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Review

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The Actinobacterium *Corynebacterium glutamicum*, an Industrial Workhorse

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Starting as a glutamate producer, *Corynebacterium glutamicum* has played a variety of roles in the industrial production of amino acids, one of the most important areas of white biotechnology. From shortly after its genome information became available, *C. glutamicum* has been applied in various production processes for value-added chemicals, fuels, and polymers, as a key organism in industrial biotechnology alongside the surprising progress in systems biology and metabolic engineering. In addition, recent studies have suggested another potential for *C. glutamicum* as a synthetic biology platform chassis that could move the new era of industrial microbial biotechnology beyond the classical field. Here, we review the recent progress and perspectives in relation to *C. glutamicum*, which demonstrate it as one of the most promising and valuable workhorses in the field of industrial biotechnology.

Keywords: *Corynebacterium glutamicum*, industrial microbe, synthetic biology, metabolic engineering

Introduction

Corynebacterium glutamicum was isolated in the search to identify a natural glutamate producer by researchers of the company Kyowa Hakko in 1956 [70]. *C. glutamicum* is a gram-positive, non-endotoxin, non-sporulating, and generally recognized as safe (GRAS) host that has been widely used for the industrial production of L-glutamate and L-lysine. The publication of the *C. glutamicum* ATCC 13032 genome sequence by two independent groups [43, 50] in 2003 could be regarded as the beginning of a new era for *C. glutamicum* in the field of industrial microbiology and biotechnology. The release of the complete genome sequence provided a platform that would allow better understanding and easier engineering of *C. glutamicum*. Heretofore, the progress in metabolic engineering based on the development and integration of genetic engineering tools, systems biology, and omics-based global analysis techniques brought *C. glutamicum* forward from among the many other workhorses used in industry [10, 149]. During the last decade, more than 100 studies reporting on the development and application of high-throughput technologies such as genomics, transcriptomics, proteomics, and metabolomics have been published. The annual number of both publications and patents increased quickly after the complete genome information was released (Fig. 1A). First, transcriptome analysis using DNA microarray was established in the early 2000s [35, 84, 89, 147]. Later, approaches followed for the analysis of global transcriptional regulatory networks in *C. glutamicum* [8, 124, 149], the analysis of industrial producer strains [19, 35, 67, 74, 128], and the analysis of fermentation processes [15, 55]. In addition to the application of high-throughput sequencing in transcriptome analysis, the development of RNA-seq [107] as an alternative to DNA microarray and capillary DNA sequencing allowed faster, deeper, and more precise insights into understanding the transcriptional regulatory mechanisms [92], function, and occurrence of small RNAs [87]. RNA-seq has also been applied in the analysis of adaptive evolution [78] and lysine producer strains [64]. The development and application of proteomics analysis tools such as 2-D PAGE for high-throughput, high-resolution

