Amino Acid Production

MSG production fermentors. The volume of each is 63,420 gallons and the height is about 100 ft tall. Hofu, Japan.(from http://smccd.net/accounts/case/biol230/indfer.html)

The amino acid industry provides illustrations of how one rationally selects and manipulates microorganisms for producing a marketable product. It also provides some insight into unforeseen problems that may arise with large industries.

Background

The amino acid business is a multi-billion dollar enterprise. All twenty amino acids are sold, albeit each in greatly different quantities (Table 1). Amino acids are used as animal feed additives (lysine, methionine, threonine), flavor enhancers (monosodium glutamic, serine, aspartic acid) and as specialty nutrients in the medical field. Glutamic acid, lysine and methionine account for the majority, by weight, of amino acids sold. Glutamic acid and lysine are made by fermentation; methionine is made by chemical synthesis. The major producers of amino acids are based in Japan, the US, South Korea, China and Europe.

Many microbe-based industries have their origins in traditions that go back hundreds or thousands of years. The amino acid industry has its roots in food preparation practices in Japan. Seaweeds had been used for centuries there and in other Asian countries as a flavoring ingredient. In1908, Kikunae Ikeda of Tokyo Imperial University isolated the flavor enhancing principle from the seaweed *konbu* (also spelled *kombu, Laminaria japonica;* related to kelp) as crystals of monosodium glutamate (MSG). Adding MSG to meat, vegetables and just about any other type of prepared food makes it savory, a property referred to as *umami*. Soon after Ikeda's discovery, and recognizing the market potential of MSG, Ajinomoto Co. in Japan began extracting MSG from acid-hydrolyzed wheat gluten or defatted soybean and selling it as a flavor enhancer.

The production of MSG via "fermentation" grew out of the ashes of WWII in Japan. Around 1957, Japanese researchers led by S. Kinoshita at Kyowa Hakko Kogyo Co. isolated soil bacteria that produced large amounts of **glutamic acid**. Producing strains were found by inoculating soil isolates in a grid pattern on duplicate Petri plates. The colonies were allowed to grow and one set of duplicates was killed with UV irradiation. The killed plate was overlaid with soft agar containing a *Leuconostoc mesenteroides*. Since *L. mesenteroides* required glutamic acid for growth, it only grew in the vicinity of colonies that had excreted glutamate. Potential glutamate producers were then picked from the duplicate, unkilled, plate.

Members of the *Actinobacteria* in the genus *Corynebacterium* (originally named *Micrococcus glutamicus*) were the most effective producers. Over the years, various glutamate-producing bacteria have been isolated and classified as *Arthrobacter, Brevibacterium,* or as members of other genera, but recent work has shown that almost all of these strains belong to the genus *Corynebacterium*. Wild-type cultures produced up to 10 g/l glutamic acid. Yields were quickly improved by process engineering and by developing over-producing mutants. Yields are now in excess of 100 g per liter.

The isolation of bacterial glutamate-producers led to the development of large-scale manufacture of MSG from cheap sugar and ammonia rather than from more expensive extracts of plants or animals. In the early 1960s, workers at the same company found that *C. glutamicum* homoserine auxotrophs (see below) produced lysine thus providing the first viable fermentation process for lysine production. Today these bacteria produce well over 1,000,000 metric tons of MSG and 600,000 metric tons of lysine annually.

Table 1. Estimated global production of amino acids (1996)*

Amino acid	Amount (ton/y)	Process	Uses
L-glutamate	1,000,000	Ferm.	Flavor enhancer
D, L. Methionine	350,000	Chemical	Food , Feed Pharm.
L-Lysine HCL	250,000	Ferm.	Feed Supplement
Glycine	22,000	Chemical	Pharm., soy sauce
L-Phenylalanine	8,000	Ferm., Synthesis	Aspartame
L-Aspartic acid	7,000	Enzymatic	Aspartame, Pharm.
L-Threonine	4,000	Ferm.	Feed supplement
L-Cysteine	1,500	Extraction, Enzyma.	Pharm.
D, L –Alanine	1,500	Chemical	Flavor, sweetener
L- Glutamine	1,300	Ferm.	Pharmaceuticals
L-Arginine	1,200	Ferm.	Flavor, pharm.
L- Tryptophan	500	Ferm., Enzymatic	Feed suppl., Pham.
L – Valine	500	Ferm.	Pharmaceuticals
L –Leucine	500	Ferm., Extraction	Pharmaceuticals
L –Alanine	500	Enzymatic	Pharm.
L –Isoleucine	400	Ferm.	Pharmaceuticals
L – Histidine	400	Ferm.	Pharmaceuticals
L – Proline	350	Ferm.	Pharmaceuticals
L – Serine	200	Ferm.	Pharmaceuticals
L - Tyrosine	120	Extraction	Pharmaceuticals

*From Ikeda, M. 2003. Adv. Biochem. Eng. Biotech. 79:1-35.

