POTENTIAL ROLE OF BIOTECHNOLOGY IN FOOD AND HEALTH PROBLEMS OF ASEAN COUNTRIES

GHAZALLY ISMAIL

Faculty of Science and Natural Resources
Universiti Kebangsaan Malaysia
Sabah Campus
Kota Kinabalu, Sabah, Malaysia

ABSTRACT

This article aims to present an overall assessment of the potential contributions of biotechnology to ASEAN's food and health problems. It has become clear that applied biology particularly biotechnology has taken great strides in improving the living standards of people in developed countries. With the vast natural resources still available and considerably large pool of manpower expertise, developing countries like ASEAN too can hope to achieve similar success in biotechnology. Current research and development efforts in biotechnology may lead to increased food production, standard of nutrition and health of ASEAN people. Biotechnological advances with promise for wider use and felt most important for developing countries are identified with an important criteria considered: that the biotechnology can be beneficially employed in developing ASEAN countries to merit research and development for future use. With this type of information, politicians and government officials who make or influence decisions on research and development can evaluate the importance of biotechnology in ASEAN countries with greater perspective.

Today science and technology bring us high hopes and anticipation. The right to live and the needs for survival of mankind no longer remain acceptable as the minimum. Developing countries are slowly forging ahead in utilizing existing scientific knowledge to improve the living standards of their people; providing increasing amenities to meet their basic needs. Science and technology have increased the basic and minimum standard of living which man aspires for. It is the advance of science and technology that has made communication easier and possible. Despite all these, half of the world's population is poor, hungry and sick. They are deprived of their basic needs necessary for living. A great percentage of the people living in the developing countries are still cut off from the main stream of social and economic progress. For most people in these countries, life has loftier goals than just living. In the context of the present-day world, living is meaningless unless it is accompanied by freedom from hunger, disease and poverty.

Inevitably man has become more conscious of what science and technology can do for him and all over the world people are clamouring for a share of these benefits. In a comprehensive consideration, one can regard science and technology as belonging to three categories: primary, middle level and sophisticated science and technology. The primary level of science and technology is that which has permeated into the majority of populations in the form of traditional skills. These, for instance, include the traditional

Figure 1: **HOW RECOMBINANT DNA WORKS IN GENETIC ENGINEERING:** Foreign gene is introduced into the bacterium *Escherichia coli* on a plasmid vector to yield a clone of bacteria carrying the gene.

techniques of farming and agricultural production. Middle level science and technology is that which can provide more inputs into the basic needs of the population. These include the provision of improved communication, transportation, food and health care systems. Sophisticated technology refers to advanced technology which is not readily adaptable by the less affluent countries for indigenous use. This category includes aircraft, satellite and nuclear technology. It is often asserted that developing countries would be judicious to stay clear from this sophisticated or glamorous advance technology which are now within the reach only of most advance countries. Thus the question frequently asked is what category of science and technology must developing countries like ASEAN place their priority to develop?

With the vast natural resources still available and considerably large pool of manpower expertise, development of middle level science and technology by ASEAN countries is not only desirable but essential. We are now in a period of especially rapid progress in applied biology; specifically biotechnology which is often regarded as a revolution in the category of the middle level science and technology. Today all over the world, there is great excitement over the vast untapped potentials from biotechnological techniques of recombinant DNA and hybridomas.

Recombinant DNA or gene splicing technique is basic to much of the progress in biotechnology. In concept it is very simple but in practice it is complex. A major objective of recombinant DNA technique is to insert a foreign gene for a desired product into an organism. The foreign gene, if successfully inserted, must then be expressed more frequent than the native genes of the host organisms. Thus the organism, will be *manufacturing the desired product more abundantly than products of its own or native gene*. The organism used for this purpose is a mutant of a common bacteria found in the gut, *Escherichia coli*. This is because a large body of information and know-how has been accumulated about *E. coli* than any other bacteria. A favourite *E. coli* that serves most purpose in genetic engineering is a mutant *E. coli* that is unable to synthesise the amino acid tryptophan. In these *E. coli* there exist circular forms of DNA called plasmids which contain a gene for making tryptophan. A foreign gene can be spliced into these tryptophan plasmids in such a manner that both genes will be expressed simultaneously. These plasmids may be introduced into bacterial cells and grown in a culture containing no tryptophan. When this *E. coli* survive and multiply in such culture, the mechanism is such that the newly introduced plasmid will replicate 20 to 40 times within each bacterial cell (Figure 1). Further, when in a culture without added tryptophan, the demand for synthesizing tryptophan by the organism will be great, thus the gene for it and simultaneously the foreign gene introduced next to it are preferentially expressed by the bacteria (Demain and Solomon, 1981).

