ADVANCES IN BIOTECHNOLOGY

Volume III

Fermentation Products

General Editor
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PREFACE

Biotechnology is a multidisciplinary field which developed from interrelated activities in Biology, Chemistry and Engineering. It has its roots in microbial chemistry and technology as practiced in the fermentation industry for several decades. In recent years, revolutionary advances in genetic engineering, fused-cell technology, immobilized systems, etc. and the necessity for better utilization of renewable resources, improvement of health care and management of the environment have focused attention on biotechnology. In recognition of this rapid evolution, the International Union of Pure and Applied Chemistry (IUPAC) has decided to change the name of the conference series "International Fermentation Symposium" which it has sponsored every four years since 1960 to "International Biotechnology Symposium" commencing with the 1984 conference. The main title of the present publication reflects this change.

"Fermentation Products" is the third volume of a three-volume series entitled "Advances in Biotechnology - Recent International Developments". The series is based on the proceedings of the Sixth International Fermentation Symposium (IFS-6), which was held in London, Ontario, Canada, July 20-25, 1980. The first volume deals with "Scientific and Engineering Principles" and the second volume with "Fuels, Chemicals, Foods and Waste Treatment". It is hoped that students and researchers in industrial microbiology, biochemical engineering and related areas, as well as managers of biotechnology, will find in "Advances in Biotechnology" a valuable source of information.

In this volume, we present 87 of the 112 papers which were submitted on the fermentation of Antibiotics (Section II), Amino Acids, Vitamins and Nucleotides (Section III), Microbial Enzymes (Section IV), Products from Immobilized Cells and Enzymes (Section V), Mycotoxins (Section VI), Biopolymers (Section VII), and Bioconversions (Sections VIII and IX). Twelve papers which deal with recent developments in the field of secondary metabolism (functions of secondary metabolites in the producing organisms, roles of plasmids in the biosynthesis of secondary metabolites and protoplast fusion in industrial microorganisms) are included in Section I. We also have included the invited keynote address of Professor Hamao Umezawa "Problems and Trends in the Development of New Antibiotics and Other Useful Microbial Products".

The remaining manuscripts were either withdrawn by the authors or rejected on the basis of peer evaluation or editorial policy on manuscript length, subject matter or revision deadlines. Because of the international origin of the manuscripts submitted, more attention was given by the reviewers to the technical merit of the manuscripts than to the correct use of the English language. We have made no changes in technical content unilaterally, but editorial modifications were often necessary to achieve consistent style and format of presentation.

A companion volume to this series entitled "Current Developments in Yeast Research" is based on papers presented at the Fifth International Symposium on Yeasts (ISY-5) which was held in London, Canada concurrently with IFS-6. The Table of Contents of "Current Developments in Yeast Research" has been included at the end of this volume as a service to our readers.

In the preparation of Volume III, invaluable assistance was received from persons too numerous to identify individually. Special mention should be made of the Editorial Board members (named elsewhere in this volume) who reviewed the manuscripts. We also wish to thank our colleague, Dr. S.N. Sehgal, who reviewed and revised several manuscripts. Finally, we thank our secretary, Micheline Reeves, for her dedicated assistance in preparing the manuscripts for publication.

March, 1981
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Claude Vézina
Kartar Singh

GUEST EDITORIAL

PROBLEMS AND TRENDS IN THE DEVELOPMENT OF NEW ANTIBIOTICS AND OTHER USEFUL MICROBIAL PRODUCTS

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ABSTRACT

Antibacterial antibiotic research entered its second era in about 1957 to develop novel chemotherapeutic agents useful in treatment of resistant infections. Successful results have been obtained by the studies with β-lactam and aminoglycoside antibiotics on the basis of biochemical mechanism of resistance. These studies will continue also in the future. Antimicrobial antibiotic research was expanded to include antitumor antibiotics in about 1953 and enzyme inhibitors with medicinal activities in about 1969. Studies of derivatives and analogs of adriamycin, bleomycin etc. have led to the development of more effective anti cancer chemotherapeutic agents. The continuation of screening with newly devised procedures will also provide us with effective anticancer agents. Genetics of the production of secondary metabolites is being studied and microorganisms have been shown to be the treasury of compounds with various medicinal activities. Development of microbial products with new medicinal activities is growing to be a big research area, as shown by the finding of small molecular weight immunomodulators. Taking the author's studies as examples, the development of useful antibiotics and other microbial products was described and discussed.

