biotechnology of amino acid production

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Preface

In 1957 several novel bacteria with high productivity of L-glutamic acid were found in various sources, and a new fermentation method called L-glutamic acid fermentation commenced in the field of applied microbiology. Since this discovery many investigators have undertaken studies on the microbial production of other amino acids, including L-lysine, L-isoleucine, L-leucine, L-valine, L-threonine, L-aspartic acid L-alanine, L-serine, L-arginine, L-tryptophan, L-phenylalanine, L-proline and L-histidine. Over the past quarter of a century, great advances have been made in breeding microorganisms capable of hyperproduction of these amino acids.

In 1972 the first monograph on amino acid fermentation, *The Microbial Production of Amino Acids* edited by Yamada *et al.* (Kodansha), was published. This was a significant publication in the field of industrial microbiology which helped to establish amino acid fermentation subsequent to antibiotics fermentation.

Over the past few decades, microbiologists and biochemists have introduced molecular biology and genetic engineering to the field of applied and industrial microbiology. Amino acid fermentation is the practical result of biotechnology which integrates microbiology, biochemistry and chemical engineering.

This volume discusses the general aspects of amino acid fermentation fundamental to industrial use. The microorganisms and their biosynthetic pathways and related enzymes are described in detail in order to clarify the regulatory mechanism of the microbial metabolism. In particular, the various amino acids are treated separately in individual chapters, including descriptions of the development of bio-

chemical processes. The book covers general and specific aspects of industrial microbiology research, past, present and future.

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An Overview of the Microbial Production of Amino Acids

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Since the discovery of monosodium glutamate (MGS) as a seasoning by Kikunae Ikeda in 1908, demand in the food industry has increased steadily throughout the world. Formerly, it was industrially obtained from wheat, soybean and other plant proteins or from Steffen's waste. However, the fact that appreciable quantities of L-glutamic acid may be synthesized and accumulated in bacterial culture broth was first reported in Japan in 1957, and one company soon began its commercial production by a fermentative process, to be followed later by various manufacturers throughout the world.

Stimulated by this success, systematic research on amino acid biosynthesis by microorganisms quickly developed. In 1959, the Association of Amino Acid Fermentation was established by the researchers in this field, and it held annual meetings and issued a biannual bulletin in Japanese. In 1963, the name of the Association was changed to the "Association of Amino Acid and Nucleic Acid" to accommodate the new results on the fermentative production of nucleic acid-related substances such as 5'-inocinic acid and 5'-guanylic acid which were reported in 1959 and produced commercially since 1961. As commemoration publications, *The Microbial Production of Amino Acids* and *Microbial Production of Nucleic Acid-Related Substances* were published in 1972 and 1976, both by Kodansha, Tokyo. The Association merged with the Japanese Association of Industrial Fermentation in 1979. The April and October issues of its bulletin, "Fermentation and Industry" were published as special issues under the former title *Amino Acid and Nucleic Acid*. At the time of the merger, *Bibliography of Amino Acid and Nucleic Acid Fermentation* was published in 1980 covering original reports and reviews issued from 1958 to 1979.

The trends in research concerning amino acid fermentation have been summarized by the numbers of papers published as shown in Table

I. Predominant interest focused on glutamic acid fermentation. Lysine and tryptophan were also subjects of keen interest. At present, most of the usual amino acids can be produced by microbial or enzymatic methods, although these methods compete economically with

TABLE I. Recent Trend of Research Concering Biotechnology of Amino Acid Production