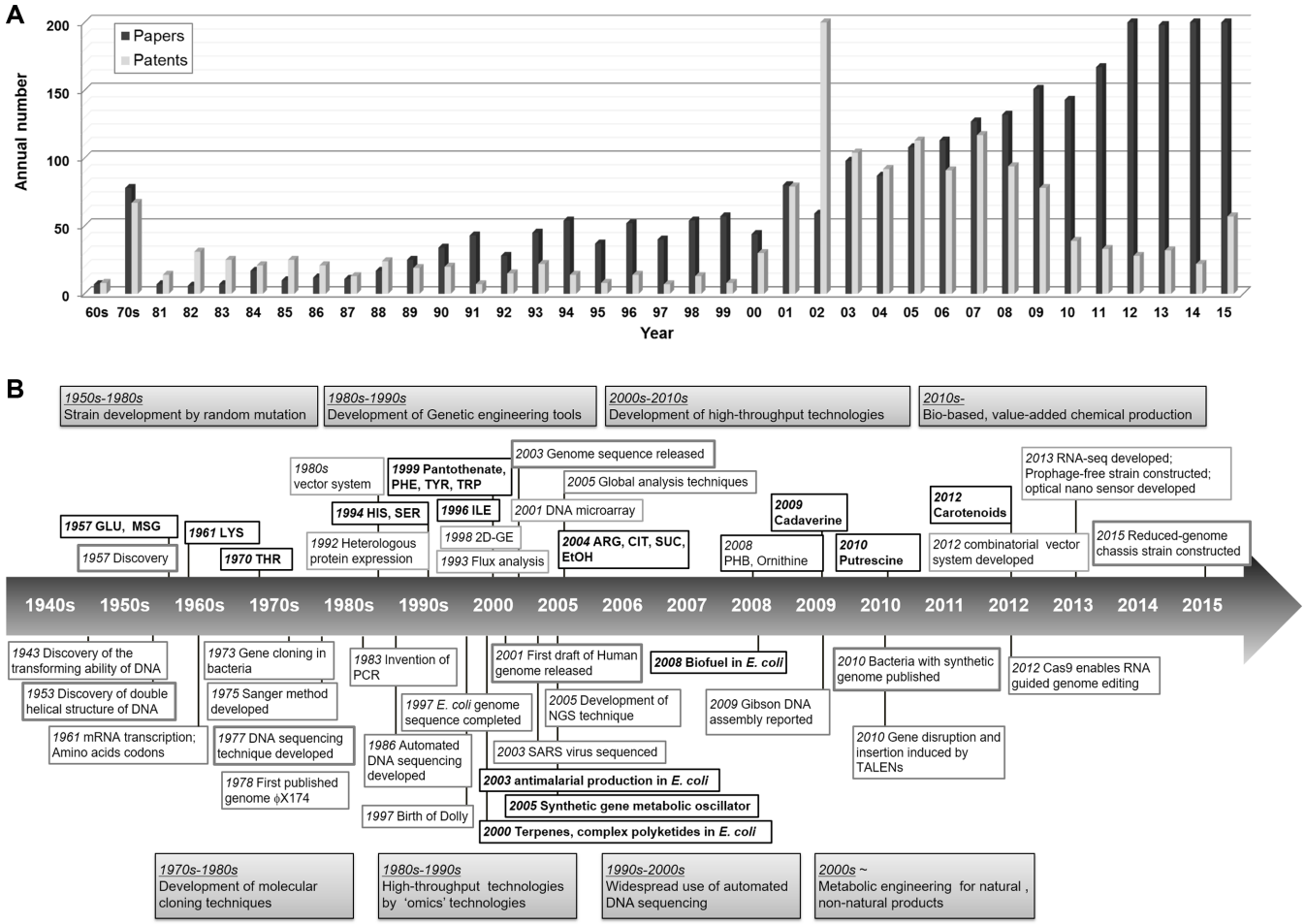

Fig. 1. A brief history of *Corynebacterium glutamicum*.
(**A**) The annual number of reports relevant to *C. glutamicum.* (**B**) Historical and technical milestones in *C. glutamicum* (upper), together with milestones in biotechnology (lower). Boxes: technical and historical events in biotechnology (gray); fermentation or metabolic engineering in microbes (black); trends in microbial biotechnology (gray-filled). Remarkable historical events are highlighted in bold.

proteome separation and MALDI-TOF MS provided a proteome reference map with comprehensive coverage of proteins [12, 37, 115]. Here, we summarize the studies and achievements relating to *C. glutamicum*, including recent progress in the field of bio-based production of value-added chemicals and recombinant proteins. Selected cases of achievements relating to *C. glutamicum* are provided in this mini-review.

Timeline of *C. glutamicum* as an Industrial Microbe

After isolation as a glutamate producer, much effort was made to understand *C. glutamicum* as well as to develop this microbe for industrial purposes. Up to 2015, over 2,700 papers and 1,700 patents have been reported relating to *C. glutamicum.* Among those reports, we selected and summarized the annual number of the reports in Fig. 1A and historical and technical milestones in Fig. 1B.

History of *C. glutamicum* Strains (Isolation, Engineering, and Future)

In the 1950s, Abe and colleagues at Kyowa Hakko carried out taxonomical studies on 208 glutamate-producing strains, isolating what was designated as *Micrococcus glutamicum*, but which later came to be known as *C. glutamicum* [1, 70]. These strains and numerous other strains were assorted to the genera *Brevibacterium*, *Microbacterium*, *Micrococcus*, or *Arthrobacter*. Later, owing to progress in taxonomy (*e.g.*, 16S rDNA sequence analysis and FT-IR spectral comparison), many of the formerly mis-classified strains were re-classified as *C. glutamicum* [80, 93]. Among the first-isolated strains, ATCC13032, a wild-type strain of *C. glutamicum*,

was mostly studied for examination of its physiological properties, metabolism, development of genetic tools, and engineering of *C. glutamicum.* Another widely used type strain, *C. glutamicum* R, was first applied for cultivation under conditions of oxygen deprivation. The R strain was then engineered in order to produce ethanol and organic acids such as succinate, lactate, and acetate, using its anaerobic metabolism under oxygen-deprived conditions. Meanwhile, many industrial *C. glutamicum* strains (screened or engineered) were also studied. To date, 227 type strains, including ATCC13032, have been deposited at American Type Culture Collection (ATCC; http://www.actt.org). In addition, 50 type strains can be found at StrainInfo (http://www.straininfo.net/taxa/569). Recently, AR strains that were mutated and metabolically engineered to enhance the production of arginine [103], genome-reduced strains initiated from prophage-free strain MB001 [7, 138], and a minicells-generating strain that can be applied in drug delivery systems [77] have been reported. Genome information of the first-published complete genome sequence of *C. glutamicum* has been made available at CoryneRegNet (http://www.coryneregnet.de) and KEGG (http://www.kegg.jp).

To date, genome assembly and annotation reports of 14 strains (9 complete genomes; 5 draft genomes) can be found in the genome database at NCBI (http://www.ncbi.nlm.nih.gov/genome/genomes/469). Reported genome assembly and annotation in NCBI are shown in Table 1. The small cryptic plasmids such as pBL1 and pAG3 were also found in *C. glutamicum* and other coryneform bacteria (Table 2).

Benefits of *C. glutamicum* as an Industrial Host

C. glutamicum has many fundamental physiological properties that make it an important industrial workhorse. These properties are listed as follows: (i) GRAS, generally *C. glutamicum* is recognized as a safe strain for human; (ii) fast growth to high cell densities [32]; (iii) genetically stable owing to the lack of a recombination repair system [90]; (iv) limited restriction-modification system [143]; (v) no autolysis and maintenance of metabolic activity under growth-arrested conditions [44]; (vi) low protease activity favoring recombinant protein production [59]; (vii) plasticity of metabolism and strong secondary metabolism properties [148]; and (viii) broad spectrum of carbon utilization (pentoses, hexoses, and alternative carbon sources); stress-tolerance to carbon sources [59, 113]. Taken together, these physiological properties render *C. glutamicum* accessible to manipulation and cultivation in robust industrial conditions.

Toolbox

Numerous physiological and genetic techniques were developed to understand and manipulate *C. glutamicum* after genome sequences of several strains were made available [43, 50, 157], and genetic engineering tools have also been developed that are now available to be used in the manipulation of *C. glutamicum.* Historical events of toolbox development for *C. glutamicum* are briefly shown in Fig. 1B.

