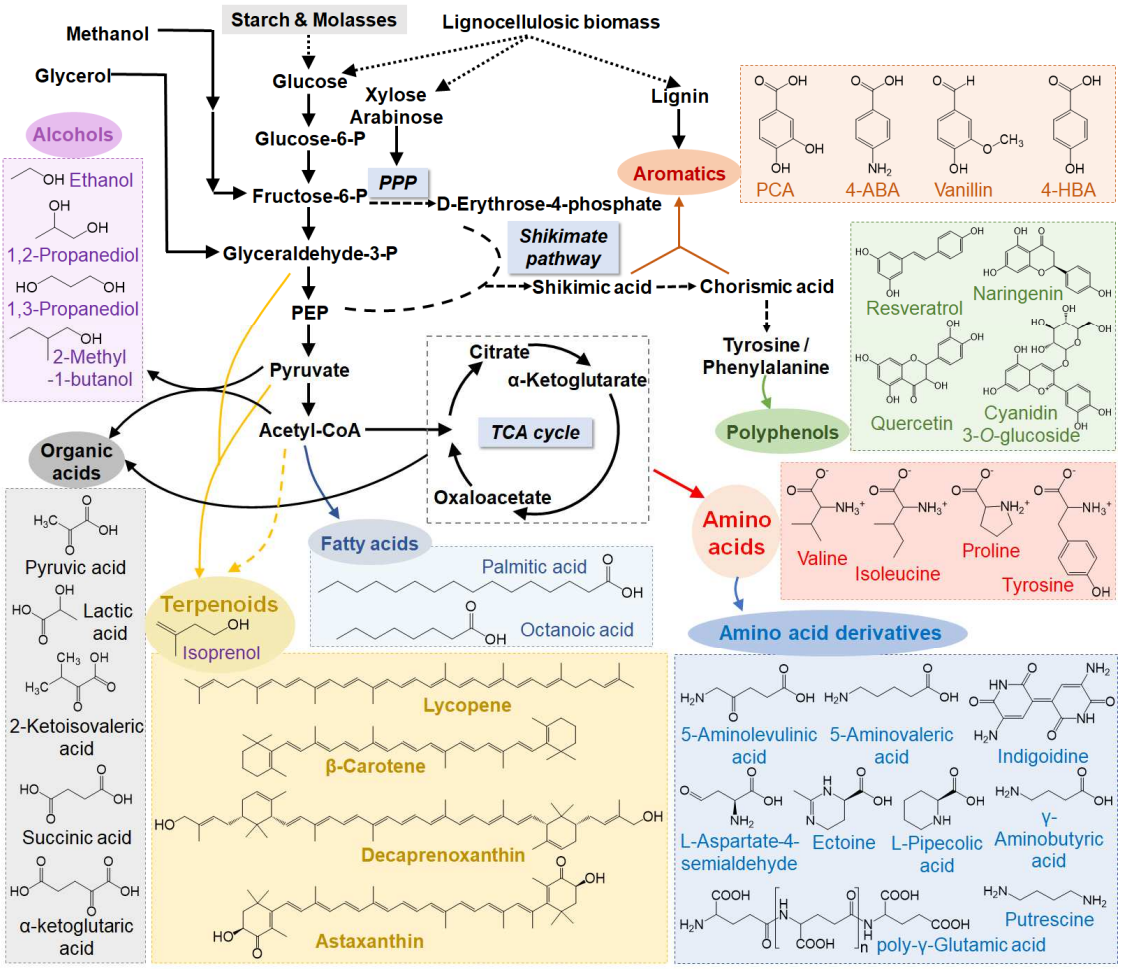

Starch & Molasses
Lignocellulosic biomass
Methanol
Glycerol
Glucose
Xylose
Arabinose
Lignin
Alcohols
Ethanol
1,2-Propanediol
1,3-Propanediol
2-Methyl -1-butanol
Glucose-6-P
Fructose-6-P
PPP
D-Erythrose-4-phosphate
Aromatics
PCA
4-ABA
Vanillin
4-HBA
Shikimate pathway
Glyceraldehyde-3-P
Shikimic acid
Chorismic acid
PEP
Pyruvate
Citrate
α-Ketoglutarate
TCA cycle
Oxaloacetate
Acetyl-CoA
Tyrosine / Phenylalanine
Polyphenols
Resveratrol
Naringenin
Quercetin
Cyanidin 3-O-glucoside
Organic acids
Pyruvic acid
Lactic acid
2-Ketoisovaleric acid
Succinic acid
α-ketoglutaric acid
Amino acids
Valine
Isoleucine
Proline
Tyrosine
Fatty acids
Palmitic acid
Octanoic acid
Terpenoids
Isoprenol
Lycopene
β-Carotene
Decaprenoxanthin
Astaxanthin
Amino acid derivatives
5-Aminolevulinic acid
5-Aminovaleric acid
Indigoidine
L-Aspartate-4-semialdehyde
Ectoine
L-Pipecolic acid
γ-Aminobutyric acid
Putrescine
poly-γ-Glutamic acid

