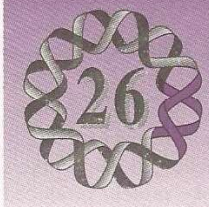
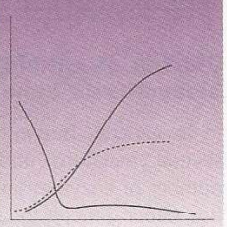


Microbial Production of Amino Acids



The first commercial production of the amino acid, glutamic acids, was started in Japan in as early as 1908. It was Ikeda (of Japan) who first identified that glutamic acid (in the form of monosodium glutamate) possesses taste-enchancing properties. In the early days, monosodium glutamate (MSG) was extracted from the vegetable proteins (wheat and soy). It was only in 1957, the large scale industrial production of MSG by using microorganisms commenced. Today, commercial production of amino acids is one of the biggest industries worldover with an annual increase in the demand by about 10%.

Glutamic acid continues to be ***largest producer*** among the amino acids, followed by lysine, methionine, threonine and aspartic acid.

AMINO ACID PRODUCTION — GENERAL CONSIDERATIONS

Some general considerations on the commercial applications of amino acids, their production methods, and the development of strains of microorganisms for improved amino acid production are briefly described.

COMMERCIAL APPLICATIONS OF AMINO ACIDS

Amino acids have a wide range of applications. The proportionate use of amino acids is given in the next column.

Food industry — 65%
Feed additives — 30%
Pharmaceutical — 5%

The individual L-amino acids (except glycine) along with their production methods and uses are given in ***Table 26.1***. The applications are broadly discussed hereunder.

Food industry

Amino acids are used either alone or in combination, as flavour enhancers. ***Monosodium glutamate is the most frequently*** used in food industry. Glycine and alanine also enhance taste and flavour. Tryptophan, in association with histidine, acts as an antioxidant to preserve milk powder. For the preservation of fruit juices, cysteine serves as an antioxidant.

Aspartame, a dipeptide (aspartyl-phenylalanine methyl ester) produced by a ***combination of aspartic acid and phenylalanine***, is about 200 times sweeter than sucrose. It is used as a low-calorie artificial sweetener in soft-drink industry.

There are certain essential amino acids that are deficient or limiting in plant proteins. These include lysine, methionine, threonine and tryptophan. Addition of the deficient amino acid(s) improves the nutritional quality of human foods as well as animal feeds. Thus, bread enriched with lysine, soy products supplemented with methionine are of better nutritional value. Methionine added soy

bean meal is a better feed for pigs and other animals.

Pharmaceutical industry

The amino acids can be used as medicines. Essential amino acids are useful as ingradients of infusion fluids, for administration to patients in post-operative treatment.

Chemical industry

Amino acids serve as starting materials for producing several compounds. Glycine is used as a precursor for the synthesis of ***glyphosate*** (a herbicide), while threonine is the starting material for the production of azthreonam (another herbicide). Poly-methylglutamate is utilised for manufacturing synthetic leather. Some amino acids in the form of N-acyl derivatives are useful for the preparation of cosmotics.

METHODS FOR PRODUCTION OF AMINO ACIDS

The industrial production of amino acids is carried out by one or more of the following three processes.

1. **Extraction :** Amino acids are the building blocks in protein structure. The proteins can be subjected to hydrolysis, and the requisite amino acids can be isolated e.g. cysteine, tyrosine, leucine.

2. **Chemical synthesis :** Chemical synthesis results in a mixture of D- and L-amino acids. Most of the amino acids required for commercial

Table 26.1 Amino acids along with their production methods and applications

*Amino acid**	*Preferred production method*	*Application(s)*
Glutamic acid	Fermentation	Flavour enhancer
Lysine	Fermentation	Feed additive, infusion solution
Methionine	Chemical synthesis	Feed additive
Threonine	Fermentation	Feed additive
Phenylalanine	Fermentation	Aspartame production
Aspartate	Enzymatic synthesis, extraction	Aspartame production
Glycine	Chemical synthesis	Sweetener, food additive
Arginine	Fermentation, extraction	Infusions, therapy for liver diseases, cosmotics
Valine	Fermentation, extraction	Infusions, pesticides
Tryptophan	Extraction, immobilized cells	Infusions, antioxidant
Isoleucine	Extraction, fermentation	Infusions
Alanine	Extraction, enzymatic	Flavour enhancer
Leucine	Extraction, enzymatic	Infusions
Proline	Extraction	Infusions
Serine	Extraction, chemical synthesis	Cosmotics
Histidine	Extraction, fermentation	Therapy for ulcers
Asparagine	Extraction, chemical synthesis	Diuretic
Glutamine	Fermentation	Therapy for ulcers
Cysteine	Extraction	Infusion
Tyrosine	Extraction, enzymatic	Infusion
Ornithine	Extraction, enzymatic	Therapy for liver diseases