Genome manipulations. Owing to advancements in recombinant DNA technology, various tools for genome manipulation of *C. glutamicum* have been developed since

Table 1. Genome assembly of *C. glutamicum* strains reported in NCBI.

Strain	Size (Mb)	GC%	Level[a]	Accession (RefSeq/GenBank)	Gene	Protein	Plasmid
ATCC 13032	3.3094	53.8	●	NC_003450.3/BA000036.3	3057	2959	-
R	3.3633	54.1	●	NC_009342.1/AP009044.1	3149	3011	pCGR1:NC_009343.1/AP009045.1
ATCC 13032 substr. K051	3.3094	53.8	●	NC_020519.1/HE802067.1	3108	3016	-
SCgG1	3.35062	53.9	●	NC_021351.1/CP004047.1	3097	2990	-
SCgG2	3.35062	53.9	●	NC_021352.1/CP004048.1	3096	2988	-
MB001	3.07925	54.2	●	NC_022040.1/CP005959.1	2885	2795	-
ATCC 21831	3.19289	54.19	●	NZ_CP007722.1/CP007722.1	2973	2822	Unnamed:NZ_CP007723.1/CP007723.1
AR1	3.16249	54.09	●	NZ_CP007724.1/CP007724.1	2949	2802	Unnamed:NZ_CP007725.1/CP007725.1
B253	3.22931	54.28	●	NZ_CP010451.1/CP010451.1	2984	2819	Unnamed:NZ_CP010452.1/CP010452.1
DSM 20300 = ATCC 13032	3.28271	53.8	●	NC_006958.1/BX927147.1	3081	2981	-
S9114	3.26289	53.9	◐	AFYA01	3022	2936	-
ATCC 14067	3.31108	54.1	◐	AGQQ02	3162	3008	-
Z188	3.28383	53.9	◐	AKXP01	3051	2964	-
SYPS-062	3.21486	54	◐	JXBH01	3075	2921	-
SYPS-062-33a	3.21199	54	◐	JYEG01	3077	2924	-

[a]Symbols: ●, complete genome sequence; ◐, draft genome sequence (scaffold or contig).

Table 2. Native plasmids of *C. glutamicum* registered in NCBI.

Plasmid	RefSeq	GenBank	Size (kb)	GC%	Protein	Gene
pAG1	NC_001415.1	AF121000.1	19.75	59	19	19
pAG3	NC_004533.1	AY172684.1	4.6	51.9	6	6
pBL1	NC_010243.1	AF092037.1	4.45	53.4	1	1
pCG2	NC_004534.1	AY172685.1	6.76	50.9	6	6
pCG4	NC_004945.1	AF164956.1	29.37	52.1	31	31
pCGR1	NC_009343.1	AP009045.1	49.12	53.9	67	67
pCGR2	NC_013726.1	AB525231.1	24.24	53.9	20	20
pGA2	NC_004535.1	AY172687.1	19.22	50.6	15	15
pAM330	NC_001385.1	D00038.1	4.45	53.3	2	2
pSR1	NC_001456.1	Z22927.1	3.05	57	2	2
pTET3	NC_003227.1	AJ420072.1	27.86	54.9	27	27
pXZ10142	NC_002099.1	X72691.1	2.44	57	5	5
pXZ10145.1	NC_001791.1	U85507.1	4.89	59.6	6	6
pXZ608	NC_004941.1	AF479770.1	5.95	56	1	1
Unknown	NC_002115.1	X03987.1	4.46	53.4	-	-
Unnamed	NZ_CP007723.1	CP007723.1	16.81	51.4	12	12
Unnamed	NZ_CP007725.1	CP007725.1	16.81	51.4	12	12
Unnamed	NZ_CP010452.1	CP010452.1	21.78	51	13	14

the 1990s [36, 40, 120, 139]. Basic tools, such as gene integration, replacement, and disruption for genome engineering of *C. glutamicum* were developed and established from a conjugation system of *E. coli* using mobilizable (*mob*-carrying) vectors [120]. Attempts were made to enhance the transfer efficiency and overcome the restriction-modification system (*e.g.*, *dam-*, *dcm-*, *mcrBC-* mutants) [114]. Single-crossover recombination [143] and double-crossover recombination (markerless method) were developed for gene replacement or disruption methods via events [114]. Cre/*loxP*-mediated recombination systems to rearrange large genome regions have been reported [129-132]. Recently, Unthan *et al.* [138] reported that combinatory deletions of irrelevant large genes would decrease the genome size to 2,561 kbp.

Promoters and plasmids. In addition to the research on the screening of native promoters of *C. glutamicum*, there has been development of promoters using mutation and selection for engineering purposes, particularly in strong and constitutive promoters (*e.g.*, P_{fbp}, P_{sod}, and P_{tuf}) [99]. Most inducible promoters and vectors were adopted from the inducible expression systems of *E. coli* promoters (*e.g.*, P_{lac}, P_{tac}, and P_{trc}) and *lac* operator-repressor. Even leaderless fully synthetic promoters have been recently reported [153]. Promoters used in engineering of *C. glutamicum* are summarized in Table 3. Attempts to tightly regulate protein expression induction by IPTG have been made numerous times using different vectors such as pEKEx1, pXMJ19, and pVWEx1 [27, 45, 105]. Owing to the low IPTG permeability of *C. glutamicum*, IPTG inducible expression systems tend to display a lower level of expression in *C. glutamicum* than those in *E. coli* [104]. However, a recent study reported that an engineered *C. glutamicum* carrying the DE3 region from *E. coli* BL21 (DE3) showed tight regulation as well as controllable expression using IPTG with a homogenous population [72].