Figure 1

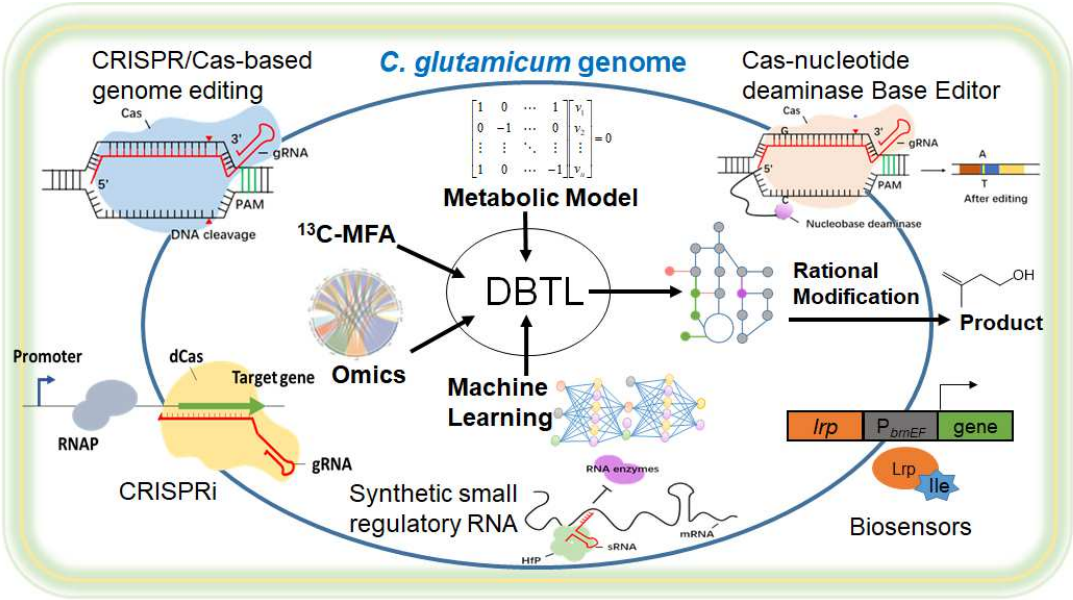
