Adv Biochem Eng Biotechnol
DOI: 10.1007/10_2016_24

Discovery and History of Amino Acid Fermentation

Shin-ichi Hashimoto

Abstract There has been a strong demand in Japan and East Asia for L-glutamic acid as a seasoning since monosodium glutamate was found to present umami taste in 1907. The discovery of glutamate fermentation by *Corynebacterium glutamicum* in 1956 enabled abundant and low-cost production of the amino acid, creating a large market. The discovery also prompted researchers to develop fermentative production processes for other L-amino acids, such as lysine. Currently, the amino acid fermentation industry is so huge that more than 5 million metric tons of amino acids are manufactured annually all over the world, and this number continues to grow. Research on amino acid fermentation fostered the notion and skills of metabolic engineering which has been applied for the production of other compounds from renewable resources. The discovery of glutamate fermentation has had revolutionary impacts on both the industry and science. In this chapter, the history and development of glutamate fermentation, including the very early stage of fermentation of other amino acids, are reviewed.

Keywords Amino acid fermentation, *Corynebacterium glutamicum*, Glutamate fermentation, L-Amino acid, Metabolic engineering, Strain breeding

Contents

S.-i. Hashimoto (✉)
Kyowa Hakko Bio Co. Ltd., 1-9-2 Ohtemachi, Chiyoda-ku, Tokyo 100-0004, Japan
e-mail: shinichi.hashimoto@kyowa-kirin.co.jp

Amino acid fermentation is a huge industry. More than 5 million metric tons of amino acids (more than 2 million metric tons of glutamate, around 2 million metric tons of lysine, etc.) are manufactured annually all over the world [1]. Amino acids find application in a wide range of fields: seasoning, animal feed, medicine, and starting material for chemicals including pharmaceuticals. A number of scientific paper and patent on amino acid fermentation have been published every year. These studies have been contributing to widen our knowledge on the science of metabolism, genetics, and physiology.

The discovery of glutamate fermentation by *Corynebacterium glutamicum* in 1956 was the inception of amino acid fermentation. This discovery was a major breakthrough not only for glutamate production but also for research, demonstrating that amino acid fermentation is possible. In this chapter, the history of amino acid fermentation (glutamate fermentation is mainly focused on) before and after its discovery is reviewed.

1 Prehistory of Glutamate Fermentation

Although glutamate was isolated from gluten in 1866, its major applicational value was found in 1908 by Kikunae Ikeda [2]. He studied taste presenting substance derived from kelp, whose soup has traditionally been used as a basic seasoning in Japan, and identified the monosodium salt of L-glutamate as an *umami* substance. An entrepreneur, Saburosuke Suzuki, collaborated with Dr. Ikeda and commercialized the discovery; he began selling monosodium glutamate (MSG) as a new seasoning "AJI-NO-MOTO®" in 1908 (Fig. 1).

Because umami is a traditional taste component in Japan, the new product got a great sales success. However, there were several difficulties in manufacturing MSG, for which wheat gluten was hydrolyzed using hydrochloric acid and L-glutamate was isolated following the recrystallization as the monosodium salt. Because there were scarce materials that could tolerate acidic conditions under high temperature during hydrolyzation in the early twentieth century, obtaining suitable vessels for hydrolyzation was a big issue. After several trials and errors, a certain type of ceramic pot was found to be suitable. Hydrogen chloride gas released during the hydrolyzation process was very hazardous. Furthermore, the

Fig. 1 The first product of umami seasoning, "AJI-NO-MOTO®." The photo is reprinted under the kind permission of Ajinomoto Co., Inc.

formation of a large volume of waste (the remaining fraction of the hydrolyzate) presented additional issue [3].

Therefore, there was a strong demand for a new manufacturing process for MSG or L-glutamate. During the mid-1950s, a decade after the end of World War II, the economy and life of people in Japan was returning to normal. Corresponding with the social situation, the demand for MSG had increased rapidly; the production of MSG by Ajinomoto Co., Inc jumped from 6,662 metric tons in 1955 to 13,586 metric tons in 1959 [4].

Several attempts were made to establish a new method for manufacturing L-glutamate. Because of the necessity of optical resolution, chemical synthesis was not advantageous while it was used commercially for a particular period. Chemical synthesis coupled with enzymatic resolution was a feasible method [5]. Izaki

et al. have reported specific degradation of D-glutamate by *Aerobacter* [6]. The optical resolution of *N*-acyl-DL-glutamate by D-specific acylase activities derived from *Aspergillus tamarii* and *Penicillium vinaceum* presented additional approach [7].

Since glutamate is biosynthesized from 2-oxoglutarate through amination, it was natural to pursue the process that consisted of microbial production of 2-oxoglutarate and microbial amination of the oxoacid.

The earliest report on fermentative production of 2-oxoglutarate can be found in 1946; *Pseudomonas fluorescens* accumulated 16–17 g of the acid per 100 g of glucose [8]. The yield was increased to 41 g of the acid per 100 g of glucose [9].

Formation of small amounts of glutamate in the presence of 2-oxoglutarate and ammonium salts was reported in *Clostridium* [10], *Escherichia coli* [11], *Bacillus subtilis* [12], *P. fluorescens* [13], and *Pseudomonas ovalis* [14]. Attempts to perform the conversion using amino acids as amino donors were also reported [13, 15].

The combination of 2-oxoglutarate fermentation and amination of the oxoacid appears to be just one step away from direct fermentation of glutamic acid (direct fermentation refers to the process of producing the product from a low-cost carbon source, such as sugar, and a nitrogen source, such as ammonia, through the cultivation of microorganisms). It was known that accumulation of small amounts of glutamate occurred in the cultivation medium of certain bacteria. Morrison and Hinshelwood and Dagley et al. have observed very small amount of accumulation of the amino acid in the cultivation broth of *E. coli* and *Aerobacter aerogenes* [16, 17]. Thorne et al. have reported that *B. anthracis* formed glutamate (2 g/L) under the conditions in which polyglutamate formation was hampered [18]. Asai et al. screened for a glutamate producer and found *Micrococcus varians* to be the best producer although the accumulation was far lower (2.9 g/L at the optimal efficiency) for industrial application [19].

2 Discovery and Industrialization of Glutamic Acid Fermentation

M. J. Johnson, an emeritus professor at the University of Wisconsin, described the situation in 1955, “there is, in many quarters, great interest in fermentative glutamic acid production” [20]. However, there was a strong notion that it is irrational to expect a microorganism to accumulate a large quantity of the amino acid extracellularly because (1) glutamate is an essential component for the organism, (2) excretion of glutamate is an economical loss for the cell because the biosynthesis of the amino acid is energetically expensive, and (3) secretion of glutamate would not be expected to have suppressing effect against surrounding microorganisms like antibiotic dose. Researchers in Kyowa Hakko Kogyo Co., Ltd. have overcome this conceptual obstacle.

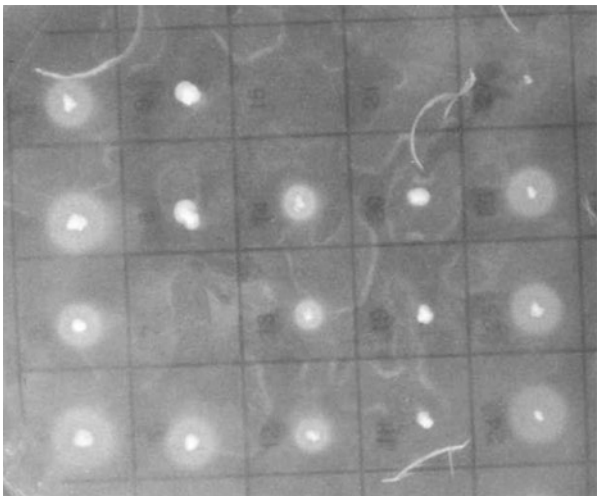

Fig. 2 Bioassay screening of glutamate-producing microorganisms. Glutamate productivity of the test strain can be estimated by the scale of the halo formed around the strain. The photo is reprinted under the kind permission of Kyowa Hakko Bio Co. Ltd.

