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Glutamic Acid Production by Fermentation: A Comprehensive Review

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ABSTRACT

Fermentation process has been initiated for amino acid production through the discovery of L-glutamic acid overproducing microorganism (Micrococcus glutamicus or Corynebacterium glutamicum) by a Japanese scientists' group headed by Kinoshita in 1956. Since then, several trials have been made to commercialize the process through isolation, characterization and strain improvements from various sources. A detailed account of chronological development in this field, including biosynthesis and metabolic regulation studies in some potential microorganisms is also given in the review. The importance for culture conditions for L-glutamic acid production and its optimization using empirical and statistical strategies have been summarized in association with the downstream methods for recovering L-glutamic acid from fermentation broth.

Keywords: *Fermentation, L-glutamic acid,Micrococcus glutamicus,Corynebacterium glutamicum, metabolic, downstream*

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INTRODUCTION

Glutamic acid ($C_5H_9NO_4$; MW: 147.13g/mol) is one of the proteinogenic, non-essential amino acids having tremendous commercial importance[68]. It was discovered by the German chemist Karl Heinrich Ritthausen from hydrolysis of wheat gluten (for which the name derived) by sulfuric acid in 1866. But its major applications were first focused by Kikunae Ikeda [36]. He first identified glutamate as the umami substance from Konbu (kelp). During the next year, monosodium salt of glutamic acid was the first commercially sold as seasoning '*AJI NOMOTO*' in 1909 [1]. At that moment four basic modalities of tastes (sweet, sour, salty and bitter) were recognized. After a century, umami was recognized as the fifth basic modality of taste. As the newly perceived taste of MSG is a traditional taste in Japan, the new product gained a huge commercial advantage. Initially, glutamic acid was prepared from acid hydrolysis of wheat gluten using hydrochloric acid. Wheat contains about 30% glutamic acid. However, due to several limitations, this method was not popularized. Trials have been also made for chemical synthesis of glutamic acid, but it produced racemic mixture of glutamic acid from which separation and isolation of stereo-specific isomer is very difficult. Successful industrial production of metabolically active L-isomer of glutamic acid was started in 1958 by *Kyowa Hakko Kogyo* by fermentation using newly isolated microbial strain *Micrococcus glutamicus* [44].

In the early days, in Western countries, people did not understand umami and monosodium glutamate (MSG) was recognized as a flavor enhancer instead of a seasoning. Its taste was described as meaty, delicate, savory, subtle, etc. In 1990s, umami receptors were first identified on the tongue and since then, scientists accepted it as a basic modality of taste [15, 50, 79]. Human colostrum also contains high glutamic acid, indicating umami is the first taste experienced by newborns [66].

Glutamic acid is the most abundant amino acid with a wide range of industrial applications. Global market of glutamic acid is gradually expanding and almost getting double in every decade with scope of new uses and progress in new technologies [47]. Glutamic acid is widely used as seasoning for a long past [46]. However, a controversy raised from a report of an American Scientist, Dr. Olney (1969) who claimed

some neurotoxic effects of MSG overuse in mice [62]. However, Dr. Takasaki (1978) found no pathogenic changes in pregnant, lactating or weaning mice fed with MSG [76]. Finally, United States Food and Drug Administration (FDA) declared MSG as 'Generally Recognized As Safe' (GRAS) for food application [29]. It is also used as a food additive, animal feed, flavoring agent for fast foods as well as for soft drinks [21, 51, 77, 84]. Very recently, its therapeutic applications as an anticancer agent, treatment of hair loss, treatment of wrinkle as anti-aging agent, treatment in mood deprivation, manufacture of various organic chemicals raised its market value [18, 20, 34, 67]. The estimated global market (in 2014) for L-glutamic acid and Monosodium glutamate (MSG) was more than 3million tons each and is likely to surpass 4million tons for each of the product with revenue collection for L-glutamic acid more than USD 15.5billions (CAGR of about 7.5%) in 2023 (Report ID: GM1225). As per the report, Asian Pacific is the highest consumer of both L-glutamic acid (64%) and MSG (88%) in 2014 with moderate growth in North America. China and India experience rapid urbanization with huge demands for fast food industries which is likely to be associated with such huge demands. Asian countries (China, Vietnam, Indonesia and Thailand) appear to be the major producers of L-glutamic acid and MSG most likely because of huge availabilities of raw materials like cane molasses and tapioca with inexpensive labor cost. Japan, Taiwan and South Korea are expected to show sluggish growth rates. Middle East (such as Saudi Arabia, UAE and Qatar, etc) and African Countries are almost totally dependent on exports.

Its biologically active L-isomer was first successfully produced in industrial scales by fermentation in 1958 at a plant of*Kyowa Hakko Kogyo* [36]. Since then several trials have been made to scale up the process, emphasizing on strain improvement, process parameters and downstream recovery from production media [2-5, 10, 13, 25, 39]. Tremendous technological developments in the field of industrial microbiology evoke a potential improvement in L-glutamic acid fermentation [19, 47]. The present review attempt to summarize the overall progress in L-glutamic acid fermentation in last few decades emphasizing the technological issues associated to its industrial production.

PRE-FERMENTATION HISTORY

Karl Henrich Leopold Ritthausen a German organic chemist and professor of chemistry at University of Bonn, when working with gliadin, identified α-aminoglutaric acid or glutamic acid from hydrolysis of wheat gluten (hence the name glutamic acid) in 1866 [68]. Later on in 1908 Dr. Kikunae Ikeda, a Japanese chemist and professor of Tokyo Imperial University, produced monosodium glutamate from kelp like seaweed called kombu and identified as first umami seasoning [36]. In the next year, its large scale production was started with the help of Saburosuke Suzuki II, the progenitor of Ajinomoto co. Inc., under the broad name of AJI-NO-MOTO® [68]. The process was successfully continued around next 50years. However, this suffers from few drawbacks. Huge cost of raw materials as well as the scarcity of the availability of acid tolerant materials under high temperature made the process difficult to operate. Though the use of ceramic pots minimized the acid tolerance difficulty, but the hydrochloric acid vapor evolved in this method adversely affected the workers and this issue made the establishment of such industries in locality. Moreover, formation of huge waste (remaining fraction of the hydrolysate) raised another issue. After World War II, the demand for MSG increases several folds. Its production between 1955 and 1959 became almost double by *Ajinomoto Co. Inc*[68].

GLUTAMIC ACID PRODUCTION METHODS

Large scale production of glutamic acid was started in December, 1908.It was the first challenge of industrial production of amino acid without any prior experience. The early production method was dependent on hydrolysis of protein (extraction), isolation and purification [68].

