L-Lysine Production

-Dr. Ekta Khare
Department of Microbiology
Institute of Biosciences & Biotechnology,
CSJM University, Kanpur

L-Lysine

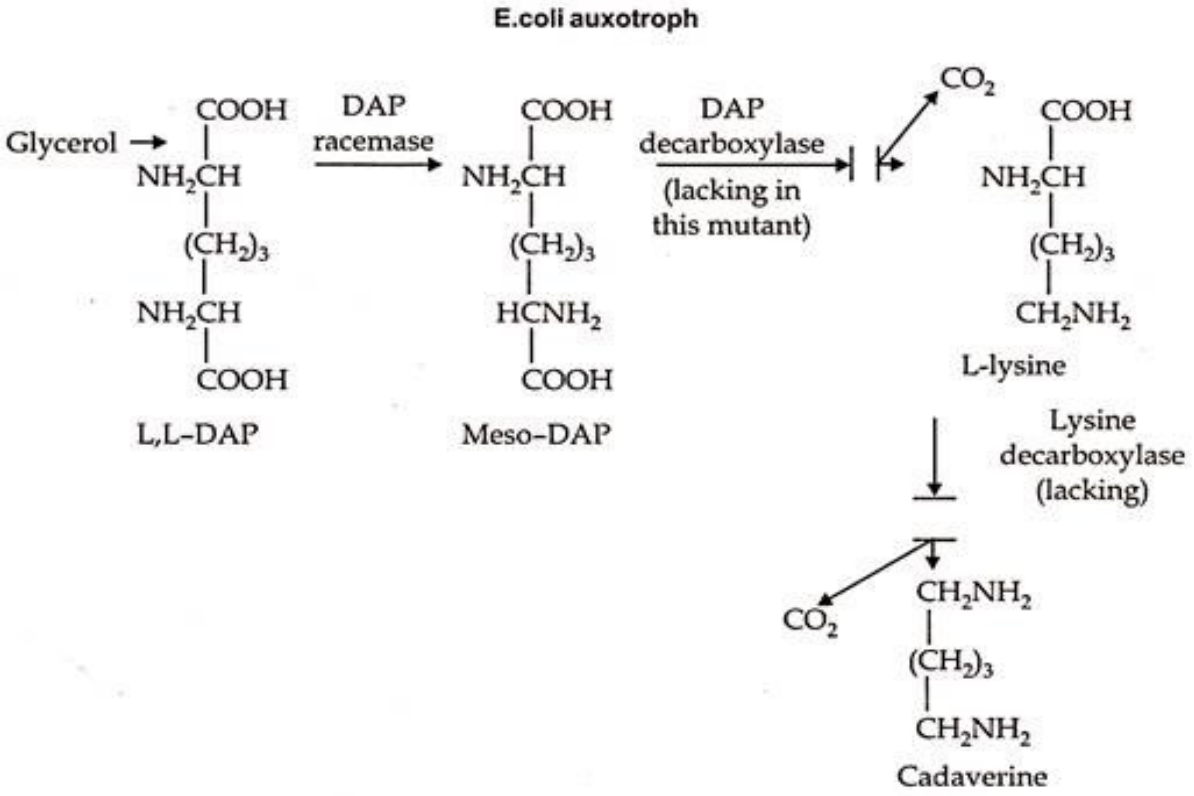
- L-Lysine is an essential amino acid as it is not synthesized by humans and hence has to be obtained from the diet.
- Cereals and vegetables also have low lysine content and are often fortified with lysine supplements.
- It is also produced for use as animal feed.
- Microorganisms are capable to synthesizing several amino acids, some of them in excess of their need.
- These amino acids are excreted out of the cells and this fact can be exploited in the industrial production of amino acids.
- The microbial fermentation leads to the formation of L-isomer of the amino acids, which is biologically active, whereas the chemical synthesis produces a mixture of L and D forms, which need to be separated by expensive process.
- Total world production of L-lysine is around 35,000 metric tons per year.
- **Industrially it is produced by two different fermentation methods:**

  (a) Indirect fermentation

  (b) Direct fermentation.

Indirect or Dual Fermentation

- Auxotrophic mutant of *Escherichia coli* is used in the first half of the fermentation and wild type or prototrophic *E. coli* or *Aerobacter aerogenes* is employed in the second half of the fermentation lacking Lysine decarboxylase activity.