Dr. Udaka, a researcher of Kyowa Hakko Kogyo Co., Ltd. at that time, has set the following screening method [21]. Bacteria isolated from the environment were replicated on nutrient agar plates and several types of defined medium plates (test plates). After colony formation, the test plates were exposed to UV radiation to kill the organisms. Then, soft agar medium containing the glutamate auxotroph *Leuconostoc mesenteroides* was overlaid on the test plates. The halo of growth development around the colony on the test plates indicated glutamate excretion by the colony (Fig. 2).

After screening *only* approximately 500 isolates, they found the superior strain, *Corynebacterium glutamicum* (originally reported under the name of *Micrococcus glutamicus*). The strain accumulated 10.3 g of glutamate per liter when cultivated in a flask with liquid synthetic medium with 5% glucose [22], and the accumulation was easily increased to >30 g/L [23] with >25% yield against glucose input, indicating that the strain is applicable for industrial glutamate fermentation.

When the study was scaled up, however, the researchers faced a puzzle; the bacterium produced only trace amounts of glutamate. Several months of intensive research revealed important characteristics of glutamate fermentation: the bacterium is a biotin auxotroph and produces glutamate only under biotin-limited conditions. It was assumed that the cells grew in screening and flask cultivation using a trace amount of biotin carried over from the pre-culture and, thus, resulted in biotin-limited conditions.

The first commercial fermentation of glutamate was conducted in 1958 at a plant of Kyowa Hakko Kogyo. There were still problems to be solved for commercialization, such as the downstream process, particularly the control of the crystal form. In that way amino acid fermentation took the first step.

3 Establishment of Glutamate Fermentation

Glutamate fermentation by *C. glutamicum* was first presented at the International Symposium on Enzyme Chemistry held in Japan in 1957. As the congress was the first major international scientific meeting held in Japan after World War II, the presentation attained great attention from Japanese researchers in the field of life sciences. Once the discovery of glutamate fermentation was public, several researchers and companies rushed into the research. It looks like a "gold rush," which is observed in Figs. 3 and 4.

Until 1970, Japanese researchers enthusiastically conducted research and held a monopoly on the scientific papers on glutamate fermentation (Fig. 3). Japanese research activity appeared to steady down by the 1980s; however, it showed resurgence during the 2000s. In contrast, reports from other Asian countries, particularly from China and India, showed an increase from the 1980s. Several of these studies have outlined the discovery of a new strain (most strains are under *Corynebacterium* sp.), the application of a new raw material, and improvement of the production system, suggesting a strong economical demand of glutamate fermentation in these areas. Research from Europe began to emerge from the 1990s. Most of the European research focused on the mechanism of glutamate fermentation and played an essential role in understanding the mechanism.

Figure 4 shows the "gold rush" in Japan from a different angle. A rapid increase of patents in 1960 suggests that companies began research on glutamate fermentation after the announcement of the discovery of *C. glutamicum*. Representative strains and carbon source are shown in Table 1. Most of the important aspects of the fermentation appeared before 1980, which are summarized below. These insights are closely or directly related to the elucidation of the mechanism of glutamate production discussed in [35].

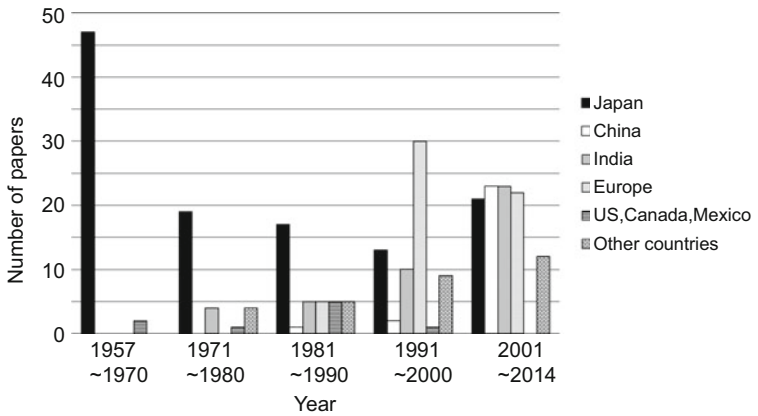

Fig. 3 Trends of scientific paper publication on glutamate fermentation in English. For each decades (except for 1957–1970 and 2001–2014), the number of papers are counted by country. Reviews, patents, and oral presentations are not included

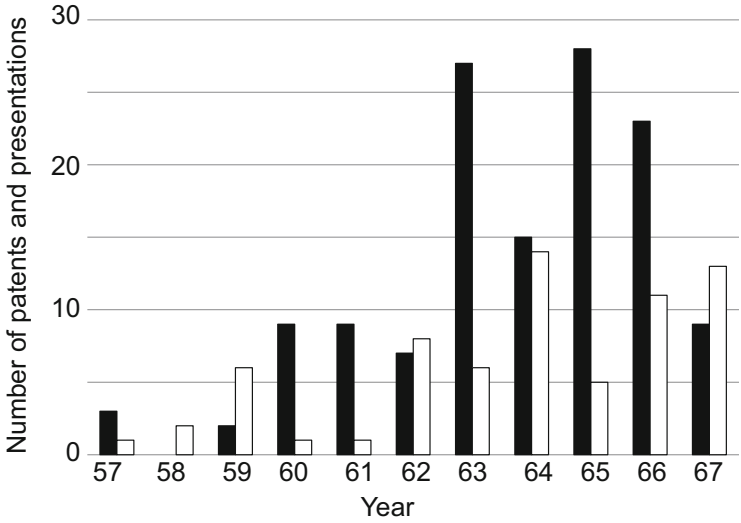

Fig. 4 Numbers of Japanese patents (*black bar*) and oral presentations (*white bar*) in the annual meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry from 1957 to 1967

Table 1 Glutamate fermentation from different carbon sources by different microorganisms

Carbon source	Microorganism	Titer (g/L)	Reference
Glucose 12%	*C. glutamicum*	30	[24]
Glucose ?%	*C. glutamicum*	195	[25]
Cane molasses 13%	*C. glutamicum*	63	[26]
Acetate 9%	*C. glutamicum*	23	[27]
Ethanol ?%	*Brevibacterium* sp.	53.1	[28]
Methanol 11%	*M. methylovora*	6.8	[29]
Methanol ?%	*B. methanolicus*	69	[30]
n-Paraffin 3%	*Corynebacterium* sp.	5	[31]
n-Hexadecane 8%	*C. hydrocarboclustus*	19.6	[32]
Benzoate ?%	*Brevibacterium* sp.	75	[33, 34]

The earliest scientific report (patents and oral presentations are not included) on each carbon source is listed. For glucose and methanol, reports of the highest titer are also shown. Symbol "?" in the carbon source indicates no description of the amount of input carbon source in the report

3.1 Producer Strain

Many species were claimed as glutamate-producing microorganisms such as *Brevibacterium lactofermentum*, *Brevibacterium flavum*, *Corynebacterium callunae*, *Corynebacterium lilium*, etc. Most of them were aerobic, gram-positive, nonacid-fast, nonspore-forming, rod-shaped, and biotin-requiring bacteria. These "new" species were categorized in the spices of *Corynebacterium glutamicum* by

thorough taxonomic investigation in later years [36–38]. Thus, the name of *C. glutamicum* is used for these bacteria hereinafter. It became a shared sense within several years after the discovery of the bacterium that the bacterium (even wild-type strain) produces glutamate at a yield of >40% against input sugar (Table 1) under appropriate conditions.