KEYWORDS

Trends in antibiotic research.

INTRODUCTION

Since 1944, I have been studying new antibiotics, and in the last 30 years, the study of antimicrobial antibiotics was expanded to the development of effective derivatives on the basis of biochemical mechanisms of resistance, the development of antitumor antibiotics and their derivatives useful in cancer treatment, and the development of enzyme inhibitors which have various biological, pharmacological and medicinal activities, and I am glad I could contribute to the opening of these new exciting research areas.

Antibiotics and small molecular weight enzyme inhibitors are secondary metabolites which are not involved in the growth of microbial cells. The studies of these secondary metabolites have indicated that microorganisms are the treasury of

organic compounds of various structures which have potential usefulness in the treatment of human, animal and plant diseases. Moreover, the reason why almost unlimited number of various organic compounds are produced by microorganisms has already been studied and is partially understood. Therefore, studies of new antibiotics and other microbial products will be continued also in the future and will stimulate progress in medical and biological sciences.

In this paper, I will review recent studies on the development of new useful secondary metabolites and their derivatives and discuss the present problems.

ANTIBACTERIAL ANTIBIOTICS AND THEIR DERIVATIVES AND ANALOGS

By 1957, resistant staphylococci and Gram negative bacteria appeared in patients and there were even infections caused by strains resistant to all drugs used at that time. Therefore, the screening of new antibacterial antibiotics was continued, and kanamycin was discovered in 1957 and gentamicin in 1963; each was introduced into clinical use. Thereafter, successful results in the development of new chemotherapeutic agents have been obtained by the study of derivatives more than by the screening for new types of antibiotics.

The isolation of 6-aminopenicillanic acid by Batchelor and others (1959) opened up the research area of the chemical synthesis of new penicillins, and the elucidation of the structure of cephalosporin C by Abraham and Newton (1961) expanded the research area of β-lactam antibiotics. Moreover, by the screening of β-lactam antibiotics, cephamycin, thienamycin etc. were discovered in culture filtrates of actinomycetes. The feedback of these screening results to chemistry stimulated the development of new types of β-lactam compounds. Thus, up to now numerous useful β-lactam compounds have been developed. For instance in Japan, 33 β-lactam compounds are marketed and soon more than 10 new β-lactam compounds will be marketed in Japan (Kuwabara, 1979): penicillin analogs: bacampicillin, apalcillin, mezlocillin, PL-385, BL-P1908, TEI-1194, TEI-2012, TA-058; cephalosporin C analogs: cefotiam (SCE963), cefsulodine (SCE129), cefaclor, cefadroxil (BL-S578), CGP-9000, cefamandole, cefonicid (SK and F75073), cefuroxime, SCE-1365, cefotaxime (HR756), ceftizoxime (FK-749, FR-13749), cefoperazone (T-1551), SM-1652, GR-20263; cephamycin analogs: YM-09330, 6059-S. Moreover, on the basis of the involvement of β-lactamases in the resistance mechanism, inhibitors of β-lactamases have been developed by chemical synthesis and by the screening of active fermentation broths: by fermentation: clavulanic acid, thienamycin, epithienamycins A, B, C and D, olivanic acids [MM4550 (MC696-SY2-A), MM13902, MM17880, MM22380, MM22381, MM22382, MM22383], PS-5; by chemical synthesis: Δ^2-deoxyclavulanic acid, CP45899, 6-β-bromopenicillanic acid, 6-α-chloropenicillanic acid sulphone; N-formimidoylthienamycin, N-acetimidoylthienamycin. One of them, clavulanic acid in clinical study was confirmed to exhibit therapeutic effect in combination with ampicillin, etc.