* *The order of amino acids is approximately in the decreasing order of their annual production; Extraction refers to the extraction of the specific amino acid from protein hydrolysates.*

applications are of L-category. However, for the synthesis of glycine (optically inactive) and some other amino acids which can be used in L- or D-form (D, L-alanine, D, L-methionine) for certain purposes, chemical methods are employed.

3. **Microbiological production : *For the large-scale production of amino acids***, microbiological methods are employed. There are three different approaches.

(a) **Direct fermentation methods :** Amino acids can be produced by microorganisms by utilising several carbon sources e.g. glucose, fructose, alkanes, ethanol, glycerol, propionate. Certain industrial byproducts like molasses and starch hydrolysate can also be used. Methanol, being a cheap carbon source, is tried for amino acid production, but with limited success.

(b) **Conversion of metabolic intermediates into amino acids :** In this approach, the microorganisms are used to carry out selected reactions for amino acid production e.g. conversion of glycine to serine.

(c) **Direct use of microbial enzymes or immobilized cells :** Sometimes resting cells, immobilized cells, crude cell extracts or enzyme-membrane reactors can be used for the production of amino acids. Some examples are given below.

Amino acid dehydrogenases from certain bacteria (e.g. *Bacillus megaterium*) can be used for the amination of α-keto acids to produce L-amino acids e.g. alanine (from pyruvate), leucine (from α-ketoisocaproic acid) and phenylalanine (from phenylpyruvate). Immobilized cells or enzyme-membrane reactors can be used.

Enzymes or immobilised cells are also employed for the production of several other amino acids e.g. tryptophan, tyrosine, lysine, valine.

STRAIN DEVELOPMENT FOR AMINO ACID PRODUCTION

The metabolic pathways, for the synthesis of amino acids by microorganisms, are tightly controlled and they operate in an economical way. Therefore, a natural overproduction of amino acids is a rare occurrence. Some strains that excrete certain amino acids have been isolated e.g. glutamic acid, alanine, valine.

In order to achieve an overproduction of any amino acid by a microorganism, methods have to be devised for the elimination of the metabolic regulatory/control processes. In fact, several amino acid-producing ***microorganisms have been developed by mutagenesis and screening programmes.***

The following are the major ways of strain development. In fact, several methods are combined to successfully develop a new strain for producing amino acids.

Auxotrophic mutation : These mutants are characterized by a lack of the formation of regulatory end product (i.e. repressor or regulatory effector). The intermediates of the metabolic pathways accumulate and get excreted.

Genetic recombination : Mutants can be developed by genetic recombination for over-production of amino acids. Protoplast fusion in certain bacteria is used for development of hybrids e.g. *Corynebacterium glutamicum* and *Bacillus flavum.*

Recombinant DNA technology : The classical techniques of genetic engineering can be used for strain development. Strains with increasing activities of rate-limiting enzymes have been developed. In one of the techniques, *E. coli* and cloning vector pBR322 were used to increase the genes for the production of amino acids e.g. glutamic acid, lysine, phenylalanine, valine.

Functional genomics : a new approach

Analysis of genomes from the wild and mutant strains of microorganisms will help in creating improved strains. Once the entire sequence of the chromosomes in the organisms (e.g. *C. glutamicum, E. coli*) is established, efforts can be made to carry out genetic manipulations for efficient overproduction of desired amino acids. ***Chip technology*** can be used to detect new mutations and consequently the fermentation processes.

L-GLUTAMIC ACID

L-Glutamic acid was the ***first amino acid to be produced by microorganisms.*** The original

