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.

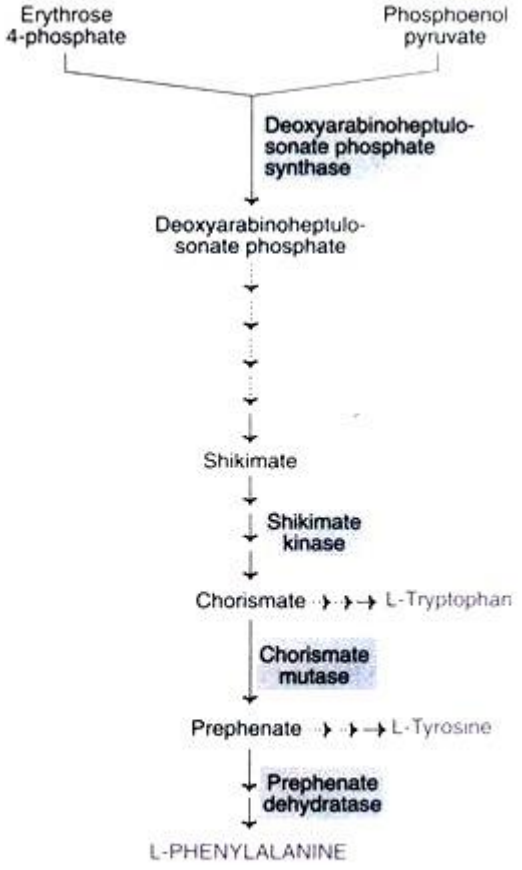


Fig. 26.7 : An outline of the pathway for the synthesis of L-phenylalanine, L-tyrosine and L-tryptophan.

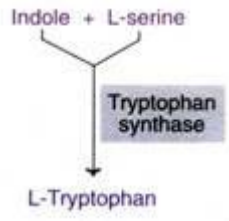
The synthetic pathway commences with the condensation of erythrose 4-phosphate with phosphoenol pyruvate to form deoxyarabinoheptulosonate phosphate (DAHP). DAHP in the next series of reactions is converted to chorismate which can form L-tryptophan. Chorismate mutase converts chorismate to prephenate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

There are three DAHP synthase enzymes in E.coli encoded by aroF, aroG and aroH. These enzymes play key role in flux control and regulation of catalytic activity in each case by one of the aromatic amino acids. About 80% of the total DAHP synthesis activity is contributed by aroG-encoded enzyme. The increased flux towards L-phenylalanine can be obtained by over expression of either aroF or aroG encoding feed-back resistant enzymes.
Further, more phe A over expression is essential which encodes the bifunctional chorismate mutase pre phenate dehydratase. A second chorismate activity is present as a bifunctional chorismate mutase – prephenate dehydrogenase. The pre A-encoded enzyme activities are inhibited by L-phenylalanine and pre A expression is dependent on the level of t-RNA. A pre phenylalanine producer obtain as per rule are tyrosine auxotrophic mutants

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.

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.



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 (deoxyarabinoheptulosonate 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.

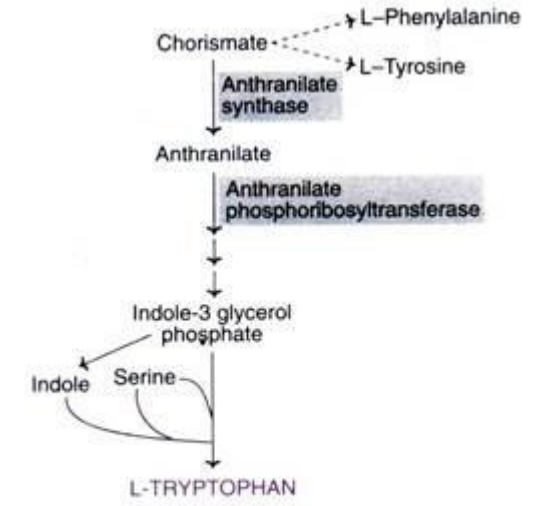


***Fig. 26.8** : Biosynthesis of L-tryptophan (For the syntheis of chorismate Refer Fig. 26.7).*