In 1967, the author was successful in the elucidation of the biochemical mechanism of resistance to aminoglycoside antibiotics (Umezawa, H. and others, 1967) and a new exciting research area was opened for the development of new aminoglycosides active against resistant strains. Resistance of many resistant strains of staphylococci and Gram negative organisms was due to 3'-phosphotransferase which transferred the terminal phosphate of ATP to the 3'-hydroxyl group of kanamycin, neomycin, paromomycin, and this enzyme reaction suggested that 3'-deoxy-derivatives would inhibit the growth of resistant strains. This was proved by chemical synthesis of 3'-deoxykanamycin A (Umezawa, S. and others, 1971) and 3',4'-dideoxykanamycin B (Umezawa, H. and others, 1971). The latter has a low ototoxicity and has been introduced into the clinic. The study of the reaction of 3'-phosphotransferase suggested that besides the elimination of

the 3'-hydroxyl group, the modification of the group which was involved in the binding to the enzyme would give derivatives active against resistant strains (Umezawa, H., 1970). Such derivatives have been synthesized (see the review by Umezawa, H., 1974 and papers presented to Aminoglycoside Symposium, Umezawa, H. and others, 1979b), and amikacin and netilomycin have been introduced into the clinic. As described by the author in a review (Umezawa, H., 1979b), the following enzymes are known to be involved in the mechanism of resistance: enzymes involved in resistance to 2-deoxystreptamine-containing antibiotics: 3'- and 2"-phosphotransferases, 2"- and 4'-adenylyltransferases, 2'- and 3-acetyltransferases, 6'-acetyltransferases; those in streptomycin resistance: 3"-phosphotransferase, 3"-adenylyltransferase, 6-phosphotransferase and 6-adenylyltransferase. All these enzymes catalyze the reaction of an aminoglycoside antibiotic with ATP or acetyl-CoA. Therefore, all enzymes should have two binding sites: one to the adenosine moiety of ATP or acetyl-CoA and the other to the aminoglycoside. It may be said that these enzymes differ from each other in the position of their two binding sites. These enzymes can be assumed, therefore, to be related to each other in their evolution. This suggests the possible future appearance of new phospho- and adenylyltransferases or acetyltransferases which transfer phosphate or AMP to the 2'-, 4"- or 6"-hydroxyl group of deoxystreptamine-containing antibiotics or acetyl group to the 1- or 3"-amino group. I, therefore, was interested in the active derivatives which had the least number of hydroxyl groups of deoxystreptamine-containing antibiotics. 3',4',4",6"-Tetradeoxyamikacin [amikacin is (1S-4-amino-2-hydroxybutyryl)kanamycin A] was synthesized and confirmed to be active against resistant strains (Umezawa, H., 1979b; Miyasaka and others, 1980). Elimination of the 5-hydroxyl group of the deoxystreptamine moiety or its epimerization does not eliminate the antibacterial activity (Umezawa, H., 1979b). Furthermore, recently Daniels and others (1979) reported that the 2'-deoxy and 2-deamino derivatives such as 1-N-(2S-3-amino-2-hydroxypropionyl)-2',3'-dideoxygentamicin B and 1-N-(2S-3-amino-2-hydroxypropionyl)-2'-desaminosisomycin had excellent antibacterial activity.

Streptomycin is the first useful streptomyces antibiotic found by Waksman in 1944. 3"-Adenylyltransferase and 3"-phosphotransferase (see the review by Umezawa, H., 1974) have been confirmed to be involved in the resistance mechanism by synthesis of 3"-deoxydihydrostreptomycin which inhibited the growth of resistant strains (Sano and others, 1976). The other enzyme, 6-phosphotransferase, was also shown to be involved in the resistance of many Pseudomonas strains (see the review, Umezawa, H., 1979b). Thus, the modification or elimination of the 6-hydroxyl group of 3"-deoxy-derivatives of streptomycin and dihydrostreptomycin is suggested in order to obtain derivatives active against resistant strains including Pseudomonas aeruginosa.

In the last 5 years, fortimicin group antibiotics [fortimicin, sporaricin, istamycin, dactimicin (SF2052)] were discovered by 4 Japanese research groups (Umezawa, H., 1979b). These aminoglycosides inhibit the growth of Gram positive and negative bacteria except Ps. aeruginosa. They undergo the action of 3-acetyltransferase. The study of their derivatives will give effective chemotherapeutic agents.