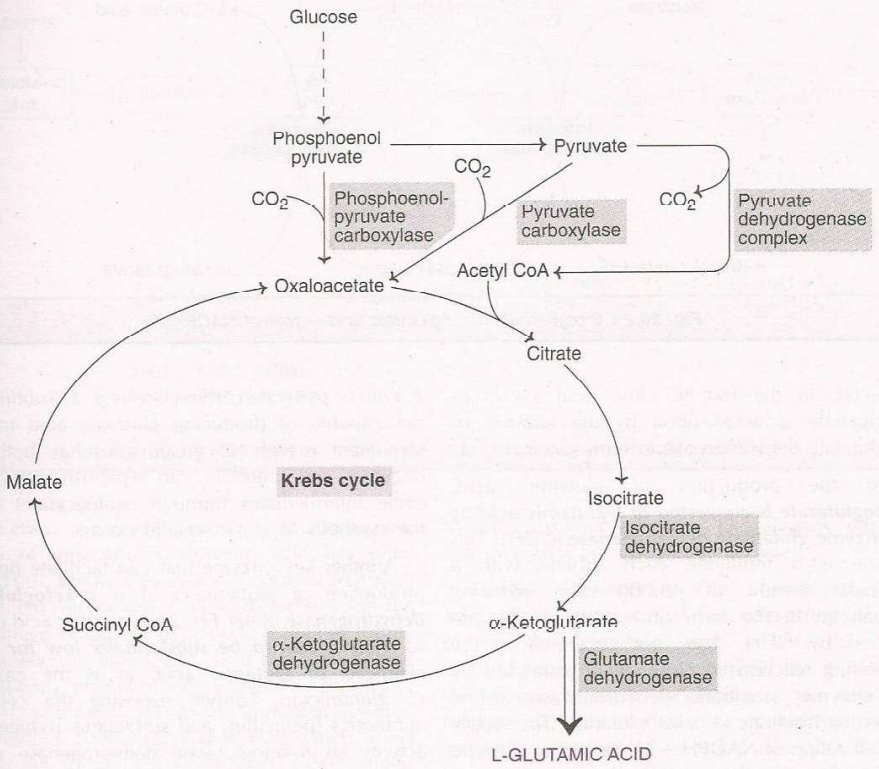


Fig. 26.1 : Biosynthesis of L-glutamic acid in Corynebacterium glutamicum.

bacterium, ***Corynebacterium glutamicum***, that was first used for large scale manufacture of glutamic acid continues to be successfully used even today. The other important organisms (although used to a lesser extent due to low yield) employed for glutamic acid production belong to genera *Microbacterium*, *Brevibacterium* and *Arthrobacter*. All these organisms have certain morphological and physiological characters comparable to *C. glutamicum*. Biochemically, ***glutamic acid-producing bacteria have a high activity of glutamate dehydrogenase and a low activity of α-ketoglutarate dehydrogenase***. They also require the vitamin biotin.

Improved production strains

Several improvements have been made, particularly in *C. glutamicum*, for improving the strains to produce and excrete more and more of glutamic acid. These include the strains that can tolerate high concentrations of biotin, and lysozyme-sensitive mutants with high yield.

Biosynthesis of L-glutamic acid

The pathway for the synthesis of glutamic acid with glucose as the carbon source is depicted in ***Fig. 26.1***. Glucose is broken down to phosphoenol pyruvate and then to pyruvate. Pyruvate is converted to acetyl CoA. Phosphoenol pyruvate (by the enzyme phosphoenol pyruvate carboxylase) can be independently converted to oxaloacetate. Both these carboxylation reactions are quite critical, and require biotin as the cofactor.

The next series of reactions that follow are the familiar citric acid (Krebs) cycle reactions wherein the key metabolite namely α-ketoglutarate is

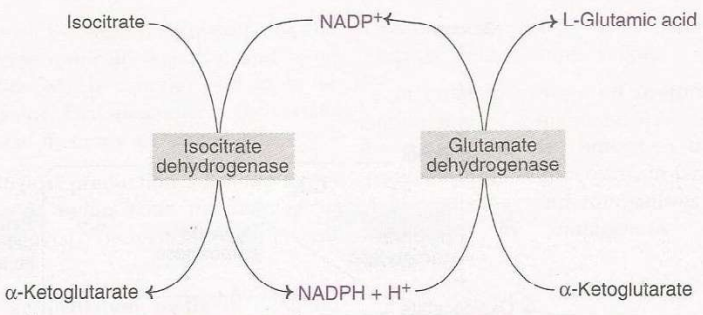


Fig. 26.2 : Biosynthesis of L. glutamic acid — role of NADP+.

produced. In the routine citric acid cycle, α-ketoglutarate is acted upon by the enzyme α-ketoglutarate dehydrogenase to form succinyl CoA.

For the production of glutamic acid, ***α-ketoglutarate is converted to L-glutamic acid*** by the enzyme ***glutamate dehydrogenase*** (GDH). This enzyme is a multimer, each subunit with a molecular weight of 49,000. The reducing equivalents, in the form of NADPH + H^+, are required by GDH. They are generated in the preceeding reaction of Krebs cycle (catalysed by the enzyme isocitrate dehydrogenase) while converting isocitrate to α-ketoglutarate. The supply and utilization of NADPH + H^+ occurs in a cyclic fashion through the participation of the two enzymes, namely isocitrate dehydrogenase and glutamate dehydrogenase (***Fig. 26.2***).