Protein hydrolysis (Extraction)

Ikeda (1908) suggested wheat gluten as a rich source of glutamine, upon hydrolysis of which yielded L-glutamic acid even up to 30g/100g of wheat gluten. In extraction method, gluten was separated from wheat flour and transferred to a Domyoji-game (ceramic pottery vessel) which was resistant to acid [36]. Hydrochloric acid was added to it and heated for 20h. The protein hydrolysates generated by this method was then filtered to eliminate humus (black residues produced from reactions of amino acids with carbohydrates). The filtrate was then returned back to Domyoji-game and concentrated for one day. It was then transferred to another Domyoji-game and stored for one month to allow it to form L-glutamic acid hydrochloride crystals. It was the only amino acid salt in the hydrolysate that exhibited low solubility in hydrochloric acid. L-glutamic acid stacks along the axis of these growing crystals which is unfavorable for accommodating other amino acids [26]. This makes the crystallization process suitable for purification of L-glutamic acid from the production medium. This unusual crystallization also unfavor other substances in the gluten hydrolysate to accommodate within the crystals or prevent their inhibition for crystallization. Though it was an effective method of glutamic acid production, however, due to

formation of corrosive acid vapor, it adversely affected exposed workers and localities which created a serious environmental issue that unfavor the establishment of these production plants within or near localities. Disposal of huge wastes also raised another environmental issue. Establishment of plants in remote areas was not also advisable due to unavailability of labors and huge transport cost. Such limitations made this process obsolete [19, 68].

Chemical synthesis

The new era of L-gutamic acid production has been introduced in the late 1950s by adopting two parallel methods of production: chemical synthesis and fermentation. Initially three chemical syntheses methods were developed, among which two were industry-university joint projects, and third one which was most accepted method used acrylonitrile, carbon monoxide and hydrogen cyanide as raw materials for strecker reaction involved in glutamic acid production. This method was industrialized by Ajinomoto Co in 1960 as acrylonitrile was easily available from polyacrylic fiber industries in the mid-1950s in Japan [86]. In this method, two limitations appeared very shortly: it produced racemic mixture of DL isomers of glutamic acid from which separation of stereo-specific L and D isomers was a long and costly method; use of hazardous as well as carcinogenic hydrogen cyanide raised a serious health issue. Due to these limitations this method was abolished in 1973 [68].

Microbial fermentation

Microbial production of L-glutamic acid was started with the discovery of soil bacterium *Micrococcusglutamicus* (later renamed as *Corynebacterium glutamicum*) in 1956 [43]. Prior to the discovery of this microbial strain, Professor Johnson, professor emeritus, University of Wisconsin focused on some interests on L-glutamic acid fermentation scattered at different segments of scientific communities, however there was a general belief that glutamic acid fermentation if irrational because: (1) it is an essential nutritional ingredient for microorganism, (2) its production requires energy, its excretion from cells is not economically viable, (3) glutamic acid does not confer any suppressing effect on surrounding microbial communities [38]. Dr. Udaka and his associates of *Kyowa Hakko Kogyo Co., Ltd*. discarded these obstacles. Under his supervision, a screening method was developed. Isolated bacterial strains were allowed to grow on nutrient agar medium. After formation of ddiscrete colonies, the plates were exposed to UV irradiations to kill the microorganisms. The test plates were the overlaid with *Leuconostoc mesenteroides* (glutamate auxotrophic strain). The halo formation around the colonies indicated glutamic acid excretion from the colonies. In this way they have screened around 500 strains among which *Micrococcus glutamicus* appeared as highest L-glutamic acid producer with accumulation of L-glutamic acid up to 10.3g/L in liquid synthetic medium contained glucose, 5% and further addition of glucose would scale up the production, suggesting that the strain could be applicable for industrial production of L-glutamic acid [43, 81].

It opened a new dimension in industrial microbiology. But suddenly researchers found that the bacterium produced a trace amount of L-glutamic acid. For searching out its cause, extensive research had been carried out and finally, it was discovered that this strain is a biotin auxotroph and required biotin limitation for maximum L-glutamic acid production [81].

The first paper on L-glutamic acid fermentation by *Micrococcus glutamicus* was presented in the year of 1957 at The International Symposia on Enzyme Chemistry held in Tokyo and Kyoto, the first major international scientific conferences after World War II with great importance in the field of life sciences considering the health status improvement among Japanese [44]. The fermentation of L-glutamic acid was first commercialized at a plant of *Kyowa Hakko Kogyo* in 1958 [44]. Until 1970, Japan showed almost monopoly in the field of research. It gradually declined in 1980s and then again resurged after 2000. Other Asian countries especially started research in this field from 1980s. Several productive strains were discovered with identificationof new raw materials and production strategies which indicated its strong economic demand. European countries started research in this field from 1990s focusing on the molecular mechanism of microbial production and extracellular excretion of L-glutamic acid [31].

A comparative analysis among different methods of glutamic acid production is summarized in Table 1.

Table 1. A comparative analysis among different methods of glutamic acid production		
Methods	**Strength**	**Weakness**
Protein hydrolysis (Extraction)	1. Industrial byproducts and waste materials can be used as raw materials which reduce its production cost [30]. 2. Use of Common reagents like hydrochloric acid and sodium hydroxide make it easier [68].	1. Scarcity of acid tolerant materials [68] 2. Evolution of hydrogen chloride gas and formation of huge volume of waste [19] 3. Very high or very low pH for protein degradation may cause low yield of glutamic acid [19] 4. Several toxic compounds also produced during the hydrolysis [70] 5. Finally, after the product formation, necessary washing to get rid of acid and alkali generates bulk of waste water [19, 36] 6. Limited raw materials (such as hair, nail, feather etc) [37]
Chemical synthesis	1. Produce bulk of racemic mixture within a short duration from which according to need enantiomeric form can be separated [52].	1. Huge price of catalysts and use of toxic cyanide compounds [89] 2. Produces racemic mixture of DL isomers [28]
Fermentation	1. Large Scale production [37] 2. Produces biologically active stereo-specific isomer [36] 3. Low cost method [19] 4. Least technical skills are required [68] 5. Easy to maintain production conditions [68] 6. Ecofriendly method [36] 7. Uses cheap and renewable raw materials [36]	1. The economy of this method depends on the cost of the crbon sources, productivity of the strain, purification cost and total fermentation yield [36] 2. Contamination of the broth with other microorganisms [36] 3. Degeneration of the productive strain (both back mutation and loss of genetic materials) [36]

Microbial strains for L-glutamic acid production

Since the discovery of *Micrococcus glutamicus*, considering the simplicity, economic viability and environmental feasibility, researchers made exhaustive research on isolation of new productive strains as well as improvements on newly isolated strains. Since 1956, many such productive strains have been identified. Most of the isolated strains were Gram +ve, non-motile, non-sporulating, rod shaped, biotin requiring, aerobic bacterium. Biochemical, morphological and taxonomical studies were done for their initial identification [85]. Recently, 16S RNA sequencing is well accepted throughout the globe in relation to the phylogenetic analysis of a newly isolated strain [55]. Major producers have been summarized in Table 2.