Yeast too have been used for recombinant DNA. In contrast to *E. coli*, yeast, which also have plasmids, can grow to very high densities. They are also stable with respect to mutations, thus *reducing the risk of the organisms reverting back to wild types* as shown to occur occasionally with *E. coli*. Because of these attractive features, the use of yeast for recombinant DNA is often preferred by some workers (Phaff 1981).

Genetech in San Francisco USA, is considered to be the leading genetic engineering company dealing with pharmaceutical business. Already the company has created more than a dozen protein products by recombinant DNA techniques, most of which are under clinical or animal tests. Products that have been created so far are listed in Table I. Advances have also been made in fermentation industries where genetically modified

microorganisms are employed to produce an increasing number of antibiotics and other specialty chemicals.

We in the ASEAN countries can hope to achieve similar success in biotechnology and substantial improvement in food production and health care can also be expected to occur in the next few years. Utilization of biotechnology for appropriate development world scientists is laid on research for the sake of research. The objective of every research undertaken must aim to meet the basic needs and comforts of people living in poorer parts of developing countries. In other words, it is necessary that research in the developing countries should be devised to suit the local needs. What is really required is appropriate application of modern scientific techniques and principles which have to be efficient and economical in the local setting. Genetic

TABLE 1
MEDICAL PRODUCTS OF RECOMBINANT DNA
CURRENTLY SUPPORTED IN BIOTECHNOLOGY RESEARCH
AND DEVELOPMENT IN ADVANCED COUNTRIES

Products	Potential Use
Insulin	Diabetes Treatment
Interferon	Virus infection and cancer Treatment
Growth Hormones	Dwarfism treatment and acceleration of wound healing
Somatostatin	Pituitary Disease Treatment
Secretin	Treatment of Digestive Problems
β - endorphins	Treatment of Pain
Urokinase	Dissolve blood clots
Clotting Factors	Treatment of hemophilia
Albumin	Blood expander
Vaccines	Prevention of infectious diseases
Hepatitis A & B	
Rabies	
Foot and Mouth	
Influenza	
Malaria	

engineering is indeed an appropriate aspect of biotechnology which ought to be viewed as a challenging task by the scientists and technologists in developing countries like ASEAN. Scientists in developing countries should investigate the scientific and technological advances of the developed countries whether they can be made applicable to the less developed regions of the world. For all intent and purposes, biotechnology adopted by a developing country should be used as a tool for the betterment of mankind in 2 basic areas: (a) to harness the natural resources and to utilize them for meeting the basic needs and comforts of mankind, in particular, to increase food production to meet the growing demands of the expanding population and (b) to have cheap medical and nutritional program to eradicate many of the dreadful communicable and endemic diseases, thus leading to improvement in the overall health of the people.

Food Production

Food is the most basic and common need for all man. The resources of a family, a nation and the world therefore must be utilised first to provide food. The widespread malnutrition is primarily due to lack of means to acquire proper food. We depend on food grains as the primary source of food energy. Rice and wheat constitute the preferred food crops supplying 40 per cent of the world's food requirement. Although rice is the staple food for nearly half the world's population, the production of these grains remains largely dependent on developed countries. Yields of most food sources are low in the tropics relative to the colder climate. Tropical agriculture, with the exception of certain plantation crops and wet rice, is less productive than temperate zone agriculture in terms of unit area per person engaged in farming (Grigg 1969). The economic developments of the last hundred years that have accompanied modern farming technologies and improvements in plant breeding have revolutionized agriculture in the temperate zone. In developing countries today, continuing efforts are being made by scientists to increase the yields of agricultural crops. For decades, plant breeders have worked diligently to obtain plant varieties having characteristics as yield, pest resistance and enhanced nutritional values. Until very recently, the payoff from plant breeding of tropical crops for rice especially and some plantation crops has been most impressive (Hutchinson 1958).