Theoretically, one molecule of glutamic acid can be formed from one molecule of glucose. In practice, the conversion efficiency of glucose to glutamic acid was found to be around 70%.

Regulation of glutamic acid biosynthesis

The essential requirement for glutamic acid production is the high capability for the supply of the citric acid cycle metabolites. This is made possible by an efficient conversion of phosphoenol pyruvate as well as pyruvate to oxaloacetate (Refer ***Fig. 26.1***). Thus, there are two enzymes (phosphoenol pyruvate carboxylase and pyruvate carboxylase) to efficiently produce oxaloacetate, while there is only one enzyme (pyruvate dehydrogenase) for the formation of acetyl CoA. Certain microorganisms which have either phosphoenol pyruvate carboxylase (e.g., *E. coli*) or pyruvate carboxylase (e.g. *B. subtilis*) are not capable of producing glutamic acid to any significant extent. *C. glutamicum* has both the enzymes and therefore can replenish citric acid cycle intermediates (through oxaloacetate) while the synthesis of glutamic acid occurs.

Another key enzyme that can facilitate optimal production of glutamic acid is ***α-ketoglutarate dehydrogenase*** (Refer ***Fig. 26.1***) of citric acid cycle. Its ***activity has to be substantially low for good synthesis of glutamic acid***, as is the case in *C. glutamicum*. Further, exposing the cells to antibiotics (penicillin) and surfactants reduces the activity of α-ketoglutarate dehydrogenase while glutamate dehydrogenase activity remains unaltered. By this way, oxidation of α-ketoglutarate via citric acid cycle can be minimised, while the formation of glutamic acid is made maximum possible.

Release of glutamic acid

Glutamic acid is ***synthesized intracellularly***, and therefore ***its release or export is*** equally important. It now appears that there is a ***carrier-mediated energy-dependent active process*** involved for the export of glutamic acid. There are several ways of increasing the membrane permeability for exporting glutamic acid.

- Biotin limitation
- Addition of saturated fatty acids
- Addition of penicillin
- Use of oleic acid auxotrophs
- Use of glycerol auxotrophs
- Addition of local anesthetics
- Addition of surfactants (Tween 40).

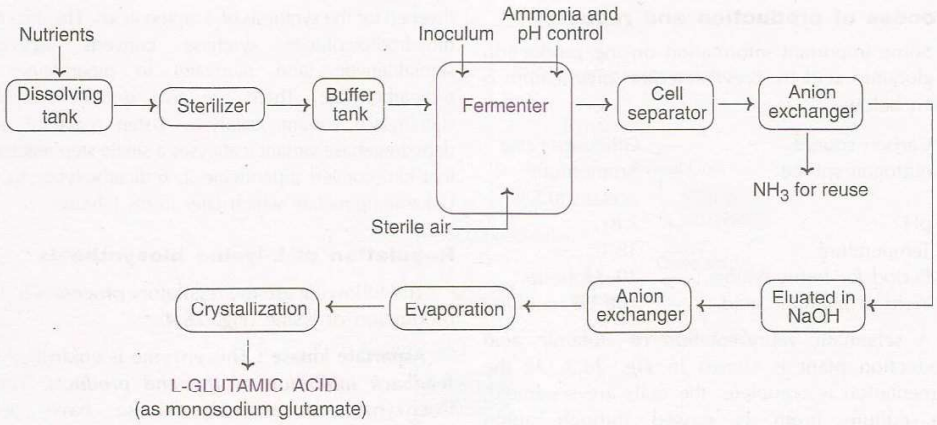


Fig. 26.3 : Diagrammatic representation of glutamic acid production plant.

The effect of biotin deficiency in facilitating the release of intracellular glutamic acid has been worked out. Biotin is an essential cofactor (required by the enzyme acetyl CoA carboxylase) for the biosynthesis of fatty acids. Due to a limited supply or deficiency of biotin, fatty acid biosynthesis and consequently phospholipid synthesis is drastically reduced. As a result, membrane formation (protein-phospholipid complex) is defective which alters permeability for an increased export of intracellular glutamic acid.

It is found that there is an alteration in the membrane composition of phospholipids in oleic acid and glycerol auxotroph mutants. This facilitates release of intracellular glutamic acid.

The knowledge on the membrane permeability of glutamic acid is successfully exploited for increased industrial production of glutamic acid.