Three general approaches are used today for making amino acids: direct chemical synthesis, fermentation and bioconversion using enzymes. Choosing between processes depends on available technology, costs of raw material, market prices and sizes, cost of running fermentation versus synthesis reactions, and the environmental impact of the process itself. Table 1 illustrates the major processes as of 1996. The processes have not changed very much since then, but the amounts made have increased at a rate of about 2-5% per year.

Amino acids are consumed in a variety of markets. The largest by volume is the food flavoring industry. MSG, alanine, aspartate, arginine are all used to improve the flavor of food. The basis of our ability to enjoy the taste of amino acids is rooted in evolution. Animals require certain amino acids for growth and nutrition. Specific taste receptors on the human tongue have been found that are G-protein coupled receptors responsive to many of the 20 L- but not D-amino acids. For human receptors, the strongest response is for glutamate, thus providing a biochemical link to what cooks have known for centuries. Interestingly, inosine mono-phosphate (IMP), another well-known flavor enhancing nucleotide also sold commercially, greatly increases the response of the receptor to amino acids.

Amino acids in an IV stock– Travasol ®	
Essential	
Leucine	730 mg
Isoleucine	600 mg
Lysine(HCl)	580 mg
Valine	580 mg
Phenylalanine	560 mg
Histidine	480 mg
Threonine	420 mg
Methionine	400 mg
Tryptophan	180 mg
Non-Essential	
Alanine	2.07 g
Arginine	1.15 g
Glycine	1.03 g
Proline	680 mg
Serine	500 mg
Tyrosine	40 mg
Acetate	88 mEq
Chloride	40 mEq
pH 6 (5-7); Total aa = 10 g	

The second largest consumer of amino acids is the animal feed industry. Lysine, methionine, threonine, tryptophan and others improve the nutritional quality of animal feeds by supplying essential amino acids that may be in low abundance in grain. Using 0.5% lysine in animal feed improves the quality of the feed as much as adding 20% soy meal. In addition, by limiting the added amino acid supplements to those required by the animal, some of the excess ammonia made via deamination reactions that is normally excreted to the environment is eliminated. The addition of microbially-produced amino acids has also increased with the onset of "Mad Cow" syndrome (BSE), a disease traced to feed supplements containing animal protein.

Finally, the pharmaceutical industry uses a variety of amino acids for making intravenous nutrient solutions for pre- and post-operative care (see Table 2). These mixtures account for a small percentage of the total volume of amino acids sold each year, but the requirement that they be highly purified provides a value-added component. In addition to these uses, amino acids are used as specialty chemicals in laboratories, in the manufacture of artificial sweeteners (aspartame) and in many other situations.

MSG

Glutamate is the most abundant free amino acid in bacterial cytoplasm. Nevertheless, in order to be useful, glutamate producers must do two things well: they must overproduce glutamate in excess of their normal metabolic needs, and they must excrete it into culture broth. The precise mechanism by which *C. glutamicum* does these things is still not completely understood despite over forty years of study. Some physiological traits, however, are clearly involved. These include biotin auxotrophy of producing strains, a marked decrease in α-ketoglutarate dehydrogenase activity during production, and a predilection for exporting glutamate, perhaps via a specific transporter.

Many of the original glutamate-excreting strains were biotin auxotrophs, and growing in biotin deficient medium was found to "trigger" glutamate production. Biotin is a cofactor (a "vitamin") used by enzymes that carboxylate substrates. One such enzyme is **acetyl-CoA carboxylase** that converts Acetyl-CoA + CO_2 to Malonyl-CoA in the first step of fatty acid biosynthesis. Biotin auxotrophs growing in biotin deficient medium were proposed to have altered membranes due to suboptimal fatty acid biosynthesis. Supporting the notion of altered permeability is the observation that growth at higher temperatures, or including detergents or cell-wall biosynthesis inhibitors like penicillin in the growth medium can also trigger excretion.