Table 2. List of microorganisms capable of overproducing L-glutamic acid		
Microorganisms	L-glutamic acid (g/L)	References
Micrococcus glutamicus	10.3	[43]
Bacillus strain 14B22	12.5	[13]
*Brevibacteriumdivaricatum*nov.sp	45	[74]
Brevibacteriumflavum 1996	35.2	[68]
Brevibacteriumflavum 1223	41.7	[68]
Brevibacteriumflavum 2247	32.6	[68]
*Brevibacteriumlactofermentum*2256	30.7	[68]
*Brevibacteriumlactofermentum*2363	21.8	[68]
*Brevibacteriumsaccharolyticum*No.2237	29.9	[68]
*Brevibacterium immariophilum*No.2237	24.3	[68]
Brevibacteriumroseum No 7	39.7	[68]
*Brevibacterium*nov.sp 3114	7.3	[68]
Bacillus megaterium No 420	32.2	[68]

Bacillus circulans No 2158	30.9	[68]
Arthrobacterglobiformis	66.2	[82]
*Brevibacteriumlactofermentum*nov.sp	64.3	[56]
Corynebacterium S10B1	5	[75]
Microbacteriumammoniaphilum	66.2	[71]
Brevibacteriumthiogenetalis No. 653	5	[60]
*Brevibacterium*sp B136	53.1	[61]
Bacillus cereus var. mycoides	5.2	[14]
*Brevibacteriumroseum*DSM20411	25	[18]
Brevibacterium strain NIABSS67	60.8	[57]
Lactobacillus plantarum MNZ	1.4	[88]
Corynebacteriumglutamicum NCIM2168	13	[72]
*Pseudomonas reptilivora*NCIM2598	16	[72]

Strain improvement strategies

The concept of strain improvement is considered as 'old biotechnology' [63].Though *Corynebacterium glutamicum* discovered as an overproducer of L-glutamic acid, however, the amount of L-gluamic acid produced by the parent strain was not sufficient enough to meet the industrial demand. Natural strains often exhibit low substrate conversion efficiency, byproducts formation and low tolerance to stress [52]. It is practically difficult to overcome these problems by only optimizing the culture conditions [87]. Genetic improvement could able to generate superior strains for such overproduction. Physical, chemical and genetic engineering tools were proved to be effective for the development of superior strains which possess: (a) capacity to utilize indigenous raw materials to minimize production cost, (b) minimized undesired by-product formation and (c) enhanced extracellular release of L-glutamic acid, (d) minimized fermentation time [69]. Practically it is difficult to obtain high L-glutamic acid yield and high tolerance simultaneously. Strain improvement gained much importance in last few decades to overcome this problem [65]. Classical strain improvement strategies involve random mutation followed by selection, gene engineering for over expression of selective genes, target gene mutation and laboratory evolution [9]. Though the above mentioned strategies studied extensively,but all these methods suffer from limitations. These methods are time consuming and laborious. Moreover, gene manipulations require sufficient information about the specific target genes. Genetic manipulations led to modifications of genotypes but the desired changes would be phenotypic [55]. Considering the desire, modern approaches for strain development include artificial transcription factor engineering, global transcription machinery engineering, genome shuffling, ribosomal engineering etc.[27]. Genome shuffling includes repeated mutagenesis followed by recursive spheroblasts fusion to obtain overproducers [27]. It has been successfully applied in different fermentation industries since 2002. In gene shuffling, potential parental library construction is the first step which involves initial screening followed by selection of suitable microorganisms. During the course of parental library construction, the strain is subjected to either single or several rouns of mutational treatments using both physical and chemical mutagens (such as UV rays, ethylmethane sulfonate/EMS, ethylenemine, N-methyl NNN-nitro-N-nitrosoguanidine/NTG etc) either single or combined exposure to develop high yielding strains [91]. Then high yielding strains are identified followed by protoplast fusion. Though this technique is very effective, however, it suffers from several limitations. Lack of appropriate screening method especially for unknown strains is one of the major problems (Magocha et al., 2018). Thus rapid identification for those strains is under constrain. Genetic stability of the modified strains is another challenge [83].

L-glutamic acid biosynthesis and its export from microbial cells

Glutamic acid bacteria can grow on sugar, ethanol, acetate etc. media, but not on hydrocarbons and methanol [40, 45, 48, 59]. The production is dependent on initial biotin concentration present in the medium. The principal pathway of L-glutamic acid production from glucose in microorganisms is shown in Fig 1a.

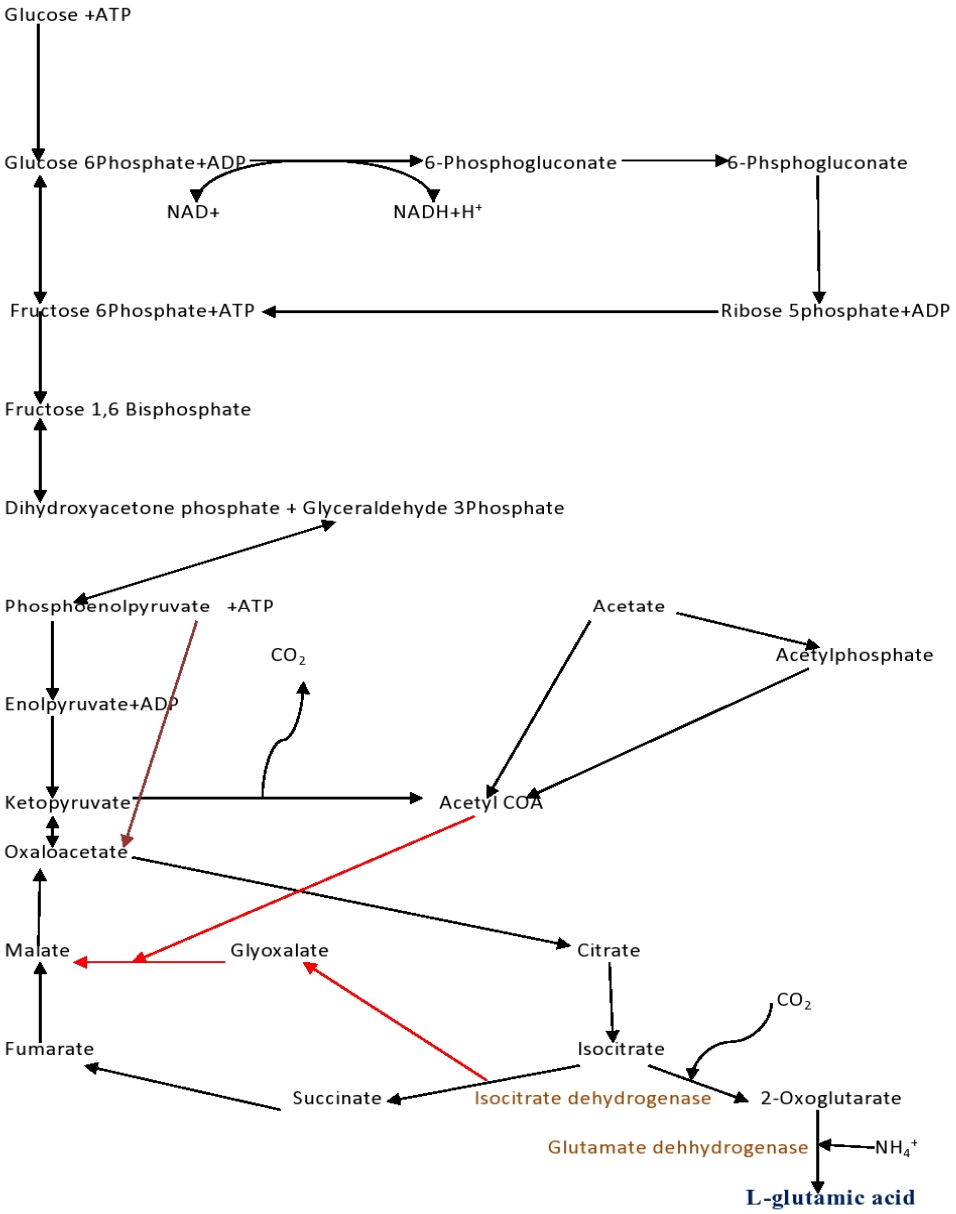

Fig 1a.**Outline of the methabolic pathways involved in L-glutamic acid synthesis in *Corynebacterium glutamicum***

