# Microbial amino acids production

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# *Microbial amino acids production*

***Zafar Alam Mahmood***

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## *9.1 Introduction*

Amino acids are the constructive components (subunits) of proteins and peptides and are thus regarded as the building blocks of life. Amino acids contain a high percentage of nitrogen (~16%), which distinguishes them from fats and carbohydrates (Mahmood 2010). Amino acids are classified into two categories: essential amino acids, which are not synthesized in the body; and nonessential amino acids, which are synthesized by the body (Table 9.1). Amino acids have great demand and application in view of their importance in the food, feed, personal care, and pharmaceutical industries as nutrients, additives, rejuvenators, and drugs. Their roles are highly prominent and specific in different applications as

***Table 9.1*** List of Essential and Nonessential Amino Acids

| S. no | Essential amino acids | S. no | Nonessential amino acids |
|---|---|---|---|
| 01 | Arginine | 01 | Alanine |
| 02 | Histidine | 02 | Asparagine |
| 03 | Isoleucine | 03 | Aspartate |
| 04 | Leucine | 04 | Cysteine |
| 05 | Lysine | 05 | Glutamate |
| 06 | Methionine | 06 | Glutamine |
| 07 | Phenylalanine | 07 | Glycine |
| 08 | Threonine | 08 | Proline |
| 09 | Tryptophan | 09 | Serine |
| 10 | Valine | 10 | Tyrosine |



well as in therapeutic potential. During the last two decades, the global demand for some essential amino acids, for example, lysine and methionine, has tremendously increased because of their extensive use in the feed, food, and pharmaceutical industries. The addition of these amino acids in animal feed not only optimizes the growth of animals but also improves the quality and quantity of meat. The availability of such animal products has certainly helped a lot in overcoming the deficiency of essential amino acids, which is quite prominent in the primary foodstuffs of underdeveloped and overpopulated areas of the globe. The demand for nonessential amino acids, for example, glutamic acid, has also significantly increased in recent years due to its extensive application in the food industry. The global demand is further expected to increase in the next decades, requiring researchers to focus more on developing advanced manufacturing techniques utilizing biotechnological tools to meet the challenging demand of various amino acids.

The aim of this chapter is to present comprehensive information on some of the abovementioned amino acids, such as lysine, methionine, tryptophan, and glutamic acid (Figure 9.1) with respect to their historical backgrounds, new challenges along with the

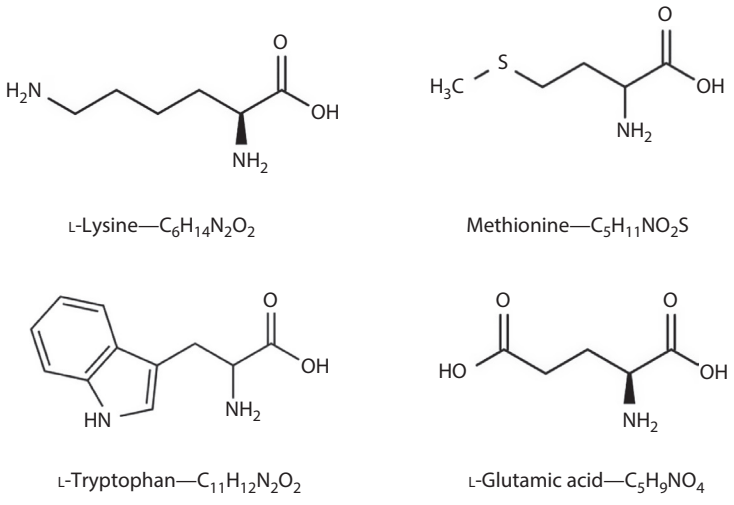


***Figure 9.1*** Structure and molecular formula of lysine, methionine, tryptophan, and glutamic acid.

utilization of biotechnological approaches and tools for increasing the production capability of bacterial strains, their industrial applications, and therapeutic roles.

The utilization of microorganisms for the development of commercial commodities is supposed to have initiated in primordial times. This has resulted in the production of fermented beverages and food stuffs but without the true concept of fermentation. It was Pasteur who introduced the word *fermentation* for the first time and negated the theory of spontaneous generation. Subsequent studies on these lines have been performed by a large number of researchers using bacteria and filamentous fungi. However, the scientific interpretation of fermentation started only when the perception and understanding of microbial physiology and metabolic processes were fully understood (Mahmood 2010).

The biotransformation of organic molecules into useful products provided a new concept in the history of fermentation and, as a result, a number of metabolites such as antibiotics and amino acids were identified. The continued studies on the subject resulted in the discovery of an immense number of pharmaceutical and biochemical products. At the same time, advancements in fermentation engineering provided great support in the production of these useful products on a commercial scale. With advanced knowledge of microbial physiology, molecular biology, and genetic engineering, the fermentation industry has grown considerably in terms of introducing some novel products, which have great industrial and medical applications. These advancements have provided further significant knowledge about these microorganisms, their isolation and modification, and their growth conditions along with the possibility to improve the productivity of the desired product through process optimization or by the addition or subtraction of a particular nutrient.

Initial progress in terms of mutating the strains and utilization of microbial physiology to develop suitable media composition for enhanced yield of desired products was the beginning of modern biotechnology. The evaluation of new biological commodities that could be used as pharmaceuticals, nutraceuticals, or cosmeceuticals has produced phenomenal progress—with industrial microbiology on one side and fermentation engineering on the other. The intensified industrial exploitation of biological practices advocates that biotechnology will be a major growing industry in the near future and that this will alter the lives and prosperity of people all over the world.

Over the last 50 years, the knowledge of microbial physiology and molecular biology has increased substantially in terms of amino acid production and its utilization in human nutrition as well as in domestic animals. The diversified application of amino acids as a raw material in the food, pharmaceutical, and cosmetic industries has played a significant role in boosting the research activities in this particular field. Older methods, such as extraction of amino acids from natural sources or production through chemical synthesis, have largely been replaced by biotechnological processes such as production through fermentation or by enzymatic catalysis. Organized research on the microbial production of amino acids apparently started during the late 1940s, and by the end of the 1950s, a number of amino acids were being produced using microbial sources. The best example is of direct L-glutamic acid fermentation using *Corynebacterium glutamicum* (Figure 9.2) in Japan by Kyowa Hakka Kogyo Company (Kinoshita et al. 1957a).

This progress had a great economic effect in the field of amino acid production and, as a consequence, L-lysine was successfully produced and commercialized using a mutant strain of *C. glutamicum* (Kinoshita et al. 1958). This has resulted in a new concept of fermentative production of amino acids with the introduction of a series of artificially derived auxotrophic or regulatory mutants for the production of other amino acids as well, such as methionine, tryptophan, and others. During the last three decades, a large number of

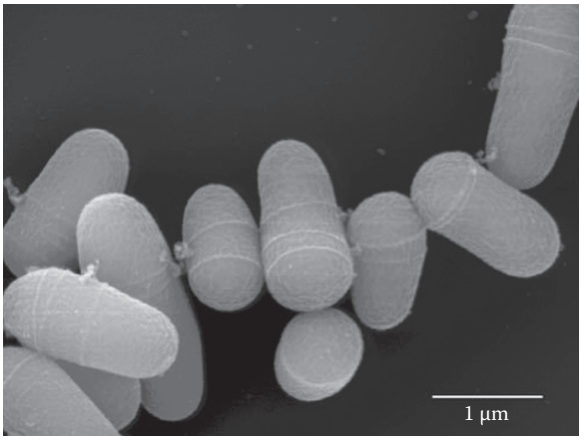


***Figure 9.2*** Electron micrograph of *C. glutamicum* ATCC 13032. (Adapted from Wittmann, C., and Becker, J. The L-lysine story: from metabolic pathways to industrial production. *Microbiology Monographs* DOI 10.1007/7171_2006_089/Published online: February 24, 2007.)



mutant strains of *C. glutamicum* have been developed to produce and meet the market demand for various amino acids. Biotechnology has certainly played a major role in the fermentative production of various amino acids.

With the development of new applications for amino acids in the feed, food, and pharmaceutical industries, enormous improvements have been made in production technology during the latter half of the twentieth century. Submerged fermentation technology plays a key role in this progress, and fermentative production of amino acids currently represent the leading products of biotechnology in terms of both volume and value. The field is very competitive and the cost of production is a major factor, which can certainly support the manufacturers to stay in the market with profitable business. For cost-effective fermentative production, many biotechnological methodologies have been applied to establish high productivity and improvement in product recovery processes. The role of genetic engineering relating to amino acid-producing strains is now being used for the development of biosynthetic and transport capacity of organisms for enhanced production. In addition, the rapid progress in genome analysis is expected to revolutionize microbial strain improvement techniques. With these advancements, the global production and consumption of amino acids is expected to increase more in the near future and thus, will have great effect on amino acid-based industries in terms of investment. Nowadays, the major producers of amino acids are based in Japan, the United States, South Korea, China, and Europe. However, some other Asian countries, such as Indonesia, Malaysia, Thailand, and Vietnam are also entering the amino acid business. The major manufacturing companies include Ajinomoto Group, Archer Daniels Midland Company, Cheil Jedang, COFCO Biochemical (Anhui) Co. Ltd., Daesang Corporation, Evonik, Global Bio-Chem Technology, Novus International Inc., Royal DSM N.V., Sekisui Medical Co. Ltd., Shandong Zhengda Linghua Biotechnology Ltd. Co., Toronto Research Chemicals Inc., Vedan, and VitaLys I/S. The global market for amino acids has been forecast to reach US$11.6 billion by the year 2015. The demand for various amino acids is expected to increase in the production of animal feed, health foods, dietary supplement products, artificial sweeteners, and cosmetics in the subsequent years (Jose 2011; Wippler 2011). The world's consumption pattern in 2011 for lysine, methionine, and tryptophan is shown in Figure 9.3, which is self-explanatory