Reduced levels of α-ketoglurate dehydrogenase during production may also be linked to membrane integrity. In corynebacteria, the enzyme has three activities on two peptides: the α-kg dehydrogenase + dihydrolipoamide S-succinyltransferase peptide and the dihydrolipoamide dehydrogenase peptide. The latter is shared with pyruvate dehydrogenase and is likely to be membrane bound and thus prone to being affected by trigger factors that alter membrane composition.

Since α-ketoglurate dehydrogenase catalyzes a step in the TCA cycle, the cycle is largely incomplete during glutamate production, a circumstance requiring that pools of oxaloacetate be filled, as carbon is lost through glutamate. Anaplerotic enzymes replace OAA; these enzymes include pyruvate carboxylase (Rxn. 1), malic enzyme (Rxn. 2), PEP carboxylase (Rxn. 3) and glyoxylate pathway enzymes.

1. pyruvate + CO_2 + ATP ← → oxaloacetate + ADP + P_i
2. pyruvate + CO2 + NADPH ← → malate + $NADP^+$ + H^+
3. phosphoenol pyruvate + CO_2 ← → oxaloacetate + P_i

The actual contribution of each anaplerotic enzyme or pathway depends on the growth conditions, production phase and a host of interacting metabolic signals. However, mutants lacking pyruvate carboxylase produce as much glutamate as wild type suggesting that PEP carboxylase is the major anaplerotic enzyme whan growing on sugars. When growing on acetate or fatty acids, the glyoxylate pathway assumes a major role in filling OAA pools, although acetate is not normally used in the industrial process.

For many years, increased membrane permeability was thought to promote glutamate excretion. Recently, however, export via a specific efflux transporter has been proposed as a mechanism for avoiding exceptionally high levels of intracellular glutamate. Such an exporter has been proposed by analogy with the LysE transporter that is responsible for lysine and arginine export in *C. glutamicum.*

Other factors contributing to glutamate overproduction include metabolic flux alterations based on cell growth limitations. The following chain of events seems likely: A triggering mechanism (biotin depletion, high temperature, cell membrane alterations by detergents, oleic acid or antibiotics) results in a decrease or

repression of α-kg dehydrogenase. The result is a redistribution of metabolites at the branch point in the TCA cycle leading from α-kg to succinyl-CoA or glutamate. The increase in glutamate levels, beyond that needed for cell growth, stimulates glutamate efflux as the cell attempts to maintain the proper level of intracellular glutamate. The result is the excretion of glutamate from the cell.

Industrial production of MSG

Although the details of MSG production remain proprietary and differ in detail from company to company, a general outline of the industrial process is known. The process is most commonly run as a **fed-batch** type, where sugar is added during the fermentation process. The reason for using fed-batch rather than batch, where all components are present at the beginning of the process, is that high sugar concentrations, more that 20% in total would otherwise need to be added to the medium. Such high levels can lead to incomplete oxidation of the sugar to lactic or acetic acids or to osmotic inhibition of growth with subsequent decrease in yields.

Small seed cultures are grown on glucose, potassium phosphate, magnesium sulfate, yeast extract and urea as a source of nitrogen. Larger cultures use cheaper sources of sugar including cane or beet molasses and starch hydrolyzates from corn or cassava. The sugar source roughly parallels the geographic location of the process. That is, corn syrup is used in the United States, tapioca (from cassava) in South Asia and cane and beet molasses in Europe and S. America. Ammonia and ammonium sulfate are used as nitrogen sources. Ammonia can also be used to control pH during fermentation. Cheap sources of vitamins and other nutrients include corn steep liquor, a by-product of cornstarch manufacture that is replete with amino acids, nucleic acids, minerals and vitamins.

To begin the process, stocks of *C. glutamicum* are used to inoculate shake flask cultures. The resulting cells are transferred to a small seed culture tank that is grown and used to inoculate a larger tank, and so on. The intermediate seed culture volumes are variable but generally in the range of 200-1000 liter, then 10,000-20,000 l and finally the production tank of about 50,000-500,000 l. The process is carefully controlled at each step such that cell density, nutrient composition, temperature, pH, aeration, agitation rates and sugar flow rates are as consistent as possible from batch to batch. Oleic acid (0.65 ml/l) might be added at the beginning of the fermentation to encourage glutamate excretion; pH is set around 8.5 with ammonia, and maintained at 7.8 during the process.

