RAMA UNIVERSITY

www.ramauniversity.ac.in

# FACULTY OF ENGINEERING & TECHNOLOGY

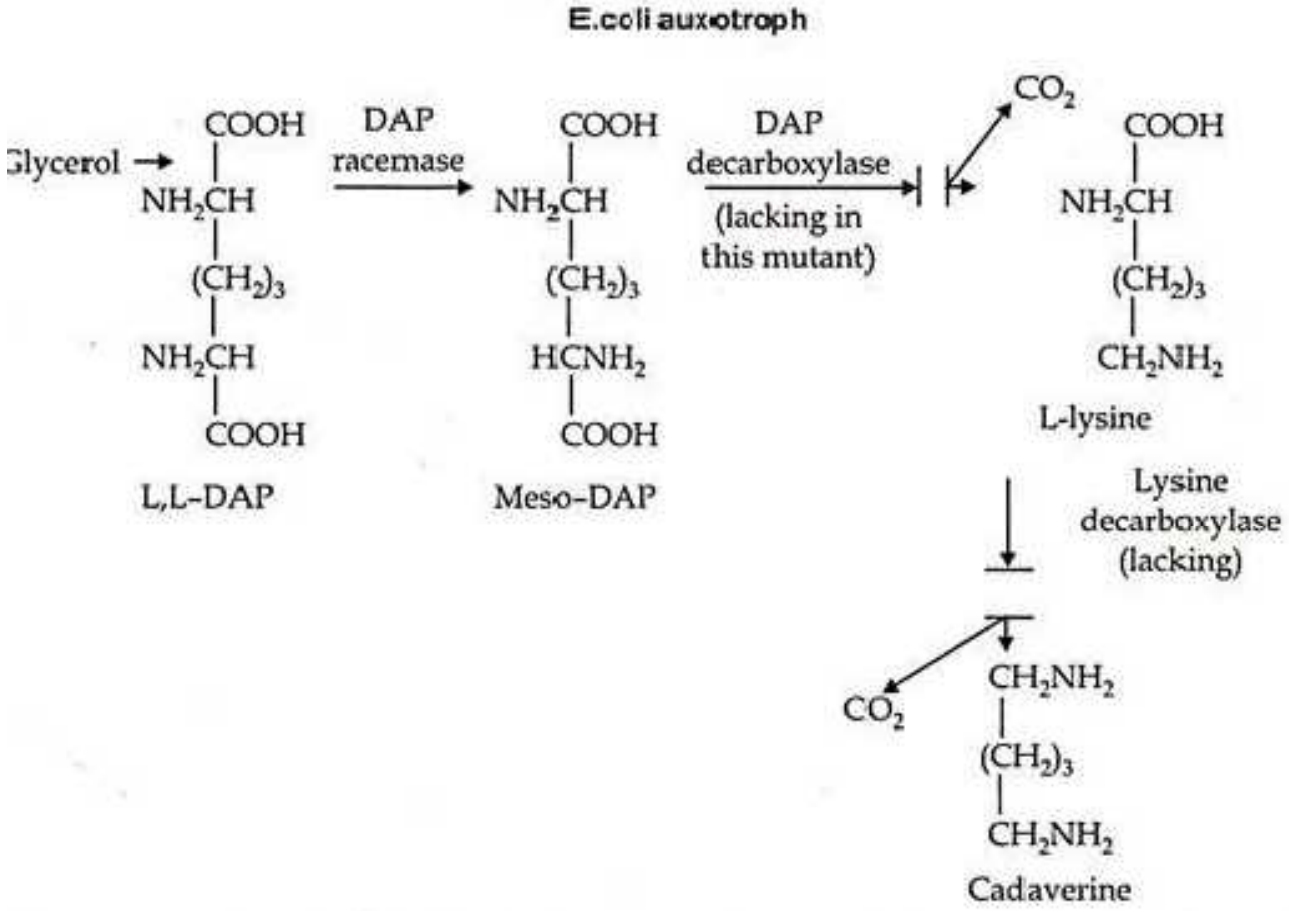
# Production of L-Lysine

•Total world production of L-lysine is around 35,000 metric tons per year.

**•Industrially it is produced by two different fermentation methods. They are:**
(a) Indirect fermentation
(b) Direct fermentation.