As described above, β-lactam compounds and aminoglycosides active against resistant strains have been developed on the basis of biochemical resistance mechanisms. In this connection, it may be said that if biochemical mechanisms of resistance to tetracyclines and macrolides are investigated in sufficient detail, then, new research areas for the development of useful derivatives and analogs will be opened. Chemotherapy of tuberculosis seems to be completed by the development of rifampicin in 1966. In contrast to antibacterial antibiotics, there has been no successful development of useful antifungal antibiotics in

the last 20 years. It is necessary to study new antifungal antibiotics, because fungal infections have become a major problem to the elderly patients with leukemia undergoing chemotherapy.

ANTITUMOR ANTIBIOTICS AND THEIR DERIVATIVES

Also in case of antitumor antibiotics, besides the screening of new antibiotics, cancer chemotherapeutic agents are being developed by the study of derivatives and analogs of those which have shown marked therapeutic effect. Adriamycin derivatives and analogs which do not cause irreversible heart muscle degeneration and have the same degree of the effect as adriamycin should be useful to increase the rate of cure for certain cancers. Therefore, the study of anthracyclines has been continued in a large scale by Arcamone (1977) who discovered adriamycin as well as many other researchers. It is said that NCI, U.S.A. has tested over 600 anthracyclines up to the present time. If anthracyclines studied in the author's institute are taken as examples, interesting results have been obtained on aclacinomycin and 4'-O-glycosidic derivatives of adriamycin.

Aclacinomycin (Oki and others, 1975) has been confirmed by Mathé (Dantchev and others, 1979) and NCI to have a markedly less cardiac toxicity in hamsters and rabbits than adriamycin, and its low cardiac toxicity to human has been shown by its clinical study. This coincidence of the experimental results with the clinical observation indicates that the animal cardiac toxicity test can provide us with the information useful to predict the degree of cardiac toxicity of new anthracyclines to patients. Aclacinomycin which had a weaker activity in prolonging the survival period of mice bearing L-1210 leukemia than adriamycin showed weaker clinical effects against human solid tumors than adriamycin, but aclacinomycin exhibited therapeutic effect on leukemia and lymphoma. It is especially noteworthy that aclacinomycin exhibited therapeutic effect against leukemia resistant to the treatment with other drugs.

Among baumycins A1, A2, B1 and B2 found in cultured broths of a daunomycin-producing strain (Komiyama and others, 1977), A1 showed a strong activity against L-1210 mouse leukemia in experiments in the author's laboratory. A1 is a 4'-O-glycosidic derivative of daunomycin. Therefore, the author studied the effect of 4'-O-glycosidic derivatives against L-1210. In general, 4'-O-glycosidic derivatives of adriamycin showed a stronger effect against L-1210 than the corresponding derivatives of daunomycin. Among these adriamycin derivatives, 4'-O-tetrahydropyranyl and 4'-O-tetrahydrofuranyl derivatives showed the strongest activity (Umezawa, H. and others, 1979a). One of the isomers of the former was shown to be more active than the latter. The cardiac toxicity test of this derivative by Mathé (Dantchev and others, 1979) indicated that it had a low cardiac toxicity similar to that of aclacinomycin. This derivative was therefore selected as worthy of further study. In the same dose this derivative showed the similar degree of activity against L-1210 as adriamycin and its maximum effect in the prolongation of the survival period was stronger than that of adriamycin, because it has a lower toxicity to mice than adriamycin and a large dose can be administered. It is certain that more effective chemotherapeutic agents than adriamycin are being developed based on the studies announced with anthracycline antibiotics.

On the basis of the mechanism of therapeutic action, more effective new bleomycins have been developed (Umezawa, H., 1976a). The structure of bleomycin proposed in 1978 (Takita and others, 1978b) has been confirmed by ^{15}N-nmr (Naganawa and others, 1979) and the mass spectroscopy. The structure of bleomycin ferrous complex proposed in 1978 (Takita and others, 1978a) has also been confirmed by nmr study of Sugiura at the Faculty of Pharmaceutical Sciences, Kyoto University and Morishima at the Faculty of Engineering, Kyoto University. The bithiazole