After 14 hours of growth, the temperature is increased from about 32-33°C to 38°. Sugar is fed in as the fermentation proceeds up to about 36 h. During the course of the fed-batch process about 160 g or more of glucose per liter, or its equivalent, is fed into the bioreactor. Glutamate concentrations are monitored at intervals and, after the process is complete, the broth is pumped from the bioreactor to recovery tanks. Yields of glutamate from a large-scale fermentation are in excess of 100 g/l. That means that a 100,000 liter bioreactor yields about 10,000 kg of glutamate. At a market price of about $1.25 per kg, a single fermenter has a value of about $12,500. Considering the costs of energy, personnel, overhead and processing, that is not much money and is a consequence of fierce competition between companies.

L-LYSINE

Soon after *C . glutamicum* was commercialized for MSG production, auxotrophs were developed that excreted commercial quantities of lysine. By 1958, Kinoshita and colleagues had shown that lysine was excreted by homoserine auxotrophs. For example, a homoserine auxotroph (strain ATCC13287) was patented in 1961 that yielded 44 g lysine per liter with a conversion efficiency of 26% from sugar (g lysine/g sugar). Further increases in yield were made incrementally with the introduction of additional amino acid and vitamin auxotrophies plus development of strains resistant to **antimetabolites**. Typical strains today provide conversion efficiencies of over 50%.

To understand why lysine is overproduced from a physiological standpoint, one must first understand the regulatory circuits that govern lysine biosynthesis. The approaches used for creating amino acid overproducers are paradigms of rational strain improvement as contrasted with approaches that rely on random mutagenesis and screening. The pathway leading to lysine (also threonine, isoleucine, methione) biosynthesis is initiated

with the conversion of aspartate to aspartyl-P via the enzyme aspartokinase (AK). The phosphorylated aspartate is then converted to aspartyl-semialdehyde (ASA) that can converted to homoserine by homoserine dehydrogenase (HSD) or to diaminopimelic acid (DAP) by a series of five enzymatic conversions, and thence to lysine. Homoserine is the starting point for making threonine and isoleucine as well as methionine. The major control points for the metabolic flux to individual amino acids occurs at the level of aspartokinase and homoserine dehydrogenase.

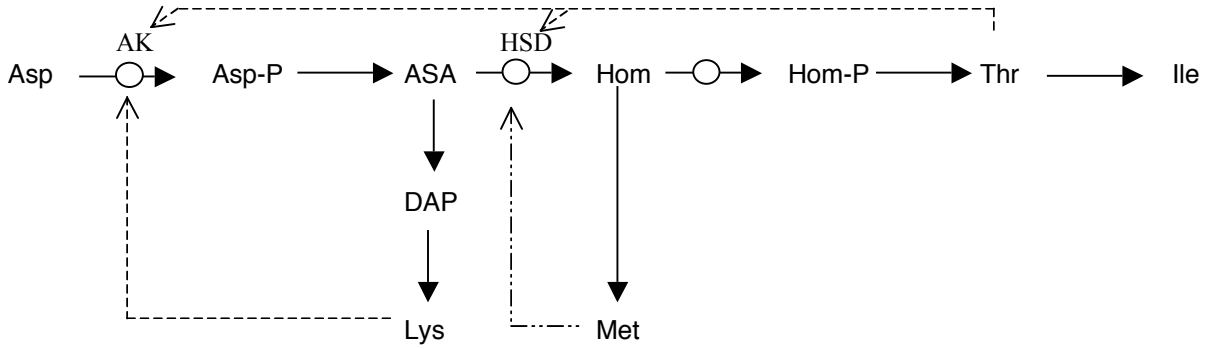


Fig. 1. Aspartate family of amino acids showing the branched pathways leading to lysine, methionine, threonine and isoleucine. Lysine plus threonine exert concerted feedback inhibition (dashed lines) on aspartyl kinase (AK) and threonine feedback inhibits homoserine dehydrogenase (HSD). Methionine represses the synthesis of HSD (dots & dashes).