Production of glutamic acid-requirements and influencing factors

The industrial production of glutamic acid is influenced by carbon sources, nitrogen sources, growth factors, pH and O_2 supply. The relevant aspects are briefly described.

Carbon sources : Either ***refined*** (glucose, sucrose, fructose, maltose) ***or unrefined*** (sugar beet molasses, sugar cane molasses) ***carbon sources*** are used. In countries like Japan, acetate (inexpensive) is utilized. Other substrates like alkanes, ethanol and methanol are less frequently used.

Nitrogen sources : The concentration of ***ammonia is very crucial for converting carbon source to glutamic acid.*** However, high concentration of ammonia inhibits the growth of the organisms. In the beginning of fermentation, ammonium salts and a low concentration of ammonia are added. During the course of fermentation, ammonia in aqueous solution is continuously fed. In this way, pH can be controlled, besides continuous supply of nitrogen source. Sometimes, urea is also used as a nitrogen source, since glutamic acid-producing bacteria possess urease that can split urea and release ammonia.

Growth factors : *Biotin* is an important growth factor and its concentration in the medium is influenced by the carbon source. For instance, a supply 5 µg of biotin per liter medium is recommended if the carbon source is 10% glucose, while for acetate as the carbon source, the biotin requirement is much lower (0.1-1.0 µg/l). Addition of L-cysteine in the medium is recommended for certain strains.

Supply of O_2 : O_2 supply should be adequately and continuously maintained. It is observed that a high O_2 concentration inhibits growth of the organisms while a low O_2 supply leads to the production of lactic acid and succinic acid. In both instances, glutamic acid formation is low.

Process of production and recovery

Some important information on the production of glutamic acid by *Brevibacterium divaricatum* is given below.

Carbon source	—	Glucose (12%)
Nitrogen source	—	Ammonium acetate (0.5%)
pH	—	7.8
Temperature	—	38°C
Period for fermentation	—	30–35 hours
Yield of glutamic acid	—	100 g/l medium.

A schematic representation of glutamic acid production plant is shown in ***Fig. 26.3***. As the fermentation is complete, the cells are separated, the culture broth is passed through anion exchanger. The glutamic acid bound to the resins is eluted in NaOH, while the ammonia released can be reused. With NaOH, glutamic acid forms monosodium glutamate (MSG) which can be purified by passing through anion exchanger. MSG can be subjected to evaporation and crystallization.

L-LYSINE

Lysine is present at a low concentration in most of the plant proteins. Being an essential amino acid, supplementation of plant foods with lysine increases their nutritional quality.

L-Lysine is predominantly produced by ***Corynebacterium glutamicum*** and to some extent by *Brevibacterium flavum* or *B. lactofermentum*.

Biosynthesis of L-lysine

The pathway for the synthesis of L-lysine is complex, and an outline of it is depicted in ***Fig. 26.4***. This metabolic pathway is also involved in the formation of 3 other amino acids, namely methionine, threonine and isoleucine.

As the glucose gets oxidised by glycolysis, phosphoenol pyruvate and pyruvate are formed. Both these metabolites can be converted to oxaloacetate, a key component of citric acid cycle. On transamination, oxaloacetate forms aspartate. The enzyme aspartate kinase converts aspartate to aspartyl phosphate which later forms aspartate semialdehyde. Aspartate semialdehyde has two fates—the biosynthesis of lysine and formation of 3 other amino acids (methionine, threonine and isoleucine). When homoserine dehydrogenase acts on aspartate semialdehyde, it is diverted for the synthesis of 3 amino acids. The enzyme dihydrodipicolinate synthase converts aspartate semialdehyde (and pyruvate) to piperideine 2, 6-dicarboxylate. There are two distinct enzymes succinylase variant (catalyses 4-step reaction) and dehydrogenase variant (catalyses a single step reaction) that can convert piperideine 2, 6-dicarboxylate to D, L-diaminopimelate which later forms L-lysine.

Regulation of L-lysine biosynthesis

The following are the regulatory processes in the production of lysine (***Fig. 26.4***).

Aspartate kinase : This enzyme is controlled by ***feedback inhibition of the end products***. Three isoenzymes of aspartate kinase have been identified-one repressed by L-methionine, the second one repressed by L-threonine and L-isoleucine, and the third one being inhibited and repressed by L-lysine. The amino acid sequence and structure of aspartate kinase have been elucidated. And by genetic manipulations, it has been possible to create mutants (of aspartate kinase) that are insensitive to feedback regulation by L-lysine.