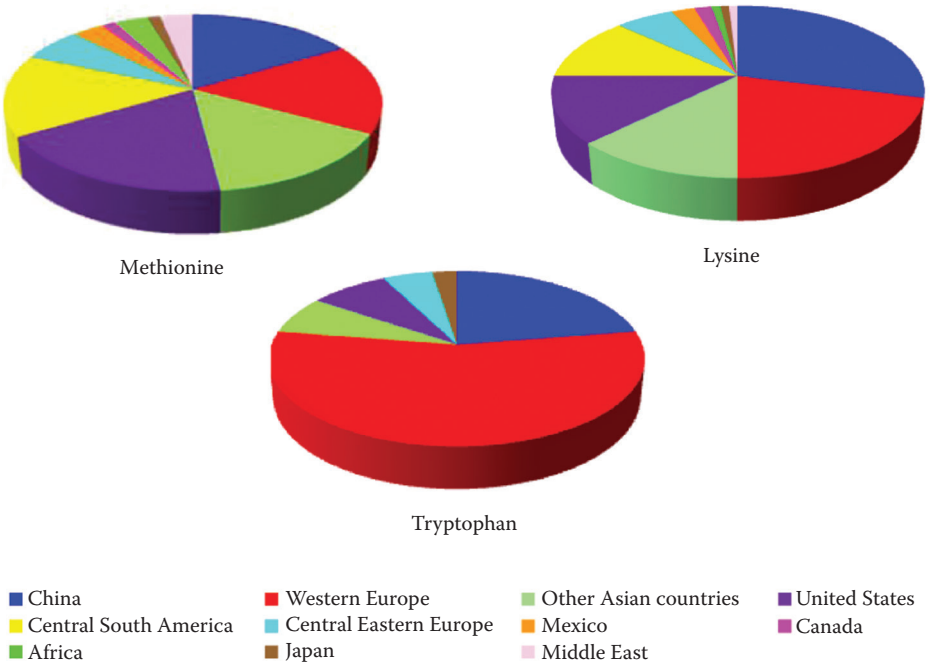


***Figure 9.3*** World's consumption of methionine, lysine, and tryptophan during 2011.

and provides a comprehensive idea about the consumption of these amino acids in different regions of the world (IHS Chemical 2012).

## 9.2 L-Glutamic acid

### 9.2.1 Historical background and new challenges

From an industrial application or commercial point of view, L-glutamic acid is one of the most important amino acids. The carboxylate ions and salts of glutamic acid are known as glutamates. Its initial discovery is quite old and was first reported in 1866 by a German scientist, Karl Heinrich Leopold Ritthausen, in wheat gluten treated with sulfuric acid (Plimmer and Hopkins 1912). In 1907, the first successful commercial production of glutamic acid was performed at Tokyo Imperial University by Kikunae Ikeda, who developed and patented a method to produce crystalline salt of glutamic acid, monosodium glutamate (MSG). Subsequently, MSG was patented by Ajinomoto Corporation of Japan in 1909. The turning point in the history of glutamic acid production was the discovery of *Micrococcus glutamicus* (later identified as *C. glutamicum* in 1957), which produces 30 g/L of L-glutamic acid in glucose medium through fermentation (Kinoshita et al. 1957b). The interest in large-scale L-glutamic acid fermentation was primarily developed due to increased demand for MSG, which was and still is used as a flavor-enhancing agent. Glutamic acid and its salt MSG represent the largest product segment within the amino acid market. In 2007, the world product volume was indicated as 1.6 million tons (Demain 2007); in 2009, the figure reached 2.0 million tons (Sano 2009), and in 2012, the figure was reported to be 2.2 million tons (Abdenacer et al. 2012).

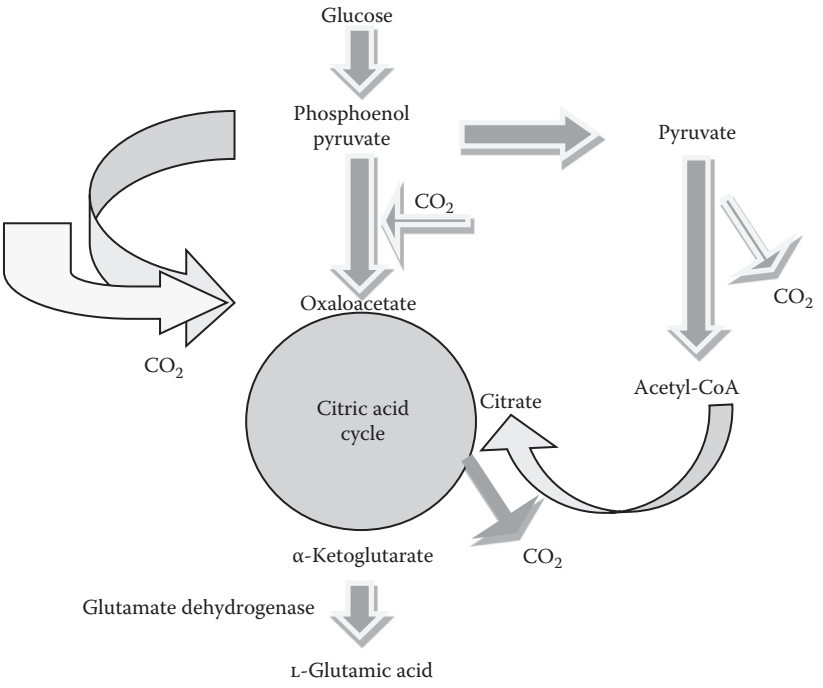


***Figure 9.4*** Biosynthesis of L-glutamic acid in *C. glutamicum* using glucose as a carbon source.

The market demand is continuously increasing and is creating a challenge for the manufacturers, either to increase the capacity of existing plants or to utilize advanced biotechnological tools to increase the production both in terms of strain improvement and modification in fermentation medium. The biosynthesis of L-glutamic acid using *C. glutamicum* involves relatively simple pathways of metabolism. Glucose is mainly metabolized through glycolytic pathways. Pyruvate and phosphoenol pyruvate play important roles in the production of oxaloacetate and then α-ketoglutarate. The α-ketoglutarate is converted to L-glutamic acid in the presence of enzyme glutamate dehydrogenase (Figure 9.4). The phosphorylation stage is quite important because the genes controlling this conversion process are located adjacent to the genes of cell wall synthesis and cell division. Therefore, any disorder in these mechanisms, cell wall synthesis or cell division, might inhibit the activity of α-ketoglutarate dehydrogenase activity. As a result, the conversion of α-ketoglutarate to glutamic acid might be affected (Eggeling and Sahm 2011).

### *9.2.2 Biotechnological approach and production*

A great deal of biotechnological tools have been used by various researchers with an understanding of microbial physiology to modify the strains for increased production of glutamic acid, especially utilizing *C. glutamicum* (Ikeda and Takeno 2013). The progresses, with respect to the capabilities of the different strains of *C. glutamicum* to produce glutamic acid, have been reported from time to time over the last few decades. This was done having comprehensive knowledge of the biology, biosynthetic pathways, mutation, nutritional requirements, and putative mechanosensitive channels associated with this organism. Microbial production of L-glutamic acid has been extensively studied by a large number of

research investigators using different strains of *Coryneform* species and the process with specific strains have been patented. The most popular *Coryneform* species include *C. glutamicum, Corynebacterium lilium, Corynebacterium herculis, Brevibacterium flavum, Brevibacterium lactofermentum, Brevibacterium divarticum, Brevibacterium ammoniagenes, Brevibacterium thiogenetalis, Brevibacterium saccharoliticum,* and *Brevibacterium roseum* (Kinoshita 1999). Other glutamic acid-producing organisms include *Escherichia coli, Bacillus megaterium, Bacillus circulans, Bacillus cereus,* and *Sarcina lutea*. Industrially, glutamic acid is usually manufactured by batch/fed-batch submerged fermentation processes using genetically modified strains of *Corynebacterium* or *Brevibacterium*. Commercial-scale production is carried out in large fermenters equipped with all the facilities to control different parameters and optimize production such as provision of cooling, measurement of dissolved oxygen and pH, and others. A typical example of the submerged fermentation process on a laboratory scale for the production of L-glutamic acid utilizing a mutant strain of *C. glutamicum* has been summarized below.

Organisms are usually maintained on Hottinger slant agar containing beef trypsin digestion, sodium chloride, dipotassium phosphate, and agar at low temperature. After subculturing, a 24-h-old culture is used to inoculate 25 mL of sterile seed medium in 250 mL Erlenmeyer flasks in triplicate. The following seed medium composition can be used: glucose (8%), $NH_4Cl$ (0.5%), corn steep liquor (0.3%), $K_2HPO_4$ (0.5%), $KH_2PO_4$ (0.5%), $MgSO_4{\cdot}7H_2O$ (0.03%), $CaCO_3$ (1.0%), and deionized water to make 100%. The pH of the medium can be adjusted to 7.2 using NaOH. The inoculated flasks are grown in an orbital shaker incubator maintained at 30°C and 230 rpm for 15 h. The entire contents of the one flask is then transferred to a 2.0 L capacity Eyla Mini Jar fermenter with 500 mL of sterile nutrient medium containing molasses (20%), $KH_2PO_4$ (0.5%), $KH_2PO_4$ (0.5%), $MgSO_4{\cdot}7H_2O$ (0.3%), urea (0.8%), $CaCO_3$ (1.0%), and deionized water to make 100%. In most cases, the optimum pH of the medium was recorded as 7.0. The fermentation is usually initiated with continuous agitation and aeration for 48 h at 30°C. At the end of the incubation period, the culture broth is separated from the cells by centrifugation at 10,000 rpm for 5 min at 4°C and the supernatant is diluted 50-fold with 7% (*v/v*) of glacial acetic acid. The diluted sample is then further centrifuged at 10,000 rpm for 5 min at 4°C. The supernatant is then finally filtered through a nylon membrane of 0.22 μm pore size and then collected for further analysis and crystallization (Khan et al. 2013a,b; Pasha et al. 2011).