Today tissue culture techniques for growing plant cells have been very successfully developed (Shepard 1980). It has been noted that tissue culture obtained from single cells when allowed to proliferate, the progeny often have a chromosome content that differs from the original. Thus it is clear that plant tissue culture technique will facilitate development of many strains of plants, some of which will hopefully prove superior than the parent plant.

Another biotechnological method of creating new plants is to bring together chromosomes from two different plant species. Work with protoplasts, that is plant cells devoid of cellulosic cell walls, applying the above breeding principles, has been shown to yield new forms (Melchers, 1980). When brought together, two protoplasts fuse and the new entity contains nuclear material from both. Thus there is a potential for the creation of novel plants. However, progress in plant genetic engineering is slow due to lack of comparable vector system, poor gene identification techniques in transformed cells and insufficient knowledge on how gene function is regulated in plants.

The growth of agricultural crop is dependent on an enormous supply of usable nitrogen, either mechanically applied as ammonia, urea or nitrate fertilizer, or naturally

produced in the soil through microbial reduction of atmospheric nitrogen. A novel approach to increase the available supply of naturally reduced nitrogen is to "engineer" nitrogen-fixing soil microorganisms which are associated with roots of plants. Most organisms cannot assimilate atmospheric nitrogen (N_2). However, a limited number of microorganisms are able to reduce atmospheric nitrogen directly to ammonia in a process called nitrogen fixation. Some microorganisms work symbiotically in nodules on the roots of plants, with the plant providing food and energy for the bacteria, which in turn, fix nitrogen from the air for their host. Bacteria that fix nitrogen in nodules on the roots of plants of the family Leguminosae (soybean, alfalfa, peanut, bean, pea, clover for example) are the *Rhizobium* species. Increasing the efficiencies of nitrogen fixation by *Rhizobium* would enable these plants to grow without addition of nitrogenous fertilizer. Because *Rhizobium* can be easily manipulated in the laboratory, introduction of appropriate nitrogen-fixation genes obtained from other soil microorganisms into *Rhizobium* has the potential to improve nitrogen fixation and perhaps to increase the yield of legume protein (Brill 1981).

Besides producing more food, it is also vital that developing countries improve their standard of nutrition. Standard modern methods of food processing have been known to contribute to increasing the nutritive value of the final product through enhanced production of essential nutrients or the synthesis of nutrients not present in the original food, e.g. cheeses. However, food processing technique like canning, freezing or dehydration with artificial heat make the preserved food too expensive for most families in the developing countries. Most cultures have traditionally used some form of microbial process to prepare and preserve food. The art of fermentation, technically defined in its broadest sense as the chemical transformation of organic compounds with the aid of microbial enzymes, is very old. In developing countries like ASEAN, people have traditionally used some form of microbial fermentation process either to preserve food that would otherwise spoil or to prepare food products preferred for their culture and taste. Fermentation favours development of a microbial flora that converts, for example, pickling cucumbers. Pickling has been the traditional method of providing a winter supply of vegetables in countries of Europe. In developing countries like ASEAN, there is less need for extended storage because in tropical areas, vegetables can be grown on a year-round basis. Pickling however can be a valuable and preferred method for taking advantage of occasional surplus production. Moreover, pickled vegetables can provide variety to the diet of people in developing countries.

Recent advances in biotechnology have generated a wave of excitement about the prospective application of novel DNA recombinant techniques in a wide range of food industry (Rose 1981). New developments are expected to take place, for instance, in the field of food microbiology when genetically modified microorganisms may be used for a variety of research and development opportunities in food processing. There may be an important role here for the microbiologists in the ASEAN in improving the preservation techniques of food resources through use of 'engineered' microorganisms which ultimately enable maintenance of better food quality control. It must be stressed that food processing is always potentially hazardous due to overgrowth of contaminating microbes, particularly when a mixed inoculum is used. To avoid this, there is a need for the introduction of pure culture fermentation methods, presumably through use of microorganisms having shorter fermentation time thus less chance for development of other undesirable contaminants.