	~1979†	'80	'81	'82	'83	'84	'85
A. Original Report							
1. Amino acid fermentation	49	3	2	4	5	4	8
Alanine	18	2	5	3	0	2	3
Arginine	46	4	3	4	4	5	7
Aspartic acid	0	0	0	0	1	0	0
Asparagine	9	1	3	0	0	1	0
Citrulline	11	1	3	4	4	3	9
Cystine, Cystein	576	7	9	19	8	4	4
Glutamic acid	21	4	3	22	4	3	2
Glutamine	0	0	1	1	1	0	0
Glycine	21	0	0	1	0	1	3
Histidine	0	0	0	0	0	1	0
Homoserine	35	1	0	0	0	0	1
Isoleucine	84	1	4	0	1	2	0
Leucine	24	1	4	4	2	0	1
Lysine	234	14	3	4	8	6	11
Methionine	25	2	2	6	5	3	12
Ornithine	13	1	0	1	1	0	0
Phenylalanine	29	0	1	3	5	5	6
Proline	28	5	3	3	1	4	5
Serine	17	2	2	8	3	2	4
Threonine	56	1	4	1	1	1	2
Tryptophan	133	4	2	9	13	8	11
Tyrosine	34	4	2	1	0	1	1
Valine	70	0	2	0	1	0	0
2. General amino acid, industrial technology, raw material	95	16	8	15	8	2	4
3. Enzyme related to amino acid biosynthesis and metabolism	178	57	161	23	36	107	120
4. Recombinant DNA	--	7	13	12	18	62	115
5. Analytical method	34	9	36	12	7	5	6
B. Review							
1. Production	112	5	19	11	50	24	28
2. Biosynthesis and metabolic control	62	6	11	5	8	1	5
3. Application	81	2	8	8	5	2	2

† Described in *Bibliography of Amino Acid and Nucleic Acid Fermentation*, Association of Amino Acid and Nucleic Acid, Japan (1980).

other methods such as hydrolysis of natural proteins and chemical synthesis.

Microbial Production of Amino Acids

In 1957, two research groups, Kinoshita *et al.* and Asai *et al.*, reported that L-glutamic acid can be produced in culture broth in appreciable quantities. Thereafter, the ability to produce glutamic acid has been found in many microorganisms, including *Corynebacterium glutamicum*, *Brevibacterium flavum*, *B. lactofermentum*, *B. thiogenitalis* and *Microbacterium ammoniaphilum*. The general characteristics of strains giving yields greater than 30% of glutamic acid from sugars were as follows: gram positive, non-sporulating, non-motile, coccal or rod-like; all required biotin for growth. Biotin was found to play a critical role in glutamic acid fermentation. Therefore, the function of biotin in glutamic acid fermentation has been studied extensively from both academic and industrial points of view. To utilize raw materials such as molasses which contain large amount of biotin, the addition of penicillin during fermentation was found to be effective. Several detergents were also found to be useful for the same purpose. The role of biotin was found to regulate the cell membrane permeability of glutamic acid through changes in cell membrane phospholipids. Based on these studies, a new type of glutamic acid-producing mutant was induced. It is a glycerol requiring mutant of *Corynebacterium alkanolyticum*, and the cell membrane permeability of glutamic acid can be regulated by the concentration of glycerol added to the medium. Glutamic acid fermentation is very interesting as a typical example whose most important characteristic is the regulation of cell membrane permeability of the fermentation product. Studies on the regulatory mechanism of glutamate biosynthesis have also been conducted in detail.

Following the success of glutamic acid fermentation, various methods have been developed for microbial production of amino acids. These may be classified as follows :

a) Methods employing wild strains (yielding glutamic acid, alanine, valine)

b) Methods employing mutants (yielding lysine, threonine, arginine, citrulline, ornithine, homoserine, tryptophan, phenylalanine, tyrosine, histidine, etc.)

c) Precursor addition method (yielding threonine, isoleucine, tryptophan, etc.)

d) Enzymatic method (yielding lysine, aspartic acid, alanine, D-*p*-hydroxyphenyl-glycine, etc.)

At first, wild strains from various environments were screened, but microorganisms which can produce amino acids in quantities sufficient to warrant commercial exploitation were extremely limited. Glutamic acid was the exception. Since amino acids are essential components of microbial cells and their biosynthesis is teleologically regulated at an optimum level, they are normally synthesized only in limited quantities. Terminal or near-terminal amino acids are under strict metabolic control, and special means must be devised to overcome such regulation in order to produce large quantities. The most successful technique so far developed involves the induction of auxotrophic mutants, analogue-resistant mutants, or combinations of these mutations. Industrial production of L-lysine was firstly accomplished by use of homoserine/threonine double auxotrophs of *Corynebacterium glutamicum*. The induction of *S*-(2-aminoethyl)-L-cysteine (AEC)-resistant mutant of *Brevibacterium flavum* as a L-lysine-producing strain greatly stimulated the use of analogue resistant mutants thereafter.