moiety of bleomycin binds selectively to the guanine moiety of DNA and the terminal amine is also involved in the binding with DNA. The oxygen molecule which binds to the ferrous ion of bleomycin ferrous complex bound to DNA is activated and this activated oxygen or free radicals produced react with the deoxyribose moiety of DNA. This reaction results in strand scission. Bleomycin when injected binds with cupric ion in blood and after penetrating into cells, the cupric ion of bleomycin copper complex is reduced and the cuprous ion thus formed is transferred to an intracellular cuprous ion-binding protein (Takahashi and others, 1977). Copper-free bleomycin thus produced in cells undergoes the hydrolysis by bleomycin hydrolase which cleaves the α-aminocarboxamide bond of the pyrimidoblamyl moiety of bleomycin. Bleomycin-copper complex is resistant to this enzyme reaction. Some copper-free bleomycin which escapes this enzyme reaction reaches the nucleus and reacts with DNA. Therefore, the modification of the α-aminocarboxamide moiety gives bleomycins resistant to bleomycin hydrolase. New bleomycins which are resistant to bleomycin hydrolase and may have a new antitumor spectrum are under development. This study has been stimulated by the fact that labeled metal complexes of bleomycin are selectively taken into human malignant tumor and used for diagnosis of cancer (Nouel, 1976).

Various bleomycins containing different terminal amines differ from one another in the degree of renal and pulmonary toxicity. New bleomycins which have lower pulmonary toxicity should produce a stronger therapeutic effect than the present bleomycin. A method of testing pulmonary toxicity to mice has been established and bleomycins which have lower pulmonary toxicity have been selected. Bleomycin PEP (pepleomycin) thus selected has been confirmed by clinical study to show a stronger therapeutic effect and lower pulmonary toxicity than the present bleomycin. Moreover, pepleomycin has been confirmed not only to be effective against squamous cell carcinoma and Hodgkin's tumor which are sensitive to the present bleomycin treatment, but also effective against prostatic carcinoma. The continuation of the study of derivatives and analogs of bleomycin or bleomycin-group antibiotics will result in the development of cancer chemotherapeutic agents which have a stronger activity and a wider anticancer spectrum than the present bleomycin.

Recently, a new antitumor antibiotic named bactobolin was found in culture filtrates of a Pseudomonas. sp. (Kondo and others, 1979). Bactobolin is structurally related to actinobolin produced by streptomyces. Bactobolin inhibited selectively B lymphocyte blastogenesis. In conforming with this, bactobolin is much more effective against L-1210 leukemia than against mouse T cell leukemia (El 4). This suggests that this antibiotic may be worthy of clinical test against B cell type lymphoma and leukemia.

More than 27 years have passed since the author reported the first successful screening results of antitumor antibiotics. In the early period of the study, culture filtrates or their extracts were tested for their effects in inhibiting Ehrlich carcinoma, sarcoma 180, Walker carcinoma or Yoshida rat sarcoma or the growth of their cells in vitro. Thereafter, in about 1960, L-1210 mouse leukemia and other experimental mouse tumors were introduced into the screening. Methods testing the mutagenic activity and the biochemical methods testing the inhibition of DNA synthesis etc. were also introduced as screening methods. Methods of testing the selective effect in inhibiting malignant transformed cells have also been used for the screening. About 10 years ago, B16 melanoma and Lewis lung carcinoma and recently colon carcinoma and human tumors inoculated to nude mice were introduced into the screening for the selection of antitumor compounds worth clinical study. Therefore, it is possible that not only from new antitumor compounds but also from known antibiotics those worth clinical study will be selected and effective cancer chemotherapeutic agents will be developed. In this connection, it should be emphasized that the development of new efficient screening

methods are still the greatest problem even at present for the development of antitumor antibiotics useful in cancer treatment.