**(a) Indirect Fermentation:**
It is also called as dual fermentation as two different microorganisms are employed in this fermentation process.

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.
Diaminopimelic acid produced in the first half of fermentation by auxotroph of E. coli, is converted into L-lysine by A. Aerogenes in the second half of the fermentation (Fig. ).
A. Aerogenes should also be deficient of lysine decarboxylase so that further decarboxylation of lysine to cadaverine is prevented and accumulation of lysine is facilitated.




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

**(b) Direct Fermentation:**

•L-lysine can also be fermentatively produced from any of the substrates directly and the process is called as direct fermentation.

•Direct fermentation processes are presently employed throughout the world for the production of L-lysine. Direct production of l-lysine from carbohydrate was developed first with a homoserine or threonine plus methionine auxotroph of Corynebacterium glutamicum.

•Production of lysine by this bacterium is regulated by the mechanism as depicted in Fig.

•The prototroph of this bacterium produces L-glutamic acid in large quantities. The same type of process was reported with a homoserine auxotroph of Brevibacterium flavum.

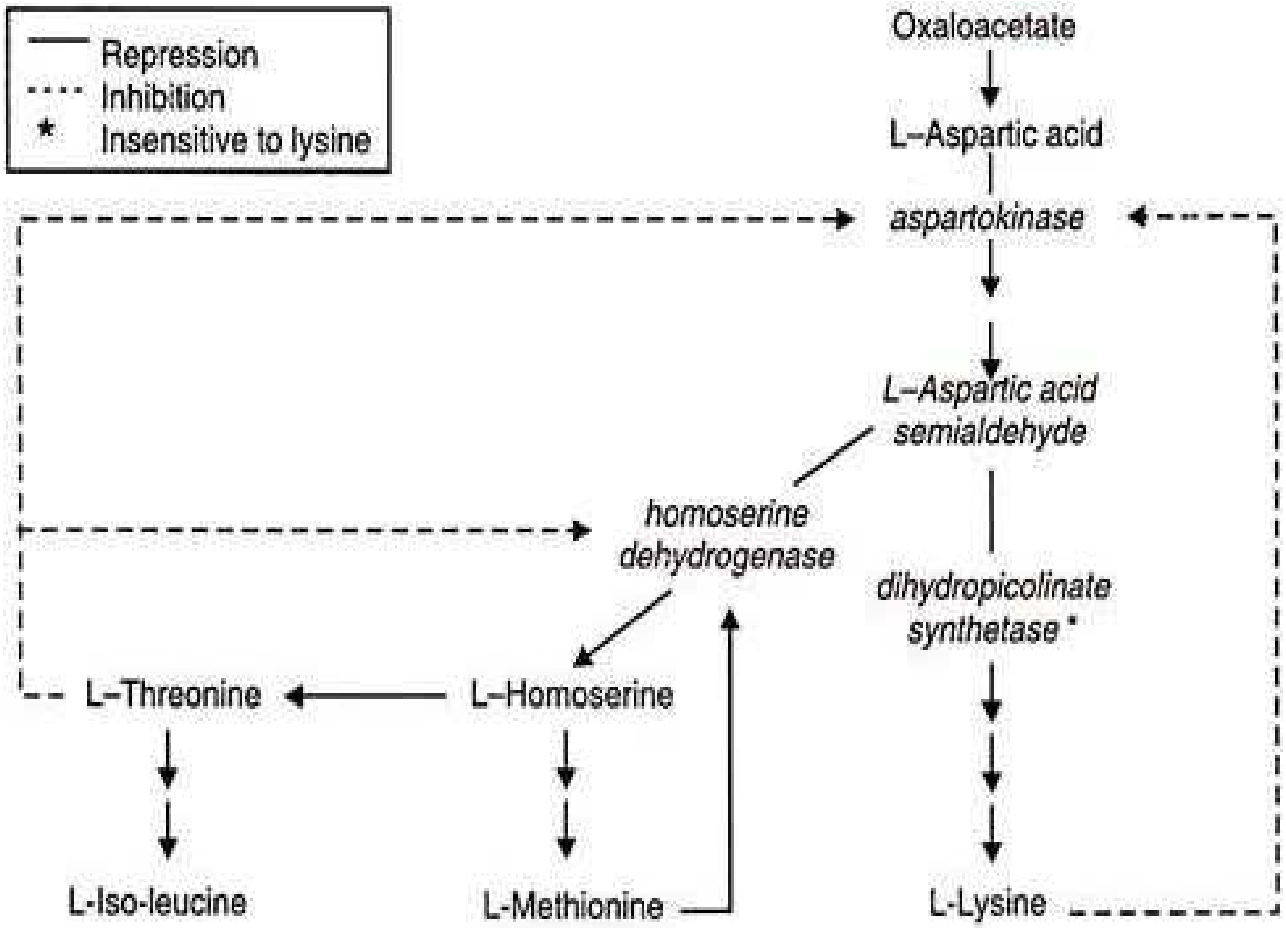



Fig. Control of L-lysine production from Corynebacterium glutamicum

•The homoserine auxotroph was later recognized as threonine sensitive mutant because growth was inhibited by the excess of threonine and the inhibition was released by the addition of methionine. This phenomenon is due to feedback inhibition of residual homoserine dehydrogenase by threonine.

•Homoserine auxotroph of other bacteria were also found to produce L-lysine but the yields were lower than that from homoserine auxotroph of Coryneform bacteria.



•Threonine and leucine auxotrophs produce fairly large amounts of L-lysine but they are inferior to the homoserine auxotroph. Other auxotrophs of Corynebacterium glutamicum and other bacteria were also inferior to the homoserine auxotroph of C. glutamicum.

• This bacterium is extensively used for the L-lysine production on commercial basis by fermentation process.

•Double auxotrophs, which require atleast one of the amino acids, threonine or isoleucine or methionine in addition the homoserine, for growth have been found highly stabilized, showing little tendency to revert the homoserine independence.