After small native plasmids were discovered in amino acid-producing corynebacteria in the 1980s (Table 2), plasmid vectors were developed [57, 112]. Based on native plasmids, vectors for various purposes (cloning, promoter activity report, and expression) were developed starting from the 1990s [27, 36, 41, 120, 139, 140]. Plasmid vectors used in the engineering of *C. glutamicum* are summarized in Table 4. Recent progress in synthetic biology (*e.g.*, commercial gene synthesis, biobrick parts) can make combinatorial assembly of various constructions possible with less effort than before.

Bio-Based Chemicals from *C. glutamicum*

C. glutamicum has had excellent success in the large-scale production of glutamate and lysine accompanied by an increase in knowledge of the metabolism and regulatory networks as well as the development of tools for genetic engineering. As such, *C. glutamicum* has become the

Table 3. Summary of promoters used in engineering of *C. glutamicum*.

Promoter	Relevant genotype or description	Reference
Inducible promoters		
P_{lac} or P_{lacUV5}	IPTG-inducible promoter	[17, 53, 72, 109]
P_{tac}	IPTG-inducible promoter	[13, 76, 81, 109]
P_{trc}	IPTG-inducible promoter	[52, 59, 82, 113]
P_{malE1}	Maltose-inducible promoter	[95]
P_{glt1}	Gluconate-inducible promoter	[95]
P_{araBAD}	L-Arabinose-inducible promoter	[58, 162]
$P_{aceA/aceB}$	Acetate-inducible promoter	[22, 28]
P_{prpB}	Propionate-inducible promoter	[79, 108]
SPLs	Synthetic promoter libraries, IPTG-inducible	[110]
Constitutive promoters		
P_{cspB}	Promoter of *cspB* gene, encoding glyceraldehyde-3-phosphate dehydrogenase	[106, 109, 111, 135]
P_{eftu} or P_{tuf}	Promoter of *tuf* gene, encoding elongation factor TU	[8, 9, 11]
P_{sod}	Promoter of *sod* gene, encoding superoxide dismutase	[8, 65, 76]
P_{180}	Isolated promoter from *C. glutamicum* genome library	[101, 102]
P_{dctA}, P_{dccT} mutants	Spontaneous mutated promoters	[155, 156]
P_{gapA} mutant	Site-directed mutated promoters for gradual modulating gene expression	[33, 34, 49, 151]
P_{dapA} mutant		[141], [71, 144]; [18], [117]
P_{gdh} mutant		[4, 29]
P_{ilvC}, P_{ilvD}, P_{ilvE} mutants		[30, 38, 76]
P_{leuA} mutant		[38]
P_{H36}, P_{I16}, and P_{L26}	Fully-synthesized and isolated promoters	[21, 94, 153]

preferred microbe in white biotechnology for the industrial production of value-added chemicals. A summary of the current status of the production of bio-based chemicals with *C. glutamicum* is shown in Table 5.

Amino Acids

Starting with L-glutamate and L-lysine, *C. glutamicum* was applied to industrial production of various amino acids during the half century after its discovery in the 1950s (Fig. 1B). Amino acids have various characteristics in terms of chemical properties, taste, and nutritional value, and thus have many potential uses, such as feed supplements, food additives, and materials for pharmaceuticals and polymers. Most L-amino acids are produced by microbial fermentation. Within a few years after the first report of L-glutamate fermentation with *C. glutamicum* [70], the microbe was also used to produce L-lysine (Fig. 1B, [91]). In the early era, strain development for amino acid fermentation depended on random mutation and selection such as analog-resistant mutant screening, which contributed to the development of many commercial and potent producers [39]. Along with the advancement in genetic engineering technology, development and application of DNA technology to *C. glutamicum* for amino acid production began in the 1980s. From the beginning until the present day, the amino acid L-glutamate has possessed the largest market share of amino acid fermentation (2.5 M tons/year), relying on *C. glutamicum* for its production. Recently, Zhang *et al.* [161] reported on a L-glutamate-producing *C. glutamicum* with the highest titer and productivity of 120 g/l and 5 g/l·h, respectively. The amino acid L-lysine represents the second largest share of the market (1.5 M tons/year). Becker *et al.* [11] reported on an efficient L-lysine hyper-producer with a titer of 120 g/l, productivity of 4.0 g/l·h, and a yield of 0.55 g/g-carbon developed through synthetic metabolic engineering of *C. glutamicum.* In the 2000s, production expanded to other amino acids such as L-arginine, L- alanine, and L-methionine, and further to derived valuable chemicals such as γ-aminobutyrate (GABA), which can be applied in functional foods, pharmaceuticals, or polyamide.

Alcohols and Organic Acids

Owing to its leading role as an amino acid producer, studies on *C. glutamicum* have focused on amino acid

Table 4. Summary of plasmid vectors used in engineering of *C. glutamicum*.