Dihydrodipicolinate synthase : This enzyme competes with homoserine dehydrogenase to act on aspartate semialdehyde. Overexpression of dihydrodipicolinate synthase has been shown to increase the production of L-lysine.

Succinylase and dehydrogenase variants : The conversion of piperideine 2, 6-dicarboxylate to D, L-diaminopimelate is carried out by these two enzymes. At the start of the fermentation, dehydrogenase variant predominantly acts, and later succinylase variant comes into picture for the biosynthesis of L-lysine.

Role of D, L-diaminopimelate : This amino acid, an immediate precursor for the synthesis of L-lysine, is also required for the synthesis of a tripeptide (L-Ala-γ-D-Glu-D, L-Dap) which is part of the peptidoglycan of cell wall. The activities of both the enzymes (succinylase and dehydrogenase) that form diaminopimelate (Dap) are important for the production of L-lysine and for the proper formation of cell wall structure.

Improved production strains

Based on the biosynthetic pathway and the regulatory steps (discussed above), certain improvements have been made in the strains of

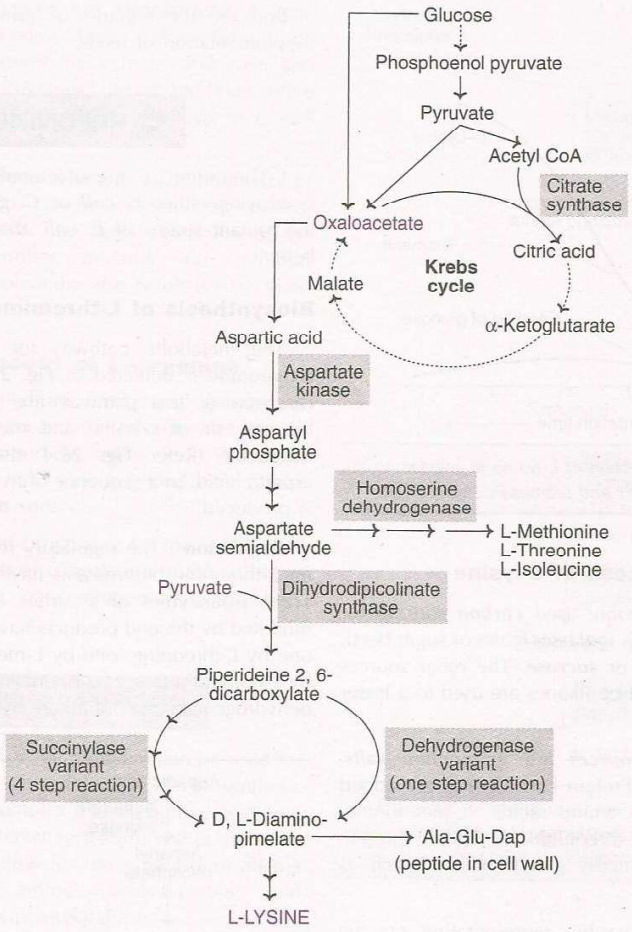


Fig. 26.4 : *Biosynthesis of L-lysine in C. glutamicum*
(Ala-Glu-Dap — Alanyl-glutamyl-diaminopimelate, a tripeptide)

C. glutamicum and *B. flavum* for overproduction of lysine.

- Mutant organisms resistant to lysine antimetabolites (e.g. b-aminoethyl-L-lysine).
- A mutant strain with an altered enzyme aspartokinase, so that it is not regulated by end product inhibition.
- A strain with a decreased homoserine dehydrogenase activity (so that diversion for the synthesis of methionine, threonine and isoleucine is minimised).
- A strain with reduced citrate synthase activity (to lower the occurrence of citric acid cycle).

Release of L-lysine

The export or release of L-lysine from the cells into the surrounding medium occurs through a ***lysine-export (LysE)*** carrier protein. It is a trans-membrane protein (mol. wt-25,400) with six segments that participate in lysine transport. The exporter system is very efficient active process to export large quantities of intracellular lysine.

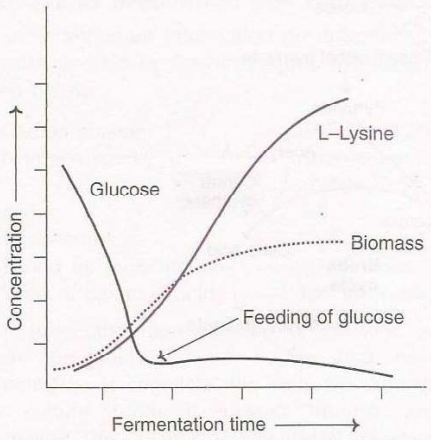


Fig. 26.5 : Production of L-lysine in relation to substrate (glucose) and biomass concentration.