Bacteria capable of producing glutamate from sugar and not belonging to *C. glutamicum* have also been reported. Chao and Foster have reported productivity of 13.5 g/L from 3% glucose by *Bacillus megaterium* [39]. It is interesting that this strain is also biotin auxotroph. Some *Arthrobacter* [40] and *Streptomyces* [41] strains were also reported to produce glutamate. Recently, it was reported that metabolically engineered *E. coli*, *Enterobacter agglomerans*, *Klebsiella planticola*, and *Pantoea agglomerans* produce significant amounts of glutamate [42, 43].

3.2 Carbon Source

As *C. glutamicum* readily utilizes glucose, fructose, and sucrose, these sugars are initially used as a carbon source. Molasses, a more economically desirable source, was mainly used after the discovery of an alternative method of biotin limitation because it contains excess amount of biotin. Ethanol and acetic acid have also been reported to provide the bacterium a good productivity. Recently, it was demonstrated that the spectrum of usable sugar of *C. glutamicum* can be expanded by expressing heterologous genes [44].

Carbohydrate obtained by petrochemistry was studied as an alternative carbon source. Shiio and Uchio have reported several kinds of bacteria capable of forming glutamate from paraffin [32]. *C. hydrocarboclustus* was reported to have relatively high productivity (Table 1). Ghosh and Banerjee have described the production from n-alkane by the *Serratia marcescens* strain [45]. In addition, aromatic compounds, such as benzoate, have been investigated as a carbon source [33, 34, 46].

Glutamate fermentation from methanol has been attempted since the 1970s [29, 47]. As *C. glutamicum* cannot assimilate methanol, various microorganisms such as *Methanomonas methylovora* [29], *Methylobacillus glycogenes* [48], and *B. methanolicus* [30] have been screened and used. The *B. methanolicus* strain M168-20(pHP13) appears to be the highest producer from methanol (Table 1). Glutamate production through photosynthesis (carbon source is CO_2) has also been investigated using algae [49, 50].

3.3 Production Conditions

As described above, biotin limitation was the crucial factor of glutamate fermentation by *C. glutamicum*. This feature made it impossible to use some economically desirable raw materials, such as molasses. Attempts to clarify the underlying

mechanism and finding alternative methods of biotin limitation have been made since a very early period. Shiio et al. first reported that biotin limitation causes the change of cellular permeability of amino acids including glutamate [51]. Reports that followed confirm the association between biotin limitation and glutamate permeability [52, 53].

Several alternative methods of biotin limitation were devised in the 1960s. One of the major breakthroughs was the addition of penicillin; adding an appropriate amount of penicillin at an early stage of cultivation triggers glutamate production under biotin excess conditions [54]. Addition of surfactant was an alternative method. Because the effect of surfactant depends on its chemical composition, specific surfactants were chosen, such as polyoxyethylene sorbitan monostearate [55, 56] and cetyltrimethylammonium bromide [57].

Penicillin inhibits cell wall synthesis. The first action point of a surfactant should be the cell surface. Putting these and the biotin effect together, the "permeability hypothesis" or the "leak model" was claimed to be the mechanism of glutamate fermentation; change in cellular permeability of glutamate caused by some treatment triggers the leak out of the amino acid, which shifts intracellular metabolism toward glutamate formation [53, 58]. The finding of the relationship between fatty acid composition in the cell membrane and glutamate productivity [59, 60] supported the notion. It was also supported by the finding that an oleic acid-requiring mutant produces glutamate under biotin-sufficient conditions [26, 61]. Based on these lines of evidences, the mechanism of glutamate fermentation seemed to have been settled around 1970. However, further contention emerged later (see below and [35]).

Conditions other than biotin were investigated in detail, but only effects of oxygen and pH are mentioned here. Glutamate production by *C. glutamicum* requires aerobic conditions. Under oxygen-insufficient conditions, the bacterium produces succinic acid and/or lactic acid [62]. Medium pH should be maintained slightly above 7.0. When pH is controlled to be acidic, around pH 5.5, the bacterium produces mainly glutamine.

4 Recent Development in Glutamic Acid Fermentation

4.1 In C. glutamicum

In general, (a) genetic modification(s) is necessary for forcing a microorganism to overproduce a certain amino acid, for example, deregulation of aspartokinase for lysine production. Glutamate production by *C. glutamicum* is different. The wild-type strain exerts high productivity under appropriate conditions as described above. Although there have been a lot of patents claiming mutations beneficial for glutamate production by the bacterium, none of them appear to improve the productivity significantly. However, since the 1990s, genes essential for glutamate

fermentation have been identified, which shed new light on the mechanism. Since the mechanism is discussed in Chap. 4, a very rough sketch is presented below.

There have been two predominant hypotheses on the mechanism. The first idea is the "permeability hypothesis" as described above. The other is the "flux hypothesis," claiming that change in carbon metabolism is the major cause of the overproduction, which is a quite common concept among other amino acid fermentations. Facts supporting each hypothesis are presented below; however, it should be noted that the two hypotheses are not necessarily exclusive.

Before mentioning on the flux hypothesis, the biosynthetic pathway of glutamate is briefly reviewed. Glutamate is formed by amination of 2-oxoglutarate. Glutamate dehydrogenase (GDH) and the coupled reactions of glutamine synthetase and glutamine synthase (GS/GOGAT system) are involved in the amination [63–67]. While cell growth can be sustained either by GDH or GS/GOGAT system, GDH is responsible for glutamate overproduction [67]. Carbon from sugar is metabolized to 2-oxoglutarate through glycolysis and part of the TCA cycle. Enzymes contributing to the flux have been clarified, although the flux around oxaloacetate is complicated. Characteristics and regulation of these enzymes have also been reported [68–73]. 2-Oxoglutarate is the important branch point, the glutamate-forming direction by amination and the glutamate-degrading direction by oxidative decarboxylation to succinate with 2-oxoglutaratedehydrogenase complex (ODHC). ODHC is the focal point in flux control.

During the 1960s, it was assumed that *C. glutamicum* had no or very low activity of ODHC [74]. This was the basis of the flux model during the early 1960s, but the permeability hypothesis became dominant as described above. Later, it was confirmed that *C. glutamicum* possessed certain ODHC activity [75]. The flux hypothesis was revived by the finding that deletion of the *odhA* gene (encoding E1o subunit of ODHC) conferred the bacterium glutamate overproductivity ([76], oral presentation was in 1996). Simultaneously it was reported that ODHC activity is reduced under glutamate-producing conditions [77]. Kim et al. have reported the supportive results [78]. Recently, it was revealed that ODHC activity is regulated by OdhI/PknG [79] and that OdhI, the inhibitor of ODHC, is induced under glutamate-producing conditions [80].

In parallel with the revival, supportive findings for the permeability hypothesis have also been accumulated. In 1989, Hoischen and Kramer have demonstrated by biochemical analysis that glutamate excretion is mediated by an active efflux system and not by simple leakage [81]. They further demonstrated the importance of alternation of membrane tension [82, 83]. Changes in the membrane components cause such alternation of membrane tension, and there have been several reports indicating the association between change in membrane composition and glutamate overproductivity [84–86].