Vector	Size (kb)	Replicon	Selection marker and reporter	Characteristics	References
Cloning vectors for integration into the chromosome					
pK18*mob*	3.8	None	Km^r, *lacZ*α	Mobilizable	[114]
pK18*mobsacB*	5.7	None	Km^r, *lacZ*α	Mobilizable, *sacB* for the selection of double-crossover event	[114]
pK19*mob*	3.8	None	Km^r, *lacZ*α	Mobilizable	[114]
pK19*mobsacB*	5.7	None	Km^r, *lacZ*α	Mobilizable, *sacB*	[114]
Promoter-probe vectors					
pEKpllacZ	9.4	pBL1	Km^r, *lacZ*α	*E. coli* β-galactosidase	[27]
pEKplCm	7.4	pBL1	Km^r, *cat*	Chloramphenicol acetyltransferase from Tn9	[27]
pUT3	7.4	pBL1	Km^r, *uidA*	*E. coli* β-glucuronidase	[6]
pET2	7.5	pBL1	Km^r, *cat*	Chloramphenicol acetyltransferase from Tn9	[140]
pSK1Cat	6.3	pCG1	Km^r, *cat*	Chloramphenicol acetyltransferase from Tn9	[101]
E. coli-C. glutamicum shuttle expression vectors					
pEKEx1	8.2	pBL1	Km^r	P_{tac}, *lacI*^{q}, IPTG inducible	[27]
pXMJ19	6.6	pBL1	Cm^r	P_{tac}, *lacI*^{q}, IPTG inducible	[45]
pCRA1	5.3	pBL1	Cm^r	P_{lac}, constitutive	[73]
pZ8-1	7	pHM1519	Km^r	P_{tac}, constitutive	[26]
pEC901	8.5	pCG1	Km^r	P_L/P_R (λ), cI857, heat inducible	[136]
pVWEx1	8.5	pHM1519	Km^r	P_{tac}, *lacI*^{q}, IPTG inducible	[105]
pSL360	6.5	pCG1	Km^r, Cm^r	P_{180}, constitutive	[101]
pTRCmob	7	pGA1	Km^r	P_{trc}, *lacI*, IPTG inducible	[82]
pTGR series (1-17)	7.5 (5)	pGA1; pNG2; pCry4	Km^r	Combinatorial assembly of promoter (*tac*, *csp*, and *sod*) and RBS (*lacZ*, *cspB*, and *sod*), constitutive	[109]
pBbEB 1; 2; 5-c	6.8; 6.0; 6.9	pBL1	Cm^r	Combinatorial assembly of promoter (*trc*, *tet*, *lacUV5*) and repressor (*lacI*, *tetR*), IPTG or aTc inducible	[52]
pCXM48 derivatives	4.84-6.22	pBL1	Cm^r	Combinatorial assembly of promoter (*tac*, *sod*, *ilv*C mutants) and RBS, IPTG inducible	[76]

aTc, anhydrotetracycline; Cm, chloramphenicol; IPTG, isopropyl β-D-1-thiogalactopyranoside ; Km, kanamycin.

biosynthetic pathways. However, fermentation of alcohols such as ethanol and isobutanol under oxygen-deprived conditions has been reported [16, 44, 49, 125, 151]. Furthermore, efforts to produce organic acids such as succinate, lactate, 2-ketoglutarate, and 2-ketoisovalerate have proven that *C. glutamicum* can also produce valuable organic acids. The possibilities for the use of *C. glutamicum* in industrial applications have also been investigated. For example, there have been reports of ethanol (119 g/l), isobutanol (73 g/l), L-lactate (95.6 g/l), D-lactate (120 g/l), and succinate (156 g/l) production under conditions of oxygen deprivation [16, 44, 49, 125, 151].

Polymers

There have been efforts made to produce polymers such as diamines (putrescine, cadavarine) and polyhydroxybutyrate (PHB) with engineered *C. glutamicum*; for example, recombinant ornithine-producing *C. glutamicum* has been used to produce diamine 1,4-diaminobutane (putrescine) and 1,5-diaminopentane (cadaverine) derived from L-ornithine by decarboxylation [19, 69, 117–119]. Recombinant *C. glutamicum* expressing PhbCAB from *Ralstonia eutropha* accumulated up to 6.0 g/l polyhydroxyalkanoate (PHA) and 0.99 g/l PHB, respectively [83, 86].

Heterologous Protein Expression in *C. glutamicum*

As mentioned above, *C. glutamicum* has been widely used as an industrial workhorse for the production of amino acids and various bio-based chemicals. Moreover, an

Table 5. Summary of the bio-based products in *C. glutamicum*.