Production process of L-lysine

The most commonly used ***carbon sources*** for lysine manufacture is ***molasses*** (cane or sugar beet), starch hydrolysates or ***sucrose***. The other sources like acetate, ethanol or alkanes are used to a lesser extent.

The ***nitrogen sources*** are ***ammonium salts***, gaseous ***ammonia***. Protein hydrolysates are added to supply certain amino acids (L-methionine, L-homoserine, L-threonine). The protein hydrolysates also supply growth factors such as biotin.

A time-course graphic representation for the formation of lysine is depicted in ***Fig. 26.5***. As is evident, a continuous supply of glucose (or other sugar) is required for sustained production of lysine. Under optimal fermentation conditions, the yield of lysine (in the form of L-lysine HCl) is 40–50 g per 100 g carbon source.

There are different recovery processes for lysine depending on its application.

- An alkaline solution containing about 50% L-lysine can be obtained after biomass separation, evaporation and filtration.
- A crystalline preparation with 98–99% L-lysine (as L-lysine HCl) can be obtained by subjecting the culture broth to ion-exchange chromatography, evaporation and crystallization.

Both the above grades of lysine are suitable for supplementation of feeds.

L-THREONINE

L-Threonine is manufactured industrially by employing either ***E. coli*** or ***C. glutamicum***. With the mutant strains of *E. coli*, the product yield is better.

Biosynthesis of L-threonine

The metabolic pathway for the synthesis of L-threonine is depicted in ***Fig. 26.6***. Some of the reactions of this pathway are common for the biosynthesis of L-lysine and methionine, besides isoleucine (Refer ***Fig. 26.4*** also). Starting with aspartic acid, in a sequence of five steps, threonine is produced.

Regulation : The regulatory reactions in *E. coli* for L-threonine biosynthesis have been elucidated. Three isoenzymes of aspartate kinase, separately inhibited by the end products have been identified-one by L-threonine, one by L-methionine and one by L-lysine. Further, two isoenzymes of homoserine dehydrogenase-one inhibited by L-threonine and

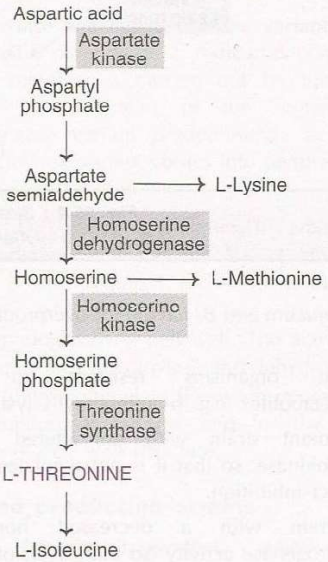


Fig. 26.6 : Biosynthesis of L-threonine in E. coli.

other by L-methionine are also known. A gene *thrABC* that encodes three polypeptides (one polypeptide possesses the activity of kinase and homoserine dehydrogenase, the second homoserine kinase and the third threonine synthase) in *E. coli* has been identified.

Improved production strains : The efficiency of the producer strains can be increased by creating *E. coli* mutants with high-level expression of the gene *thrABC*. Further, mutants with minimal production of L-isoleucine also result is high yield of L-threonine.

Production process of L-threonine

The culture medium containing glucose or sucrose, yeast extract and ammonium salts is adequate for L-threonine production. The sugar feeding has to be continued for good yield (about 60% of the carbon source).

The downstream processing for the isolation of L-threonine consists of coagulation of the cell mass (by heat), filtration, concentration by evaporation, and crystallization.

L-PHENYLALANINE

Both ***E. coli*** and ***C. glutamicum*** can be used for the production of L-phenylalanine. The biosynthetic pathway is quite complex and an outline is shown in ***Fig. 26.7***. An interesting feature is that the same pathway is responsible for the synthesis of all the three aromatic amino acids-tyrosine and tryptophan, besides phenylalanine.

The synthetic pathway commences with the condensation of erythrose 4-phosphate with phosphoenol pyruvate to form deoxy-arabinoheptulosonate phosphate (DAHP). DAHP in the next series of reactions is converted to chorismate which can form L-tryptophan. Chorismate mutase converts chorismate to prephenate which forms L-phenylalanine through the participation of prephenate dehydrogenase. Prephenate also serves as a precursor for the synthesis of tyrosine.

