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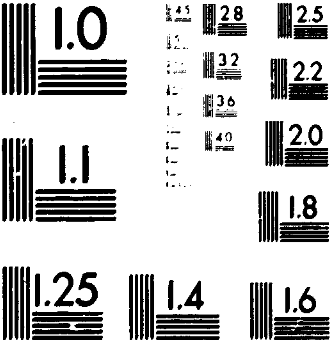
UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org



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ICGEB

THE ROLE OF THE INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY IN FOSTERING DEVELOPMENT THROUGH APPLIED MICROBIOLOGY*

Prepared by

the UNIDO secretariat

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I. Introduction

1. The advances of biotechnology made during the last decade through the introduction of recombinant DNA techniques is likely to present one of the greatest challenge to our world nations today. The application of those advanced techniques in a number of economic sectors such as food, health, energy and industry is paving the way to a wide range of opportunities combined with new dimensions of versatility, efficiency and economy.

2. In awareness of this challenge and of the opportunities advanced biotechnology may offer to developing countries, in 1981 UNIDO, assisted by an international group of leading scientists, took the initiative towards the establishment of the International Centre for Genetic Engineering and Biotechnology (ICGEB) as a "centre of excellence". In launching this initiative, a number of considerations were foremost in the minds of the people behind it. First, a well-endowed ICGEB could play a significant role in advancing the world-wide frontiers in this field. Second, it would ensure the accumulation and continuity of scientific knowledge and make it accessible to all participating countries. Third, the Centre's unique feature of transdisciplinarity would be a major factor in assisting developing countries to achieve the critical mass of technological and scientific capabilities in this frontier technology. Fourth, the forceful argument forwarded by many developing countries regarding the role the ICGEB can assume to help obviate a situation where "secrecy and monopoly" could result in a burden of excessive costs and limitations of access to the international flow of technology.

3. The Centre's activities will range from undertaking R+D (in areas such as the bioconversion of biomass, microbiology of hydrocarbons, large-scale fermentation and process development, protein engineering, the development of stress tolerance in plants, improvement of plants for nutritional contents, animal and human vaccine development) to the training of multidisciplinary teams of scientists from developing countries who will be able to carry out substantial research and development activities, including pilot plant operations. It is also envisaged that the Centre will provide other services to member countries, including making available advisory services, organizing

expert group meetings and workshops on problem-oriented topics, disseminating specialized information etc. Other important functions of the Centre should be to consider from time to time the adequacy of safety guidelines in effect and their relevance to the actual work conducted at the Centre itself and its affiliated centres in member countries. It may also, as appropriate, assume the responsibility for the development of uniform international safety standards.

4. During the Ministerial Level Plenipotentiary Meeting on the Establishment of the ICGEB, held at Madrid, 7-13 September 1983, 28 countries signed the Statutes of the ICGEB; they were later joined by 8 additional countries which makes a total of 36 countries up to date. Generous offers to host the Centre were presented by a number of countries. However, the signatories of the Statutes decided on 4 April 1984 that the Centre would consist of two equal components, one located in New Delhi, India, and the other in Trieste, Italy.

5. At present, the Preparatory Committee (in which all signatory States of the ICGEB are represented) is working towards the target of starting operational activities, including research and training in provisional facilities, at both components by the end of 1985.

6. In order to ensure the highest scientific and technological quality of the Centre's operation, a Panel of 15 emininent international scientists including three Nobel laureates, was formed at the end of 1984 to guide the establishment of the Centre's permanent facilities and R+D programme. The Panel is also to assist in the recruitment of the Centre's Director General and senior staff scientists, help in the securing of additional financial resources for scientific activities and to consider matters related to affiliated centres.

II. Highlights on the ICGEB Work Programme with Particular Reference to Applied Microbiology

7. As stated earlier, the ICGEB consists of two equal components, one located in New Delhi, India, and one in Trieste, Italy. Each of the components will have a broad capacity for basic research in general areas common to all advanced biotechnology. However, the component in Trieste is foreseen to focus its applied research efforts in the areas of industrial biotechnology processes, including conversion of biomass, hydrocarbon microbiology, large-scale process development and protein engineering. The New Delhi component will focus its applied R+D efforts on the areas of agriculture as well as human and animal health.