Product	Producer	Titer (g/l)	Productivity (g/l·h)	Yield (g/g)	Reference
Amino acids and related compounds					
L-Alaninie	R Δ*ldhA*Δ*ppc*Δ*alr*+*alaD*+*gapA*	98	3.1	0.83	[48]
L-Arginine	ATCC21831 Δ*argR*Δ*farR pgi*(A1G)P_{sod}-*tkt*ΔNCgl1221 P_{sod} -*carABargF*(rev.)P_{eftu}-*argGH*	92.5	1.3	0.4	[103]
L-Citrulline	ATCC 13032 Δ*argG*Δ*argR*+pXMJ19-*argJ*	8.5	0.1	0.11	[31]
L-Cysteine	Δ*aecD*+pVK-CEM2561 (*cysE*^{M256I})	0.29	0.004	0.029	[145]
L-Glutamate	GDK-9 Δ*ldhA*	120	5	n.d.	[161]
L-Glutamine	ATCC14067 pJC*glnA'*tac*vgb*	17.3	0.36	0.08	[83]
L-Histidine	F81/pCH99	22.5	0.19	0.15-0.2	[88]
L-Isoleucine	DR17/pECM3::*ilvA*38	21	0.7	0.24	[60]
L-Leucine	ATCC13032 Δ*ltbR*Δ*leuA*::P*tuf-leuA*_B018+IlvN$^{(G20D,I21D,I22F)}$ Δ*iolR* $P_{dapA-L1}$*gltA*	23.7	4.3	0.3	[144]
L-Lysine	ATCC13032+*lysC*^{T311I}+2x*ddh*+Δ*pck*+P_{sod}.*dapB*+2×*lysA*+P_{sod}-*lysC*+*hom*^{V59A} +P_{sod}-*pycA*^{P485S}+P_{sod}-*pycA*+*icd*^{GTG}+P_{tuf}-*fbp*+P_{sod}-*tkt-tal-zwf-opcA-pgl*	120	4	0.55	[11]
L-Methionine	MH20-22B/*hom*^{FBR}/Δ*thrB*	2.9	0.02	0.02	[102]
L-Ornithine	ATCC13032 Δ*proB* Δ*argF* Δ*argR pgi*^{GTG} *zwf*^{ATG} P_{tkt}::P_{sod} +pSY223	51.5	1.29	0.24	[65]
L-Phenylalanine	KY10865+pKF1	28	0.35	0.47	[40]
L-Proline	ATCC13032 Δ*argR* Δ*argF* +pEKEx3-*argB*$_{A49VM54V}$	12.7	0.52	0.36	[46]
L-Serine	SYPS-062 Δ*serA*Δ*sdaA* Δ*alaT* Δ*avtA* ΔC-T *ilvN*	42.6	0.44	0.21	[163]
L-Threonine	TK42/pChom93	52	0.58	0.15	[56]
L-Tryptophan	KY9218+pIK9960	58	0.73	0.25	[42]
L-Tyrosine	KY10865+pKY1	26	0.32	0.43	[40]
L-Valine	RDLP_ΔAc+GP_ilvN^{GE}C^{TM}_ΔAla +pCRB-BN^{GE}C^{TM} + pCRB-DLD	149.8	0.27	0.57	[33]
D-Arginine	ATCC13032 Δ*argR*+pVWEx2-*argB*^{A49VM54V}+pEKEx3-ArgR	0.5	0.01	0.01	[127]
D-Lysine	ATCC13032 Δ*pck pyc*^{P458S} *hom*^{V59A} 2×*lysC*^{T311I} 2×(*lysE asd dapA dapB ddh lysA*)+pEKEx3-ArgR	4.2	0.11	0.1	[127]
D-Orthinine	ATCC13032 Δ*argR* Δ*argF*+pEKEx3-ArgR	6.3	0.13	0.16	[127]
D-Serine	ATCC13032 Δ*sdaA* Δ*pabABC* (P*serABC*)+pEKEx3-ArgR	8.5	0.12	0.21	[127]
γ-Aminobutyrate (GABA)	ATCC13032+ pHGmut	38.6	0.54	0.56	[21]
Shikimate	RES167 Δ*aroK* + pXMJ19-GBTDE	11.3	0.19	0.12	[159]
Organic acids and alcohols					
Ethanol	R Δ*ldhA* Δ*ppc*+*pgi*+*pfkA*+*gapA*+*pyk*+*glk*+*fba*+*tpi*+*pdc*+*adhB*	119	2.3	0.48	[49]
Glycolate	ATCC13032 Δ*aceB icd*^{GTG} +pVWEx1-*ycdW*	5.3	0.1	0.18	[158]
Isobutanol	R P_{tac}-SD-*gapA*+P_{tac}-SD-*pyk*+P_{tac}-SD-*pfk*+P_{tac}-SD-*gpi*+P_{gapA}-*ilvBNC*^{TM} +P_{ldhA}-*kivd*+P_{gapA}-*adhP*+P_{gapA}-*ilvD*	73	1.22	2.6	[151]
Itaconic acid	ATCC13032 *icd*^{A1G}+pEKEx2-*malEcad*$_{opt}$	7.8	0.27	0.03	[98]
2-Ketoglutarate	HH109 Δ*gdh* Δ*gltB* Δ*aceA*	47.5	0.39	n.d.	[47]
2-Ketoisocaprate	ATCC13032 Δ*ltbR* Δ*ilvE* Δ*prpC1* Δ*prpC2* P_{gltA}^{mut}_L1+pJC4*ilvBNCDleuA*$^{EC-G462D}$	9.2	0.37	0.24	[5]
2-Ketoisovalerate	*aceE* A16 Δ*pqo* Δ*ppc* Δ*ilvE*+pJC4 *ilvBNCD*	35	0.79	0.15	[18]
12-Ketooleic acid	ATCC13032+pCES-L10::ADH	n.d.	1.2	74%[a]	[75]
L-Lactate	R	95.6	42.97	0.71	[96]
D-Lactate	R *ldhA*+pCRA720-*ldhA*	120	4	0.83	[97]

Table 5. Continued.