Another example of an early and progressing application of modern biotechnology in food production is the large-scale cultivation of microorganisms for use as a food source for humans and for animal feeds (Table 2). These dried cells of microorganisms, referred to as single-cell protein (SCP) so far grown in large-scale culture systems include microorganisms such as algae, bacteria, yeasts, molds and higher fungi (Davies 1974). Although these microorganisms are grown primarily for their protein contents, they are potentially very important sources of amino acids, carbohydrates, lipids, vitamins, minerals and nonprotein nitrogen materials such as nucleic acids. Microorganisms have been a component of human food since ancient times. Examples include yeasts in bread; lactic acid bacteria in fermented milks, cheese and sausage; and mold in a variety of traditional fermented foods in Asia, eg. koji (Japan) and tempeh (Indonesia and Malaysia). It should be emphasized, however, that SCP production is capital-intensive. The future prospects for large-scale SCP production of human food appear to be limited to use as protein supplements and functional protein ingredients rather than as primary sources of protein in human diets. At present, SCP process for the production of animal feed are the most attractive. This is especially so for developing countries like ASEAN who are still importing conventional animal feedstuffs such as soybean meal and fish meal at prevailing international prices. To achieve economies of scale, an SCP production facility should have a capacity of at least 50,000 tonnes per year. Many cities in ASEAN have industries that produce huge volumes of utilizable carbohydrates in wastes which should be sufficient as potential carbon and energy sources for SCP production. Current

TABLE 2
SELECTED MICROORGANISMS OF INTEREST FOR SINGLE-CELL
PROTEIN PRODUCTION

Organism	Reference
ALGAE	
<i>Scenedesmus acutus</i>	Becker, E.W. and Venkataraman, L.V. (1980)
<i>Spirulina maxima</i>	Durand-Chaster and G. Clement (1975)
BACTERIA	
<i>Rhodopseudomonas capsulata</i>	Kobayashi, M. and S.I. Kurata (1981)
<i>Cellulomonas</i> sp	Han, Y.W. et al. (1970)
<i>Methylococcus capsulatus</i>	Harwood J.H. and S.J. Pirt. (1972)
<i>Methylophilus methylotrophus</i>	Gow, J.S. et. al. (1975)
YEASTS	
<i>Candida lipolytica</i>	Evans, G.H. and J.G. Shennan (1978)
<i>Candida utilis</i>	Ridgeway, J.A. et al. (1977)
<i>Kluyveromyces fragilis</i>	Bernstein, S. et al. (1977)
<i>Saccharomyces cerevisiae</i>	Reed, G. and H.J. Peppler (1973)

research and development efforts in modern biotechnology may lead to significant improvements in SCP processes. A challenge to scientists in the developing countries may be to develop thermotolerant strains of microorganisms through genetic engineering so that requirement for cooling process during growth of microorganisms can be reduced.

HEALTH

One of the major challenges of our time is health. Good health is now widely recognized as a fundamental human right for all people. If we accept this proposition, it implies that human society must shoulder the burden and accord the highest priority to ways and means of achieving this goal.

Tropical diseases have always been a major plague of humanity. Parasitic diseases are the most widespread of all the major human diseases currently affecting about three billion people. They cause malnutrition, debilitation, blindness, disfiguring lesions and death. In spite of this, parasitic diseases have been consistently neglected for the past 40 years. Research on important diseases like malaria, filariasis and amebiasis for instance have been severely underfunded. The late Professor Jacques Monod, a Noble-Prize winner in molecular biology, called this neglect a "disgrace"; citing the mere pittance of money and effort that goes towards tropical disease research annually. It was estimated that the total worldwide annual expenditures devoted to research on all tropical diseases is about US\$30 million, the cost of building a few miles of motorways. Thus the natural lag between research discovery and application has been prolonged and continuing in the developing countries that are most affected by these diseases. The broad base of knowledge available has not yet been exploited for concerted attack against tropical diseases.

Malaria is estimated to affect some 150 million people (WHO report 30, 1977). In some regions, malaria transmission is so intense that present efforts at mosquito control and disease treatment are totally inadequate. In Africa, at least one million children die of malaria every year. In other developing countries like ASEAN, malaria had regressed before, but it is now resurgent.