SMALL MOLECULAR WEIGHT ENZYME INHIBITORS AND IMMUNOMODULATORS

By 1965, nmr has already been introduced into chemistry and the structure determination has become much easier. Therefore, the author started the screening of small molecular weight inhibitors of various enzymes. Up to now in my laboratories we have found about 50 enzyme inhibitors (Umezawa, H., 1972, 1976b, 1977). For instance, leupeptin inhibiting plasmin, trypsin and cathepsin B, antipain inhibiting trypsin, papain, cathepsins A and B, chymostatin inhibiting chymotrypsin, elastatinal inhibiting pancreas elastase, pepstatin inhibiting pepsin, cathepsin D and renin, phosphoramidon inhibiting metalloendopeptidases were discovered. Oudenone inhibiting tyrosine hydroxylase, isoflavone compounds inhibiting dopa decarboxylase, fusaric acid and dopastin inhibiting dopamine β-hydroxylase showed hypotensive effect against hypertension of spontaneously hypertensive rats. My study of enzyme inhibitors has been watched with interest by other antibiotic researchers, and the compound ML-236B which inhibited the mevalonate synthesis was found by Endo and others (1976) and has been confirmed to be useful in the treatment of cholesterinemia. An amylase inhibitor found by Schmidt and others (1977) has been reported to prevent the raise of blood sugar.

Recently I extended the study of enzyme inhibitors to immunomodulators. In 1972, the administration of a very small dose of diketocoriolin B was found to increase the number of mouse spleen cells producing antibody against sheep red blood cells (Ishizuka and others, 1972). On the other hand, diketocoriolin B inhibited Na^+-K^+-ATPase (Kunimoto and others, 1973). Therefore, I thought that diketocoriolin B might bind to the ATPase in the membrane of cells involved in immune responses. I assumed that the screening of even small molecular compounds binding to the cell membrane or cell surface would result in the finding of immunomodulators. I searched for inhibitors of enzymes on the cell surface. In this study, all aminopeptidases were found to be located not only in cells but also on the cell surface without extracellular release (Aoyagi and others, 1976). Alkaline phosphatase and esterase were also found to be located on the cellular surface. At the same time, bestatin was discovered by the study of protease inhibitors (Umezawa, H. and others, 1976a). Bestatin inhibited aminopeptidase B (Ki, 6.0×10^{-8}M) and leucine aminopeptidase (Ki, 2.0×10^{-8}M). As expected, bestatin in its wide range of doses (0.1 - 100 μg/mouse) enhanced delayed-type hypersensitivity to sheep red blood cells and oxazolone (Umezawa, H. and others, 1976b; Umezawa, H., 1979a). Its high dose, 1,000 μg/mouse, increased the number of antibody-forming cells.

Amastatin inhibiting aminopeptidase A (Ki, 1.5×10^{-7}M) and leucine aminopeptidase (Ki, 1.6×10^{-6}M) (Umezawa, H., 1979a), forphenicine inhibiting chicken alkaline phosphatase (Ki, 1.6×10^{-7}M) (Aoyagi and others, 1978) and esterastin inhibiting esterase (Ki, 1.6×10^{-10}M) were discovered (Umezawa, H. and others, 1978a). Amastatin increased the number of antibody forming cells. Forphenicine enhanced delayed-type hypersensitivity and increased the number of antibody-forming cells. Esterastin suppressed both delayed-type hypersensitivity and antibody-formation. The binding to esterase is not the cause for the suppression, because other esterase inhibitors found thereafter enhanced delayed-type hypersensitivity and antibody-formation.

Bestatin and forphenicine showed antitumor activity against solid tumors of Gardner lymphosarcoma and IMC carcinoma. These inhibitors have mitogenic effect on lymphocytes *in vivo* and *in vitro* (Ishizuka and others, 1980a, 1980b). The selective effect of bestatin on T cell blastogenesis has been confirmed biochemically by

Müller and others (1979). Bestatin has been clinically studied and the following results have been obtained by Japanese clinical investigators, Blomgren and others at Radiohemmt, Karolinska Institute, and Mathé and others at the Institute of Cancerology and Immunology, Villejuif: 1) reduced percentage of T cells in cancer patients is restored to the normal value (dose: 10 - 100 mg daily); 2) reduced NK cell activity in cancer patients is restored (30 or 60 mg daily); 3) round cell infiltration around or into the cervix cancer nest is observed after 4 - 6 weeks of bestatin treatment (30 mg daily orally); 4) bestatin (30 mg, orally daily) improved the bone marrow cell picture of cancer patients; 5) in high frequency skin reactions turned positive during bestatin treatment (30 or 60 mg daily); 6) during phase 1 study, some favourable effects of bestatin on cancer have been noted; 7) the frequency of infections seems to be lower in cases treated with bestatin than without bestatin. Bestatin has been selected by the NCI Biological Modifier Program to undergo additional in vivo and in vitro studies.