The most significant step is the assimilation of ammonium. In *Corynebacterium glutamicum*, there are two major pathways for ammonium assimilation have been identified:glutamate dehydrogenase and glutamate synthase glutamine 2-oxoglutarate aminotransferase [80]. Under excess nitrogen supply (>1mmol dm^{-3}) glutamate dehydrogenase reduces 2-oxoglutaric acid to L-glutamic acid. But under nitrogen deprivation (<1 mmol dm^{-3}), initially L-glutamic acid is amidated to glutamine which in turn further transfers amide group to 2-oxoglutaric acid by 2-oxoglutarate aminotransferase which is shown in Fig 1b.

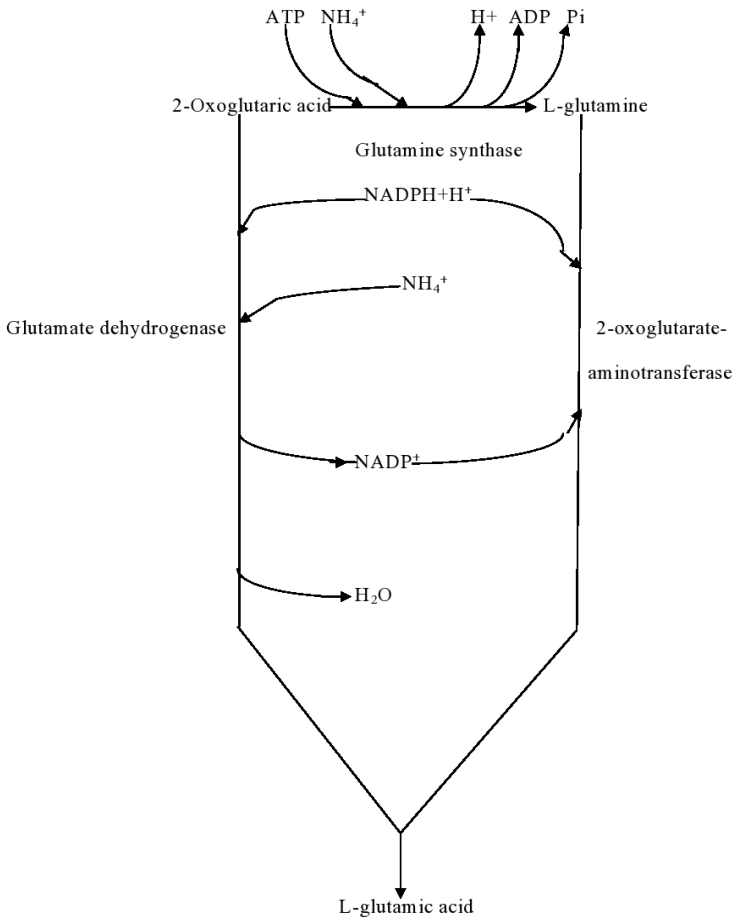

Fig1b. Synthesis of L-glutamic acid from 2-Oxoglutaric acid by glutamate dehydro and 2-oxoglutarate aminotransferase

Both these two pathways are operating simultaneously in *Corynebacterium glutamicum*.Though, initially it was believed that glutamate dehydrogenase is the key enzyme for the assimilation, but later on through deletion and overexpression of *gdh* gene, encoding glutamate dehydrogenase, revealed that glutamate dehydrogenase is mandatory for nitrogen assimilation in *Corynebacterium glutamicum*[11, 78].

Biosynthesis of L-glutamic acid in various microorganisms has been studied extensively. From radioisotopic studies followed by NMR spectroscopy revealed that there are two distinct pathways involved in L-glutamic acid production in microorganisms. Reductive carboxylation of succinate played a major role [41]. 2-oxoglutarate required for L-glutamic acid biosynthesis is produced from acetyl COA in Kreb's cycle [12]. An enzyme re-citrate synthase responsible for conversion of oxaloacetate to citrate has been reported in many microorganisms [41]. Product mediated feed-back inhibition of glutamate dehydrogenase complex is also reported [42].

L-glutamic caid secretion in *Corynebacterium glutamicum* has been well studied. It has been concluded that its secretion does not occur under normal physiological conditions; however, the efflux is based on alteration of cell membrane phospholipid synthesis which is principally mediated via biotin limitation [80]. Additional factors include: addition of surfactants, penicillin addition and limitation of membrane phospholipid synthesis in glycerol or oleic acid auxotrophic mutants [17]. A leaky membrane model was proposed to demonstrate the role of biotin on L-glutamic acid efflux by *Corynebacterium glutamicum*[68]. As per this model, lack of biotin inhibited fatty acid synthase activity which in turn, depleted fatty acid content in the membrane, altered physiological properties of the membrane which is more permeable to L-glutamic acid [80]. In addition, acetylCoA is funneled for L-glutamic acid production, but due to increased extracellular efflux, there is no feedback inhibition leading to continuous extracellular release of L-glutamic acid [58]. Clement and his co-worers (1984) hypothetized an alternative model known as 'The Carrier Inversion Model'. As per this model, L-glutamic acid efflux is mediated via 'permease' which under normal physiological machinery, but under biotin limitationor surfactant addition, it uncouples its

energy source from proton motive force, leading to release of L-glutamic acid from the bacterial cells down the electrochemical potential [17]. But this model was ruled out by another hypothesis proposed in 1989. As per this model, lipid state of the membrane is not only the parameter for determining L-glutamic acid efflux, it can be secreted under biotin limitation against chemical gradient where the secretion operating system is not driven by membrane potential, pH and other ionic gradients [32].