The genes responsible for the formation of the regulatory enzymes of L-phenylalanine have been identified. By employing genetic manipulations, strains for improved production of L-phenylalanine have been developed.

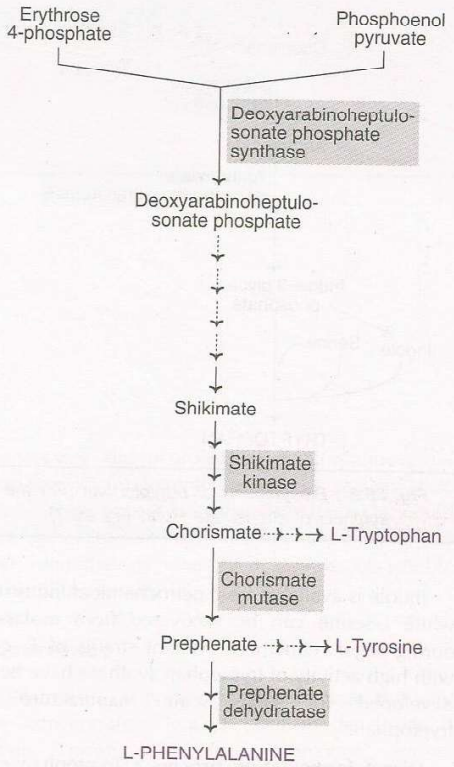
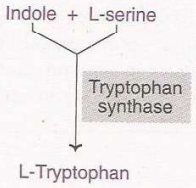


Fig. 26.7 : An outline of the pathway for the synthesis of L-phenylalanine, L-tyrosine and L-tryptophan.

L-TRYPTOPHAN

There are different ways of synthesizing L-tryptophan-chemical, enzymatic and fermentation methods. At present, large scale manufacture of tryptophan is carried out by using the enzyme ***tryptophan synthase*** of ***E. coli***. Tryptophan synthase combines indole with L-serine to form tryptophan.



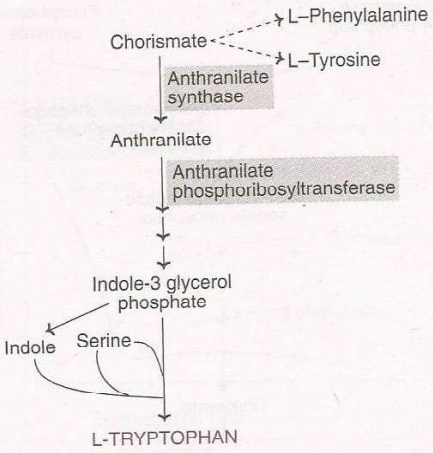


Fig. 26.8 : Biosynthesis of L-tryptophan (For the syntheis of chorismate Refer Fig. 26.7).

Indole is available from petrochemical industries while L-serine can be recovered from molasses during sugar refinement. Mutant strains of *E. coli* with high activity of tryptophan synthase have been developed for large scale manufacture of tryptophan.

Direct fermentation process : Tryptophan can also be produced by fermentation employing *C. glutamicum*, or *E. coli*. For the biosynthetic pathway, refer ***Fig. 26.7***. Mutant strains of both these organisms have been developed for increased yield of tryptophan.

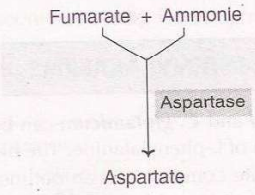
Mutant strains for overproduction L-tryptophan

The production of tryptophan by *C. glutamicum* was increased by introducing a second gene encoding ***anthranilate synthase***, a key enzyme in its biosynthesis (***Fig. 26.8***). Further, genes encoding other important enzymes (deoxyarabino-heptulosonate phosphate synthase, anthranilate phosphoribosyltransferase) were also be modified. The result is that the pathway becomes insensitive to feedback inhibition by end products, leading to an overproduction of L-tryptophan.

L-ASPARTIC ACID

There is a growing demand for aspartate, as it is a ***component of aspartame*** (an artificial sweetener), besides its use as a food additive, and in pharmaceutical preparations.

The preferred method for aspartate production is enzymatic in nature. The enzyme aspartase converts fumarate and ammonia to aspartate. Although this reaction is reversible, aspartate formation is favoured.



The aspartase of *E. coli* is used. It is a tetramer with a molecular weight 196,000. This enzyme is quite unstable. Immobilization of aspartase in polyacrylamide or carrageenan that enhances the stability of the enzyme is commonly used.

Immobilized *E. coli* cells with good activity of aspartase are also used for aspartate production.