Microbial production of amino acids

Amino acids market demand is increasing since the production of monosodium glutamate (MSG) started in 1907. Amino acids are used in many industrial applications as bulk biochemical to produce a wide range of products such as animal feed additives, flavor enhancers in human nutrition or as ingredients in cosmetic and medical products.

Amino acids can be produced by different processes such as extraction from protein hydrolysates, chemical synthesis or enzymatic and fermentation pathways with the aid of microorganisms. In particular, the fermentation process is becoming one of the most promising processes for amino acids commercial production because of the new genetic engineering tools applied to maximize yield, specificity and productivity of the target compounds.

The most important method for producing amino acids microbiologically is by direct fermentation. What method is used in any particular situation depends on factors such as process economics, the available raw materials, market size, the environmental regulation operating in the place of production, etc.

The production of amino acids by fermentation was stimulated by the discovery of an efficient L-glutamic acid producer *Corynebacterium glutamicum*. Auxotrophic and regulatory mutants of glutamic acid producing bacteria are used for the commercial production of all amino acids outside L-glutamic acid and L-glutamine, which are produced by the wild type of these organisms (Table 1).

Table 1. Amino acids produced from wild type and mutant strains of bacteria

Wild-type		*Auxotrophic Mutants*		*Regulatory Mutants*	
L-glutamic acid	1	L-citrulline	1	L-arginine	1,2,3
L-valine	1	L-leucine	1	L-histidine	1,3,5
		L-lysine	1	L-isoleucine	1,3,5
		L-ornithine	1	L-leucine	5,6
		L-proline	3	L-lysine	3
		L-threonine	4	L-methionine	1
		L-tyrosine	1	L-phenylalanine	1,3
				L-threonine	1,3
				L-tryptophan	1,3
				L-tyrosine	1,3
				L-valine	6

1 = *Corynebacterium glutamicum* 2 = *Bacillus subtilis*
3 = *Brevibacterium flavum* 4 = *Escherichia coli*
5 = *Serratia marcescens* 6 = *Brevibacterium lactofermentum*

Production of glutamic acid by wild type bacteria

(i) Organisms:

Wild type strains of the organisms of the four genera mentioned above are now used for the production of glutamic acid. The preferred organism is however *Corynebacterium glutamicum.* The properties common to the glutamic acid bacteria are:

(a) they are all Gram-positive and non-motile;

(b) they require biotin to grow;

(c) they lack or have very low amounts of the enzyme α ketoglutarate which is formed by removal of CO_2 from isocitrate formed in TCA cycle (citric acid cycle). Since α-ketoglutarate is not dehydrogenated, it is available to form glutarate by reacting with ammonia.

(ii) Conditions of the fermentation:

The composition of a medium which has been used for the production of glutamic acid is as follows (%): glucose, 10; corn steep liquor 0.25; enzymatic casein hydrolysate 0.25; K_2HPO_4 0.1, $MgSO_4$.$7H_2O$, 0.25; and urea, 0.5. It should be noted that besides glucose, hydrocarbons have served as carbon sources for glutamic acid production. The optimal temperature is 30°C to 35°C, and a high degree of aeration is necessary.

(iii) Biochemical basis for glutamic acid production:

(a) Glutamic acid production is greatest when biotin is limiting. When biotin is optimal, growth is luxuriant and lactic acid instead of glutamic acid is excreted. The optimal level of biotin is 0.5 mg per gram of dry cells.

(b) The isocitrate-succinate part of the TCA cycle (Fig. 1) is needed for growth. It is only after the growth phase that glutamic acid production becomes optimal.

(c) An increase in the permeability of the cell is necessary so as to permit the outward diffusion of glutamic acid, essential for high glutamic acid productivity. This increased permeability to the acid can be achieved in the following ways:

(i) ensuring biotin deficiency in the medium

(ii) treatment with fatty acid derivatives,

(iii) ensuring oleic acid deficiency in mutants requiring oleic acid (C_{16}–C_{18}), and

(iv) addition of penicillin during growth of glutamic acid bacteria.