AK is feedback inhibited by the accumulation of threonine plus lysine. Homoserine dehydrogenase is feedback inhibited by threonine and its synthesis is repressed by methionine.

Mutations that lead to the overproduction of lysine include HSD^-, HSD^{leaky}, and **lysine analogue resistant** mutants. HSD^- mutants are homoserine auxotrophs that effectively lack the ability to make threonine, methionine and isoleucine, thus eliminating the feedback inhibition of AK by threonine. HSD^{leaky} mutants make an HSD that is less effective at making homoserine so that threonine does not accumulate to levels sufficient to shut down AK. The benefit of the latter mutants is that additional amino acids do not need to be added to growth media to make up for their complete lack of synthesis in HSD null mutants.

Analogue resistant mutants resist the feedback inhibition effects of allosteric effectors. In the case of AK, mutants were derived that resisted analogues of lysine and/or threonine. To derive such mutants, mutagenized cultures are incubated in the presence of the analogue; those that grow "resist" the analogue and are screened for their ability to make lysine. The rationale behind the approach is that wild-type AK recognizes the lysine analog 2-aminoethyl-L-cysteine (AEC) as lysine. The enzyme is inhibited as if lysine had accumulated in the cell and the cell fails to grow due to lysine starvation. Analogue resistant mutants have AKs that do not recognize AEC as lysine; these enzymes remain uninhibited, continue to make aspartyl-P and then lysine and the cells continue to grow. Such mutants, since they no longer respond to intracellular lysine levels, continue to synthesize lysine at high levels. Other types of auxotrophs, for example for leucine and alanine also overproduce lysine since their biosynthetic pathways compete for pyruvate.

In 1996, the gene encoding a membrane protein involved in the active export of lysine– *lysE* – was cloned and sequenced. It is responsible for exporting lysine up a concentration gradient eventually leading to the accumulation of as much as 1 mole per liter of lysine. Mutations in this gene cause lysine to accumulate in the cytoplasm but not in the extracellular medium. Introducing the gene into a multi-copy plasmid in *C. glutamicum* leads to increases in the rate of efflux of intracellular lysine.

Lysine Fermentation

The industrial production of L-lysine is carried out in a manner similar to that for L-glutamate production. Sugar cane or beet molasses is used as the substrate for production. If the biotin level is low in the starting material it must be added to a level of about 30 ug/l. If an auxotroph for any amino acid is used, that amino acid

must be present so that the cells may grow but should not be present at high enough concentration to exert inhibitory physiological effects such as feedback inhibition of the pathways used for production.

L-THREONINE

L-Threonine production has been accomplished by genetically manipulating strains of *E. coli* and stands as a good example of rational design of industrial organisms. The initial strains constructed for his purpose were derived 1969 as mutants resistant to the L-threonine analogue, α-amino-β-hydroxyvaleric acid (AHV) by Shiio & Nakamori in Japan. Since that time several strains have emerged as production strains based on a combinations of auxotrophic and analogue resistant mutations that have the collective effect of funneling carbon to L-threonine production.

Detailed understanding of threonine metabolism in *E. coli* was essential for rationally designing threonine overproducers. The regulation of threonine biosynthesis in *E. coli* is more complex than that in *C. glutamicum.* Unlike *Corynebacterium, E. coli* has three aspartate kinases, AKI, AKII and AKIII. Two (AKI and AKII) are multidomain proteins that also have homoserine dehydrogenase activity responsible for the third step of the pathway. AKI is feedback inhibited by threonine and its synthesis is repressed by a combination of threonine and isoleucine. The synthesis of AKII is repressed by methionine. AKIII is feedback inhibited and repressed by lysine.