Genetic findings supporting the permeability hypothesis have also been accumulated. Kimura et al. have described the *dtsR* gene that restores detergent (Tween 40) sensitivity [87]. DtsR has a high similarity to the β-subunit of methylmalonyl-CoA carboxyltransferase, and it is suggested to be the biotin enzyme involved in fatty acid biosynthesis. The disruption of *dtsR* causes oleic acid auxotrophy and

glutamate production [88]. The *ltsA* gene was found to code a gene whose disruption causes the cell to be lysozyme sensitive and glutamate productive [89, 90]; however, the physiological role of the gene product is not known. Nakamura et al. found a mechanosensitive channel Ncgl1221 for glutamate excretion [91–95]. Mutation of the gene caused glutamate overproduction even in the presence of intact OdhA [92, 94].

The production mechanism still remains elusive, but it will be comprehensively understood in the near future.

Because glycolysis and the TCA cycle are the major carbon metabolic pathways to glutamate, the relation between energy metabolism and glutamate production is a point to be investigated. In this respect, Yokota et al. have reported that mutants decreasing H-ATPase activity at below 1/4 the level of the wild type produce less glutamate [96].

4.2 In Other Bacteria

It was a strong support of the flux hypothesis that the disruption of *odhA* confers *E. coli* glutamate productivity [43, 74]. Since glutamate productivity by the disruption of the gene has known in other bacteria [42], at least, reducing the flux through ODHC appears to be generally applicable.

Glutamate excreted in the medium is reimported by the glutamate import system in *C. glutamicum* [97, 98] or other bacteria, such as *E. coli* [99]. Thus, an increase in glutamate in the medium exerts a negative effect on glutamate synthesis through the regulation of biosynthetic enzymes. When glutamate dissolved in the medium forms crystals, it no longer has further effect on the regulation. Since isoelectric point of glutamate is around pH 5.5, glutamate concentration dissolved in the medium is lowest at the pH area. Thus, if a bacterium that grows and produces glutamate in this pH, it must be advantageous for glutamate production by reducing the negative effect of glutamate in the medium. Based on this idea, several bacteria were found and constructed as the producers [100]. The entire genome sequence of one strain has recently been published [101].

5 Amino Acid Fermentation

Whether it is a natural expectation or not, researchers in Kyowa Hakko Kogyo Co., Ltd. thought that *C. glutamicum* might produce other amino acids immediately after the discovery of glutamate fermentation. They started delivering mutants for amino acid producers. The attempt quickly resulted in the second amino acid fermentation; a mutant requiring arginine accumulated ornithine [24]. This was the first report releasing regulation on amino acid biosynthesis by amino acid auxotrophy. Further, in the next year, the researchers reported lysine-producing mutants of

C. glutamicum based on the same idea [102]. Earliest reports on each of the amino acid fermentations are listed in Table 2.

The other important method liberating regulation is delivering analogue-resistant mutants. Although it was known since the 1950s that analogue resistance confers amino acid overproductivity [106, 108], the first clear and quantitative example was presented by Sano and Shiio [103]. They demonstrated that an *S*-(2-aminoethyl)-L-cysteine-resistant mutation makes aspartokinase of *C. glutamicum* insensitive to feedback regulation by lysine, resulting in lysine production. Since obtaining resistant mutants (positive screening) is easier than obtaining auxotrophic mutants (negative screening), this method was a very powerful tool for amino acid fermentation.

Producer breeding was not limited to *C. glutamicum*. Other bacteria, such as *E. coli* and *S. marcescens*, were manipulated and used as amino acid producers. In these enterobacteria, destroying the degrading activity of the target amino acid was important at some times. For example, the construction of the threonine producer of *S. marcescens* was initiated by getting a mutant incapable of metabolizing threonine [116]. Destruction of the amino acid-degrading ability and deregulation of the biosynthetic pathway can be regarded as the general strategy of strain improvement.

Table 2 Earliest scientific reports on each of the amino acid fermentations

Amino acid	Type of producer	Titer (g/L)	Reference
Ornithine	*C. glutamicum* A-mutant	26.2	[24]
Lysine	*C. glutamicum* A-mutant	14	[102]
	C. glutamicum R-mutant	32	[103]
Tyrosine	*E. coli* R-mutant	?	[104]
Tyrosine, phenylalanine	*C. glutamicum* R-mutants	2.2	[105]
Methionine	*E. coli* R-mutant	?	[106]
	C. glutamicum A- and R-mutant	2	[107]
Histidine	*E. coli* R-mutant	?	[108]
	C. glutamicum R-mutant	7	[109]
Valine	*Aerobacter cloacae* strain isolated by screening	12	[110]
Threonine	*E. coli* A-mutant	3.7	[111]
Proline	*C. glutamicum* A-mutant	11.4	[112]
Tryptophan	*C. glutamicum* A- and R-mutant	2	[113]
Isoleucine	*S. marcescens* R-mutant	6.7	[114]
Leucine	*S. marcescens* A- and R-mutant	13.5	[115]

Patents and oral presentations are not included. For lysine, the first report of auxotrophic mutant and that of the analogue resistant mutant are shown. For methionine and histidine, the first reports describing the titer are also shown. A-mutant, auxotrophic mutant; R-mutant, analogue resistant mutant. Symbol "?" in titer indicates no description on the titer in the report

Since the 1980s, recombinant DNA technology has been available and applicable for strain improvement. Gene dosage effect is the most instant use of the technology. A gene coding for a key enzyme is amplified by cloning on a multicopy plasmid. An increase in the expression results in the increase of the activity, enhancing the metabolic flux to the desired direction. Combining conventional mutation and recombinant DNA technology enables the construction of a producer strain for the amino acid whose biosynthesis is under multiple and complex regulation, such as tryptophan [117].

Discovery of an amino acid exporter was noteworthy. The lysine exporter in *C. glutamicum* was first predicted using biochemical analysis [118, 119] and then confirmed genetically [120]. The finding is interesting not only because it is counterintuitive but also because it raises a new key concept in amino acid fermentation. Currently, over eight kinds of amino acid exporters have been identified in *C. glutamicum* [121, 122] and *E. coli* [123–127].

Knowing carbon flux in the producer microorganism is important for strain improvement. Carbon distributions between the hexose monophosphate pathway (HMP) and the pentose monophosphate pathway (PPP) and around the oxaloacetate supply have been the focal points of research interest. ^{13}C-NMR analysis combined with metabolic balance analysis has been widely used for this purpose, and much insight has been accumulated [128–130]. One of the earliest finding of the analysis is that the carbon distribution through HMP and PPP in *C. glutamicum* is different between the glutamate producer and the lysine producer [131]. The carbon flux distribution between HMP and PPP was 8:2 in the glutamate producer, whereas it was 4:6 in the lysine producer, suggesting that the flux is controlled by the necessity of NADPH. Subsequently, this finding led to cofactor engineering [132–134].

6 Feature Prospective

At the end of the twentieth century, the whole genome sequence determination was initiated in various kinds of organisms including *E. coli* [135] and *C. glutamicum* [136]. Genome data enabled a new way of strain improvement termed as "genome breeding" [137–139] that creates a minimal set of mutations beneficial for production by (1) comparative analysis of whole genomes of the wild-type strain and a producer strain obtained from the wild-type via successive classical mutations, (2) defining beneficial mutations, and (3) assembling them in the wild-type background. This enables the construction of a producer strain with high productivity without undesirable traits of the classical producer mutant, such as slow growth and stress sensitiveness.

Glutamate fermentation has been used mainly for production of MSG, a seasoning. However, it may take part in wider areas of application because glutamate is expected to be one of the basic chemicals from renewable resources [140]. Thus, glutamate fermentation yet retains enough potential for contributing for the quality

of human life in addition to its historical role in modern fermentation industry and applied microbiology.

Acknowledgments The author would like to acknowledge Ajinomoto Co., Inc. and Kyowa Hakko Bio Co. Ltd. for kind permission of reprinting historical photos. The author would like to express sincere thanks to Dr. M. Hayashi for helpful support.