Four species of mosquito-borne malaria parasites can infect man: *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. They provoke different forms of disease and react differently to antimalarial drugs. There are ways of treating individual cases of malaria and ways of preventing them. But no single method has been developed that succeeds in protecting all of the people at risk or treating all those affected by the disease. In vast regions of the world, the parasites have become resistant to some of the major drugs. For instance, *P. falciparum* resistance to 4-aminoquinolines occurs in some parts of Asia and these resistant strains have progressed westwards to reach the Indian subcontinent. Clearly, the existing malarial drugs are insufficient for the operational demands of a control programme, and there has not been a great fundamental progress since the synthesis of chloroquine more than 40 years ago.

Filariasis is another widespread disease which in various forms affects about 200 million people throughout the world. There are at least eight filarial parasites that infect man. In ASEAN countries, the two filarial parasites *Wuchereria bancrofti* and *Brugia malayi* are transmitted by mosquitoes. The adult worms live in the lymphatic vessels and lymph nodes, obstructing the flow of lymph causing inflammation and swelling (elephantiasis) of the arms, legs and genitals.

There is a score of other parasitic diseases, most of them caused by organisms that prey on man — his liver, blood, heart, brain and gut. There are roundworms and flatworms that invade the intestine, hookworms that feed on the membrane of the small bowel; single cell amoeba that attack the intestine and liver.

Considering all the efforts that have been made by the World Health Organization (WHO) and the United Nations Development Programme (UNDP), it is clear that there is one aspect which, although of high priority, is not receiving adequate attention; that is the technical tools to control many of these diseases in the developing countries. New effective tools are needed. Now there is a wide range of existing knowledge on biotechnology from developed countries that can be coordinated and exploited to bring change and benefit in health of people from developing countries. Genetic engineering technique has not yet been applied in tropical disease research in a methodical, purposeful fashion which can make it possible to unravel some of the fundamental riddles of tropical diseases and to develop the powerful tools in combating or eradicating the diseases. Genetic engineering certainly has a paramount role to play in achieving this goal.

Immunization has permitted the control of many viral and bacterial infections. Yet till today there are no effective vaccines against the majority of tropical parasitic diseases. One of the important steps in developing a vaccine involves methods for measuring the protective effect of a potential vaccine preparation. These methods make use of antigens, the particular component of the causative parasite which, when introduced into an organism triggers a specific immune response. The immune response to most tropical parasitic diseases has been shown to occur and are specific for the infecting parasites. For instance the protective properties of antiplasmodial antibodies in malaria have been well established (Greenblatt and Jacobsen, 1977). Therefore, there are strong indications that vaccines could be developed against many of the parasitic disease if only the antigens recognised by the host on the parasite surface can be identified.

Identification and isolation of surface components of parasites for vaccine preparation can reasonably be expected with the success following production of monoclonal antibodies; the technique for making single specific antibodies (Figure 2). Monoclonal antibodies provides the cell biologist with methodology that renders practical the production of highly specific probes for analysis of structure-function relationships of complex antigen. These antibodies are directed against a single distinct component of the antigen or the immunodeterminant group. By studying the protective ability of immunization with the various components of the antigen, a researcher may come to a conclusion that the whole antigen is not needed for the development of protective immunity. Small protective determinants when used as a vaccine possess various advantages over usage of complex antigenic determinants. In particular simple antigens will be free of nonessential structure(s) that may give rise to toxic response in the host.

Given a source of the parasite antigens, it is virtually certain that one or more of these antigens can be adopted for use in vaccine preparation. The development of vaccines could permit the control of parasitic diseases and perhaps the eradication of some of them. To realise this goal, it may require the input of many disciplines, such as molecular biology, genetics and cellular immunology, in addition to parasitology. In other words, frontier disciplines of biomedical sciences must be associated with parasitology in a concerted effort at developing vaccines.

Fig. 2

Another goal in efforts to combat tropical diseases is to develop sufficient immunological knowledge of the disease with a view to perfecting diagnostic methods for the disease. In circumstances involving infection with highly pathogenic parasites, the health of the host is threatened. When this occurs, there is a pressing need to rapidly and specifically identify the infecting organisms. With tropical diseases, this need becomes of paramount importance. Several hundred million people are affected by tropical diseases and many more are at risk. Most of these diseases are chronic, which adds to the diagnostic problem.