Product	Producer	Titer (g/l)	Productivity (g/l·h)	Yield (g/g)	Reference
Oleic acid	ATCC13032 *fasR20 fasA63*^{up} *fasA2623*	0.21	n.d.	n.d.	[134]
Palmitic acid	ATCC13032 *fasR20 fasA63*^{up} *fasA2623*	0.05	n.d.	n.d.	[134]
Pantothenate	ATCC 13032 Δ*ilvA* P-*ilvE*M3+pJC1*ilvBNCD*+(Ptrc-*panBC*)x2	1.8	0.04	0.04	[30]
1-Propanol	ATCC13032 Δ*hdpA*Δ*ldh*+pEKEx3-*mgsA-yqhD-gldA*+pVWEx1-*ppdABC*	0.72	0.01	0.02	[123]
1,2-Propanediol	ATCC13032 Δ*hdpA*Δ*ldh*+pEKEx3-*mgsA-yqhD-gldA*	4.56	0.09	0.14	[123]
Pyruvate	ATCC13032 Δ*aceE* Δ*pqo* Δ*ldhA* ΔC-T *ilvN* Δ*alaT* Δ*avtA*	44.5	0.49	0.72	[150]
Succinate	R Δ*ldhA* pCRA717	146.3	3.19	0.92	[97]
Polymers					
1,4-Diaminobutane (putrescine)	ATCC13032 Δ*argR* Δ*argF*+pVWEx1-*speC-5′21-argF*	19	0.55	0.16	[117]
1,5-Diaminopentane (cadaverine)	ATCC13032+*lysC*^{T311I}+2×*ddh*+Δ*pck*+P$_{sod}$-*dapB*+2×*lysA* +P$_{sod}$-*lysC*+*hom*^{V59A}+P$_{sod}$-*pycA*^{P485S}+P$_{sod}$-*pyc*A+*icd*^{GTG}+P$_{tuf}$-*fbp* +P$_{sod}$-*tkt-tal-zwf-opcA-pgl*+P$_{tuf}$-*ldcC*^{opt}+ΔNCgl1469+Δ*lysE*+P$_{sod}$-cg2893	103	1.5	0.32	[19]
Ectoine	ATCC13032 *lysC*^{T311I}+P$_{tuf}$-*ectABCD*+Δ*lysE*	4.5	0.28	0.24	[9]
Poly-hydroxyalkanoate (PHA)	ATCC13869 + pPS-C′ *AB* + pVC7-*AB*	6	n.d.	n.d.	[86]
Poly-hydroxybutyrate (PHB)	9114 + *phbCAB*	0.99	n.d.	n.d.	[82]
Poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV)	ATCC13869 + pPS-C′ *AB* + pVC7-*AB*	0.47	n.d.	n.d.	[86]

n.d., not determined.

Numerical values in titer, yield, and productivity were adapted or calculated based on values in the references.

[a]Maximum conversion yield.

increasing number of reports indicate *C. glutamicum* to have great potential as a platform microbe for heterologous protein expression (Table 6). Owing to the many intrinsic attributes of *C. glutamicum*, a broad range of different approaches has been used to investigate its potential as a host for the expression of heterologous proteins. There are several successful examples showing that heterologous expression in *C. glutamicum* enhanced the production of amino acids and other value-added chemicals, or allowed the utilization of various carbon sources via metabolic engineering approaches.

Intrinsic Advantages of *C. glutamicum* as a Protein Expression Host

C. glutamicum has intrinsic advantages as a microbial cell factory for protein production. A low level of extracellular protease activity and the presence of two native protein secretion mechanisms, the general secretory (Sec-dependent) pathway and the twin-arginine translocation (TAT-dependent) pathway have been known to enhance the secretion of homologous and heterologous proteins. Proteome analyses of *C. glutamicum* showed that approximately 10% of the proteins coded by wild-type *C. glutamicum* could be secreted [51]. Many species belonging to *Corynebacterium* are known to possess low protease activity unlike many other soil bacteria such as those of the *Streptomyces* and *Bacillus* genera. It has been identified that *C. glutamicum* has only one extracellular protease coding gene in its genome, the extracellular trypsin-like protease gene *etpr* (cg1243, cgR1176) [157]. To date, two well-identified independent secretion pathways have been utilized to enhance the secretion of proteins in *C. glutamicum*: the Sec-dependent and TAT-dependent pathways. Overexpression of components of the Sec and the TAT pathways, such as Sec(E/Y)DF and TatABC, has been suggested as a method to reduce molecular bottlenecks [63]. Moreover, the signal peptides type I and II have also been investigated to enhance secretion efficiency, such as signal sequences 29, 11, and 8 for Sec, Tat (type I), and lipoprotein (type II), respectively [142].

Heterologous Protein Expression

As shown in Table 6, heterologous expression in *C. glutamicum* has been reported. Amylases from other soil bacteria such as *Bacillus amyloliquefaciens*, *Geobacillus stearothermophilus*, *Streptomyces bovis*, and *Streptomyces*

Table 6. Summary of the heterologous expression cases in *C. glutamicum*.

