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RECENT PROGRESS IN MICROBIAL PRODUCTION OF AMINO ACIDS

by Hitoshi Enei
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Preface to the Series

Modern technology has a great impact on both industry and society. New technology is first created by pioneering work in science. Eventually, a major industry is born, and it grows to have an impact on society in general. International cooperation in science and technology is necessary and desirable as a matter of public policy. As development progresses, international cooperation changes to international competition, and competition further accelerates technological progress.

Japan is in a very competitive position relative to other developed countries in many high technology fields. In some fields, Japan is in a leading position; for example, manufacturing technology and microelectronics, especially semiconductor LSIs and optoelectronic devices. Japanese industries lead in the application of new materials such as composites and fine ceramics, although many of these new materials were first developed in the United States and Europe. The United States, Europe, and Japan are working intensively, both competitively and cooperatively, on the research and development of high-critical-temperature superconductors. Computers and communications are now a combined field that plays a key role in the present and future of human society. In the next century, biotechnology will grow, and it may become a major segment of industry. While Japan does not play a major role in all areas of biotechnology, in some areas such as fermentation (the traditional technology for making "sake"), Japanese research is of primary importance.

Today, tracking Japanese progress in high-technology areas is both a necessary and rewarding process. Japanese academic institutions are very active; consequently, their results are published in scientific and technical journals and are presented at numerous meetings where more than 20,000 technical papers are presented orally every year. However, due principally to the language barrier, the results of academic research in Japan are not well known overseas. Many in the United States and in Europe are thus surprised by the sudden appearance of Japanese high-technology products. The products are admired and enjoyed, but some are astonished at how suddenly these products appear.

With the series *Japanese Technology Reviews,* we present state-of-the-art Japanese technology in five fields:

Electronics,
Computers and Communications,
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Biotechnology.

Each tract deals with one topic within each of these five fields and reviews both the present status and future prospects of the technology, mainly as seen from the Japanese perspective. Each author is an outstanding scientist or engineer actively engaged in relevant research and development.

We are confident that this series will not only give a bright and deep insight into Japanese technology but will also be useful for developing new technology of our readers' own concern.

As editor in chief, I would like to acknowledge with sincere thanks the members of the editorial board and the authors for their contributions to this series.

Toshiaki Ikoma

Preface

With special emphasis on recent advances, this book describes the aspects of the microbial method of amino acid production according to the following methods: (1) the breeding of amino acid-producing microorganisms, (2) the direct fermentation method, (3) the precursor addition method, (4) the enzymatic method, and (5) the biochemical engineering aspects of microbial production of amino acids.

Contents

CHAPTER 1

Introduction

Amino acids are the basic building units of protein, and essential nutrients for living organisms. Demand for amino acids has been therefore spread over many areas such as medicines, food additives, feed additives and fine chemicals. As a result, amino acid industries grow and have come to occupy an important role in world chemical industries. The annual production of amino acids in the world has an estimated value of about US $\$2.0 \times 10^9$ or over 650,000 t in 1986[1]. Monosodium glutamate, a well-known flavor enhancer, is still the predominant product, with a worldwide annual production of about 340,000 t. About 250,000 t of DL-methionine and 70,000 t of L-lysine are produced for animal feed additives. The demand for other amino acids is small, mostly in the pharmaceutical field. However, the demand will grow with progress in production technology to reduce costs, and on further expansion of their uses (see Table 1-1): for example, tryptophan and threonine have been (Table 1-1) used as animal feed additives recently.

Production technology of amino acids are classified into: (1) the extraction method from protein-hydrolyzate, (2) chemical synthesis and (3) microbial methods. Production methods differ individually, depending on the kinds of amino acids. Early on, the extraction method was used mainly, Next, the synthetic method and the microbial method were introduced and each amino acid had come to be manufactured by the most advantageous method. Microbial process, however, is dominant in industrial production, especially when optically active compounds are needed. Therefore, many amino acids are currently produced in commercial quantities through the microbial method. Microbial method can be divided into three processes: (a) direct fermentation of amino acids from carbon and nitrogen sources such as glucose and urea, (b) precursor addition method that converts a supplemented precursor to a

Table 1-1. Annual production of amino acids in the world (1986)[1].

Amino acids	Production (T/Y)		Production methods				
	Japan	World	F	E	C	C+R	Ex
Glycine	3,500	6,000			●		
L-Alanine	150			●		●	
DL-Alanine	1,500				●		
L-Aspartic acid	2,000	4,000		●			
L-Asparagine	30			●			●
L-Arginine	700	1,000	●				●
L-Cysteine	300	1,000		●			●
L-Glutamic acid	80,000	340,000	●				
L-Glutamine	850		●				
L-Histidine	250		●				
L-Isoleucine	200		●				
L-Leucine	200						●
L-Lysine	30,000	70,000	●	●			
L-Methionine	150					●	
DL-Methionine	30,000	250,000			●		
L-Ornithine	70		●				
L-Phenylalanine	1,500	3,000	●			●	
L-Proline	150		●				●
L-Serine	60		●				●
L-, DL-Threonine	200		●		●		
L-, DL-Tryptophan	250		●	●	●	●	●
L-Tyrosine	60						●
L-Valine	200		●			●	

(F) Fermentation, (E) Enzymatic method, (C) Chemical method
(R) Optical resolution of DL-amino acids, (Ex) Extraction

corresponding amino acid during cultivation, and (c) enzymatic conversion of economical substrates into amino acids.

Almost all amino acids are produced economically at commercial production by the direct fermentation from cheap carbon sources such as glucose, sucrose, cane molasses, beet molasses and so on. In this method, the breeding of amino acid-producing microorganisms is the important factor to get the successful yield of amino acids from carbon sources. Since the synthesis of amino acids is restricted to the amounts needed for cell growth within normal wild-type microbial cells and the wasteful overproduction of amino acids is prevented by feedback inhibition and repression, various genetic mutants, free from the regulatory mechanisms, are used in direct fermentation. Recently, many techniques for the breeding of amino acid-producers have been developed remarkably in artificial mutation, genetic manipulation in vivo, DNA recombination, and so on.

On the other hand, some limited kinds of amino acids are now produced commercially by the precursor addition method and the enzymatic method. These methods are a better means to avoid the undesirable regulation of amino acid synthesis within cells, but they need to use the low cost substrates converted to the corresponding amino acids. Recently, the application of these methods tends to increase for commercial production with the progress of processes to synthesize chemical substrates. In addition, recent progress in the technology of enzyme or cell immobilization made these processes even more convenient.

In order to industrialize the microbial processes for the production of amino acids, the techniques of large-scale cultivation were required. These problems concerning biochemical engineering include: (1) maintenance of pure culture (sterilization of equipment media and air); (2) automatic control of pH, temperature and foaming; and (3) agitation and aeration. These technologies were developed during the course of industrialization of penicillin fermentation, and successfully applied to the fermentation of amino acids.

With a special emphasis on recent advances, this paper describes the aspects of the microbial method of amino acid production according to the following items; (1) the breeding of amino acid-producing microorganisms, (2) direct fermentation method, (3) precursor addition method, (4) enzymatic method, and (5) biochemical engineering aspects of microbial production of amino acids.

For a more detailed background, there are excellent reviews of the subject by Aida et al.[2], Yamada et al.[3], Tsunoda and Okumura[4], Kisumi[5], Kinoshita[6], Nakayama[7], and Yamada[8].

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