•It is possible not only to prevent reversion of the culture to a wild type, but also to produce lysine in higher yields since many of the microorganisms are double mutants in the homoserine pathway.

# Fermentation Process of L-Lysine

This process consists of four stages.

**They are:**

(i) Preparation of inoculum,
(ii) Preparation of medium,
(iii) Fermentation process,
(iv) Harvest and recovery,

**(i) Preparation of Inoculum:**

Suitable and high yielding mutant strain of C. glutamicum usually (strain 901) is used from the stock culture for the production of inoculum. Seed cultures are raised twice, in which two different media are used.

**The medium for first seed culture contains:**

| | |
|---|---|
| Glucose | 2% |
| Peptone | 1% |
| Meat extract | 0.5% |
| NaCl | 0.25% |

The medium is prepared in tap water.

**The medium for second seed culture contains:**

| | |
|---|---|
| Cane molasses | 5% |
| $(NH_4)_2SO_4$ | 2% |
| Corn steep liquor | 5% |
| $CaCO_3$ | 1% |

It is also prepared in tap water. This prepared inoculum is employed for fermentation.

**(ii) Preparation of Medium:**

The medium with the following composition is used as fermentation medium. Reducing sugar (expressed as inverted cane molasses), 20%, Soyabean meal hydrolysate (as weight of meal before hydrolysis with $6NH_2SO_4$ 1.8% and neutralization with ammonia water) are dissolved in tap water and sterilized.

**(iii) Fermentation Process:**

The fermentation is carried out at 28°C and is allowed upto 60 hours. The amount of growth factors, homoserine or threonine and methionine should be appropriate for the production of L-lysine and suboptimal quantity to support the optimal growth. The biotin concentration in the medium should be greater than 30 mg per liter.

## (iv) Harvest and Recovery:

The same process of recovery of L-lysine that is employed in indirect fermentation process is also used in this process.
Mutant strains of Bacillus licheniformis are also employed for the production of L-lysine. The mutant strains were obtained by the introduction of both analogue-resistance and auxotrophy.

The medium containing 10% cane molasses is used. A temperature of 40°C is suitable for L-lysine production. The sporulation activity which reduces yield, can be suppressed by the addition of certain antibiotics like tetracycline and chloramphenicol. These mutants yield approximately 30 mg of L-lysine per ml of carbon source used.

L-lysine is also produced by enzyme process. Racemase mixture of D and L-amino-caprolactum can be transformed by the L-α-aminocaprolactum hydrolase to lysine.