Metabolic engineering for L-glutamic acid overproduction

Metabolic engineering integrates system biology, synthetic biology and evolutionary engineering [49]. It utilizes omics data for strain improvement. Integration among system biology, evolutionary biology and synthetic biology could able to explore the area of industrial strain development by metabolic engineering 7, 16, 73]. Metabolic engineering strategies have successfully applied now a day for overproduction of amino acids involving *Corynebacterium glutamicum* and *Escherichia coli* [53]. L-glutamic acid covers approximately two third of the total amino acids markets. Since its discovery, strain improvement strategies have becoming a central focus in fermentation of amino acids [53]. It could be achieved either by rational intuitive approaches or by the systematic and rational random approaches. The former approaches use mainly classical metabolic engineering techniques whereas later includes omics based metabolic approaches where no target gene was identified [49]. L-glutamic acid fermentation was initiated under biotin-limitation with *Micrococcus glutamicus*[43]. Lateron it has been found that several non-productive strain could also produce L-glutamic acid on addition of penicillin and surfactants [22-24]. Based on these early studies, current advancements consider alterations of intracellular metabolic pathways and correlation between structure-function relationships of cellular envelops [6, 32, 64]. Under triggering conditions, 2-oxoglutarate is converted to L-glutamic acid by glutamate dehydrogenase instead of forming succinyl Co-A. Biotin limitation and surfactant addition could alter the pattern of fatty acids and phospholipids syntheses which led to improved production of L-glutamic acid [53]. In addition, anapleurotic pathway of PEP-pyruvate-oxalate is essential for enhanced carbon flux for L-glutamic acid overproduction [8]. Fig 2 depicts the outline of metabolic engineering in *Corynebacterium glutamicum* for L-glutamic acid production. So, metabolic engineering could able to prove valuable information for enhanced intracellular synthesis of L-glutamic acid followed by its extracellular export.

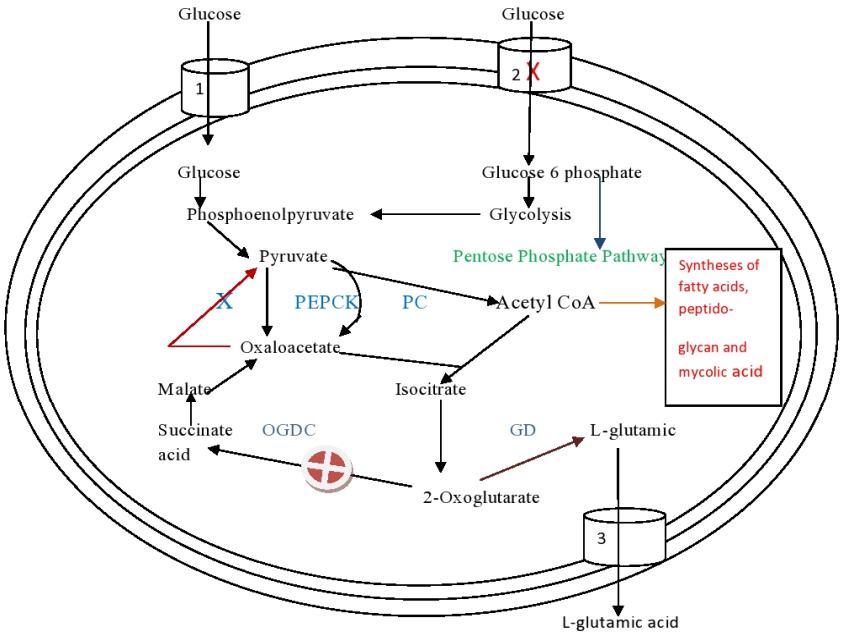

Fig2. Metabolic engineering of *Corynebacterium glutamicum* for L-glutamic acid overproduction (1: Nonphosphotransferase; 2: Phosphotransferase; 3: NCgl1221; PEPCK: Phosphoenolpyruvate carboxykinase; PC: Pyruvate carboxylase; GD: Glutamate dehydrogenase; OGDC: Oxoglutarate dehydrogenase complex)

Recovery of L-glutamic acid from production medium

The separation of L-glutamic acid hydrolysate from its sodium salt is done by isoelectric crystallization with or without prior separation of microbial cells/cell fractions. Though the presence of biomass in the broth minimizes crystallization and potentiates formation of p-forms of crystal [35, 54, 90]. In addition,

after crystallization, the broth contains tiny amount (1-2%) of glutamic acid hydrolysate at isoelectric pH [90]. Compare to monosodium glutamate, glutamic acid hydrochloride shows higher solubility in water which ensures its loss during purification process. Several rounds of mineral acid treatments followed by washing are required to remove the salt formed [47]. After filtration, the calcium glutamate is bleached by activated vegetable carbon instead of activated carbon for the production of food grade L-glutamic acid. Then calcium glutamate is evaporated at 70^0C (0.57atm) to approximately 37% concentration. It was then acidified with concentrated sulfuric acid followed by removal of calcium sulphate precipitate by continuous filtration and again sent back for reuse. The filtrate was treated with activated carbon for bleaching. L-glutamic acid was then evaporated out to concentrate in evaporator made up of stainless steel double effect evaporator. Liquid-liquid extraction using immiscible solvent is also preferred [47].

CONCLUSION

This review pointed out the advantages of fermentation over other methods of glutamic acid production. It is environmentally benign and economically viable as it utilized low cost raw materials. Moreover, with the advancement of biotechnology and genetic engineering several high yielding strains were already generated which would able to utilize residues and waste products as raw materials that do not compete with human diet. Process optimization using statistical tools and computerized technologies not only give accuracy but also minimize total number of experimental trials and hence reduce time cost.

Improvement in down-stream recovery methods also favor the overall hike in total amount of purified product with reduction in processing cost. Improvements in down-stream process also minimize by-product formation and hence purification of L-glutamic acid becomes easier and low cost.

Finally, combination of modern biotechnology including genetic engineering and mathematical modeling with simulations for designing the production plants at early stages optimizes the product formation and minimizes the rate of uncertainty to almost near zero.

AUTHORS' CONTRIBUTIONS

The corresponding author conceived the idea and the co-authors provide him necessary information and technical supports.

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CONFLICT OF INTEREST

The authors clearly declare there is no technical or financial conflict of interest.

REFERENCES

1. Ajinomoto**, (**2003). Encyclopedia of Amino Acids. http://www.ajinomoto.com/amino (accessed: October 2012)
2. Alharbi, N.S., Kadaikunnan,S., Khaled,J.M., Almanaa, T.N., Innasimuthu,G.M., Rajoo,B., Alanzi, K.F. and Rajaram, S.K., (2019). Optimization of glutamic acid production by *Corynebacterium glutamicum* using response surface methodology. Journal of King Saud University – Science, 32(2),1403-1408. https:// doi. org/10.1016/j.jksus.2019.11.034
3. Amin, G., Shahaby, A.F. and Allah, A.M.K.,(1993). Glutamic acid and by-product synthesis by immobilized cells of bacterium *Corynebacterium glutamicum*. Biotechnol. Lett.15(11), 1123-1128. https:// doi. org/ 10.1007/ BF00131201
4. Amin, G.,(1994). Continuous production of glutamic acid in a vertical rotating immobilized cell reactor of the bacterium *Corynebacterium glutamicum.*Biores.Technol.47(2), 113-119.https:// doi. org/10.1016/0960-8524(94)90108-2
5. Aoki, R., Wada, M., Takesue, N., Tanaka, K. and Yokota, A.,(2005). Enhanced glutamic acid production by a H^+-ATPase defective mutant *Corynebacterium glutamicum*. Biosci. Biotechnol. Bioeng. 69(8), 1466-1472. https:// doi. org/ 10. 1271/ bbb. 69.1466
6. Asakura, Y., Kimura, E., Usuda, Y., Kawahara, Y., Matsui, K., Osumi, T. and Nakamatsu, T., (2007). Alteredmetabolic flux due to deletion of odhA causes L-glutamate overproduction in*Corynebacterium glutamicum*. Appl Environ Microbiol 2007;73:1308e19.
7. Bassalo, M.C., Liu, R., Gill, R.T.,(2016). Directed evolution and synthetic biology applicationsto microbial systems. Curr Opin Biotech 39:126e33.
8. Becker, M., B€orngen, K., Nomura, T., Battle, A.R., Marin, K., Martinac, B., Krämer, R., (2013). Glutamate efflux mediated by *Corynebacterium glutamicum* MscCG, *Escherichiacoli* MscS, and their derivatives. BBA-Biomembranes 1828(4):1230e40.
9. Biot-Pelletier, D., Martin, V.J., (2014). Evolutionary engineering by genome shuffling.Appl. Microbiol. Biotechnol. 98, 3877–3887.