Since the discovery of the original strain of *C. glutamicum*, a large number of strains, which are capable of producing significant quantities of glutamic acid, have been developed through the process of genetic engineering. Yields have now exceeded 100 g/L and even more with modified strains compared with original wild strains, which produced only 10 g/L. Most original glutamic acid-producing strains of *C. glutamicum* were observed as biotin auxotrophs. Thus, growing such strains in a medium deficient in biotin was found to "trigger" glutamic acid production. The biotin-deficient medium has the property of altering the cell membrane of the organism because of suboptimal fatty acid biosynthesis and is thus responsible for increased production. In view of this hypothesis, it is believed that some other physicochemical factors such as fermentation at higher temperatures, the addition of surfactants (e.g., Tween 40) or antibiotics (e.g., penicillin) in the culture medium can also trigger the excretion of glutamic acid. These factors have been linked to a triggering mechanism resulting in a decrease or repression of the enzyme α-ketoglutarate dehydrogenase. Thus, finally causing redistribution of metabolites at the branch point in the tricarboxylic acid (TCA) cycle leading from α-ketoglutarate to succinyl-CoA or glutamate (Figure 9.4). However, this mechanism has limitations, therefore, a further increase or overproduction of glutamate can be achieved through optimization of the metabolic flux

by tuning the glutamate dehydrogenase activity (Asakura et al. 2007). L-Glutamic acid production was also achieved through immobilized cells of *C. glutamicum*. Using more than 93 g/L, good yields were achieved through batch fermentation but with low productivity (3.8 g/L/h), whereas 73 g/L of glutamic acid was recovered through continuous fermentation with high productivity of approximately 29.1 g/L/h (Amin and Al-Talhi 2007). A number of other studies have also been published, documenting the role of immobilized cells in the production of L-glutamic acid. It has been reported that immobilization of microbial cells in biological processes can occur either as a natural phenomenon or through artificial processes. Although attached cells in their natural habitat exhibit significant growth, the artificially immobilized cells also allow restructure of growth. The authors reported obtaining 48.5 g/L of L-glutamic acid through the immobilized cells of a mutant strain of *C. glutamicum* (Pasha et al. 2011; Prasad et al. 2009). The use of immobilized cells for the production of L-glutamic acid and some other amino acids is progressing rapidly and is expected to have some more valuable and useful data for commercial exploitation in the near future.



### 9.2.3 *Industrial application and therapeutic role*

The greatest application of glutamic acid and its salt is in the food industry as a flavor enhancer. A considerable quantity of free glutamic acid, or its salt MSG, is present in different food products, for example, in different kinds of cheeses and soy sauces. In addition, because glutamate is a key compound in the cellular metabolism, it therefore serves as a unique brain fuel and performs some other important functions such as detoxification of ammonia, as a hepatoprotective agent (glutamine), to aid in peptic ulcer healing, and others (Chaitow 1985; Zareian et al. 2012).

One of the leading roles of glutamic acid in pharmaceuticals is that of a neurotransmitter. L-Glutamic acid is widely distributed in the central nervous system (CNS) and is reported to act as a neurotransmitter. Furthermore, it may have a stimulating effect on the metabolism of the cerebral cortex thus improving mental performance and memory function. The concentration of glutamic acid in the CNS is higher than any other commonly recognized neurotransmitter. In addition, the biochemical and electrophysiological data also suggest that glutamic acid or its analogue acts as an excitatory neurotransmitter of CNS. There is a high concentration of *N*-methyl-D-aspartate (NMDA) receptor in the hippocampus (Bloom 2001). The blockage of NMDA receptors can greatly affect the memory and overall mental performance of an individual. Glutamic acid and aspartic acid have the capability to combine with NMDA receptors thus increasing cation conductance, depolarizing the cell membrane, and deblocking the NMDA receptors. The ultimate results of these biochemical features lead to improvements in memory function and mental performance (McEntee and Crook 1993).

## 9.3 *L-Lysine*

### 9.3.1 *Historical background and new challenges*

L-Lysine is one of the leading and most exploited amino acid among the essential amino acids list. It has vast applications and role in human and animal nutrition. The systematic research to explore the possibility of amino acid production through microorganisms started during the late 1940s, and during the later part of the 1950s, some fruitful results were reported (Mitchell and Houlahan 1948; Windsor 1951). In continuation, a number

of research investigators published their findings that L-lysine can be synthesized from α-aminoadipic acid by yeast and *Neurospora* mold, or from diaminopimelic acid (DAP) by *E. coli* (Davis 1952; Dewey and Work 1952; Mitchell and Houlahan 1948; Windsor 1951). However, microbial production of L-lysine through the decarboxylation of DAP by Chas Pfizer and Company Inc. in the United States was regarded as the first commercial scale production (Casida and Baldwin 1956). A modified process (Figure 9.5) utilizing a single organism, which produced DAP and converted it to L-lysine, was then simultaneously discovered and reported (Kita et al. 1958).

The introduction of L-glutamic acid fermentation using *C. glutamicum* had a great economic effect in the field of L-lysine fermentation. Further research in this direction resulted in the development of an efficient mutant of *C. glutamicum* for the commercial production of L-lysine (Kinoshita et al. 1957b, 1958). This modified method was also successfully used to produce some other amino acids (Mahmood 2010). Despite the commercial challenges, the production of L-lysine using mutant strains brought about a new concept in the fermentative production of amino acids. With the emergence of new biotechnological approaches and tools, more focus was given to improving the strains and taking artificially derived auxotrophic and regulatory mutants that were resistant to feedback inhibition. Using these genetically modified organisms, increased production of L-lysine was achieved by controlling the biosynthetic pathways. The biosynthetic block most conducive for effective L-lysine accumulation in auxotrophic mutants was observed to be those requiring homoserine or threonine plus methionine for the growth of organisms (Figures 9.6 and 9.7).

Modifications in the biosynthetic pathway extended one of the biggest advantages by producing some other amino acids along with L-lysine using *C. glutamicum*, *B. flavum*, and *B. lactofermentum*.