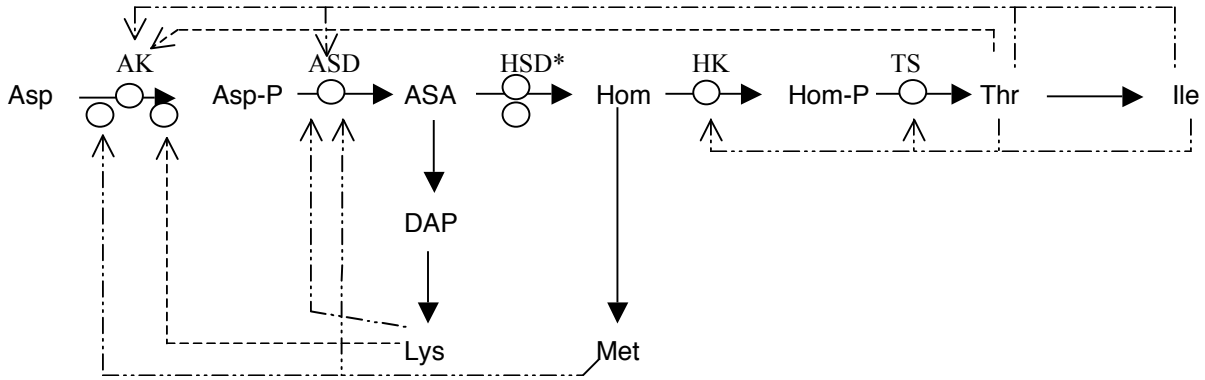


Fig. 1. Aspartate family of amino acids in *E. coli*. *HSD is part of the multidomain proteins AKI and AKII are is under the same regulatory controls as AKI and AKII.

The second step of the pathway is catalyzed by aspartate semialdehyde dehydrogenase (ASD). Its expression is repressed by an accumulation of high levels of lysine, threonine and methionine, with lysine being the most effective. The last two enzymes, homoserine kinase (HK; *thrB*) and threonine synthase (TS; *thrC*) are coexpressed along with AKI (*thrA*) as part of the *thrABC* operon. This operon is controlled by transcriptional attenuation. A 21 amino acid long leader peptide between the promoter and first gene has seven theronines and four isoleucines. The rate of transcription dictates whether the genes are transcribed. If the rate of transcription is fast (lots of charged trp-tRNA and ile-tRNA) then transcription is terminated; if it is slow, leading to pausing of the ribosome during translation, transcription continues and the enzymes are made.

Aside from these regulatory mechanisms, other systems also affect threonine production. These include the breakdown of threonine by threonine deaminase (*ilvA*) or threonine dehydrogenase (*tdh*), and transport systems that control the uptake or efflux of threonine from the medium.

With such a complex regulatory scheme, several approaches have been taken to “open up” the pathway to threonine production. These generally involve deriving auxotrophs for amino acids that

might compete for carbon flow through the pathway, or analogue resistant mutants that have enzymes that have lost the ability to be feedback inhibited by the product of the pathway.

Analogue resistant mutants of *E coli* were derived by 1969 using the threonine analogue α-amino-β-hyroxyvaleric acid (AHV). This compound has been used for virtually all subsequent work. In addition to analogue resistance that removed the feedback inhibition on AKI, auxotrophs were made that required isoleucine. By placing the threonine operon onto multicopy plasmids and introducing it into such a strain, strains were obtained that produced about 65 g/l of threonine with yields of 48% (g thr/g sugar). A production strain that was made by a collaboration between Russian and Japanese workers with the following genotype:

Genotype and phenotype of Strain B-3996	
relA$^+$	Gene encododing stringent factor synthesis (ppGpp) that enhances transciption of amino acid biosynthesis operons
thrC	Mutant inactivating threonine synthase
*ilvA**	Mutant threonine deaminase reducing levels to ca. 1% WT; removes attenuation of thr operon
suc$^+$	Ability to use sucrose introduced
rht23A	Mutant enhancing *rht* expression involved in amino acid eflux
tdh	Mutant lacking threonine dehydrogenase; decreases breakdown of threonine
*ppc**	PEP carboxylase insensitive to aspartic acid; increases flow of carbon to OAA and asp
pVC40	RSF1010 plasmid containing complete threonine operon with *thrA** (ATI) mutation

L-ASPARTATE & L-ALANINE

L-Aspartate is used in foods and pharmaceuticals. The production of aspartate from immobilized *E. coli* cells has been done since 1973. Cells immobilized in various gels including polyacrylamide or κ-carrageenan, polyurethane has been the method of choice.

Aspartic acid is made by the enzyme aspartate ammonia lyase (aspartase) that carries out the following reaction in presence of ammonium fumarate:

$$^-OOCCH{=}CHCOO^- + NH_4^+ \leftrightarrow {}^-OOCCH_2CH(NH_3^+)COO^-$$

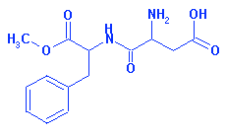
Once immobilized, the cells are quite stable retaining aspartase activity for well over 600 days even at 37°C. The process is carried out at pH 8.5 with ammonium fumarate as the substrate.