Infectious diseases are generally diagnosed by four methods: (i) microscopic examination of tissue specimens and exudates (ii) culture methods with the use of selective growth media that allow amplification of small numbers of causative organisms, (iii) immunological identification in tissues or body fluids of antigens associated with specific diseases or (iv) measurement of specific antibodies produced by the patient as a result of infection with an organism. Given the complexity of infectious tropical diseases, it is not surprising that no one diagnostic method mentioned above has proved optimal for all situations.

It has been only 8 years since the discovery of a method to produce large amounts of a specific antibody was first reported. Already, monoclonal antibody technique has made a major impact on clinical procedure making diagnosis of a variety of medical conditions possible. These specialized antibodies are now used to detect microorganisms, hormones and tumour-associated substances. In the past, many diagnostic tests have already used antibodies to detect diseases. However, those antibodies were uncharacterized mixtures of antibodies taken from animals injected with the target substance. Every time an animal is injected with foreign material, a different mixture of antibodies results. But with the new technique, large amounts of a single antibody can be made at will. It is now possible to clone immortal cell lines that continuously and reproducibly produce specific antibody molecules for use in diagnosis of various diseases. Now people talk of a new era in medicine and a billion dollar market. More than 20 clinical tests using monoclonal antibodies have now been approved by the Food and Drug Administration, USA and many are already on the market.

In most cases the monoclonal antibody tests, compared with conventional tests, offer greater sensitivity, specificity and speed. Often, these tests also can avoid the use of costly instruments or radioactive isotopes. Presently, hybridoma technique in ASEAN countries is still in its infancy and most ASEAN's investigators have not met with much success in utilizing this technique. Monoclonal antibodies for some parasitic antigens have been produced by several investigators (Table 3). An opportunity to collaborate with these scientists in developed countries where hybridoma technique has been well established should certainly increase the chance of success. Monoclonal antibody technique thus has provided an opportunity to re-evaluate the role of immunological methods for the diagnosis of infectious diseases (Kaplan et al, 1982).

CONCLUSION

Given adequate resources and a careful selection of priorities, new knowledge in biotechnology can readily alter the course of the uphill fight against food and health problems in ASEAN countries. Scientists in developing countries should investigate the

scientific and technological advances of the developed countries whether they can be made applicable to the less developed regions of the world. Biotechnological advancement today certainly can be identified as having sufficient potential for developing countries like ASEAN to merit research and development for future use.

TABLE 3
MONOCLONAL ANTIBODIES IN PARASITIC DISEASES

Diseases	Monoclonal antibodies (Reference)
Malaria	
-- Sporozoites	Yoshida et al. (1980)
-- Merozoites	Freeman et al. (1980) Perrin et al. (1981)
-- Gametes	Rener et al. (1980)
Toxoplasma	Handman et al. (1980) Sethi et al. (1980)
Trypanosoma	Pearson et al. (1981) Lyon et al (1981)
Taenia	Craig et al. (1980)
Echinococcus	Craig et al. (1980)
Mesocestoides	Mitchell et al. (1979)
Schistosoma	Verwaerde et al. (1979) Hillyer et al. (1980)

REFERENCES

- Becker, E.W. and Venkataraman L.V., (1980). In "Algae Biomass Production and Use", eds. Shelef, G. and Soeder, C.J., Elsevier/North-Holland, New York, 35.
- Bernstein, S., Tzeng, C.H. and Sisson, D., (1977). Biotechnological Bioengineering Symposium (No. 7), 1.