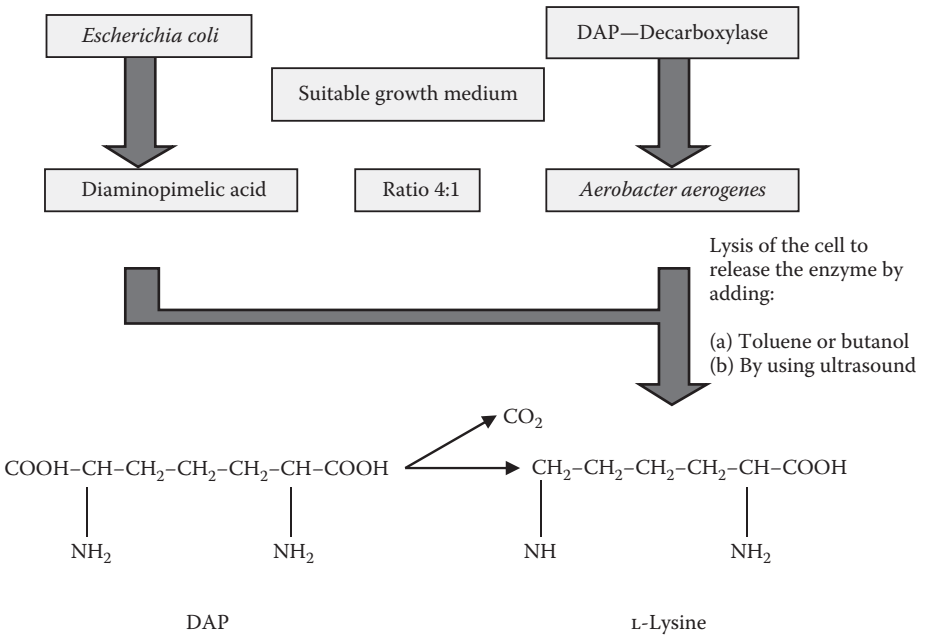


***Figure 9.5*** Production of L-lysine through the conversion of its immediate precursor DAP (two-step process).

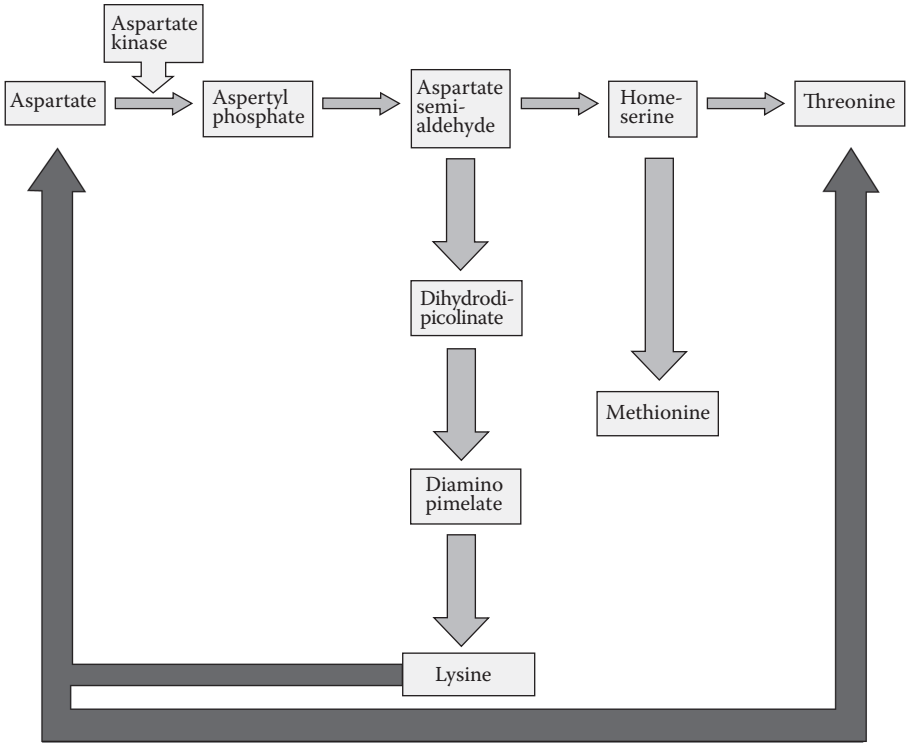


***Figure 9.6*** Regulatory pathways of L-lysine biosynthetic pathways in *C. glutamicum*.

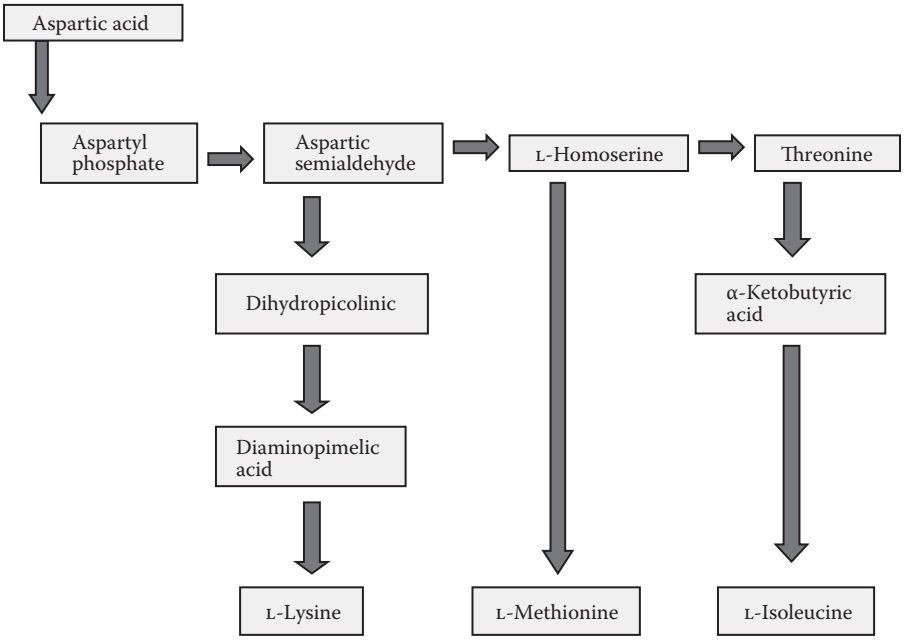


***Figure 9.7*** Production of L-lysine by the auxotrophic mutants (one-step process).

The isolation of auxotrophs can be carried out by using penicillin selection techniques followed by ultraviolet radiation (Adelberg and Myers 1953; Nakayama and Kinoshita 1961). The identification of growth factors (such as vitamins and amino acids) of the auxotrophs can be made auxanographically (Pontecorvo 1949). Further advancements in the production of L-lysine were achieved by introducing analogue-resistant *C. glutamicum* and *B. flavum*. An L-lysine analogue, *S*-(2-aminoethyl)-L-cysteine (AEC), is the best example of developing such analogue-resistant strains. The development of an AEC-resistant strain is primarily based on the treatment of a suitable homoserine auxotroph in a suitably complete culture medium containing meat extract, peptone, yeast extract, sodium chloride, and agar. The medium is supplemented with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine in phosphate buffer. The treatment time is short (~30 min) and at a very low temperature (0°C). This is followed by the treatment of cells with AEC after washing with saline in a minimal medium supplemented with glucose as a carbon source, inorganic salts, and amino acids, for example, L-threonine and DL-methionine. It is now evident that auxotrophy and resistance to feedback inhibition, when genetically combined into a single strain (i.e., mutants resistant to AEC), results in increased production of L-lysine. The overproduction of L-lysine might be due to the starvation of threonine, which decreases the feedback inhibition of aspartate kinase, the first key enzyme in L-lysine biosynthesis (Figure 9.6). The genetic induction of an auxotropic or regulatory mutant that can overproduce L-lysine is of course extremely important. However, at the same time, process optimization (especially the medium composition and the addition of certain growth factors and culture conditions) is also indispensable for the commercial production of L-lysine.

The worldwide increased demand for L-lysine has certainly created a challenging situation for researchers to further improve the existing strains or develop new genetically modified organisms capable of producing exceptional quantities of L-lysine. The major producers of L-lysine in the world are Ajinomoto (with manufacturing facilities in Japan, France, Italy, United States, Brazil, China, and Thailand), Archer Daniels Midlands (United States), Changchun Dacheng (China), Cheil Jedang (with facilities in China, Indonesia, and Brazil), Global Biochem Technology (China), and COFCO Biochemical (China). In terms of tonnage, these major manufacturers along with other companies produced 1,755,300 tons of L-lysine in 2011 and the forecast for 2013 is around 2,376,300 tons, which is expected to reach 2,518,000 tons, corresponding to around US$5.9 billion by the end of 2018. China is the biggest producer and will continue to dominate as the world's largest L-lysine-producing country accounting for approximately 65% of the world lysine production capacity (IHS Chemical 2012; Wippler 2011).

Based on the demand for L-lysine, which is still increasing, meeting the production demand with cost-effective raw materials and recovery processes is quite challenging and will play a major role in its success. In the fermentative production of L-lysine, all raw materials are natural or biologically available substances. No harmful by-products have been found in L-lysine fermentation. On the contrary, useful substances remain in the spent-broth from which many by-products could be recovered. The spent-broth still contains various useful substances including organic and inorganic nitrogen compounds, phosphorus compounds, and potassium salts, which could be used as animal feed additives or fertilizers. A recent process that involves the spray-drying of the whole fermented broth at the end of the incubation period has also been reported to produce feed-grade L-lysine hydrochloride very successfully (Eggeling and Sahm 2011). This will certainly reduce the cost of production of feed-grade L-lysine as well as the disposal of waste material.

### 9.3.2 Biotechnological approach and production

Despite tremendous advancements in the production of L-lysine, researchers are still trying to develop a cost-effective fermentation process using new biotechnological methodologies. Also, biotechnology companies are continuously seeking novel research developments and trying to use multifaceted management models and business approaches toward achieving market leadership in the field of L-lysine and other amino acids production. Along with highly productive strains, the use of cost-effective raw materials and recovery processes has now become highly essential for different manufacturers to compete and stay in the market. Genetically modified strains, which have been introduced for the production of L-lysine, can produce as much as approximately 170 g/L. With the use of metabolic engineering approaches, more advanced and targeted developments can be made to further improve the L-lysine biosynthetic pathway and its secretion. Control over these dynamics will certainly warrant effective precursor delivery and ensure the energy requirement of the cells. It has been indicated that along with the genetic engineering of central metabolic pathways, biosynthetic pathways, and transport systems, energy metabolism and osmoregulation are also gaining interest among researchers as new targets to be engineered for the overproduction of L-lysine. Furthermore, strains other than *C. glutamicum,* such as *Bacillus methanolicus* (methylotropic bacteria) are also gaining interest for the production of L-lysine using methanol and lignocellulose as an alternate raw material (Brautaset and Ellingsen 2011).

With the progress in molecular biology and genetic engineering, some focus has been driven to ensure an optimal carbon and energy flow within the central metabolism (CCM) of bacterial cells to achieve optimized metabolic production. A specific example is that of *C. glutamicum* in which the CCM involves glycolysis, the pentose phosphate pathway, and the TCA along with the anaplerotic and gluconeogenic reactions. It is expected that the in-depth knowledge of CCM along with the specific enzyme activities of the pathways will certainly provide an opportunity to create cell factories ideal for the production of not only L-lysine but also some other industrially important metabolites as well (Papagianni 2012).

In the industrial production of L-lysine, right from the selection of bacterial strains, the media composition, process optimization, and recovery of the end product have a great significance on the overall yield and economics of the process. A balance between carbon and nitrogen sources along with the nutritional requirements of the strain and the addition of certain precursors should be taken into consideration. In addition, a sufficient supply of oxygen to satisfy the cell's oxygen requirement is highly essential for increased yield of L-lysine. On an industrial scale, submerged fermentation is highly effective and is the most widely used method worldwide. The major raw materials such as starch, glucose, molasses, and others used in L-lysine fermentation are quite common and are used in other fermentation industries as well. The most commonly used organism for L-lysine fermentation is *C. glutamicum*. Therefore, to understand its production, a specific example has been mentioned below using a genetically modified strain of *C. glutamicum*.