References

1. Ajinomoto Group HP (2014) http://www.ajinomoto.com/jp/ir/pdf/FY13_Data_J.pdf
2. Ikeda K (1908) Japanese Patent 14805
3. Sano C (2009) History of glutamate production. Am J Clin Nutr 90(3):728S–732S
4. Ajinomoto Group HP. http://www.ajinomoto.com/jp/aboutus/history/story/story07.html
5. Greenstein JP (1954) The resolution of racemic α-amino acids. In: Anson ML et al (eds) Advances in protein chemistry. Academic Press, New York, pp 121–202
6. Izaki K, Takahashi H, Sakaguchi K (1955) Studies on the metabolism of D-amino acid in microorganisms. Part 1. Degradation of D-glutamic acid in genus *Aerobacter*. Bull Agric Chem Soc Japan 19:233–239
7. Michi K, Nonaka H (1954) Enzymatic resolution of racemic amino acids. Part 2. Resolution of DL-glutamic acid and DL-valine. Nippon Nogei Kagaku Kaishi 28:346–349
8. Lockwood LB, Stodola FH (1946) Preliminary studies on the production of α-ketoglutaric acid by *Pseudomonas fluorescens*. J Biol Chem 164:81–83
9. Koepsell H, Stodola FH, Sharpe ES (1952) Production of α-ketoglutarate in glucose oxidation by *Pseudomonas fluorescens*. J Am Chem Soc 74:5142–5144
10. Nisman B, Raynaud M, Cohen GN (1947) Mecanisme de la formation des acides amines chez les bacteries a partir de lammoniaque et des acides α-cetoniques. C R Hebd Séances Acad Sci 225:700–701
11. Adler E, Hellström V, Günther G, v Euler H (1938) Uber den enzymatischen Abbau und Aufbau der Glutaminsaure. Hoppe-Seylers Z Physiol Chem 225:14–26
12. Konikova AS, Kritzman MG (1948) Formation of amino nitrogen from ammonia and α-keto-acids by suspension of *Bacillus subtilis*. Biokhimiya 13:39–41
13. Feldman LI, Gunsalus IC (1950) The occurrence of a wide variety of transaminase in bacteria. J Biol Chem 187:821–830
14. Otsuka S, Yazaki H, Hasegawa H, Sakaguchi K (1957) Fermentative production of L-glutamic acid from α-ketoglutaric acid and ammonium salt. Bull Agric Chem Soc Japan 21:69–70
15. Housewright RD, Thorne CB (1950) Synthesis of glutamic acid and glutamyl polypeptide by *Bacillus anthracis*. I. Formation of glutamic acid by transamination. J Bacteriol 60:89–100
16. Dagley S, Dawes EA, Morrison GA (1950) Production of amino-acids in synthetic media by *Escherichia coli* and *Aerobacter aerogenes*. Nature 165:437–438
17. Morrison GA, Hinshelwood C (1949) Nitrogen utilisation and growth of coliform bacteria. Part III. Nitrogen utilisation and lag phase. J Chem Soc 1949:380–384
18. Thorne CB, Gomez CG, Blind GR, Housewright RD (1953) Synthesis of glutamic acid and glutamyl polypeptide by *Bacillus anthracis*. III. Factor affecting peptide production in synthetic liquid media. J Bacteriol 65:472–478
19. Asai T, Aida K, Oishi K (1957) On L-glutamic acid fermentation. Bull Agric Chem Soc Japan 21:134–135
20. Johnson MJ (1955) Unit process review. Ind Eng Chem 47:1872–1875
21. Udaka S (1960) Screening method for microorganisms accumulating metabolites and its use in the isolation of *Micrococcus glutamicus*. J Bacteriol 79:754–755

22. Kinoshita S, Udaka S, Shimono M (1957) Studies on the amino acid fermentation. Part I. By various microorganisms. J Gen Appl Microbiol 3:193–205
23. Kinoshita S, Udaka S, Akita S (1957) Japanese Patent Sho 32-8698
24. Kinoshita S, Nakayama K, Udaka S (1957) The fermentative production of L-ornithine (preliminary report). J Gen Appl Microbiol 3:276–277
25. Li Z, Chen N (2012) Study on the directional breeding of high glutamic-acid production strain GTS190 and the control on its fermentation. Environ Sci Mater Eng 2:1029–1033
26. Kanzaki T, Isobe K, Okazaki H, Motizuki K, Fukuda H (1967) L-Glutamic acid fermentation. Part I. Selection of an oleic acid-requiring mutant and its properties. Agric Biol Chem 31: 1307–1313
27. Tsunoda T, Shiio I, Mitsugi K (1961) Bacterial formation of L-glutamic acid from acetic acid in the growing culture medium. I. Cultural conditions. J Gen Appl Microbiol 7:18–29
28. Oki T, Sayama Y, Nishimura Y, Ozaki A (1968) L-Glutamic acid formation by microorganisms from ethanol. Agric Biol Chem 32:119–120
29. Oki T, Kitai A, Kouno K, Ozaki A (1973) Production of L-glutamic acid by methanol-utilizing bacteria. J Gen Appl Microbiol 19:79–83
30. Brautaset T, Jakobsen OM, Degnes KF, Netzer R, Naerdal I, Krog A, Dillingham R, Flickinger MC, Ellingsen TE (2010) *Bacillus methanolicus* pyruvate carboxylase and homoserine dehydrogenase I and II and their roles for L-lysine production from methanol at 50 °C. Appl Microbiol Biotechnol 87:951–964
31. Imada Y, Takahashi J, Yamada K (1967) Production of L-glutamic acid from hydrocarbons: incorporation of molecular oxygen. Biotech Bioeng 9:45–54
32. Shiio I, Uchio R (1969) Microbial production of amino acids from hydrocarbons. IV. L-glutamic acid production by *Corynebacterium hydrocarboclastus* R-7. J Gen Appl Microbiol 15:65–84
33. Yamamoto M, Nishida H, Inui T, Ozaki A (1972) Microbial production of amino acids from aromatic compounds. I. Screening of aromatic compounds-assimilating bacteria. J Ferment Technol 50:868–875
34. Yamamoto M, Nishida H, Inui T, Ozaki A (1972) Microbial production of amino acids from aromatic compounds. II. Production of L-glutamic acid from benzoate. J Ferment Technol 50:876–883
35. Hirasawa T, Wachi M (2016) Glutamate fermentation-2: mechanism of L-glutamate overproduction in *Corynebacterium glutamicum*. Adv Biochem Eng Biotechnol. doi:10.1007/10_2016_26
36. Liebl W, Ehrmann M, Ludwig W, Schleifer KH (1991) Transfer of *Brevibacterium divaricatum* DSM 20297^T, "*Brevibacterium flavum*" DSM 20411, "*Brevibacterium lactofermentum*" DSM 20412 and DSM 1412, and *Corynebacterium lilium* DSM 20137^T to *Corynebacterium glutamicum* and their distinction by rRNA gene restriction patterns. Int J Syst Bacteriol 41:255–260
37. NCBI Taxonomy (2014) *Corynebacterium glutamicum*. http://www.metalife.com/NCBI%20Taxonomy/1718
38. Yamada K, Komagata K (1972) Taxonomic studies on coryneform bacteria. V. Classification of coryneform bacteria. J Gen Appl Microbiol 18:417–431
39. Chao K-C, Foster JW (1959) A glutamic acid-producing *Bacillus*. J Bacteriol 77(6):715–725
40. Roy DK, Chatterjee SP (1982) Production of glutamic acid by an *Arthrobacter* sp. I. Identification and nutritional requirement in relation to glutamic acid production. Acta Microbiol Pol 31:153–158
41. Chatterjee S, Chatterjee SP (1982) A glutamic acid-producing *Streptomyces* sp. Folia Microbiol 27:116–120
42. Moriya M, Izui Y, Ono E, Matsui K, Ito H, Hara Y (2000) Japanese Patent P2000-106869A
43. Tujimoto N, Kikuchi Y, Kurahashi O, Kawahara Y (1995) US Patent 5378616A