Position of metabolic block in the L-lysine metabolic pathway of an *E.coli* auxotroph which accumulates diaminopimelic acid (DAPA) during growth on glycerol

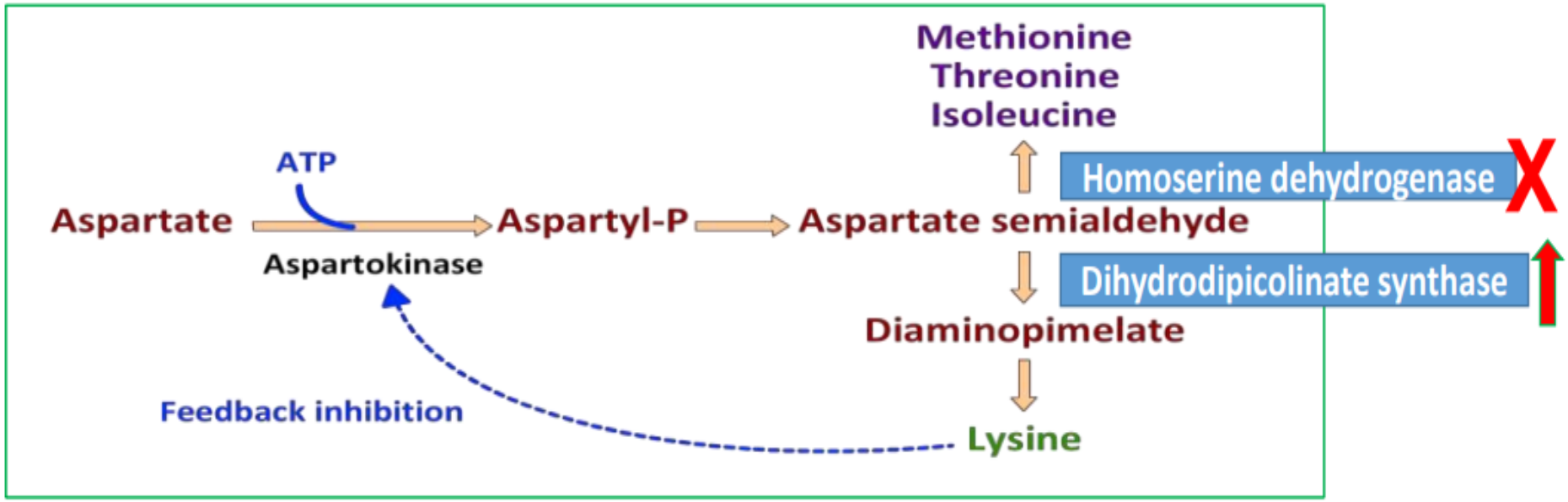
Fermentation conditions

- In *E. coli* auxotroph, DAP decarboxylase mediated metabolic block occurred, which accumulates DAP during growth on glycerol and releases considerable quantity in the culture broth.
- Because of the metabolic block, this organism requires small amount of L-lysine for growth and accumulates DAP in media.
- The *Aerobacter aerogenes* auxotrophic cell, also used in this fermentation, employ a similar pathway except that the DAP decarboxylase is both present and active and through mutation, lack lysine decarboxylase.
- Firstly, *E. coli* is grown in molasses based medium containing glycerol, corn-steep liquor, and $(NH_4)_2HPO_4$ to produce diaminopimelic acid (DAP).
- Apart from glycerol, ethanol or alkanes supplemented with soybean hydrolysates can also be used as carbon sources.
- The temperature is maintained at 28ºC.
- After three days of fermentation under optimized conditions of temperature and pH in aerated stirred tank rectors, the entire fermentation broth is then incubated with *Aerobacter aerogenes* at 35ºC, which decarboxylates the DAP to L-lysine.

Direct Fermentation

- Another industrial method utilizes one step single strain approach.
- For this, *Micrococcus glutamicus* or *Corynebacterium glutamicum* or *Brevibacterium* are used
- Production of L-lysine is controlled at the level of the enzyme aspartokinase, homoserine dehydrogenase and dihydrodipicolinate synthase.
- The auxotroph requires L-homoserine and biotin or a mixture of L-threonine along with L-methionine and biotin for growth, and produces greater than 20 g/L of L-lysine in fermentation broth.
- L-lysine is not destroyed by the organism during the fermentation, because organism lacks the ability to produce enzyme named L-lysine decarboxylase.
- In this method Aspartate is used as the precursor.
- In the biochemical pathway leading from aspartate to lysine; lysine can feedback-inhibit activity of the enzyme aspartokinase causing cessation of lysine production.