The studies of immunomodulators is a recent successful example indicating that the screening study using a quantitatively exact method can give the compounds which have the aimed bioactivity.

GENETICS OF PRODUCTION OF SECONDARY METABOLITES AND THE SCREENING

Generally, it is said that microorganisms in nature compete with each other and produce antibiotics to suppress the growth of their competitors. However, all enzyme inhibitors have had no significant antimicrobial activity. The study of the reason why numerous various organic compounds are produced by microorganisms as secondary metabolites may help in the development of new useful microbial products.

From 1963 to 1967, when we worked to increase the fermentation yield of kasugamycin, I felt that the improvement of antibiotic - producing strains should be studied from the fundamental view point. I, therefore, studied the possible involvement of plasmid in the production of antibiotics. Then, the kasugamycin-producing ability was eliminated by acriflavine treatment (Okanishi and others, 1970). In 1975, Hopwood reported the involvement of plasmid in the production of methylenomycin (Kirby and others, 1975; Wright and Hopwood, 1976). In the case of a chloramphenicol-producing strain, we observed that the genes involved in chloramphenicol biosynthesis are located in chromosome and there was plasmid gene which increased the production (Akagawa and others, 1979). The treatment of an aclacinomycin-producing strain with acriflavine gave many mutants which markedly decreased the ability to produce anthracyclines, suggesting a possible presence of plasmid gene which increases the production yield. The leupeptin-producing ability of a mutant was transferred to a leupeptin-nonproducing mutant by conjugation (Umezawa, H. and others, 1978b).

On the basis of the structures, antibiotics can be divided into various groups, each containing a characteristic structural part common to the same group. Antibiotics of the same group are produced by strains belonging to different species or by strains belonging to different genus or family. For instance, 2-deoxystreptamine-containing compounds are produced by strains belonging to streptomyces, nocardia, micromonospora and eubacteria. On the basis of these facts, I proposed that gene involved in the biosynthesis of a characteristic structural part of a group of antibiotics, for instance, the 2-deoxystreptamine-producing gene, is widely distributed among various microorganisms. Many kanamycin-producing mutants obtained by acriflavine treatment produce kanamycin in media to which 2-deoxystreptamine was added, suggesting the presence of a gene involved in 2-deoxystreptamine biosynthesis (Hotta and others, 1977; Umezawa, H., 1977).

For the production of antibiotics I proposed as follows (Umezawa, H., 1977): a gene involved in the biosynthesis of a characteristic structural part of a group of antibiotics was generated; the characteristic structural part which has no cytotoxicity is transformed to final products which are released extracellularly; in different strains, this is transformed to different final products; in some or many cases, there are plasmid genes which increase the production yield of final products; in some cases, plasmid genes are involved in the biosynthesis of the characteristic structural part.

In the development of new useful antibiotics and other microbial products, whether successful results are obtained or not is dependent on the strains examined and the screening method. In the case a traditional screening method is employed, strains which are examined should be collected freshly from nature or selected from the genus and family which have not yet been examined. Microorganisms from new sources such as marine mud etc. are also worth examination. As shown by discovery of new β-lactam antibiotics in the last 10 years, if a sensitive screening method is employed, then new types of a known group of antibiotics can be discovered.

As discussed in the paragraph of antitumor antibiotics, in the case of the development of cancer chemotherapeutic agents, it is still necessary to establish new screening methods on the basis of cancer biology and biochemistry. As shown by the study of immunity-enhancing compounds, if an exact screening method is established, it is reasonable to search for the microbial products with the aimed bioactivity. Parallel to the progress of biochemistry of diseases, the development of microbial products which have new useful medicinal activities will grow to a great research area extended from antibiotic studies. The application of gene engineering to the development of new useful products is a present interesting study. In the coming era, it will be successful to produce new genes artificially for the development of new antibiotics and other microbial products.

In the last part of this paper, it may be necessary to discuss the development of microbial secondary metabolites useful in agricultural areas. Kasugamycin and validamycin are used for prevention of rice plant diseases. Besides medical antibiotics, an enormous amount of a polyether antibiotic is used as feed supplement. Parallel to the progress in biological sciences, the area of the development of new useful antibiotics and other microbial products will be expanded.

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