44. Schneider J, Niermann K, Wendish VF (2011) Production of the amino acids L-glutamate, L-lysine and L-arginine from arabinose by recombinant *Corynebacterium glutamicum*. J Biotechnol 154:191–198
45. Ghosh BB, Banerjee AK (1986) Production of methionine and glutamic acid from *n*-alkanes by *Serratia marcescens* var. *kiliensis*. Folia Microbiol 31:106–112
46. Revillas JJ, Rodelas B, Pozo C, Martinez-Toledo MV, Lopez JG (2005) Production of amino acids by *Azotobacter vinelandii* and *Azotobacter chroococcum* with phenolic compounds as solo carbon source under diazotrophic and adiazotrophic conditions. Amino Acids 28: 421–425
47. Ju DK (1976) Studies on the production of amino acids by methanol-utilizing bacteria. Misaengmul Hakhoechi 14:8–16
48. Motoyama H, Anazawa H, Kastumata R, Araki K, Teshiba S (1993) Amino acid production from methanol by *Methylobacillus glycogenes* mutants: isolation of L-glutamic acid hyper-producing mutants from *M. glycogenes* strains, and derivation of L-threonine and L-lysine-producing mutants from them. Biosci Biotechnol Biochem 57:82–87
49. Bagchi SN, Rao NS (1997) Sustained production of amino acids by immobilized analog-resistant mutant of a cyanobacterium *Anacystis nidulans* BD-1. J Microbiol Biotechnol 7: 341–344
50. Matsunaga T, Nakamura N, Tsuzaki N, Takeda H (1988) Selective production of glutamate by an immobilized marine blue-green alga, *Synechococcus* sp. Appl Microbiol Biotechnol 28:373–376
51. Shiio I, Otsuka S, Takahashi M (1962) Effect of biotin on the bacterial fermentation of glutamic acid. I. Glutamate formation and cellular permeability of amino acids. J Biochem 51:56–62
52. Kimura K (1963) The effect of biotin on the amino acid biosynthesis by *Micrococcus glutamicus*. J Gen Appl Microbiol 9:205–212
53. Otsuka S, Miyajima R, Shiio I (1965) Comparative studies on the mechanism of microbial glutamate formation. II. Effect of biotin. J Gen Appl Microbiol 11:295–301
54. Phillips T, Somerson NL (1963) US Patent 3,080,297
55. Takinami K, Okada H, Tsunoda T (1964) Biochemical effect of fatty acid and its derivatives on L-glutamic acid fermentation. Part II. Effective chemical structure of fatty acid derivatives on the accumulation of L-glutamic acid in biotin sufficient medium. Agric Biol Chem 28: 114–119
56. Takinami K, Yoshii H, Tsuri H, Okada H (1965) Biochemical effect of fatty acid and its derivatives on L-glutamic acid fermentation. Part III. Biotin-Tween 60 relationship in the accumulation of L-glutamic acid and the growth of *Brevibacterium lactofermentum*. Agric Biol Chem 29:351–359
57. Oshima K, Tanaka K, Kinoshita S (1964) Studies on L-glutamic acid fermentation. XIII. Effect of surface active agents on the conversion of citrate to L-glutamate with the resting cells of *Micrococcus glutamicus*. J Gen Appl Microbiol 10:333–342
58. Shiio I, Otsuka S, Katsuya N (1963) Cellular permeability and extracellular formation of glutamic acid in *Brevibacterium flavum*. J Biochem 53:333–340
59. Shibukawa M, Ohsawa T (1966) L-Glutamic acid fermentation with molasses. Part VI. Effect of the saturated-unsaturated fatty acid ratio in the cell membrane fraction on the extracellular accumulation of L-glutamate. Agric Biol Chem 30:750–758
60. Shibukawa M, Kurima M, Okabe S, Ohsawa T (1968) L-Glutamic acid fermentation with molasses. Part X. On the difference in mechanisms for the bacterial extracellular accumulation of L-glutamate between fatty acid derivative and penicillin. Agric Biol Chem 32: 641–645
61. Okazaki H, Kanzaki T, Doi M, Sumino Y, Fukuda H (1967) L-Glutamic acid fermentation. Part II. The production of L-glutamic acid by an oleic acid-requiring mutant. Agric Biol Chem 31:1314–1317

62. Oishi K, Aida K, Asai T (1961) Studies on amino acid fermentation. Part VIII. On the mechanism of conversion of L-glutamic acid fermentation to succinic acid fermentation. J Gen Appl Microbiol 7:213–226
63. Ertan H (1992) Some properties of glutamate dehydrogenase, glutamine synthetase and glutamate synthase from *Corynebacterium callunae*. Arch Microbiol 158:35–41
64. Ertan H (1992) The effect of various culture conditions on the levels of ammonia assimilatory enzyme of *Corynebacterium callunae*. Arch Microbiol 158:42–47
65. Kimura K (1962) The significance of glutamic dehydrogenase in glutamic acid fermentation. J Gen Appl Microbiol 8:253–260
66. Sung H, Tachiki T, Kumagai H, Tochikura T (1984) Production and preparation of glutamate synthase from *Brevibacterium flavum*. J Ferment Technol 62:371–376
67. Sung H, Takahashi M, Tamaki H, Tachiki T, Kumagai H, Tochikura T (1985) Ammonia assimilation by glutamine synthetase/glutamate synthase system in *Brevibacterium flavum*. J Ferment Technol 63:1–5
68. Mori M, Shiio I (1985) Purification and some properties of phosphoenolpyruvate carboxylase from *Brevibacterium flavum* and its aspartate-overproducing mutant. J Biochem 97: 1119–1128
69. Shiio I, Ozaki H, Ujigawa K (1977) Regulation of citrate synthase in *Brevibacterium flavum*, a glutamate-producing bacterium. J Biochem 82:395–405
70. Delaunay D, Daran-Lapujade P, Engasser JM, Goergen JL (2004) Glutamate as an inhibitor of phosphoenolpyruvate carboxylase activity in *Corynebacterium glutamicum*. J Ind Microbiol Biotechnol 31:183–188
71. Eikmanns BJ, Rittmann D, Sahm H (1995) Cloning, sequence analysis, expression, and inactivation of the *Corynebacterium glutamicum icd* gene encoding isocitrate dehydrogenase and biochemical characterization of the enzyme. J Bacteriol 177:774–782
72. Sato H, Orishimo K, Shirai T, Hirasawa T, Nagahisa K, Shimizu H, Wachi M (2008) Distinct roles of two anaplerotic pathways in glutamate production induced by biotin limitation in *Corynebacterium glutamicum*. J Biosci Bioeng 106:51–58
73. Shiio I, Ujigawa K (1978) Enzymes of the glutamate and aspartate synthetic pathways in a glutamate-producing bacterium, *Brevibacterium flavum*. J Biochem 84:647–657
74. Shiio I, Otsuka S, Takahashi M (1961) Significance of α-ketoglutaric dehydrogenase on the glutamic acid formation in *Brevibacterium flavum*. J Biochem 50:164–165
75. Shiio I, Ujigawa-Takeda K (1980) Presence and regulation of α-ketoglutarate dehydrogenase complex in a glutamate-producing bacterium *Brevibacterium flavum*. Agric Biol Chem 44: 1897–1904
76. Asakura Y, Kimura E, Usuda Y, Kawahara Y, Matsui K, Osumi T, Nakamura T (2007) Altered metabolic flux due to deletion of *odhA* cases L-glutamate overproduction in *Corynebacterium glutamicum*. Appl Environ Microbiol 73:1308–1319
77. Kawahara Y, Takahashi-Fuke K, Shimizu E, Nakamatsu T, Nakamori S (1997) Relationship between the glutamate production and the activity of 2-oxoglutarate dehydrogenase in *Brevibacterium lactofermentum*. Biosci Biotechnol Biochem 61:1109–1112
78. Kim J, Hirasawa T, Sato Y, Nagahisa K, Furusawa C, Shimizu H (2009) Effect of *odhA* overexpression and *odhA* antisense RNA expression on Tween-40-triggered glutamate production by *Corynebacterium glutamicum*. Appl Microbiol Biotechnol 81:1097–1106
79. Schultz C, Niebisch A, Gebel L, Bott M (2007) Glutamate production by *Corynebacterium glutamicum*: dependence on the oxoglutarate dehydrogenase inhibitor protein OdhI and protein kinase PknG. Appl Microbiol Biotechnol 76:691–700
80. Kim J, Hirasawa T, Saito M, Furusawa C, Shimizu H (2011) Investigation of phosphorylation status of OdhI protein during penicillin- and Tween-40-triggered glutamate overproduction by *Corynebacterium glutamicum*. Appl Microbiol Biotechnol 91:143–151
81. Hoischen C, Kramer R (1989) Evidence for an efflux carrier involved in the secretion of glutamate by *Corynebacterium glutamicum*. Arch Microbiol 151:342–347