Organisms are usually maintained at a low temperature, around 6°C to 8°C, on nutrient agar slants on a monthly transfer schedule. A 24-h culture, produced by subculturing from stock culture, is usually used for seed, followed by fermentation. A synthetic or natural medium that has the ability to fulfill the requirements of the particular strain can be used. In commercial-scale starches, molasses and glucose are mostly used as the carbon source. Care must be taken to create a balance between carbon and nitrogen sources such as corn steep liquor, soybean cake acid hydrolysate, yeast extract, peptone, and the like, along with the addition of inorganic salts such as $KH_2PO_4$, $K_2HPO_4$, $MgSO_4{\cdot}7H_2O$,

$FeSO_4{\cdot}7H_2O$, $ZnSO_4{\cdot}7H_2O$, $MnSO_4{\cdot}7H_2O$, $(NH_4)_2\,SO_4$, and others. Other necessary nutrients are biotin and vitamin $B_1$. In some cases, the production of L-lysine can be enhanced by using the leucine fermentation liquor (0.2%–15%) in the production medium. In most cases, the optimum pH of the medium has been recorded as 7.2 and temperature at 30°C. The seed stage cultivation requires around 24 h, whereas the fermentation stage is complete by approximately 96 h. After this, harvesting is done and the product, L-lysine, is recovered using some suitable and economical method. More details on the subject can be obtained through the case study available at the end of the chapter.

### *9.3.3 Industrial application and therapeutic role*

L-Lysine has one of most diversified applications and roles in feed, food, and pharmaceutical industries. It is the most important amino acid for monogastric animals and is regarded as the first limiting amino acid for pigs and the second limiting amino acid for poultry. Therefore, it is an important additive to animal feed for optimizing the growth of pigs and chickens. The metabolic fate of L-lysine is highlighted in Figure 9.8.

In the food industry, L-lysine is used in a number of dietary or nutritional supplements that are popularly used by athletes, weight lifters, bodybuilders, and even some individuals to boost their energy level and protect their muscles from deterioration. Nutritional supplements with a high quantity of L-lysine are available in various dosage forms such as syrups, film-coated tablets, capsules, and powder for instant drinks. L-Lysine is required by the body to synthesize L-carnitine, which is a substance required for the conversion of fatty acids into energy. L-Lysine also helps in calcium absorption and collagen formation, which are important for muscle and bone health. It also supports or acts as a precursor in the synthesis of enzymes, antibodies, and some hormones as well.

Additionally, L-lysine is also recommended for the treatment of some viral infections, for example, herpes simplex, cold sores, shingles, and human papillomavirus infections such as genital warts and genital herpes. It has also been reportedly used in the management of migraines and in some other types of pain and inflammation. Its deficiency may cause severe health problems, growth and development problems, formation of kidney stone, low thyroid hormone production, asthma, and chronic viral infection. Symptoms include nausea, fatigue, dizziness, anemia, loss of appetite and energy, inability to concentrate, irritability, bloodshot eyes, hair loss, growth retardation (especially in children), and reproductive disorders. The reported therapeutic dosage of L-lysine for the maintenance of an antiherpes effect is 500 to 1500 mg, and up to 3000 mg in active stages in a divided dosage with a low-arginine diet. The human body's daily requirement for L-lysine is 103 mg/day for infants, 1600 mg/day for children, 800 mg/day for adult males, and 500 mg/day for adult females (Balch and Balch 1990; Chaitow 1985; Mahmood 1996, 2010).

## *9.4 Methionine*

### *9.4.1 Historical background and new challenges*

The discovery of glutamic acid-producing bacterium, *C. glutamicum*, eventually led to fermentation processes for producing various amino acids. However, during the early phase, not much attention was given to the fermentative production of methionine. There is a severe dearth of scientific publications relating to methionine production by microorganisms. Until 1967, very few references were available on the subject. In this period, a leucine-requiring strain of *Ustilago maydis* was reported to accumulate 6 g/L of methionine on

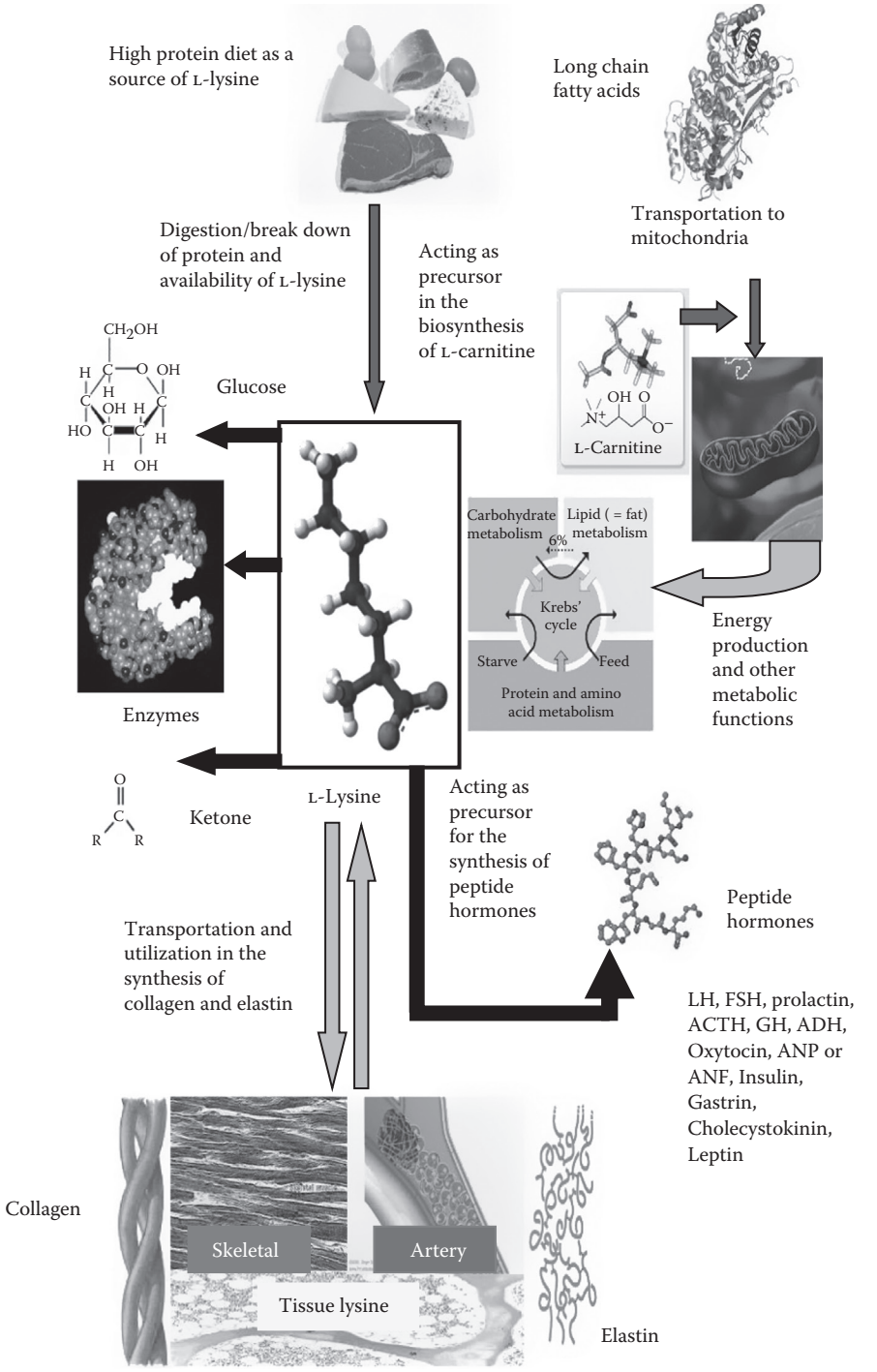


***Figure 9.8*** Metabolic fate: application and role of L-lysine.

a synthetic medium, and a strain of *Pseudomonas* sp. G-132-13 accumulated 13.2 g/L of methionine (Dulaney 1967).

During 1990 to early 2001, a number of constructive contributions were made by various researchers relating to the commercial production of methionine (Odunfa et al. 2001; Pham et al. 1992; Umerie et al. 2000). However, the fermentative process still needs more elaborate studies for its reproducibility and better yield before being implemented on a large scale. Currently, methionine is produced either by chemical synthesis or by enzymatic hydrolysis of proteins. Both processes are quite expensive. Chemical synthesis delivers a mixture of dextro and levo rotatory methionine, whereas enzymatic hydrolysis of proteins produces a complex mixture from which methionine needs to be separated through an expensive recovery process. Methionine isomers produced through chemical reactions can be resolved with the help of fungal enzyme aminoacylase using continuous-flow immobilized enzyme bioreactors (Tosa et al. 1967). Nevertheless, the chemical manufacturing process is still not suitable because of the hazardous nature of chemicals such as acrolein, methyl mercaotan, and ammonia, which are used during different manufacturing stages (Fong et al. 1981).

It is really challenging for the researchers as well as the manufacturers to overcome the problems associated with chemical synthesis. They need to find an optimum solution for these problems and look into the introduction of fermentative production using genetically modified organisms on a commercial scale. In bacteria, like other essential amino acids, methionine is synthesized from oxaloacetate-derived aspartate. Along with L-lysine, methionine is also a dominant amino acid used in animal feed. However, it is almost half of the L-lysine market volume. The global methionine market for animal feed has been reported as 850,000 tons in 2011, and is worth US$2.85 billion (Roquette 2012). Two big companies, Cheil Jedang (South Korea) and Arkema SA (France), are establishing a huge plant in Malaysia to manufacture biomethionine. The operation is expected to start in the latter part of 2013 and will produce 80,000 tons per year (Saidak 2012).

### 9.4.2 *Biotechnological approach and production*

Biotechnological tools have been applied for the production of methionine through auxotrophic, regulatory, or auxotrophic regulatory mutants. Auxotrophic mutants are generally less feasible for methionine (branched pathway amino acids) and therefore, auxotrophic regulatory are most suitable, because methionine itself will not inhibit or repress its own production. Microorganisms such as *Brevibacterium heali* can accumulate higher quantities (up to 25 g/L) of methionine (Mondal et al. 1994a,b). Regulation of methionine biosynthesis in *Corynebacterium* and *Brevibacterium* is shown in Figure 9.9.