Cells treated in one of the first three ways above have cell membranes in which the saturated to unsaturated fatty acid ratio is abnormal, therefore the permeability barrier is destroyed and glutamic acid accumulates in the medium. The major factor in glutamic acid production by wild type organism is thus altered permeability. Treatment with penicillin prevents cell-wall formation. Cell wall inhibiting antibiotics such as penicillin and cephalosporin have enabled the use of molasses which are rich in biotin for glutamic acid production.

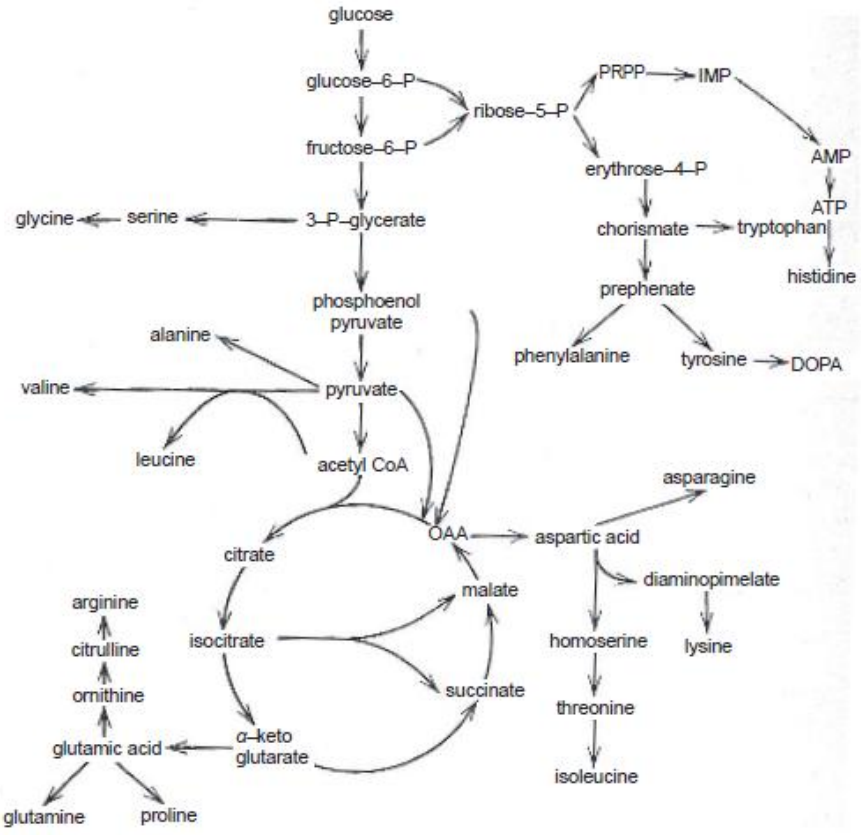

Fig. 1. Metabolic Pathways Involved in the Biosynthesis of Amino Acids from Glucose

Production of amino acids by mutants

After wild type strains of *C. glutamicum* and of other bacteria were found to accumulate glutamic acid, efforts to find in nature bacteria able to yield high amounts of other amino acids failed. The reason for this is that microorganisms avoid over-production of amino acids, producing only the quantity they require. To induce the organism to overproduce, regulatory mechanisms must be disorganized. Two major means of regulating amino acid synthesis are feedback inhibition and repression. Auxotrophic mutants and regulatory mutants are two means by which the organisms' tendency not to overproduce can be disorganized. In order to overproduce an amino acid which is an intermediate in a synthetic pathway, a mutant auxotroph is produced whose pathway in the synthesis is blocked. When this mutant is cultivated, limiting nutrient feedback and/or repression would have been removed and an overproduction of the amino acid will occur. The mutants used for the production of amino acids other than glutamic acid are produced from L-glutamic acid producing bacteria. These bacteria assimilate carbon efficiently and do not degrade the amino acid they excrete.