82. Hoischen C, Kramer R (1990) Membrane alteration is necessary but not sufficient for effective glutamate secretion in *Corynebacterium glutamicum*. J Bacteriol 172:3406–3416
83. Lambert C, Erdmann A, Eikmanns M, Kramer R (1995) Triggering glutamate excretion in *Corynebacterium glutamicum* by modulating the membrane state with local anesthetics and osmotic gradients. Appl Environ Microbiol 61:4334–4342
84. Hashimoto K-I, Kawasaki H, Akazawa K, Nakamura J, Asakura Y, Kudo T, Sakuradani E, Shimizu S, Nakamatsu T (2006) Change in composition and content of mycolic acids in glutamate-overproducing *Corynebacterium glutamicum*. Biosci Biotechnol Biochem 70: 22–30
85. Nampoothiri K, Hoischen C, Bathe B, Moeckel B, Pfefferle W, Krumbach K, Sahm H, Eggeling L (2002) Expression of genes of lipid synthesis and altered lipid composition modulate L-glutamate efflux of *Corynebacterium glutamicum*. Appl Microbiol Biotechnol 58: 89–96
86. Radmacher E, Stansen KC, Besra GS, Alderwick LJ, Maughan WN, Hollweg G, Sahm H, Wendish VF, Eggeling L (2005) Ethambutol, a cell wall inhibitor of *Mycobacterium tuberculosis*, elicits L-glutamate efflux of *Corynebacterium glutamicum*. Microbiology 151: 1359–1368
87. Kimura E, Abe C, Kawahara Y, Nakamatsu T (1996) Molecular cloning of a novel gene, *dtsR*, which rescues the detergent sensitivity of a mutant derived from *Brevibacterium lactofermentum*. Biosci Biotechnol Biochem 60:1565–1570
88. Kimura E, Abe C, Kawahara Y, Nakamatsu T, Tokuda H (1997) A *dtsR* gene-disrupted mutant of *Brevibacterium lactofermentum* requires fatty acids for growth and efficiently produces L-glutamate in the presence of an excess of biotin. Biochem Biophys Res Commun 234:157–161
89. Hirasawa T, Wachi M, Nagai K (2000) A mutation in the *Corynebacterium glutamicum ltsA* gene causes susceptibility to lysozyme, temperature-sensitive growth, and L-glutamate production. J Bacteriol 182:2696–2701
90. Hirasawa T, Wachi M, Nagai K (2001) L-Glutamate production by lysozyme-sensitive *Corynebacterium glutamicum ltsA* mutant strains. BMC Biotechnol 1:9
91. Becker M, Boerngen K, Nomura T, Battle AR, Marin K, Martinac B, Kraemer R (2013) Glutamate efflux mediated by *Corynebacterium glutamicum* MscCG, *Escherichia coli* MscS, and their derivatives. Biochim Biophys Acta 1828:1230–1240
92. Hashimoto K, Nakamura K, Kuroda T, Tabe I, Nakamatsu T, Kawasaki H (2010) The protein encoded by NCgl1221 in *Corynebacterium glutamicum* functions as a mechanosensitive channel. Biosci Biotechnol Biochem 74:2546–2549
93. Hashimoto K, Murata J, Konishi T, Tabe I, Nakamatsu T, Kawasaki H (2012) Glutamate is excreted across the cytoplasmic membrane through the NCgl1221 channel of *Corynebacterium glutamicum* by passive diffusion. Biosci Biotechnol Biochem 76:1422–1424
94. Nakamura J, Anazawa H, Katsumata R, Araki K, Teshiba S (2007) Mutations of the *Corynebacterium glutamicum* NCgl1221 gene, encoding a mechanosensitive channel homolog, induce L-glutamic acid production. Appl Environ Microbiol 73:4491–4498
95. Nakayama Y, Yoshimura K, Iida H (2012) A gain-of-function in gating of *Corynebacterium glutamicum* NCgl1221 causes constitutive glutamate secretion. Appl Environ Microbiol 78: 5432–5434
96. Sekine H, Shimada T, Hayashi C, Ishiguro A, Tomita F, Yokota A (2001) H^+-ATPase defect in *Corynebacterium glutamicum* abolishes glutamic acid production with enhancement of glucose consumption rate. Appl Microbiol Biotechnol 57:534–540
97. Burkovski A, Weil B, Kraemer R (1996) Characterization of a secondary uptake system for L-glutamate in *Corynebacterium glutamicum*. FEMS Microbiol Lett 136:169–173
98. Trotschel C, Kandirali S, Diaz-Achirica P, Meinhardt A, Morbach S, Kraemer R, Burkovski A (2003) GltS, the sodium-coupled L-glutamate uptake system of *Corynebacterium*: identification of the corresponding gene and impact on L-glutamate production. Appl Microbiol Biotechnol 60:738–742