8. Accordingly, the ICGEB work programme, now being considered by the Preparatory Committee, has been developed in a manner suitable to areas of work assigned to the two components. Although the scope of research to be undertaken is wide (includig agriculture, animal and human health and protein engineering), much of the work on applied microbiology is related to the areas of the work programme proposed for the Trieste component (as is described below).

A. Bioconversion of Biomass (lignocellulose)

9. Biomass refers to the bulk of the non-food components of plant material, including wood (especially wood waste products), residues of crop plants (stalks and stems), and many other non-crop plants. The primary components are cellulose and hemicellulose, sometimes complexed with lignin in woody material. Because of the chemical complexity and high molecular weight of this material, there have been few processes developed by which economically useful products can be made, even though the raw material is cheap and plentiful. The primary breakdown products are sugars, especially glucose, and, in the case of lignin, phenolic compounds. One may potentially obtain animal fodder from partially degraded biomass, sugars suitable for human consumption, alcohol for combustible fuels, and the possible conversion of lignin phenolics for the raw material for synthetic polymers. If successful, then, petroleum may be replaced to a large extent as a source of both combustible fuels

and the raw materials for certain plastics. It is also possible that processes could be developed to convert non-lignin biomass into food and food supplements for animals and perhaps humans.1/

10. In the beginning, this project should involve a thorough investigation of the organisms that normally produce cellulases, hemicellulases and ligninases. Although most work to date has been done on the cellulase producing fungus Trichoderma reesei, the organisms of interest include all classes of microorganisms, including those that inhabit the digestive tracts of termites and snails. Some insects and animals also produce cellulases. Concommitant with a thorough microbiological investigation will be a characterization of the enzymes involved and the genes that direct their production.

11. Once organisms, and their constituent enzymes, have been identified and characterized, there are two methods that may be used to select industrially useful strains. First, individual colonies of a microbe from a mutagenized population may be selected for their high activity of specific enzymes. This has been the classical way in which microbial engineering has been accomplished in the past. Then, genetic engineering methods may be used to enhance the production of specific enzymes or otherwise adapt previously selected strains of organisms for maximum efficiency in a particular process. In conjunction with the protein engineering programme described later in this paper, the properties of specific enzymes could be enhanced to make them more efficient and suitable for particular purposes.

12. Microbial biomass conversion programmes are now in operation. Therefore, the prospects for the ICGEB developing useful new and improved methods of biomass degradation in a short time are very good.

13. This programme would require advanced skills in microbiology, molecular biology, including recombinant DNA methods, enzymology and

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1/ UNIDO document ID/WG.382/2/Add.2, "Application of Genetic Enineering for Energy and Fertilizer Production from Biomass".

protein chemistry. It would be expected to work in close co-operation with the large-scale fermentation group and the protein engineering programme.

B. The Microbiology of Hydrocarbons

14. The study of microorganisms that can use the components of petroleum as substrates, and the use of these organisms in specific applications, is becoming an economically important area of biotechnology. Included could be many steps in the refining of crude oil (dewaxing, the removal of sulphur and metals, and the production of desired chain lengths) that now require large amounts of energy, the degradation of environmental pollutants from spilled oil and other organic compounds, and the enhanced recovery of oil from oil wells.2/

15. Strains of the genus *Pseudomonas* are known to contain enzymes which attack the components of petroleum. However, as in the programme on biomass conversion, a great deal of initial investigation of new sources of organisms (and their specific enzymes) is recommended. Sources where the environment is known to contain petroleum, such as the earth around old oil wells, should be investigated.

16. Genetic engineering can be expected to be very important to all applications of hydrocarbon microbiology and enzymology, to increase the yields of desired enzymes and to modify their properties for specific conditions. In addition, protein engineering would be an important adjunct to this programme since it could provide the means for altering the solubility and activity of enzymes in non-acqueous solvents, as well as enhancing their thermal stability and substrate specificity.