Racemase enzyme converts D-α-aminocaprolactum to L-α-aminocaprolactum (Fig.). The L-α amino-caprolactic hydrolase and racemase enzymes are obtained from the bacteria, Achromobacter obae and yeast, Cryptococcus lauranti.

•If biotin is supplied in limited quantities there will be accumulation of L- glutamic acid instead of L-lysine. Cane molasses generally supplies enough biotin.

•There will be 30-40% yield of L-lysine as monohydrochloride in relation to the initial sugar concentration. Foam production in the aerated culture can be controlled by adding suitable antifoam agent.

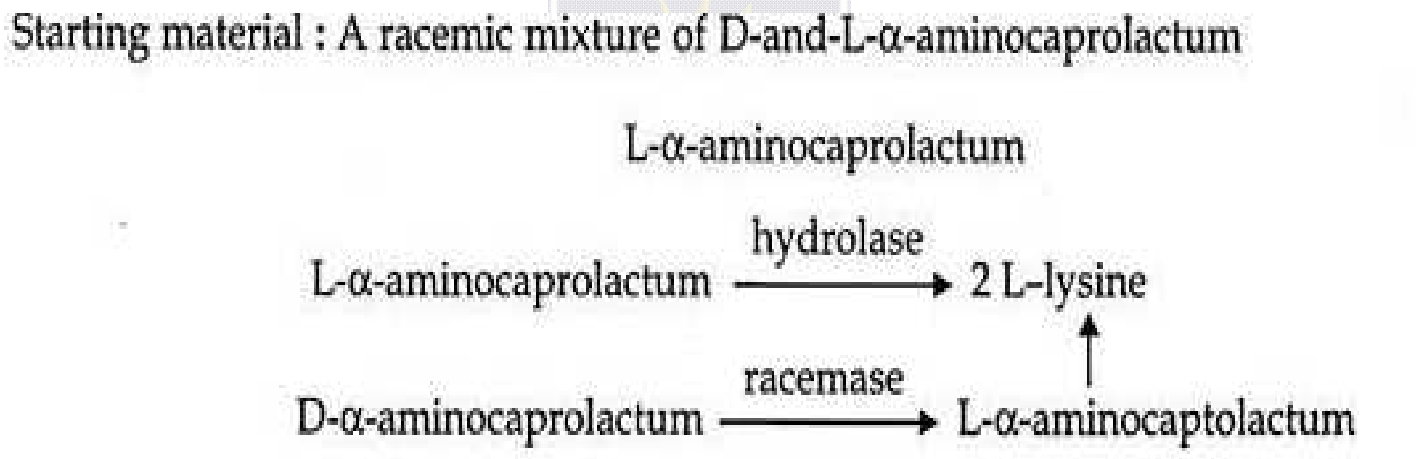



Fig. Formation of L-lysine through biotransformation by *Achromobacter* obae or *Cryptococcus lauranti*

Fermentation process of L-lysine can be described under four headings.

**They are:**
(i) Inoculum production,
(ii) Preparation of medium,
(iii) Fermentation process,
(iv) Harvest and recovery.

**(i)Inoculum Production:**

Pure inoculum of both E. coli and A. aerogenes is produced from the suitable and high yielding stock culture.

These microorganisms should lack the ability to produce diaminopimelic acid (DAPA) decarboxylase and lysine decarboxylase enzymes respectively, so that the DAPA and L-lysine produced will not be further metabolized by respective organisms.

•The cells of the organisms are separated from growth medium by centrifugation or sedimentation.

**(ii) Preparation of Medium:**

Both the inoculum and fermentation media contain glycerol, corn-steep liquor as carbon sources and ammonium hydrogen phosphate, as nitrogen source. In addition, calcium carbonate is also used in the production medium.

The levels of all of the nutrients are kept lower in the inoculum medium. Apart from supplying carbon source, the corn steep liquor also provides L-lysine required for the initial growth of auxotroph of E. coli. The pH of the medium is maintained at neutral, to slightly alkaline level (pH 8.0).

**(iii) Fermentation Process:**

Sufficient quantifermenter. Pure and required quantities of inoculum of E. coli is added (4.0%) to the fermenter. The fermentation is carried out for 3 days at 28 to 30°C temperature.