99. Deguchi Y, Yamato I, Anraku Y (1989) Molecular cloning of *gltS* and *gltP*, which encode glutamate carriers of *Escherichia coli* B. J Bacteriol 171:1314–1319
100. Sato M, Akiyoshi N (2002) Japanese Patent P2002-238591A
101. Hara Y, Kadotani N, Izui H, Katashkina JI, Kuvaeva TM, Andreeva IG, Golubeva LI, Malko DB, Makeev VJ, Mashko SV, Kozlov YI (2012) The complete genome sequence of *Pantoea ananatis* AJ13355, an organism with great biotechnological potential. Appl Microbiol Biotechnol 93:331–341
102. Kinoshita S, Nakayama K, Kitada S (1958) L-Lysine production using microbial auxotroph (preliminary report). J Gen Appl Microbiol 4:128–129
103. Sano K, Shiio I (1970) Microbial production of L-lysine. III. Production by mutants resistant to *S*-(2-aminoethyl)-L-cysteine. J Gen Appl Microbiol 16:373–391
104. Cohen GN, Adelberg EA (1958) Kinetics of incorporation of *p*-fluorophenylalanine by a mutant of *Escherichia coli* resistant to this analogue. J Bacteriol 76:328–330
105. Sugimoto S, Nakagawa M, Tsuchida T, Shiio I (1973) Regulation of aromatic amino acid biosynthesis and production of tyrosine and phenylalanine in *Brevibacterium flavum*. Agric Biol Chem 37:2327–2336
106. Adelberg EA (1958) Selection of bacterial mutants which excrete antagonists of antimetabolites. J Bacteriol 76:326
107. Kase H, Nakayama K (1975) L-Methionine production by methionine analog-resistant mutants of *Corynebacterium glutamicum*. Agric Biol Chem 39:153–160
108. Moyed HS, Friedman M (1959) Interference with feedback control: a mechanism of antimetabolite action. Science 129:968–969
109. Araki K, Nakayama K (1971) Studies on histidine fermentation. Part I. L-Histidine production by histidine analog-resistant mutants from several bacteria. Agric Biol Chem 35: 2081–2088
110. Sugisaki Z (1959) Studies on L-valine fermentation. Part I. Production of L-valine by *Aerobacter* bacteria. J Gen Appl Microbiol 5:138–149
111. Huang HT (1961) Production of L-threonine by auxotrophic mutants of *Escherichia coli*. Appl Microbiol 9:419–424
112. Yoshinaga F, Konishi S, Okumura S, Katsuya N (1966) Studies on the fermentative production of L-proline. I. Production of L-proline by an isoleucine auxotrophic mutant of *Brevibacterium flavum* 2247. J Gen Appl Microbiol 12:219–228
113. Shiio I, Sato H, Nakagawa M (1972) L-Tryptophan production by 5-methyltryptophan-resistant mutants of glutamate-producing bacteria. Agric Biol Chem 36:2315–2322
114. Kisumi M, Komatsubara S, Sugiura M, Chibata I (1972) Isoleucine accumulation by regulatory mutants of *Serratia marcescens*: lack of both feedback inhibition and repression. J Bacteriol 110:761–763
115. Kisumi M, Komatsubara S, Chibata I (1973) Leucine accumulation by isoleucine revertants of *Serratia marcescens* resistant to α-aminobutyric acid: lack of both feedback inhibition and repression. J Biochem 73:107–115
116. Komatsubara S, Kisumi M, Chibata I (1979) Participation of lysine-sensitive aspartokinase in threonine production by *S*-2-aminoethyl cysteine-resistant mutants of *Serratia marcescens*. Appl Environ Microbiol 38:777–782
117. Katsumata R, Ikeda M (1993) Hyperproduction of tryptophan in *Corynebacterium glutamicum* by pathway engineering. Nat Biotechnol 11:921–925
118. Broer S, Kraemer R (1991) Lysine excretion by *Corynebacterium glutamicum*. 1. Identification of a specific secretion carrier system. Eur J Biochem 202:131–135
119. Broer S, Kraemer R (1991) Lysine excretion by *Corynebacterium glutamicum*. 2. Energetics and mechanism of the transport system. Eur J Biochem 202:137–143
120. Vrljic M, Sahm H, Eggeling L (1996) A new type of transporter with a new type of cellular function: L-lysine export from *Corynebacterium glutamicum*. Mol Microbiol 22:815–826

121. Kennerknecht N, Sahm H, Yen M-R, Patek M, Saier MH Jr, Eggeling L (2002) Export of L-isoleucine from *Corynebacterium glutamicum*: a two-gene-encoded member of a new translocator family. J Bacteriol 184:3947–3956
122. Simic P, Sahm H, Eggeling L (2001) L-Threonine export: use of peptides to identify a new translocator from *Corynebacterium glutamicum*. J Bacteriol 183:5317–5324
123. Dassler T, Maier T, Winterhalter C, Boeck A (2000) Identification of a major facilitator protein from *Escherichia coli* involved in efflux of metabolite of the cysteine pathway. Mol Microbiol 36:1101–1112
124. Doroshenko V, Airich L, Vitushkina M, Kolokolova A, Livshits V, Mashko S (2007) YddG from *Escherichia coli* promotes export of aromatic amino acids. FEMS Microbiol Lett 275: 312–318
125. Hori H, Yoneyama H, Tobe R, Ando T, Isogai E, Katsumata R (2011) Inducible L-alanine exporter encoded by the novel gene *ygaW* (*alaE*) in *Escherichia coli*. Appl Environ Microbiol 77:4027–4034
126. Kutukova EA, Livshits VA, Altman IP, Pitysyn LR, Zyiatdinov MH, Tomakova IL, Zakataeva NP (2005) The *yaeS* (*leuE*) gene of *Escherichia coli* encodes an exporter of leucine, and the Lrp protein regulates its expression. FEBS Lett 579:4629–4634
127. Madhusudan RN, Gowrishankar J (2004) Evidence for an arginine exporter encoded by *yggA* (*argO*) that is regulated by *lysR*-type transcriptional regulator ArgP in *Escherichia coli*. J Bacteriol 186:3539–3546
128. Matsuoka Y, Shimzu K (2010) Current status of ^{13}C-metabolic analysis and future perspective. Process Biochem 45:1873–1881
129. Sahm H, Eggeling L, de Graaf AA (2000) Pathway analysis and metabolic engineering in *Corynebacterium glutamicum*. Biol Chem 381:899–910
130. Sauer U, Eikmanns BJ (2005) The PEP-pyruvate-oxaloacetate node as the switch point for carbon flux distribution in bacteria. FEMS Microbiol Rev 29:765–794
131. Ishino S, Shimomura-Nishimuta J, Yamaguchi K, Shirahata K, Araki K (1991) ^{13}C nuclear magnetic resonance studies of glucose metabolism in L-glutamic acid and L-lysine fermentation by *Corynebacterium glutamicum*. J Gen Appl Microbiol 37:157–165
132. Kabus A, Georgi T, Wendish VF, Bott M (2007) Expression of the *Escherichia coli pntAB* genes encoding a membrane-bound transhydrogenase in *Corynebacterium glutamicum* improves L-lysine formation. Appl Microbiol Biotechnol 75:47–53
133. Takeno S, Murata R, Kobayashi R, Mitsuhashi S, Ikeda M (2010) Engineering of *Corynebacterium glutamicum* with an NADPH-generating glycolytic pathway for L-lysine production. Appl Environ Microbiol 76:7154–7160
134. Wang Y, San KY, Bennett GN (2013) Cofactor engineering for advancing chemical biotechnology. Curr Opin Biotechnol 24:994–999
135. NCBI Database (2015) *Escherichia coli* str. K-12 substr. MG1655. http://www.ncbi.nlm.nih.gov/genome/167?genome_assembly_id=161521
136. NCBI Database (2015) *Corynebacterium glutamicum* ATCC13032. http://www.ncbi.nlm.nih.gov/genome/469?genome_assembly_id=166859
137. Ikeda M, Nakagawa S (2003) The *Corynebacterium glutamicum* genome: features and impacts on biotechnological progresses. Appl Microbiol Biotechnol 62:99–109
138. Ohnishi J, Mitsuhashi S, Hayashi M, Ando S, Yokoi H, Ochiai K, Ikeda M (2002) A novel methodology employing *Corynebacterium glutamicum* genome information to generate a new L-lysine-producing mutant. Appl Microbiol Biotechnol 58:217–223
139. Ohnishi J, Hayashi M, Mitsuhashi S, Ikeda M (2003) Efficient 40°C fermentation of L-lysine by a new *Corynebacterium glutamicum* mutant developed by genome breeding. Appl Microbiol Biotechnol 62:69–75
140. Werpy TA, Petersen G (eds) (2004) Top value added chemicals from biomass. In: Results of screening for potential candidates from sugars and synthesis gas, vol 1. US Department of Energy (DOE), Golden, CO. http://www.energy.gov/eere/bioenergy/downloads/top-value-added-chemicals-biomass-volume-i-results-screening-potential