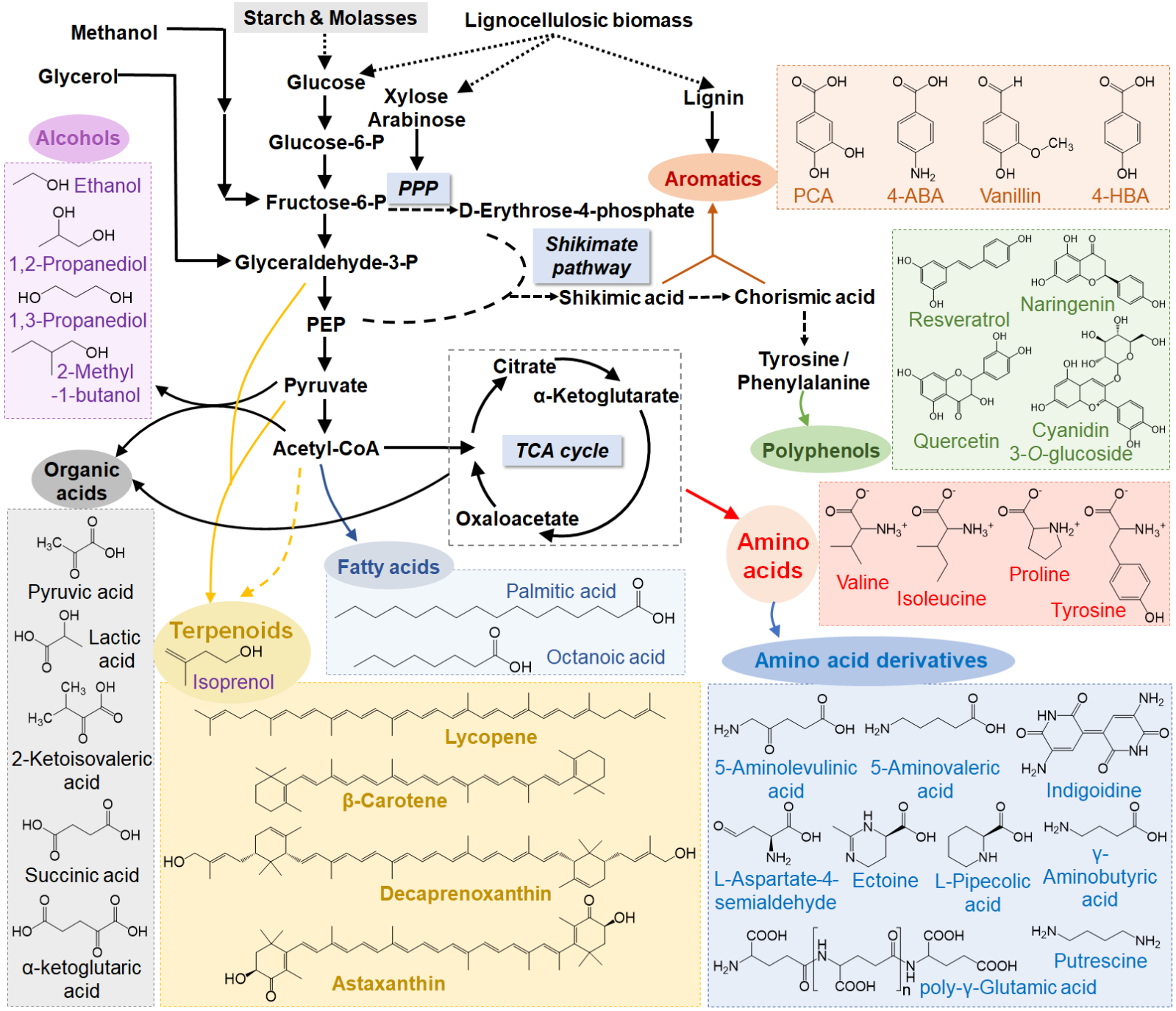
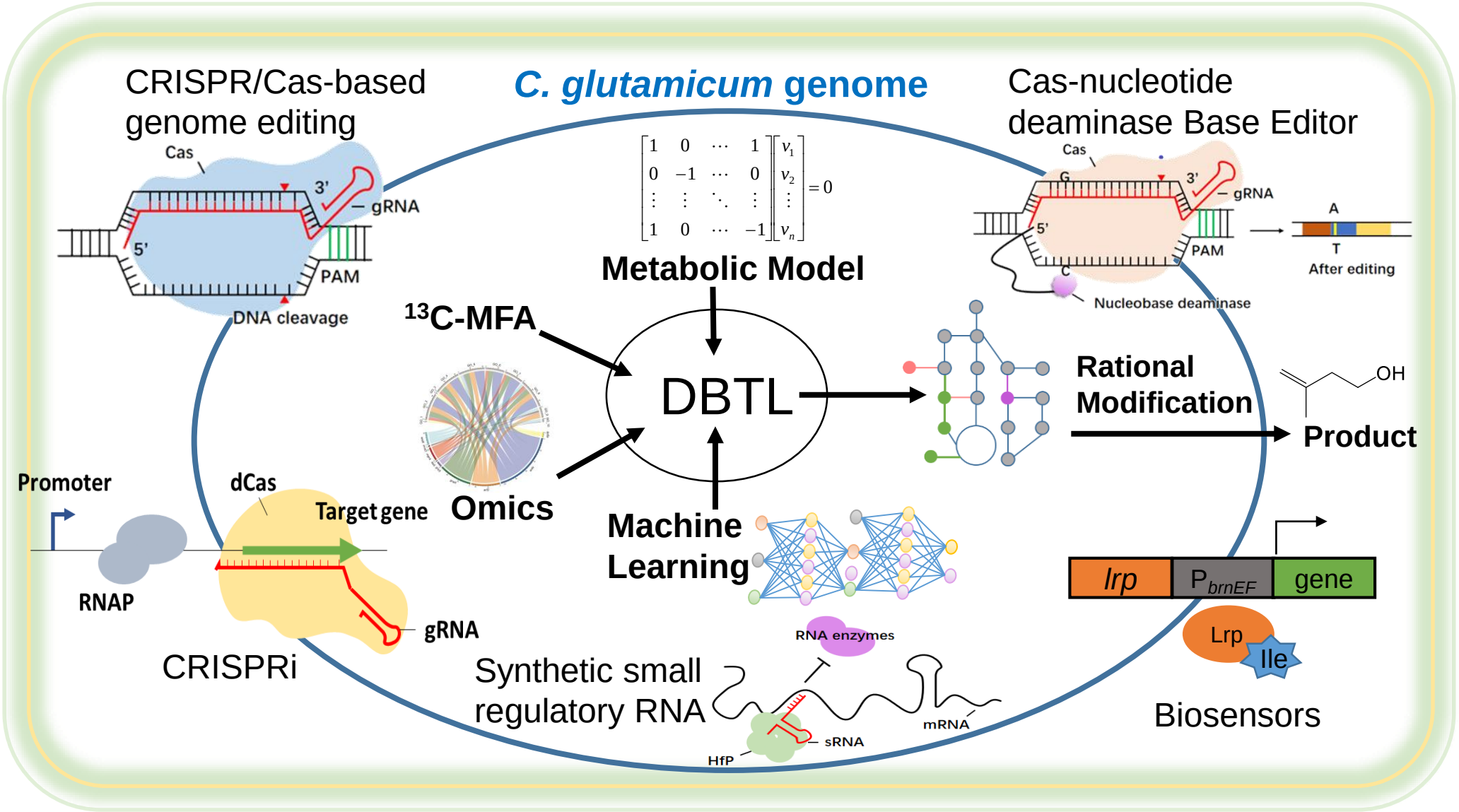