17. Plasmid engineering, by which plasmids containing different enzymes can be assembled in a single strain of bacteria, is also an aproach that has enabled the creation of an entire biochemical breakdown pathway in a single organism.

2/ UNIDO document ID/WG.382/2/Add.3, "Hydrocarbon Microbiology with Special Reference to Tertiary Oil Recovery from Petroleum Wells".

18. There are a variety of goals that could be encompassed by the basic approach to hydrocarbon microbiology described above. The development of a microbial strain that degrades a specific organic pollutant should be a relatively short-term project. Enhanced oil recovery strategies, which must take into account the specific characteristics of each source and of the petroleum to be recovered, is likely to be a rather long-term project. That is, not only must one develop strains to reduce the viscosity of the crude oil, but a means must be found for making the petroleum accessible to the organisms while at the same time preventing excessive growth. Individual processes in petroleum refining have far fewer requirements and, therefore, should be considered intermediate range projects (3-7 years).

19. This programme would investigate both the use of microorganisms themselves and their isolated enzymes for the various applications described above. However, unlike most other programmes proposed for the ICGEB, it involves the engineering of microorganisms to be used directly in the environment, as in the case of the degradation of spilled oil or residues of organic pollutants, or for enhanced oil recovery. Thus, research must not only be directed towards adapting these organisms to function efficiently in harsh environments (e.g. in sea water, high or low pH, high temperature, high pressure), but to assess the environmental and ecological safety of these organisms. That is, it may be desirable to engineer them in such a way as to function efficiently only under specific conditions and not survive in other environments where they could conceivably disrupt the local ecology. One way to do this, for example, is to restrict an organism so it can only use a specific hydrocarbon as an energy and carbon source, and cannot grow once that compound is exhausted.

C. Food Processing

20. The advent of genetic engineering has greatly increased the application of biotechnology to the food industry. It is being used in a great variety of ways, but perhaps the most far reaching is the application of enzymes to food processing. The use of enzymes is a long and well-established tradition in many societies - an obvious example is

the use of chymosin (rennet) in cheese manufacture and, of course, enzymatic activities are essential in baking, brewing, distilling and many other ancient and widespread food processes. In modern times the uses of enzymes have become more sophisticated and diversified as more enzymes have become available in partially purified forms and in large quantities. Now about 20 different enzymes are used on what amounts to industrial scale, including amylases, proteases, amyloglucesidase, chymosin, lipases, glucose oxidase, pectinase, glucose isomerase etc.

21. The processing and presentation of foods using applied microbiology has been a part of virtually every culture for thousands of years; in the making of beer and wine, cheese, yoghurt and other dairy products, to preserve vegetables such as sauerkraut and the many kinds of kim chee, and in the making of bread. In all of these cases, strains of yeast or bacteria produce enzymes that convert the sugar and starch into alcohol or organic acids that keep foods from spoiling, sometimes for long periods of time without refrigeration. Techniques of genetic manipulation and strain selection can result in higher yields of enzymes or more efficient enzymes thereby to improving the economics and efficiency of processing foods, and perhaps increasing the range of foods that can benefit from such presentation methods. It is important to remember that in much of the developing world, there is a lack of refrigeration. Thus, it is far more appropriate to enhance the processes already in use than to attempt to put in place a whole new technology that is both expensive, complex and inappropriate for widespread use in many countries.

22. Genetic engineering will affect the food processing industry by making a much larger range of enzymes available to it on a larger scale and at a reduced cost. The total market in these afore-mentioned enzymes is approaching US$ 300 million and is expanding rapidly. They are used to add value to food, for example, by improving storage properties as in the conversion of milk to cheese or fruit to fruit juices or wine, or by improving the nutrient value as in the conversion of cellulose to glucose. Some enzymes have become extremely important in the manufacture of new foods or food additives. The best known example of this is use of a series of enzymatic activities, especially glucose isomerase, to produce high fructose syrup from maize. This new sweetener now accounts

for about half of the United States' sweetener market, displacing cane and beet sugar and seriously affecting sugar production in both the United States and elsewhere.