Product	Strain	Titer	Protein / signal peptide	Reference
Amino acids and related compounds				
D-Arginine	ATCC13032	0.5 g/l	*Pseudomonas taetrolens* ArgR, broad-substrate racemase	[127]
D-Lysine	ATCC13032	4.2 g/l	*P. taetrolens* ArgR, broad-substrate racemase	[127]
D-Ornithine	ATCC13032	6.3 g/l	*P. taetrolens* ArgR, broad-substrate racemase	[127]
D-Serine	ATCC13032	8.5 g/l	*P. taetrolens* ArgR, broad-substrate racemase	[127]
L-Phenylalanine	ATCC13032	15.76 g/l	*Escherichia coli* YddG, *olT2-ppgK*	[160]
γ-Aminobutylate (GABA)	ATCC13032	38.6 g/l	*Lactobacillus brevis* glutamate decarboxylase; *E. coli* GAD mutant	[21, 121, 122]
Heterologous proteins				
α-Amylase	ATCC13032; ATCC13869; ATCC21798; R	0.78 g/l	*Bacillus amyloliquefaciens* AmyE, BamP; *Geobacillus stearothermophilus* AmyE; *Streptomyces bovis* AmyA; *Streptomyces griseus*/ native; CspA; 98 Sec SPs; 10 Tat SPs	[20, 126, 133, 146, 154]
Antigen 85A	BI15; CGL1017	n.q.	*Mycobacterium tuberculosis* 85A / native; $CspB_{Cg}$	[111]
cAbHuL22 VHH	ATCC13032	1.57 g/l	Camelid heavy-chain antibody fragment against human lysozyme	[154]
Cellulase	ATCC13869	5 U/ml	*Streptomyces halstedii* Cel1	[2]
Endoglucanase	ATCC21798	35 U/ml	*Cellulomonas fimi* CenA / native	[100]
Endoxylanase	ATCC13032	746 mg/l	*Bacillus* sp. XynA; *Streptomyces coelicolor* XynA / $PorB_{Cg}$	[3, 152]
Exoglucanase	ATCC21798	0.3 U/ml	*Cellulomonas fimi* Cex / native	[100]
GFP	ATCC13032; YDK010; R	2.8 g/l	*Aequorea victoria*, *A. coerulescens* AcGFP1 / $TorA_{Ec}$; IMD_{Ag}; $CgR0949_{Cg}$	[62, 146]
Fab fragment of human anti-HER2	ATCC13869; YDK010	57.6 mg/l	Human heavy chain, AY513484; light chain, AY513485 / $CspA_{Ca}$	[85]
Epidermal growth factor protein	YDK010	0.156 g/l	Human / $CspA_{Ca}$	[23]
IFN-gamma	AS019	n.q.	Ovine gamma interferon / native	[13]
Protease	AS019	2.5 mg/l	*Dichelobacter nodosus* BprV; *Bacillus subtilis* AprE	[14]
SAM-P45 protease	ATCC13869	78 U/l	*Streptomyces albogriseolus* / $CspA_{Ca}$	[61]
M18 scFv	ATCC13032	68 mg/l	Murine / $PorB_{Cg}$	[3, 153]
Protein glutaminase	ATCC13869; YDK010	1.24 g/l	*Chryseobacterium proteolyticum* PG / IMD_{Sm}; $TorA_{Ec}$	[62, 63]
SNase	AS019/R163	20 mg/l	*Staphylococcus aureus* nuclease / native	[81]
Sorbitol-xylitol oxidase	ATCC13032	n.q.	*S. coelicolor* SoXy / $TorA_{Ec}$	[116]
Transglutaminase	ATCC13869; YDK010	0.881 mg/l	*Streptomyces mobaraensis* MTG; *S. albogriseolus* pro-MTG / $CspA_{Cg, Ca}$; $CspB_{Cg}$; IMD_{Sm}; $TorA_{Ec}$	[24, 25, 61-63]
Xylanase	ATCC13869; R31	20 U/ml	*S. halstedii* Xys1; *Aspergillus nidulans* XlnA / native; $Xys1_{Sh}$	[2]
Organic acids and alcohols				
Ethanol	R	119 g/l	*Zymomonas mobilis* Pdc and AdhB	[49]
D-Lactate	R	120 g/l	*E. coli* XylA and XylB; Dldh from *E. coli* and *Lactobacillus delbrueckii*	[59, 97]
Isobutanol	R	73 g/l	*E. coli* AdhP, *Lactococcus lactis* KidV, and *Saccharomyces cerevisiae* Adh2	[151]
Itaconate	ATCC13032	7.8 g/l	*Aspergillus terreus* CAD1, *E. coli* MalE	[98]
1-Propanol	ATCC13032	0.72 g/l	*E. coli* GldA, MgsA, YqhD and *Klebsiella oxytoca* PpdABC	[123]
1,2-Propanediol	ATCC13032	4.56 g/l	*E. coli* GldA, MgsA, YqhD	[123]
Succinate	R	11.9 g/l	*E. coli* XylA, XylB	[59]

Table 6. Continued.

Product	Strain	Titer	Protein / signal peptide	Reference
Biopolymers				
Acrylamide	ATCC13032	425 g/l	*Rhodococcus rhodochrous* NhhBAG	[54]
1,4-Diaminobutane (putrescine)	ATCC13032	19 g/l	*E. coli* AdiA, SpeA, SpeB, SpeC, SpeF	[117, 119]
1,5-Diaminopentane (cadaverine)	ATCC13032	88 g/l	*E. coli* CadA, CadB, LdcC; *Hafnia alvei* CadA, Ldc	[19, 66–69, 94]
Ectoine	ATCC13032	4.5 g/l	*Pseudomonas stutzeri* EctABCD	[9]
12-Ketooleic acid	ATCC13032	74 %[a]	*Micrococcus luteus* ADH	[75]
PHA	ATCC13869	6.0 g/l	*Ralstonia eutropha* PhbCAB	[86]
PHB	9114	0.99 g/l	*R. eutropha* PhbCAB	[82]

n.q., not quantified.

[a]Maximum conversion yield.

griseus were successfully expressed in various *C. glutamicum* strains [20, 126, 133, 146, 154]. Numerous cases have shown that heterologous expression enhanced the production of natural or non-natural compounds in *C. glutamicum;* for example, *Pseudomonas taetrolens* ArgR enabled the production of D-amino acids in an engineered *C. glutamicum* strain [127]. More recently, a synthetic *Pseudomonas stutzeri* ectoine gene cluster enabled the production of ectoine, a chemical chaperone that has a stabilizing effect on biological molecules in *C. glutamicum* [9].

Concluding Remarks and Outlook

Best known for its use over a period of decades in industry as a glutamate and lysine producer, *C. glutamicum* has been widely used in various applications and is considered as one of the most preferred microbes used for metabolic engineering. A number of approaches for enhancing secretion efficiency have been identified, such as the use of signal peptides. However, *C. glutamicum* has some disadvantages when compared with *E. coli*, such as a much lower transformation efficiency and a low number of available expression systems. To overcome these issues, a remarkable effort should be made to develop expression systems and secretion machinery.

Taken together, we still need to develop the toolbox that will allow us to gain a deeper understanding and increase production speed. However, we can surely expect further progress for *C. glutamicum* as a microbial value-adding producer workhorse in the field of microbial biotechnology.

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