Immobilized *Pseudomonas dacunhae* cells can convert aspartate to alanine using the pyridoxal-phosphate dependent **aspartate β-carboxylase**. The reaction proceeds as follows:

$$^-OOCCH_2CH(NH_3^+)COO^- \leftrightarrow CH_3CH(NH_3^+)COO^- + CO_2$$

This process was industrialized in Japan in 1982 and is used today for making L-alanine.

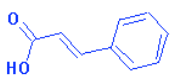
Aspartame

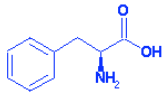


Aspartame

Aspartame is the trade name applied to a dipeptide: L-aspartyl-L-phenylananine methyl ester. It was discovered apparently by accident in 1965 by James Schlatter, a chemist working at G.D. Searle & Co. looking for new treatments for gastric ulcers. As the story goes, he spilled some intermediate on his hand and later licked his finger to pick up a piece of paper and noticed the intense sweet flavor. Aspartame is about 180 times sweeter than sucrose and, gram for gram, has about as many calories. Thus, much less is needed to provide the equivalent sweetness of sucrose. Aspartame is sold under a variety of trade names including Nutrasweet®, originally made by Searle & Co., Equal, Spoonful and Measure).

A process for making phenylalanine was developed and patented by Genex was based on older work that had demonstrated the production of phenylalanine from cinnamic acid plus ammonium ions by the activity of phenylalanine ammonia-lyase. Although known for many years, the process had not been used due to the instability of the enzyme and its inhibition of by the substrate *t*-cinnamic acid. Genex managed to improve the stability by carrying out the bioconversion under anaerobic, static conditions, and using strains of the yeast *Rhodotorula rubra*.





trans-cinnamic acid L-phenylalanine

The process of phenylalanine production is described in U.S. Patent #4,584,269. An initial aerobic growth phase is used to grow the yeast cells on complex medium that includes yeast extract. After growth, cells are induced to produce the enzyme by adding a substrate such as L-, or D, L-phenylalanine or tyrosine, casein or blood hydrolyzates. Once the enzyme has been induced, whole suspended or immobilized cells are then incubated with slow agitation under anaerobic conditions and in the presence of t-cinnamic acid (25 g/l) plus ammonium ions. Alternatively, cinnamic acid can be added as a fed batch to the stirring cells yielding about 42.7 g/l of phenylalanine.

Other amino acids

All amino acids may be produced by fermentation. Whether they will or not depends on the costs of competing technologies such as chemical synthesis or extraction from protein sources. Bacterial strains that produce amino acid are, with some exceptions, mainly derived from *Corynebacterium* sp., *Bacillus* sp. or *E. coli.* Strains used in production are wild-type natural overproducers, auxotrophic or regulatory mutants that have altered feedback inhibition pathways, or derepressed enzyme synthesis, and/or genetically engineered organisms that have multiple copies of genes encoding rate-limiting enzymes.

Table 3. Amino acid yields and producing strains*

Amino acid	Yields (% of sugar)	Organism	Genotype (Example)
L-glutamate	45-55	*C. glutamicum* (*Brevibacterium lactofermentum*)	*dtsR,* low αkg-dehydrogenase
L-Lysine HCL	40-50	*C. glutamicum*	AEC^r, Rif^r, Sm^r, AU^r,
Glycine			
L-Phenylalanine	20-25		
L-Aspartic acid			
L-Threonine	40-50	*Escherichia coli* KY10935	Thr-N, AHV^r, AEC^r, Asp^r, Lys^r, Hse^r, Thr^r, Met$^-$, altered L-Thr transport systems
L-Cysteine			
L –Alanine	45-55		
L- Glutamine			
L-Arginine	30-40	*C. glutamicum* (*C. acetoacidophilum*)	ArgHX^r, TA^r, AU^r, [MFA+CBZA]^r, Cys^r, AC^r
L- Tryptophan	20-25		
L – Valine	30-40		
L –Leucine			
L –Alanine			
L –Isoleucine	20-30		
L – Histidine	15-20		
L – Proline			
L – Serine	30-35		
L - Tyrosine			