23. It is easy to see, therefore, that genetic engineering and biotechnology are going to have a very large impact on developing countries. The purpose of this part of the research programme of the ICGEB is to undertake research projects which will help the developing countries to adapt and apply the new technology to relevant aspects of agriculture, forestry and food production. This will mean emphasizing research on plants, and on the processing and preservation of food products which are important, but which are not being carried out in developing countries. Some research will be concentrated on novel methods of breeding plants, including clonal propagation from tissue culture and genetic engineering using recombinant DNA. Other research will be directed towards applications of genetic engineering for the production of enzymes for the food processing industry.

D. Large-Scale Fermentation and Process Development

24. In applied microbiology, the desired product can fit into one of the three following categories:

- (a) be an ample quantity of the microbial biomass itself (as in single cell protein);
- (b) be a constituent of the grown organism;
- (c) be a particular compound produced by the organism and secreted into the medium.

25. In all cases, it requires the growth and harvesting of the organism on a scale that is hundreds or thousands of times greater than is done in the laboratory. The equipment and facilities are, therefore, necessarily much different and special problems are posed, especially in the precise

control of the growth conditions and compositon of the media, sterilization, and harvesting the products.3/

26. The ICGEB will need fermentation facilities to support many of its programmes both at the Trieste and New Delhi components. However, because of cost limitations, a full scale pilot plant is appropriate for only one site. Trieste was chosen since it is absolutely essential for most industrial microbiology. Nevertheless, it is important that the facility be made to be generally adaptable to a wide variety of conditions and different organisms. It can be expected to be used not only to support the specific projects to be undertaken at the Trieste component, but for the development of scale-up of the manufacture of some of the products resulting from activities in the New Delhi facilities (including vaccines, soil microorganisms and cloned antibodies).

27. For each organism studied, large-scale process optimization will be carried out, based upon laboratory data as a starting point. Processes unique to specific organisms or products may also need to be developed, including such methods as immobilized antibodies as a separation tool, large scale ion exchange chromatography, gel filtration and electrophoresis, and methods involving immobilized cells.

28. An especially important function of the pilot plant and process development programme will be to provide process development services to affiliated centres or to developing countries that request assistance in adapting specific technology to large scale production or to processes of direct application. The scientists and engineers at the ICGEB must thus maintain design flexibility to respond to many applications.

29. The pilot plant should contain several fermentors of different designs, ranging from 100 to 1500 litres capacity, supported by advanced computerized control technology, and equipment for the harvesting of cells, the purification and fractionation of microbial products or components, their sterilization, packaging and storage. The construction

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3/ UNIDO document ID/WG.382/2/Add.1, "Selective Application of Advanced Biotechnology for Developing Countries".

of the pilot plant facility should be phased in, along with the rest of the ICGEB's programmes, during the first three years of operation, beginning with the smaller fermentors.

30. The pilot plant facility will be an important adjunct to the Trieste component's programmes in biomass conversion, hydrocarbon microbiology, and protein engineering, all of which will at times require the production of large quantities of biological material. As the programmes at New Delhi reach maturity, requiring large scale production, close liaison between the two branches will be required.

31. Although the programme's primary function will be to render practical the research results of other programmes in the ICGEB, it may at times also produce rare or expensive reagents or cells needed by the ICGEB or its affiliated laboratories. It should also be noted that this facility will be an important component in the training programmes of the ICGEB, both in the production methods for biotechnology, and in the appreciation of necessary safety practices.

32. In addition to the permanent pilot plant facilities of the ICGEB, it is planned that in the third year of ICGEB operations, a project will begin to design and construct mobile field fermentors. These units will be an important part of the ICGEB's training functions, since instruction in fermentation technology can be brought directly to people in remote areas of the world, thus reaching many more people than is possible to train at the ICGEB facilities.