Regulatory mutants are also used for producing methionine (Kumar et al. 2003; Kumar and Gomes 2005). Recently, a new approach to screening for methionine-producing bacteria was reported using methionine auxotrophs of *E. coli* (Ozulu et al. 2012), whereas another study relating to the production of L-methionine using *B. cereus* isolated from soil samples was also reported (Dike and Ekwealor 2012). The regulation of methionine biosynthesis in *E. coli* is shown in Figure 9.10.

In *Corynebacterium* and *Brevibacterium*, the biosynthesis mechanism for methionine production is quite simple (Figure 9.9) as compared with the biosynthesis mechanism in *E. coli* (Figure 9.10) in which all enzymes are inhibited or repressed by the end product.

Most researchers reported approximately 10% glucose or 5% maltose as the carbon source supplemented with inorganic salts and different concentrations of biotin and vitamin $B_1$ for the fermentative production of L-methionine (Banik and Majumdar 1975; Kase

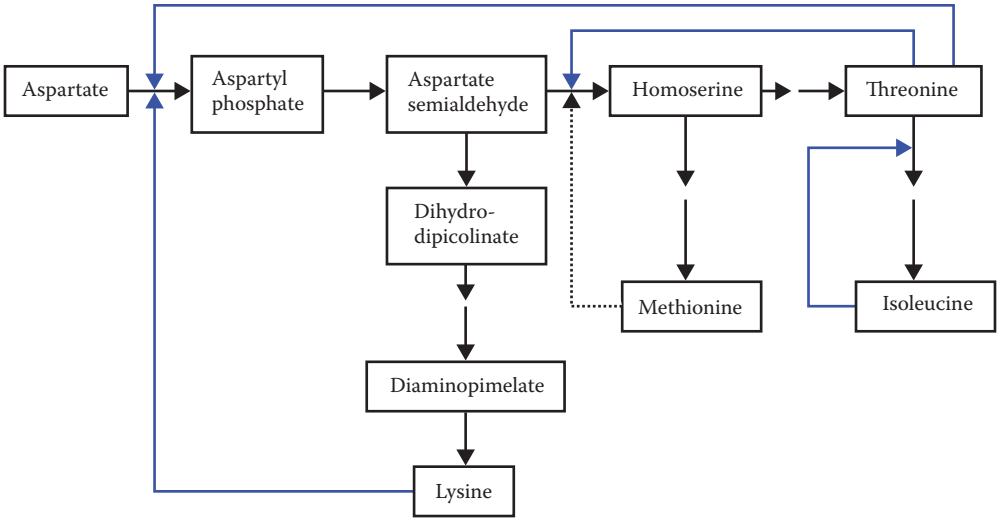


***Figure 9.9*** Regulation of methionine biosynthesis in *C. glutamicum*. The blue arrows show inhibition and dotted arrows represent repression.

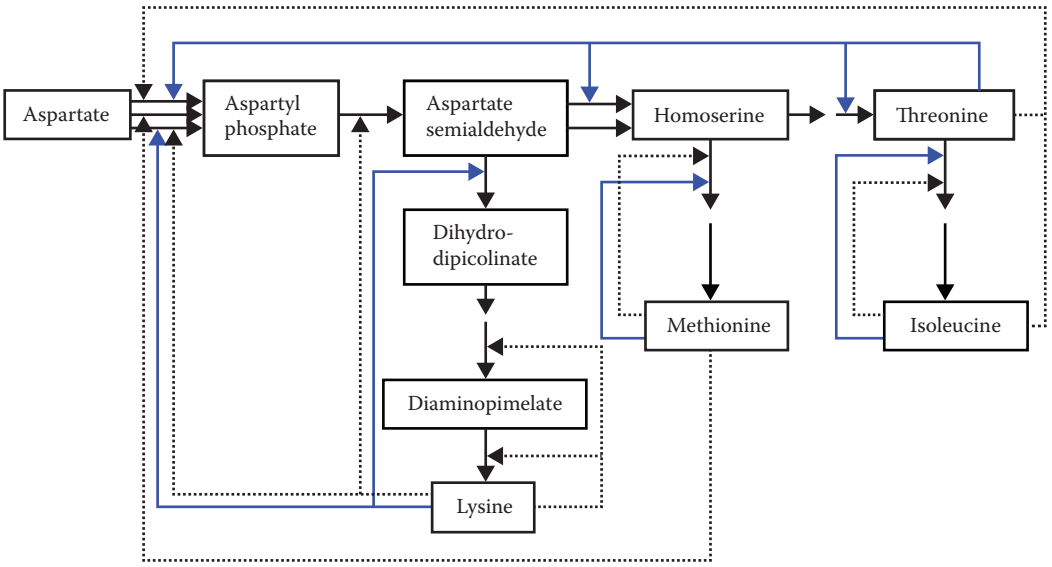


***Figure 9.10*** Regulation of methionine biosynthesis in *E. coli*. The blue arrows show inhibition and dotted arrows show repression.

and Nakayama 1975). Recovery of the product is based on treatment with ion exchange resins. Crystalline methionine can be obtained by concentrating under vacuum, treating with absolute alcohol, and drying at a low temperature of approximately 4°C for 24 h (Kumar and Gomes 2005).

### 9.4.3 Industrial application and therapeutic role

The application of methionine is mostly reported in some pharmaceutical and livestock formulations. Because it is an essential amino acid, it is therefore required in the diets of humans and in livestock. Methionine is an excellent natural lipotrophic agent that processes and eliminates fats from the liver and acts as a natural detoxifying agent, removing heavy metals from the body and excess histamine from the brain. In addition, it has antioxidant properties as well and thus protects the body against free radicals (Chaitow 1985). Plant proteins are frequently deficient in methionine and, consequently, an exclusively vegetarian diet may fail to meet nutritional requirements. Methionine deficiency has been linked to the development of various diseases and complications. These include toxemia, childhood rheumatic fever, muscle paralysis, hair loss, depression, schizophrenia, Parkinson's liver deterioration, and impaired growth (Chaitow 1985). These symptoms appear due to deficiencies that can be overcome by incorporating methionine in the diet (Parcell 2002). Methionine is extensively used in the poultry and livestock industries (Funfstuck et al. 1997; Neuvonen et al. 1985).

## 9.5 Tryptophan

### 9.5.1 Historical background and new challenges

Until 1967, no scientific reports were available relating to the microbial direct production of tryptophan. During this period, more attention was given by researchers looking into the possibility of tryptophan production through the conversion of anthranilic acid, coupling of indole and serine, transamination of 3-indolepyruvic acid, and conversion of β-indolyllactic acid (Dulaney 1967). The chemical method to produce tryptophan was the first method used for industrial-scale manufacturing (Sidransky 2001). This was followed by production through enzymatic reaction and fermentation (Potera 1991). A variety of microorganisms such as *Bacillus subtilis, Pseudomonas aeruginosa,* and others containing tryptophan synthase (TSase; EC 4.2.1.20) or tryptophanase (TPase; EC 4.1.pp.1) can be used for enzymatic reaction. *E. coli* contains both tryptophan synthase and tryptophanase (Austin and Esmond 1965; Hamilton et al. 1985). With the introduction of efficient strains of *Corynebacterium* and *E. coli*, now tryptophan is largely produced by fermentation. The market for tryptophan is not big. The 2012 figure indicates the production of 8,400 tons, and the main companies involved are Ajinomoto, Cheil Jedang, and Evonik. However, China is expected to dominate the market (Dong 2013; Gunderson and Koehler 2012).

### 9.5.2 Biotechnological approach and production

The initial commercial production method used was based on chemical synthesis. However, by the end of 1980, other methods such as production through enzymatic reaction and fermentation were also included in view of the increased demand of tryptophan, especially by the feed and pharmaceutical industries. Thus, continuous attempts are now being made, especially utilizing the biotechnological approach to meet the desired production

demand. Among different options, metabolic engineering for increased L-tryptophan production has been given special attention. The results were encouraging when *E. coli* and *Corynebacterium* were used. The tryptophan biosynthetic pathway in *E. coli* is shown in Figure 9.11.

Overproducing strains of *E. coli* and *C. glutamicum* containing overexpressed genes have been developed, which can produce 40.2 g/L of tryptophan on suitable medium containing glucose as a carbon source in 40 h (Shen et al. 2012). A genetically engineered strain of *E. coli* was also reported to produce approximately 10.15 g/L of L-tryptophan in 48 h (Gu et al. 2012). It is believed that further research using more advanced biotechnological tools will be able to increase the productivity of tryptophan.

To understand the phenomenon of tryptophan fermentation, a brief method has been highlighted, which reflects the use of a genetically modified strain of *C. glutamicum* that is capable of producing tryptophan. Organisms are usually maintained on a monthly transfer schedule on nutrient agar slants supplemented with 0.5% sodium chloride. A seed medium containing glucose (20.0), peptone (5.0), yeast extract (1.0), sodium chloride (0.5), $KH_2PO_4$ (0.5), $K_2HPO_4$ (1.5), $MgSO_4$ (0.5), $NH_4SO_4$ (5.0), and calcium carbonate, (5.0 g/L) can be used. Optimum pH was observed at 6.8. Cultivation can be done in Erlenmeyer flasks of 250 mL capacity containing 30 mL of the above sterilized medium. Inoculation can be done with a loopful of culture grown on nutrient agar slant for 24 h. The flask should be shaken at 200 rpm at 30°C for 24 h in an orbital shaker-incubator to prepare the seed culture.