10. Bisht, D., Yadav, S.K., Darmwal, N.S.,(2013). Optimization of immobilization conditions by conventional and statistical strategies for alkaline lipase production by *Pseudomonas aeruginosa* mutant cells: Scale-up at batch scale bioreactor level. Turk. J. Biol.37(4), 392-402.https:// doi.org/10.3906/biy-1209-19
11. Bormann-El Kholy, E.R., Eikmanns, B.J., Gutmann, M. and Sahm, H.(1993). Glutamate dehydrogenase is not essential for glutamate formation by *Corynebacterium glutamicum*. ApplEnvironMicrobiol 59(7),2329–2331.
12. Buckel, W., (2001). Unusual enzymes involved in five pathways of glutamate fermentation. Appl Microbiol Biotechnol 57,263–273.
13. Chao, K.C. and Foster, J.W., (1959). A glutamic acid producing *Bacillus*. J. Bacteriol.77(6), 715-725.
14. Chattopadhyay, S.P., Banerjee, A.K., 1978. Production of L-glutamic acid by *Bacillus* sp. Folia Microbiol. 23, 469-480. https:// doi.org/ 10. 1007/ BF02885578
15. Chaudhuri, N., Landin, A.M., Roper, S.D., (2000). A metabotropic glutamate receptor variant functions as a taste receptor. Nat. Neurosci. 3(2), 113-119.https:// doi. org/10.1038/ 72053
16. Chubukov V, Mukhopadhyay A, Petzold CJ, Keasling JD, Martín HG.,(2016). Synthetic and systems biology for microbial production of commodity chemicals. NpjSyst Biol Appl 2:16009.https://doi.org/10.1038 /npjsba.2016.9
17. Clement, Y, Lanneelle, G.,(1986). Glutamate excretion mechanism in *Corynebacteriumglutamicum*: Triggering by biotin starvation or by surfactant addition. J.Gen.Microbiol. 132(4), 925-929. https://doi.org/10.1099/ 00221287-132-4-925
18. Confield, C.A., Bradshaw, P., (2019). Amino acids in the regulation of aging and aging related diseases.Translational Medicine of Aging 2, 20-89. https:// doi. org/10.1016/ j. tma. 2019.09.001
19. D'Este, M., Alvarado-Morales, M. and Angelidaki, I., (2018). Amino acid production focusing on fermentation technologies- A review. Biotechnology Advances. 36(1), 14-25.https:// doi. org/10.1016/ j. biotechadv.2017.09.001
20. Dutta, S., Nagarajan, K., Ray, S., (2013). Glutamic acid as anticancer agent: An Overview. Saudi Pharmaceutical Journal 21(4), 337-343.https:// doi. org/10.1016/ jsps. 2012. 12. 007
21. Fontanille, P. and Larroche, C., (2010). Production of food Additives, in : Pandey, A., Soccol, C.R., Gnansounou, E., Larroche, C.,Nigan, P.S.N., Dussap, C.G. (Eds.), Cpmprehensive Food Fermentation and Biotechnology, Asian Pub, pp. 281-304.
22. Ganguly, S. and Banik A.K. (2010). Induced mutation and selection of high yielding strain of *Micrococcus glutamicus* for glutamic acid production. J. Indian Chem.Soc., 87(6): 717-721
23. Ganguly, S. and Banik A.K. (2012).Selection of suitable carbon and nitrogen sources on growth and production of L-glutamic acid by a mutant *Micrococcus glutamicus* AB100. J. Indian Chem.Soc., 89(11): 1-5
24. Ganguly, S. (2020). Role of penicillin G and surfactants on extracellular secretion of L-glutamic acid as a function of membrane permeability. Indian J. Applied and Pure Bio., 35(1): 1-6
25. Gavin, J.J., Umbreit, W.W.,(1965). Effect of Biotin on Fatty Acid Distribution in *Escherichiacoli*. J. Bact.,80(2), 437-443.
26. Giacometti, T., (2009). Free and bound glutamate in natural products, in: Filer,LJ, Garattini, S., Kare ,M.R., Wurtman, R.J.(Eds.), Glutamic acid: advancesin biochemistry and physiology. Raven Press,New York, pp 25–34.
27. Gong, J., Zheng, H., Wu, Z., Chen, T., Zhao, X., 2009. Genome shuffling: progress andapplications for phenotype improvement. Biotechnol. Adv. 27, 996–1005.
28. Groger, H., (2003). Catalytic enantios elective Strecker reaction and analogous syntheses. Chem. Rev. 103(8), 2795-2827.https:// doi. org/10. 1021/ cr 020038p
29. Hanlon, P.R., Frestedt, J. and Magurany, K., (2017). GRAS from ground up: Review of the Interium Pilot Program for GRAS notification. Food and Chemical Toxicology, 105: 140-150.https:// doi. org/j.fct 2017. 06.042
30. Hauer, B., Breuer, M., Ditrich, K., Habicher, T., Keβeler, M., Sturmer, R., Zelinski, T., 2004. Industrial methods for the production of optically active intermediates. Angew. Chem. Int. Ed. 43(7), 788-824.https:// doi. org/ 10. 1002/ anie. 200300599
31. Hirasawa, T., Wachi, M., (2016). Glutamate fermentation 2: mechanism of L-glutamic acid overproduction in *Corynebacterium glutamicum*. Adv. Biochem. Eng. Biotechnol., https://doi.org/ 10.1007/10_2016_26
32. Hoischen, C. and Kr¨amer, R.,(1989). Evidence for an efflux carrier systeminvolved in the secretion of glutamate by *Corynebacteriumglutamicum*. Arc. Microbiol.151:342–347.
33. Hoischen, C and Kr¨amer,R.,(1990). Membrane alteration is necessary but not sufficient for effective glutamate secretion in *Corynebacterium glutamicum*. J. Bacteriol.172(6):3409e16.
34. Hosking, A.M., Juhasz, M., Mesinkovska, N.A., (2019). Complementary Alternative Treatments for Alopecia: A Comprehensive Review. Skin Appendage Disord 5, 72-89.https:// doi. org/ 10. 1159/ 000492035
35. Huang, S., Wu, X., Yuan, C., (1995). Application of membrane filtration for glutamic acid recovery. J. Chem. Technol. Biotechnol. 64(2), 109-114.
36. Ikeda, K., (1908). A production method of seasoning mainly consists of salt of L-glutamic acid. Japanese patent 14805.
37. Ivanov, K., Stoimenova, A., Obreshkova, D., Saso, L., (2014). Biotechnology in the production of pharmaceutical industry ingredients: amino acids. Biotechnol. Biotechnol Equip. 27(2), 3620-3626.https:// doi. org/ 10.5504/ BBEQ. 2012.0134
38. Johnson, M.J., (1955). Unit process review. Ind. Eng. Chem. 47(1), 1872-1875.
39. Kikuchi, M., Nakao, Y.,(1972). Change in Permeability to L-Glutamic Acid the Glycerol Auxotroph GL-21. Agricultural and Biological Chemistry, 36(7), 1135-1140. https:// doi.org/ 10.1080/00021369.1972.10860388