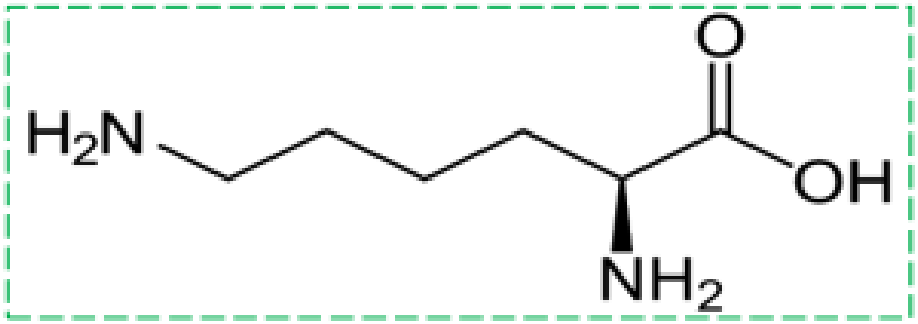
Control of L-lysine production in *Corynebacterium glutamicum*

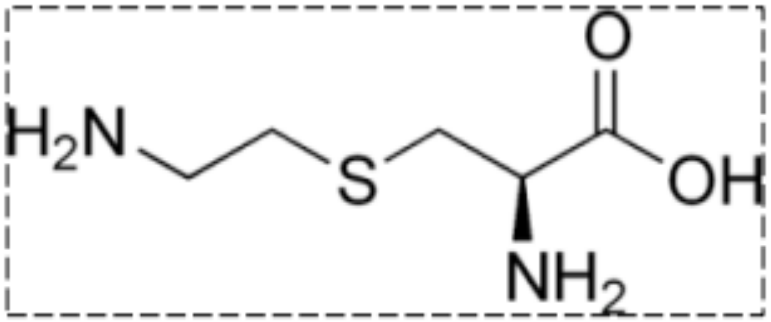


C. glutamicum genetically engineered mutant

- To achieve higher production values, mutants of *C. glutamicum* are used in which aspartokinase enzyme is no longer subject to feedback inhibition.
- Homoserine dehydrogenase enzyme is under metabolic block by S-aminoethyl cysteine (AEC) and over expression of Dihydrodipicolinate synthase enzyme.
- Such AEC-resistant genetically engineered mutants have been generated and by repeated culturing in a medium containing lysine analog S-aminoethyl cysteine (AEC), is identical to lysine in structure, except that a sulfur atom replaces a methyl (-CH2) group.
- AEC binds to the allosteric site of aspartokinase (wild type) and competitively inhibits homoserine dehydrogenase activity of the enzyme.
- However, AEC-resistant genetically engineered mutants synthesize a modified form of aspartokinase, whose allosteric site no longer recognizes AEC or lysine but block homoserine dehydrogenase.
- In such mutants, feedback inhibition of this enzyme by lysine is nearly eliminated and over produce L-lysine.
- A typical AEC-resistant mutant of *C. glutamicum* can produce over 60 g of lysine per litre in industrial fermenters.

Lysine





S-aminoethyl cysteine

Batch Fermentation

- Industrial production is carried out as batch process in aerated stirred tank reactors.
- A variety of carbon sources such as sugarcane molasses, acetate, ethanol with soybean hydrolysates can be used.
- Gaseous ammonia or ammonium salts are used as nitrogen source and urea along with other growth factors such as L-homoserine or L-threonine and L-methionine.
- The addition of ammonia and urea also helps in maintaining a neutral pH around 7.
- It is critical to maintain biotin content in the medium >30 μg/L for optimal lysine production and lower concentrations result in the accumulation of L-glutamate instead of L-lysine.
- To shorten the lag phase during fermentation larger ~ 10% inoculum is used.
- Lysine production starts in the early log phase of growth and through the stationary phase and a maximum of production 40-45 g/L lysine is achieved in 60h.

Recovery

- Lysine is recovered by acidification of the cell free fermentation broth to pH 2.0 with hydrochloric acid.
- This acidified mixture is then passed through cation exchange column where L-lysine gets adsorbed in the ammonium form.
- Bound L-lysine is then eluted from column with dilute ammonia solution.
- The eluate is re-acidified and crystallized as L-lysine hydrochloride.