CRISPR/Cas-based genome editing
C. glutamicum genome
Cas-nucleotide deaminase Base Editor
Cas
gRNA
PAM
DNA cleavage
Metabolic Model
13C-MFA
DBTL
Omics
Machine Learning
Rational Modification
Product
OH
Nucleobase deaminase
After editing
Promoter
dCas
Target gene
RNAP
gRNA
CRISPRi
Synthetic small regulatory RNA
RNA enzymes
sRNA
mRNA
Hfq
lrp
P bmEF
gene
Lrp
Ile
Biosensors

Figure 2


Methanol
Glycerol
Starch & Molasses
Lignocellulosic biomass
Glucose
Xylose
Arabinose
Lignin
Glucose-6-P
PPP
Fructose-6-P
D-Erythrose-4-phosphate
Shikimate pathway
Glyceraldehyde-3-P
Shikimic acid
Chorismic acid
PEP
Pyruvate
Acetyl-CoA
Citrate
α-Ketoglutarate
TCA cycle
Oxaloacetate
Alcohols
Ethanol
1,2-Propanediol
1,3-Propanediol
2-Methyl -1-butanol
Aromatics
PCA
4-ABA
Vanillin
4-HBA
Tyrosine / Phenylalanine
Polyphenols
Resveratrol
Naringenin
Quercetin
Cyanidin 3-O-glucoside
Organic acids
Pyruvic acid
Lactic acid
2-Ketoisovaleric acid
Succinic acid
α-ketoglutaric acid
Fatty acids
Palmitic acid
Octanoic acid
Amino acids
Valine
Isoleucine
Proline
Tyrosine
Terpenoids
Isoprenol
Lycopene
β-Carotene
Decaprenoxanthin
Astaxanthin
Amino acid derivatives
5-Aminolevulinic acid
5-Aminovaleric acid
Indigoidine
L-Aspartate-4-semialdehyde
Ectoine
L-Pipecolic acid
γ-Aminobutyric acid
Putrescine
poly-γ-Glutamic acid


CRISPR/Cas-based genome editing
Cas
3'
gRNA
5'
PAM
DNA cleavage
C. glutamicum genome
Metabolic Model
13C-MFA
DBTL
Omics
Machine Learning
Cas-nucleotide deaminase Base Editor
Cas
G
3'
gRNA
5'
PAM
C
Nucleobase deaminase
A
T
After editing
Rational Modification
OH
Product
Promoter
dCas
Target gene
RNAP
gRNA
CRISPRi
Synthetic small regulatory RNA
RNA enzymes
mRNA
sRNA
HfP
lrp
P brnEF
gene
Lrp
Ile
Biosensors