The precursor addition method was successfully applied to L-threonine and L-isoleucine. For the former, the added substance was D-threonine in the case of *Serratia marescens*. For L-isoleucine, α-aminobutyric acid was added in the case of *Bacillus subtilis* etc. The enzymatic method involves microbial transformations and microbial enzymes. L-Aspartic acid can be produced almost quantitatively from fumaric acid and ammonia using aspartase of *E. coli*. Enzymatic production of L-lysine has been successful. DL-α-Amino-ε-caprolactum is converted almost completely to L-lysine using L-α-amino-ε-caprolactum hydrolase and α-amino-ε-caprolactum racemase of *Cryptococcus laurentii* and *Achromobacter obae*, respectively, at the same time in the same vessel. DL-5-(*p*-hydroxyphenyl) hydantoin can be transformed to D-5-(p-hydroxyphenyl) hydantoin by asymmetric hydrolysis with hydantoinase of *Pseudomonas putida*. Under a slightly alkaline condition, residual L-form is transformed to D-form by spontaneous racemization. As a result, all DL-5-(*p*-hydroxyphenyl) hydantoin can be quantitatively converted to *N*-carbamoyl-D-*p*-hydroxyphenyl-glycine, which is further converted to D-*p*-hydroxyphenylglycine by chemical decarboxylation with HNO_2 and HCl. This process has been industrialized and the product widely used as a raw material of semisynthetic antibiotics.

The present volume surveys all essential aspects of the biotechnology of amino acid production. Chapter 1 covers microbial and biochemical fundamentals, such as breeding of amino acid-overproducing mutants, intracellular genetic recombination, recombinant DNA method and related enzymes. Chapter 2 deals with biochemical engineering and fermentation technology, such as raw material, oxygen supply, automatic

analysis and computer control. The production of individual amino acids is described in detail in Chapter 3. Chapter 4 is devoted to the production of amino acid-related substances. So far as is known, no comparable treatise has yet been compiled in English.

Future Prospects

Any consideration of the future prospects of mass industrial production of amino acids by microbial or enzymatic processes cannot ignore the rival methods of chemical synthesis and protein hydrolysis. For example, DL-methionine, which is effectively used to strengthen animal fodder in large quantities, is produced by chemical synthesis. However, biotechnological methods have the imoprtant advantage of directly yielding the optically active L-form of amino acids.

Both amino acid fermentation and nucleic acid fermentation have been investigated mostly in Japan, and they are representative in the fermentation industry. Such unique techniques as the utilization of various kinds of mutants, increased permeability of cell membrane, artificial regulation of microbial metabolism, and the combination of enzymatic reaction and chemical synthesis have been developed. Over the past decade, significant progress has been made in the utilization of immobilized enzymes and cells for the microbial production of amino acids. Half-life of aspartase activity of immobilized *E. coli* cells increased to 680 days in a continuous enzymatic reaction system. Breeding of amino acid-overproducing strains has reached a state of very high development. Application of intracellular genetic recombination to strain improvement has been successful in several cases and recombinant DNA method and cell fusion method have been effectively applied to strain improvement in amino acid fermentation. It is reported that in the USSR L-threonine has been industrially produced by *E. coli* whose productivity of L-threonine was improved by recombinant DNA technique in 1979. The establishment of host-vector systems in genera *Brevibacterium* and *Corynebacterium* will further open new prospects in this field.

A principal factor which will control the future development of the amino acid fermentation industry is the exploitation of new uses for the amino acids produced. Efforts in this direction will be necessary in conjunction with basic research in the biotechnology of amino acid production.

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