Fermentation medium may be prepared from molasses (30%), corn-steep liquor (0.7%), $KH_2PO_4$ (0.05%), $K_2HPO_4$ (0.15%), $MgSO_4 \cdot 7H_2O$ (0.025%), $(NH_4)_2SO_4$ (1.5%), and calcium carbonate (1%). A mixture of the following ingredients should be added in grams per liter: vitamin $B_1$ (1000.0), biotin (50.0), L-phenylalanine (200.0), and L-tyrosine (175.0). The pH of the seed and fermentation media remain unchanged, that is, adjusted to 6.8, whereas 1.0 mL of 20% silicon RD in deionized water is added as antifoam. For the laboratory scale, mini jar fermenters of 2 L capacity containing 1 L of the medium can be used. Serialization can be affected by autoclaving for 15 min at 15 psi. After cooling, the fermenter is inoculated

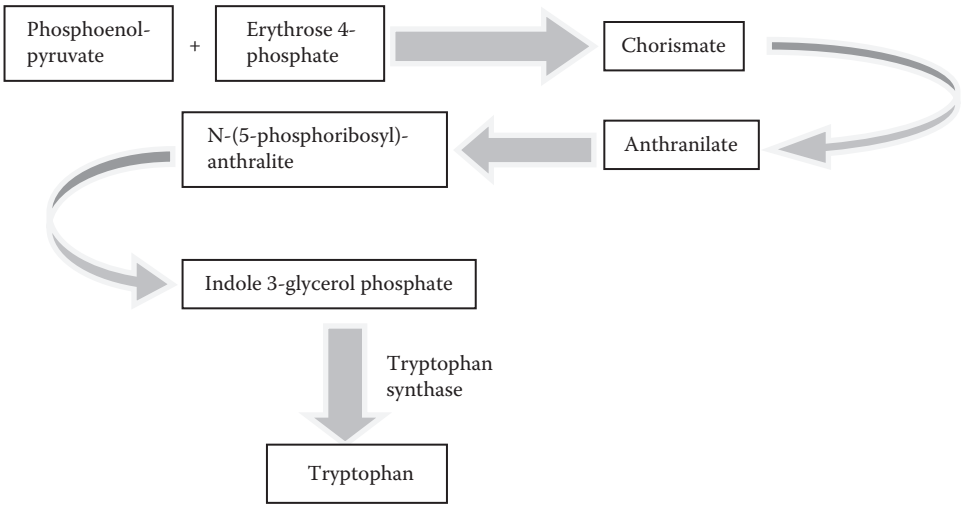


***Figure 9.11*** Tryptophan biosynthetic pathway in *E. coli*.

by one seed flask and stirred at 400 rpm with an air flow of 1.0 vvm (air volume/liquid volume per minute) at 30°C. The fermenters can be harvested after 72 h. Product recovery is usually done using ultracentrifugation at around 10,000 rpm, followed by treatment with cation exchange resin and decolorization with activated carbon. After further centrifugation, the mixture can be subjected to drying under a vacuum dryer. The product yield usually ranges between 10 and 25 g/L.

### *9.5.3 Industrial application and therapeutic role*

Tryptophan has a wide range of applications in the feed and pharmaceutical industries. As an essential amino acid with a unique indole side chain, which indicates its use as a precursor for a number of neurotransmitters in the brain, for example, serotonin, melatonin, and niacin associated with appetite, sleep, mood, and pain perception. Its application in the chemical synthesis of some antidepressant drugs and in the treatment of schizophrenia is quite prominent (Chaitow 1985; Porter et al. 2005; Van der Heijden et al. 2005).



## *9.6 Case study: production of L-lysine on glucose and molasses medium supplemented with fish meal under submerged condition in mini jar fermenter*

The importance of L-lysine has been highlighted by many researchers based on its application in the food, feed, and pharmaceuticals industries. The deficiency of L-lysine can greatly affect the growth and development of bone in children; in adults, L-lysine helps calcium absorption and maintains nitrogen balance. Therefore, it may also help prevent bone loss associated with osteoporosis. Also, it plays a role in enhancing the defense mechanisms of the body, especially acting against cold sores and herpesviruses, and as an aid in antibodies, hormones, enzymes, and collagen formation; its benefits in the repair of tissues also cannot be ignored (Mahmood 2010).

The demand for L-lysine is thus continuously increasing, asking investigators to meet the challenge by increasing the production capacity using high-yield bacterial strains and the best-possible economical source of raw material, especially carbon and nitrogen for its production. This case study is also part of this program. In the current experiment, L-lysine production was evaluated with two different types of media using a homoserine auxotrophic mutant of *C. glutamicum* FRL no. 61989, which is also resistant to AEC. The auxotrophic and regulatory mutants are good options for increased L-lysine production. Isolation of the auxotrophic mutant is based on treatment of the wild strain of *C. glutamicum* with ultraviolet radiation followed by a penicillin selection method, whereas AEC-resistant strains were developed by treating the homoserine auxotrophic mutant with *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, followed by growing the cells in a medium supplemented with AEC. Colonies that appeared on the surface of the agar plate during 2 to 7 days of incubation were picked up as AEC-resistant mutants (Mahmood 1996).

### *9.6.1 Cultivation of* C. glutamicum *FRL no. 61989*

The organism was maintained on a monthly transfer schedule on nutrient agar slants. A seed medium of following composition in grams per liter was used: glucose (30.0),

peptone (10.0), yeast extract (5.0), sodium chloride (3.0), $KH_2PO_4$ (0.5), $K_2HPO_4$ (1.5), $MgSO_4$ (0.5), $NH_4SO_4$ (0.5), sodium acetate (0.1), and 25.5 μg/L of biotin. The pH was adjusted to 7.2. Erlenmeyer flasks of 250 mL capacity containing 30 mL of the sterilized medium were inoculated with a loopful of culture grown on nutrient agar slant for 24 h. The flasks were shaken at 200 rpm at 30°C for 24 h in an orbital shaker-incubator to prepare the seed culture.

Fermentation media I and II with the following composition in grams per liter were used for comparative studies in terms of yield and cost affectivity. Medium I contains glucose (110.0), yeast extract (5.0), peptone (10.0), and meat extract (5.0). Medium II contains molasses (400.0), fish meal (25.0), and corn steep liquor (5.0). Both media were supplemented with the following ingredients in grams per liter: $KH_2PO_4$ (0.5), $K_2HPO_4$ (1.5), $MgSO_4{\cdot}7H_2O$ (0.5), $(NH_4)_2SO_4$ (15.0), calcium carbonate (10.0), $MnSO_4{\cdot}4H_2O$ (0.01), ferrous sulfate $4H_2O$ (0.01), sodium acetate (2.0), and vitamin $B_1$ (0.001). The concentration of biotin was 125.0 and 25.0 μg/L in media I and II, respectively. The pH was adjusted to 7.2 for both media, and 1.0 mL of 20% silicon RD in deionized water was added as antifoam.

One liter of media I and II was transferred separately to 2.0 L capacity mini jar fermenters and serialized by autoclaving for 15 min at 15 psi. The respective fermenters containing media I and II were inoculated with the contents of one seed flask and stirred at 400 rpm with an air flow of 1.0 vvm at 30°C. The fermenters were harvested after 96 h.

### 9.6.2 *Isolation, extraction, and purification of L-lysine*

After the incubation period, the temperature of the fermenters was increased to 60°C for 15 min and the liquid culture was transferred to separate 3.0 L capacity Erlenmeyer flasks. Broths were centrifuged at 10,000 rpm using a high-speed centrifuge for 20 min to separate cells and other precipitated materials, and then subjected to ion exchange method for separation and purification. The supernatants from both fermenters were adjusted to pH 2.0 using 6N HCl and passed through a separate column 30 cm in length and 6 cm in diameter, packed with 250 g of Amberlite IR-120, a strong cation exchange resin. The rate of flow of supernatant through the column was fixed at approximately 100 drops/min to adsorb the L-lysine. After this, 250 mL of deionized water was passed twice through both columns and eluted with 500 mL of dilute ammonia. The whole liquid collected from each column was then passed through Amberlite IRC-50, a weak cation exchange resin. The column size, quantity of resin, and rate of flow of liquid were kept the same. Finally, columns were washed twice with a 250 mL quantity of deionized water and the solution was adjusted to pH 5.5 and treated with 10 g/L of activated carbon for 6 h. The slurry containing carbon and lysine was filtered under vacuum and concentrated to approximately 30% under reduced pressure and left overnight at 10°C to develop crystals of L-lysine. The material was filtered and, after washing with cold deionized water, the crystalline material was dried in a vacuum dryer.

The purity of the material was checked by performing thin-layer chromatography in a mixture of *n*-propanol and strong ammonia solution (67:33), using ninhydrin as a spray reagent as well as by comparing the infrared absorption spectrum with standard L-lysine. The purity was recorded as 98.9% and 98.2%, respectively. An assay for L-lysine was also performed and the endpoint was determined potentiometrically. The average of three experiments produced 67.80 and 65.20 g/L of L-lysine monohydrochloride by glucose and molasses medium with a corresponding yield ($Y_{p/s}$) of 0.6116 $gg^{-1}$ (61.6% conversion efficiency) and 0.592 $gg^{-1}$ (59.2% conversion efficiency), respectively.