(

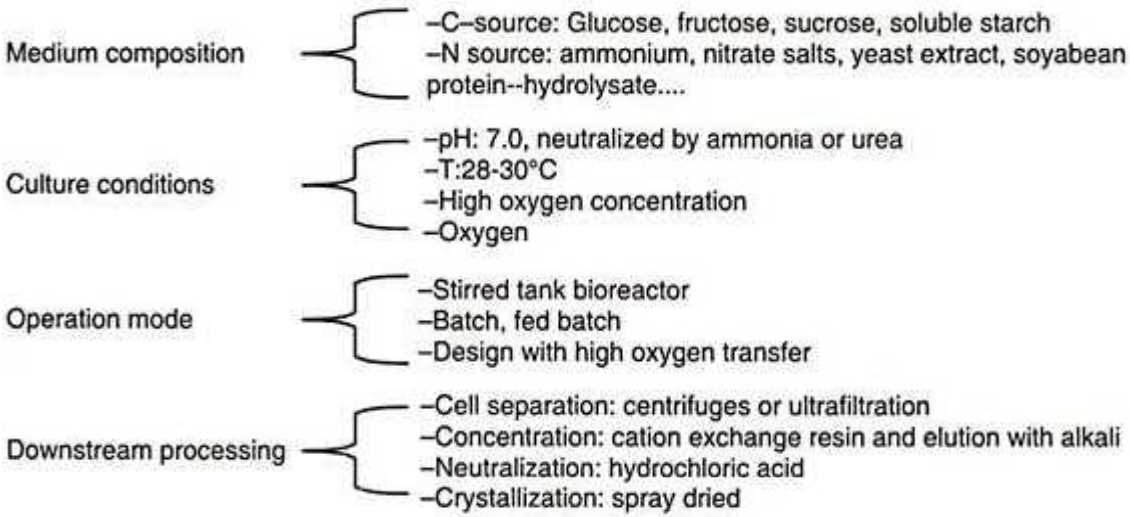



**Fig.** : Some important features of L-lysine fermentation

The level of L-lysine quantity provided to E. coli is very important, because providing low quantities, less than optimum, results in the back mutation and more quantities results in the feedback control of lysine biosynthesis both of which badly affect the yield.ties of sterilized medium is fed into the

•Through a sequence of enzymatic steps during first stage of fermentation glycerol is converted into L, L-diaminopimelic acid, which is partially converted into D, L-isomer and mesodiaminopimelic acid by the action of diaminopimelic acid racemase enzyme.

•The above-mentioned metabolites accumulate in the fermentation broth because auxotrophic E. coli lacks diaminopimelic acid decarboxylase enzyme. Hence, it cannot be converted into L-lysine. The broth contains approximately 40% L, L-isomer and 60% mesoisomer of diaminopimelic acid.



•In the second half of the fermentation, 1-2 days old culture of A. aerogenes is added to the fermentation broth formed at the end of first fermentation process. The microorganism is allowed to grow for one day at 24°C.

After sufficient growth occurs toluene is added to the fermentation broth which causes lysis of cells of A. aerogenes, due to which the enzyme diaminopimelic acid decarboxylase is liberated into the fermentation broth.

By this time most of the L, L-diaminopimelic acid is converted into meso-diaminopimelic acid by the action of diaminopimelic acid racemase enzyme. The meso-diaminopimelic acid is completely converted into L-lysine by the action of diaminopimelic acid decarboxylase. Some of the important features of lysine fermentation are depicted in Fig.

**iv) Harvest and Recovery:**

After sufficient quantities of L-lysine is formed, lysed bacterial cells are removed from the fermentation broth by centrifugation.

**The L-lysine is obtained in pure form after acidification by any one of the following separating processes:**

1. Precipitation at the isoelectric point
2. Ion exchange chromatography
3. Electrophoresis
4. Extraction with organic solvents

**Uses of L-Lysine:**

**L-lysine is useful in many fields:**

1. L-lysine is an essential amino acid required for the human nutrition.
2. It is used as supplementary for cereal proteins.
3. Protein quality of certain foods like wheat (based foods) is improved by addition of L-lysine which results in the improved growth and tissue synthesis.
4. It is used as a nutraceutical.