- Brill, W.J. (1981). "Agricultural Microbiology", *Scientific American* 245 (3), 146.
- Craig, P.S., Mitchell, G.F., Cruise, K.M. and Rickard, M.D. (1980). *Aust J. Expt. Biol. Med. Sci.*, 58, 338.
- Davies, P., ed. (1974). *Single-cell protein*. New York: Academic Press.
- Demain, A.L. and Solomon, N.A. (1981). "Industrial Microbiology", *Scientific American*. 245 (3), 42.
- Duran-Chastel, H. and Clement G., (1975). *Proceedings of the 9th International Congress of Nutrition, Karger, Basel, Switzerland*. 3, 85.
- Evans, G.H. and Shennan J.G., (1978). *Chem. Engng. News*, 56 (38), 12.
- Freeman, R.R., Trejchosiawicz, A.J. and Cross, G.A.M. (1980). *Nature* 284, 366.
- Gow, J.S., Littlehailes J.D., Smith S.R.L., Walter R.B., (1975). "Single Cell Proteins II", eds. Tannenbaun S.R., and Wang D.I.C., MIT Press Cambridge, Mass, 375.
- Greenblatt, L. and Jacobson R.L. (1977). "Malaria-Immunology and Immunopathology", eds. Spira D.T., and Greenblatt G.L., S. Karger AG. Basel, Switzerland, 94.
- Grigg, D.B. (1969). "The agricultural regions of the world", Cambridge University Press. Cambridge University, Cambridge, England.
- Han, Y.W., Dunlop C.E., and Gallihan C.D., (1970). *Food Tech.*, 25, 130.
- Handman, E., Goding, J.W. and Remington, J.S. (1980). *J. Immun.*, 124, 2578.
- Harwood, J.H. and Pirt S.J., (1972). *J. Appl. Bact.*, 35, 597.
- Hillyer, G.B. and Pelley, R.R. (1980). *Amer J. Trop. Med. Hyg.*, 29, 582.
- Hutchinson, J.B. (1958), "Genetics and Plant Improvement of Tropical Crops", An Inaugral Lecture. Cambridge University, Cambridge, England.
- Johnson, I.S. (1983). "Human Insulin from Recombinant DNA Technology", *Special Issue of Science. Biotechnology*. 219, (4585), 632.
- Kaplan, H.S., Olson L. and Ranbitschek A. (1982). "Monoclonal Antibodies in Clinical Medicine", eds. McMichael, A.J. and Fabre J.W., Academic Press, 1982, 17.
- Kobayashi, M. and Kurata, S.I., (1981). *Process Biochem.*, 13 (9), 27.

21. Lyon, J.A. Pratt, J.M., Travis, R.W., Doctor, B.P. and Olenick, J.G., (1981). *J. Immun.*, 126, 134.
22. Melchers, G. (1980). "Advances in Protoplas Research", eds. Ferenczy, L. and Farkas, G.L., Akasemiai Kiado, Budapest, 283.
23. Mitchell, G.F., Cruise, K.M., Chapman, C.B., Anders, R.F. and Howard, M.C., (1979). *Aust. J. Expt. Biol. Med. Sci.*, 57, 287.
24. Pearson, T.W., Kar, S.K., McGuire, T.C. and Lundin, L.B. (1981). *J. Immun.*, 126, 823.
25. Perrin, L.H., Ramirez, E., Lambert, P.H. and Miescher, P.A. (1981), *Nature* 289, 301.
26. Phaff, H. (1981). "Industrial Microorganisms", *Scientific American*. 245 (3), 52.
27. Reed, G. and Peppler, H.J., (1973). "Yeast Technology", Westport, Conn.
28. Rener, J., Carter, R., Rosenberg Y. and Miller, L. H. (1980). *Proc. Natl. Acad. Sci. USA*. 77, 6767.
29. Ridgeway, J.A. Jr., Lappin, T.A., Benjamin, B.M., J.B. Corns and Akin, C., (1977). *Food Engng.*, 49 (6), 95.
30. Rose, A.H. (1981). "The Microbiological Production of Food and Drink", *Scientific American* 245 (3), 94.
31. Sethi, K.K., Endo, T. and Brandis, H. (1980). *J. Parasitolgy*. 66, 192.
32. Shepard J.F. (1980). "Emergent Techniques for the Genetic Improvement of Crops", Rubenstein, I., eds. Grengenbach, R., Phillips, R.L., and Green, C.E., 185 Univ. of Minnesota Press, Minneapolis.

Talmadge, K., Stahl, S. and Gilbert, W., (1980. *Proc. Natl. Acad. Sci., U.S.A.*, 77, 3369.
34. Verwaerde, C., Grzych, J.M., Bazin, H., Capron, M. and Capron, A. (1979). *C.R. Acad. Sci. Paris* 289, 275.
35. Yoshida, N., Nussenzweig, R.S., Patocnjak, P., Nussenzweig, V. and Aikawa, M. (1980). *Science*, 207,71.