### 9.6.3 *Effect of media composition and fermentation parameters on L-lysine production*

A complex microbiological process is observed to be involved in the fermentation of L-lysine, which is greatly influenced by several biochemical and physical parameters. Therefore, in-depth knowledge of bacterial physiology and biochemistry is essential to achieve high yield of the product by choosing the most suitable carbon and nitrogen source along with the fermentation parameters optimization. In the present case study, after several sideline studies, the nutritional requirement and fermentation parameters were established for a strain of *C. glutamicum* FRL no. 61989 and accordingly applied for a final comparative study to observe the production capacity on glucose and molasses medium. The strain FRL no. 61989 was developed by treating a homoserine auxotroph with AEC.

During industrial production of L-lysine, the choice of raw material, especially the carbon and nitrogen source, largely depends on economic consideration. Therefore, it is essential to determine the optimum level of carbon and nitrogen source depending on the nutritional characteristics of the production culture. In the present study, a method that utilizes shake flask experiments with medium containing different concentrations of sugar supplemented with a sufficient quantity of nitrogen source to satisfy the demand imposed by increasing the carbon level was used. An analysis of the amount of L-lysine produced and the amount of sugar remaining after the process provides the data necessary to calculate the percentage yield of product. The sugar concentration, which provides the maximum percentage yield of L-lysine, can be treated as the optimum concentration of sugar to further proceed (Daoust 1976). In addition, the overall method used for the extraction and purification of the product including the fermentation time, yield, and treatment of waste material should also be taken into consideration. Similarly, the optimization of

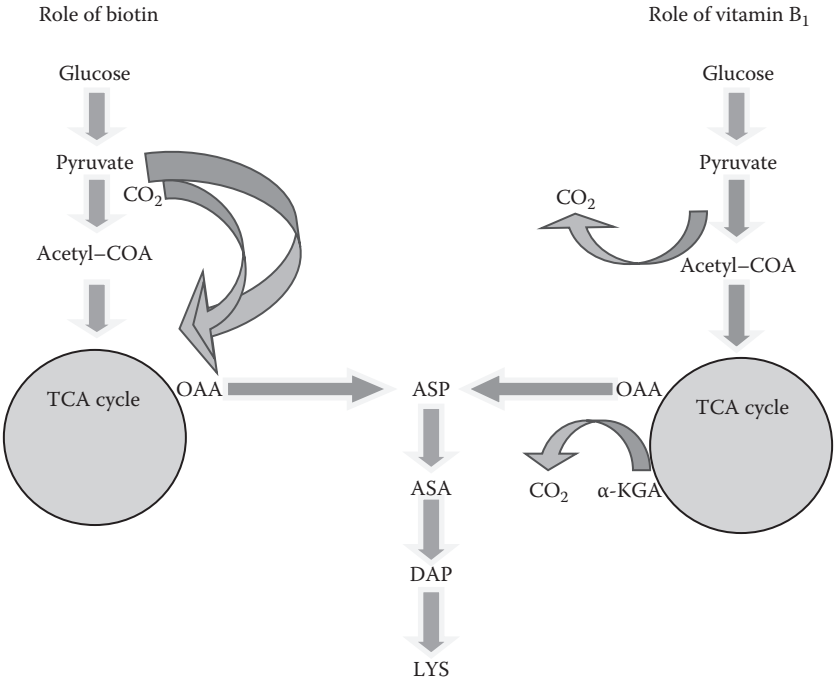


***Figure 9.12*** Effect of biotin and vitamin $B_1$ on L-lysine production.

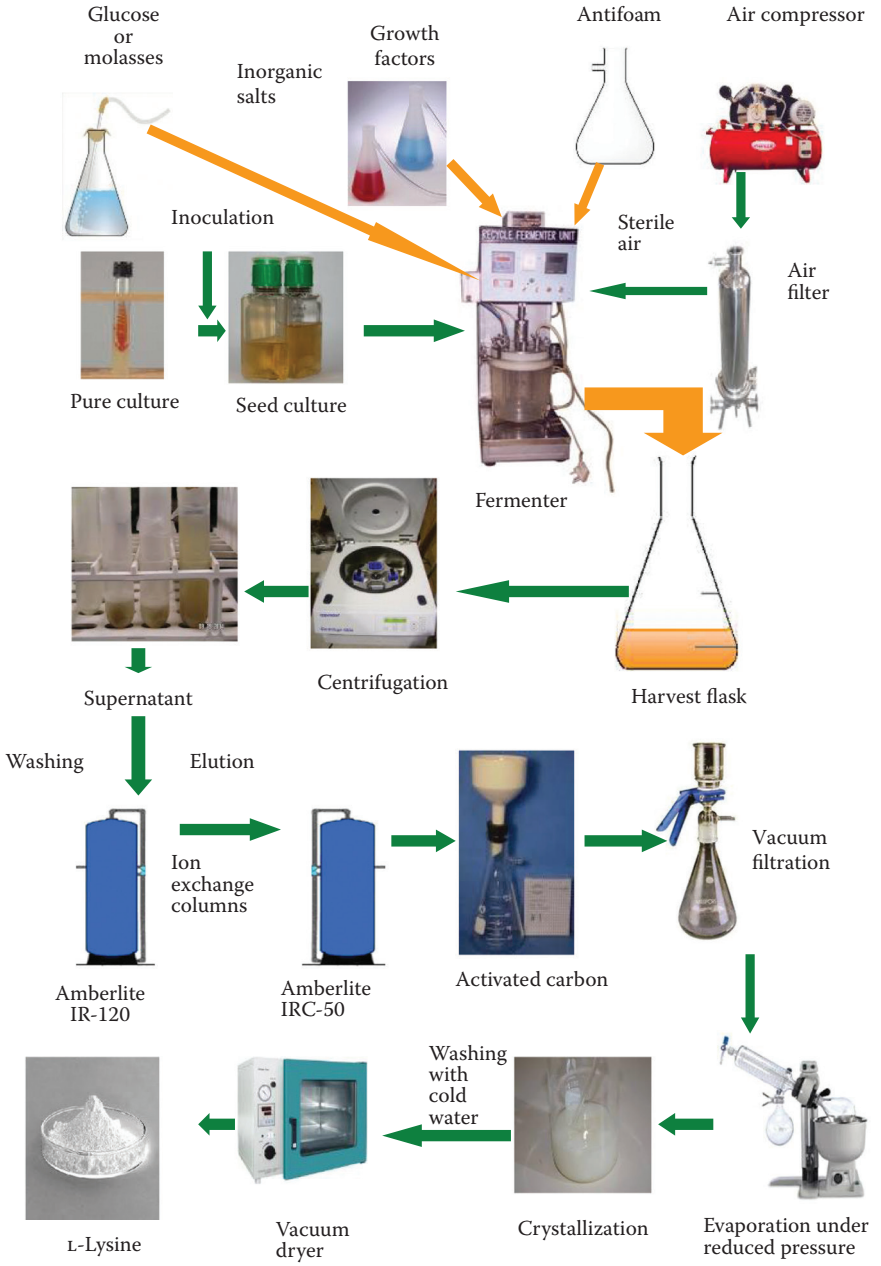


***Figure 9.13*** Laboratory-scale production of L-lysine.

culture conditions in relation to oxygen supply and carbon dioxide removal have played a key role in the scale-up and economic production of L-lysine. These factors determined both the rate of cell growth and product formation.

Because of the present case study, our finding is that the yield of L-lysine is more or less similar on glucose and molasses medium. Therefore, comparing the cost of glucose and molasses, the composition of medium II justified the use of molasses as a carbon source

for the production of L-lysine. Furthermore, because molasses contain a sufficient quantity of biotin, the cost of the product can therefore be further reduced by decreasing the concentration of biotin in the molasses medium. Similarly, the use of fish meal as a nitrogen source was also justified in view of the high cost of soybean protein acid hydrolysate, yeast extract, peptone, and meat extract. Hence, a substantially less expensive L-lysine fermentation can be carried out using such reasonably low-cost materials.

The concentrations of biotin and vitamin $B_1$ were established as 125 µg/L and 1.0 mg/L, respectively. The roles of biotin and vitamin $B_1$ in amino acid fermentation are quite established. Biotin is reported to be involved in the oxidation of glucose and in the synthesis of proteins as well as in cell permeability (Tosaka et al. 1979), and as a carbon dioxide carrier of covalent bonds (Moss and Lane 1971). Furthermore, enzymes containing biotin provide aspartic acids, an important intermediate that leads to the synthesis of L-lysine (Tosaka and Takinami 1978). Vitamin $B_1$ is reported to take part in the oxidative decarboxylation of pyruvate and α-ketoglutaric acid in the bacterial system, leading to increased formation of acetyl-CoA and oxaloacetic acid, thus taking part in the increased formation of L-lysine (Koike and Reed 1960; Mahmood 1996). The effect of biotin and vitamin $B_1$ is highlighted in Figure 9.12. Also, the overall production mechanism of L-lysine on the laboratory-scale is presented in Figure 9.13.

## 9.7 Conclusion

The microbial production of amino acids has gained significant attention in recent years because of its extensive application in food, feed, and pharmaceutical industries. Therefore, evaluation of the topic in view of recent biotechnological advancements is fully justified for increased production to meet the market demand. Some very specific amino acids such as glutamic acid, lysine, tryptophan, and methionine were selected for review in this chapter, and which are of considerable importance because of the role they play in our daily lives. It is expected that the selection of cost-effective raw materials and the utilization of a biotechnological approach will certainly bring a revolution with the introduction of new microbial strains capable of producing increased quantities of the desired amino acids. Also, an in-depth knowledge of submerged fermentation for the production of amino acids will have a significant contribution. With this approach, the chapter has been written to provide comprehensive information on the production, application, and market dimensions covering four important amino acids for both students and researchers interested or working on such topics. It is expected that the information cited will help in analyzing and understanding the dynamics of the topic and to overcome the challenges expected during process design and optimization for amino acid fermentation.



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