40. Kim M, Le H, McInerney MJ, Buckel W. (2013). Identification and characterization of *re*-citrate synthase in *Syntrophus aciditrophicus.* J Bacteriol 195,1689–1696.
41. Kim, M., Le, H.M., Xie, X., Feng, X., Tang, Y.J., Mouttaki, H., Mclnerney, M.J., Buckel, W., (2015). Two pathways for glutamate biosynthesis in syntrophic bacterium *Syntrophus acidophicus*. Appl. Environ. Microbiol. 81(24), 8434-8444.
42. Kimura, M., (2003). Metabolic Engineering of L-glutamic acid . Adv. Biochem. Eng. 79, 37-57.
43. Kinoshita, S., (1957). Studies on The Amino Acid Fermentation. Part I. Production of L-glutamic acid by various microorganisms. J. Gen. Appl. Microbiol. 3(3), 193-205.
44. Kinoshita, S., (1972). Amino acid production by the fermentation process. Nature 240, 211.
45. Kinoshita, S., Udaka, S. and Shimono, M.(1985). Glutamic acid bacteria,in *Biotechnology of Industrial Microorganisms*, ed by Demain Aand Solomon NA. Benjamin Cumings, London, pp 115–142
46. Kobayashi, K., Tanaka, M., Tanabe, S., Yatsukawa, Y., Tanaka, M., Suzuki, T.,(2018). Distinguishing glutamic acid in foodstuff and monosodium glutamate used as seasoning by Stable carbon and nitrogen ratios. Heliyon 4(9), e00800.https:// doi. org/10.1016/ jheliyon. 2018.e00800
47. Kumar, R., Vikramachakravarthi, D., Pal. P., (2014). Production and purification of glutamic acid: A critical review towards process intensification. Chemical Engineering and Processing 81, 59-71.https:// doi. org/10.1016/jcep. 2014. 04.012
48. Lee, J.W., Kim, T.Y., Jang,Y.S., Choi, S., Lee, S.Y.,(2011). Systems metabolic engineering for chemicals and materials. Trends Biotechnol 29(8):370e8.
49. Lee, J.W., Na, D., Park, J.M., Lee, J., Choi, S., Lee, S.Y.,(2012). Systems metabolic engineering of microorganisms for natural and non-natural chemicals. Nat Chem Biol8(6):536e46.
50. Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M., Adler, E., (2002). Human receptor for sweet and umami taste. Proc. Natl. Acad. Sci. USA. 99(7), 4692-4696.https:// doi. org/10.1016/10. 1073/ pnas. 072090199
51. Loiger, J., (2000). Function and importance of glutamate for savory foods. The journal of Nutrition 130(4), 915S-920S.https:// doi. org/10.1093/jn/130.4.915S
52. Magocha, T.A., Yun, J., Zabed, H., Yang M., Yun, J., Zhang, H. and Qi, X. (2018). Improvement of industrially important microbial strains by genome shuffling: Current status and future prospects. Bioresour. Technol. 257, 281-289.
53. Ma, Q., Zhang, Q., Xu, Q., Zhang, C., Li, Y., Fan, X., Xie, X. and Chen, N., (2017).Systems metabolic engineering strategies for the production of amino acids.Systems and Synthetic Biotchnology 2(1): 1 10.http://dx.doi.org/10.1016/j.synbio.2017.07.003
54. Mizoguchi, N., Hara, M., Ito, K., (1996). Method of optical resolution for glutamate salt or glutamicacid. Japanese patent 9971.
55. Morales, S.E., Cosart, T.F., Johnson, J.V., Holben, W.E., (2009). Extensive Phylogenetic analysis of a soil bacterial community Illustrates Extreme Taxon Evenness and the Effects of Amplicon Length, degree of converge, and DNA Fractionation on classification and Ecological parameters. Appl. Environ. Microbiol. 75(3), 668-675. https:/doi. org/ 10.1128/ AEM. 01757-08
56. Motozaki, S., Tsunoda, T., Zushi, Okumura,S., Okada,H., Kawasaki, Ozaki, A., Ishikura,T.,(1964). Process for Producing L-glutamic acid by bacterial fermentation. United States Patent 3, 128, 237.
57. Nadeem, S., Niaz, B., Muzammil, H.M., Rana, S.M., Rajoka, M.I., Shakoori, A.R., (2011). Optimizing carbon and nitrogen sources for L-glutamic acid production by *Brevibacterium* strain NIABSS67. Pakistan J. Zool.,43(2), 285-290
58. Nampoothiri, K.M., Pandey, A., (1999). Fermentation and recovery of L-glutamic acid from cassava starch hudrolysate by ion-exchange resin column. Rev.Microbiol. 30(3), 258-264. https:// doi.org/ 10. 1590/ s0001-37141999000300013
59. Nampoothiri, M., Hoischen, C., Bathe, B., Mockel, B., Pfefferle, W.,Krumbach, K., Sahm, H. and Eggeling, L., (2002). Expression of genesof lipid synthesis and altered lipid composition modulatesL-glutamate efflux of *Corynebacterium glutamicum*. Appl. Environ. Microbiol.,58(1),89–96.
60. 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. Agr. Biol. Chem. 31(11), 1314-1317
61. Oki, T., Sayama, Y., Nishimura, Y., Ozaki, A., (1968). L-glutamic acid fermentation by Microorganisms from ethanol. Agr. Biol. Chem. 32(1), 119-120
62. Olney, J.W., (1969). Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science 164(3880),719-721. https://doi. org /10.1126/ science.164. 3880.719
63. Parekh, S., Vinci, V.A., Strobel, R.J.(2000). Improvement of microbial strains and fermentation processes. Appl. Microbiol. Biotechnol. 54, 287-301.
64. Radmacher, E., Stansen, K.C., Besra, G.S., Alderwick, L.J., Maughan, W.N., Hollweg, G.,Sahm,H., Wendisch,V.F., Eggeling, L., (2005).Ethambutol, a cell wall inhibitor of *Mycobacterium tuberculosis*, elicits Lglutamateefflux of *Corynebacterium glutamicum*. Microbiology 151:1359e68.
65. Qi, X., Zhang, H., Magocha, T.A., An, Y., Yun, J., Yang, M., Xue, Y., Liang, S., Sun, W.,Cao, Z., (2017). Improved xylitol production by expressing a novel d-arabitol dehydrogenasefrom isolated Gluconobacter sp. JX-05 and co-biotransformation of whole cells. Bioresour. Technol. 235, 50–58.
66. Ramirez, I., De Santiago, S., Tovar, A.R., Torres, N., (2001). Amino acid intakeduring lactation and amino acids of plasma and human milk. Adv. Exp. Med. Biol. 501, 415-421.https:// doi. org/10. 1007/ 978-1-4615-1371-1 52