AC = ammonium chloride; AEC = S-(2-aminoethyl)-L-cysteine; AHV = α-amino-β-hydroxyvaleric acid; ArgHX^r = L-arginine hydroxamate; Asp = L-aspartate; AU = 6-azauracil; Cys = L-cysteine; Hse = L-homoserine; Lys = L-Lysine; Met = L-Methionine; [MFA+CBZA]^r = monofluoroacetic acid – N-CBZ-L-arginine; Rif = rifampicin; Sm = Streptomycin; TA = β-(2-thiazolyl)-D,L-alanine; Thr = L-Threonine; Thr-N = Nonutilization of thr as N-source;

*From Ikeda, M. 2003. Adv. Biochem. Eng. Biotech. 79:1-35.

Controversies

Although adding a natural product like an amino acid to food might seem a simple matter, some major controversies have arisen. For example, the story of aspartame manufacture has become something of a touchstone for industrial relations in the biotechnology industry. Both aspartic acid and phenylalanine are made by fermentation. The discoverer of aspartame, G. D. Searle Co., lacked the fermentation capacity to make either phenylalanine or aspartic acid and so purchased the components from Japanese amino acid manufacturers. In 1983, as demand grew, Searle contracted with Genex Corp., a fledgling U.S. biotechnology company, to provide L-phenylalanine through a new bioreactor process. The agreement was to be a major boon to both companies. At the time (1984), Nutrasweet accounted for about half of Searle's revenue. In 1985, Searle decided not to renew its contract with Genex Corp. This decision proved to be a fatal blow to Genex because they had invested a considerable amount of money into production facilities for phenylalanine, on the expectation that Searle would continue to buy the amino acid. Lawsuits quickly followed and Searle's reputation dimmed in the public and business communities.

The health effects of aspartame have also been constroversial. After consumption, aspartame is metabolized as its component amino acids, aspartate and phenylalanine, plus methanol. This fact has led to concerns about its safety, particularly when consumed by young children. Aspartate can be metabolized as glutamate and may lead to neurological effects such as seen with MSG consumption. Phenylalanine, although an essential amino acid required in the diet, is a toxin for individuals lacking the enzyme to metabolize tyrosine, that is, those who suffer from phenylketonuria.

The consumption of some single amino acids has led to concerns over the health effects of consuming pure amino acids. For example, high levels of MSG in foods have been linked to "Chinese Food Syndrome" that includes symptoms resembling a heart attack, an understandably disconcerting side effect. Some people seem more sensitive to high levels of dietary glutamate than others. Glutamate is an excitatory neurotransmitter in the central nervous system; high levels can lead to feelings of anxiety as it competes with the related inhibitory neurotransmitter gamma-amino butyric acid.

Tryptophan has also had its share of controversies. In the 1980s, the company Showa Denko K.K. began making tryptophan using genetically engineered bacteria. The approach was to incorporate multiple copies of genes involved in tryptophan biosynthesis. Tryptophan from these organisms entered the market in 1988. Its sale was permitted since the product had been purified and tryptophan had been on the market for several years. The "recombinant" product, however, was soon linked to 37 deaths, the permanent disabling of 1500 more people and an unknown number of lesser illnesses. The problem was not discovered immediately because the tryptophan produced by recombinant organisms was not labeled as such.

Victims had elevated numbers of eosinophils and myalgia (muscle pain) in a condition known as "eosinophilia myalgia syndrome" or EMS. Over time additional symptoms developed includiing paralysis, neurological problems, skin cracking, heart problems, memory and cognitive deficits, headaches, light sensitivity, and fatigue, death and long term disability.

Eventually, the engineered bacterium was found to have produced not only tryptophan but also a dimerization product of tryptophan, a toxin called EBT (1,1'-ethylidene-bis-tryptophan). Increased tryptophan levels in the cell allowed intermediates to react with each other to produce the toxin. The toxin constituted less than 0.1% of the total weight of the product. Exacerbating the problem was the marketing by supplement manufacturers of tryptophan tablets and powders as sleep aids, antidepressants or for weight loss programs.

Despite these problems, the demand for amino acids remains strong and growing, particularly as the packaged food industry gains strength through societal demand, and the demand for food supplements continues unabated. Amino acids are now incorporated into everything from body building supplements and health-food drinks to nutrient solutions used for intravenous feeding or maintenance.

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