67. Saleem, S., Shaukat, F., Gul, A., Arooj, M., Malik, F., (2017). Potential role of amino acids in pathogenesis of Schizophrenia. International Journal of Health Sciences 11(3), 63-68.
68. Sano, C., (2009). History of glutamate production. Am. J. Clin. Nutr.90, 728S-732S. https://doi.org/10.3945/ajcn.2009.27462F.
69. Saxena, S., 2015. Strategies of strain improvement of industrial microbes, in: Applied Microbiology. Springer India, India, pp. 151-171.
70. Sereewatthanawut, I., Prapintip, S., Watchiraruji, K., Goto, M., Sasaki, M., Shotipruk, A., (2008). Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis. Bioresour. Technol. 99(3), 555-561.https://doi.org/j.biortech.2006.12.030
71. Shibukawa, M., Ohsawa, T., (1966). L-glutamic acid fermentation with molasses. Part VI. Effect of saturated and unsaturated fatty acid ratio in the cell membrane fraction of the extracellular accumulation of L-glutamate. Agr. Biol. Chem. 30(8), 750-758
72. Shyamkumar, R., Moorthy, I.M.G., Ponmurugan, K., Baskar, R., (2014). Production of L-glutamic Acid with *Corynebacterium glutamicum* (NCIM 2168) and *Pseudomonas reptilivora* (NCIM 2598): A Study on Immobilization and Reusability.Avicenna J. Med. Biotechnol., 6(3), 163-168
73. Soyer, O.S., O'Malley, M.A.(2013). Evolutionary systems biology: what it is and why it matters. BioEssays 35(8):696e705.
74. Su, Y.C., Yamada, K., (1960). Studies on L-glutamic Acid Fermentation. Part II. Fermentative Production of L-glutamic acid from Glucose by *Brevibacterium divaricatum* nov.sp. Bull. Agr. Chem. Soc. Japan 24(2), 140-146.
75. Takahashi, J., Kobayashi, K., Imada, Y., Yamada, K., (1965). Effect of Corn steep liquor and thiamine on L-glutamic acid fermentation of hydrocarbons. IV. Utilization of hydrocarbons by microorganisms. Appl. Microbiol. 13(1),1-4
76. Takasaki, Y., (1978). Studies on brain lesions after administration of monosodium glutamate to mice II absence of brain damage following administration of monosodium glutamate in the diet. Toxicology 9(4), 307-318.https:// doi. org/10.1016/0300-483x(78)90014-8
77. Tennant, D.R., (2018). Review of Glutamate Intake from both food additive and non-additive sources in the European union. Ann. Nutr. Metab. 73(Suppl.5), 21-28.https:// doi. org/10.1159/000494778
78. Tesch, M., de Graaf, A.A. and Sahm, H.(1999).*In vivo* fluxes inthe ammonium-assimilatory pathways in *Corynebacterium glutamicum* studied by 15N nuclear magnetic resonance.*ApplEnvironMicrobiol.*65(3),1099–1109.
79. Tiziana, P., Sabrina, M., Simone, T., Lanfranco, S.C., (2017). A survey on the presence of free glutamate in food stuffs,with and without added monosodium glutamate. Food Chem. 34(3), 1712-1717.https:// doi. org/ 10. 1016/ j. foodchem 2007. 03. 034
80. Tryfona, T. and Bustard, M.T.,(2004). Mechanistic understanding of the fermentative L-glutamic acid overproduction by *Corynebacterium glutamicum* through combined metabolic flux profiling and transmembrane transport characteristics.J Chem Technol Biotechnol. 79(12), 1321-1330.
81. Udaka, S., (1960). Screening methods for microorganisms accumulating metabolites and its use in the isolation of *Micrococcus glutamicus*. J. Bacteriol. 79, 754-755.
82. Veldkamp, H., den Berg, G.V., Zevenhuizen, L.P.T.M., (1963). Glutamic acid production by Arthrobacter globiformis. Antonie Van Leeuwenhoek 29, 35-51. https:// doi. org/ 10.1007/ BF02046037
83. Wang, S., Duan, M., Liu, Y., Fan, S., Lin, X., Zhang, Y., (2017a). Enhanced production offructosyltransferase in *Aspergillus oryzae* by genome shuffling. Biotechnol. Lett. 39,391–396.
84. Wu, G., Bazer, F.W., Dai, Z., Li, D., Wang, J., Wu, Z., (2014). Amino Acid Nutrition in Animals: Protein Synthesis and Beyond. Annu. Rev. Anim. Biosci. 2, 387-417.https:// doi. org/10.1146/annurev-animal-022513-114113
85. Yamada, K., Komagata, K., 1972. Taxonomic studies on Coryform bacteria. V. Classification of coryform bacteria. J. Gen. Appl. Microbiol. 18(6), 417-431. https:/doi. org/ 10.2323/jgam.18.417
86. Yoshida,T., (1970). Industrial manufacture of optically active glutamic acidthrough total synthesis. Chem. Ing. Tech.42, 641–4.
87. Yun, J., Yang, M., Magocha, T.A., Zhang, H., Xue, Y., Zhang, G., Qi, X., Sun, W., (2018).Production of 1, 3-propanediol using a novel 1, 3-propanediol dehydrogenase fromisolated *Clostridium butyricum* and co-biotransformation of whole cells. Bioresour.Technol. 247, 838–843.
88. Zareian, M., Ebrahimpour, A., Bakar, F.A., Mohamed, A.K.S., Forghani, B., Kadir, M.S. B.A., Saari, N., (2012). A glutamic acid producing lactic acid bacteria isolated from Malaysian Fermented food. Int. J. Mol. Sci. 13(5), 5482-5497
89. Zuend, S.J., Coughlin, M.P., Lalonde, M.P., Jacobsen, E.N., (2009). Sealeable calalytic asymmetric strecker syntheses of unnatural alpha amino acids. Nature. 461(7266), 968-970.https:// doi. org/10.1038/nature08484
90. Zhang, X., Lu, W., Lu, H., Ren, H., Cong, W., (2007). Recovery of glutamic acid from isoelectric supernatant using electrolysis. Sep. Purif. Technol. 55(2), 274-280.
91. Zhang, Y., Liu, S., Du, Y., Feng, W., Liu, J., Qiao, J., (2014). Genome shuffling of *Lactococcus lactis* subspecies lactis YF11 for improving nisin Z production and comparative analysis. J. Dairy Sci. 97, 2528–2541.

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