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UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

Genetic Engineering and Bio-technology Monitor

Distributed free to a targeted audience in developing countries

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CONTENTS

A. NEWS AND EVENTS

UNIDO's activities in biotechnology*

Recognizing that the first order of impacts of technological advances in biotechnology and genetic engineering will be felt in the industrial sector, UNIDO started giving attention to the subject more than 10 years ago, as part of its programme of sensitizing developing countries on the potential and implications of advances in these technologies. One of its earliest steps was to promote an International Centre for Genetic Engineering and Biotechnology. This effort itself generated several other activities relating to various aspects of the promotion and development of genetic engineering and biotechnology in developing countries. As a result, promotional and technical cooperation activities in this field have increased substantially over the years. The activities are carried out by the Industrial Technology Development Division in the Department of Industrial Promotion, Consultations and Technology and in the Industrial Technology Operations Division of the Department of Industrial Operations.

A programme approach to the subject is being adopted. The following table provides information on the programme elements and also examples of activities under each element.

Programme element

(a) Identification of national and regional R and D priorities, monitoring of technological trends, sensitization of policy makers, scientists and technologists and the development of databases and bio-informatics network;

Example: Studies of: policies and programmes in selected developing countries, global trends in biotechnology, etc.

Development of a bio-informatics activity including a workshop in the USSR.

Genetic Engineering and Biotechnology Monitor.

(b) National biotechnology policy formulation;

Example: Biotechnology programmes in several African and Asian countries and advisory services.

(c) Research cooperation between institutions of industrialized and developing countries;

Example: Programmes for research cooperation for vaccines, bioreagents, bioremediation of oil and enhanced oil recovery, lactic acid fermentation.

(d) Transfer of technology through investment promotion and technological cooperation at the enterprise level;

Example: Expert group meeting on commercialization of biotechnology. Expert group meeting on the application of biotechnology to food processing in Africa.

(e) Monitoring regulatory issues, such as patenting and biosafety; formulation of safety guidelines for biotechnology;

Example: Development of a voluntary international code of conduct on the release of genetically modified organisms into the environment. Advisory Services.

(f) National institutional capability building through strengthening of R and D, management and maintenance of bio-medical instrumentation, and production infrastructure; the International Centre for Genetic Engineering and Biotechnology (ICGEB);

Example: ICGEB; increasing cooperation with affiliated centres; institutional capability building in countries, such as Algeria, Egypt and the Sudan; culture collections centres.

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* This paper appeared first in Biopractice, January 1992, pp. 18-21.

UNIDO'S basic approach is that technological capabilities in genetic engineering and biotechnology should be built up by developing countries, since biotechnology is extremely relevant to them in view of the plenitude of their bioresources, and a long tradition of fermentation products. It is recognized that the development of capabilities in advanced fields such as genetic engineering can take place only over a period of time but actions have to be initiated by developing countries from now on. In what follows, only certain selected major activities are discussed as examples. These will be:

(i) The International Centre for Genetic Engineering and Biotechnology;

(ii) The Regional Network for Biotechnology in Latin America;

(iii) A voluntary Code of Conduct for Release of Genetically Engineered Organisms into the Environment;

(iv) Bioremediation and microbial enhanced recovery of oil;

(v) Fermented foods and lactic acid fermentation; and

(vi) Genetic Engineering and Biotechnology Monitor.

It is hoped that highlighting a few activities would give a better understanding of the work of UNIDO and the potential it has in the future.

International Centre for Genetic Engineering and Biotechnology

The Centre has been promoted as an intergovernmental institution by UNIDO with the help of a distinguished Panel of Scientific Advisers. Forty-three countries have subscribed to the Statutes of the Centre. More are interested in joining. Pending the entry into force of the Statutes UNIDO, the promoter, has been asked to administer the Centre as a UNIDO project. The Centre has two components, one in Trieste, Italy and the other in New Delhi, India. Work started in Trieste from 1987 and in New Delhi from the middle of 1988. The permanent premises in both components are nearing completion. The full extent of the permanent premises will be available by the middle of 1992. The New Delhi and the Trieste component have the following research groups with close coordination being maintained between them.

New Delhi	Trieste
Mammalian Biology	Molecular and Cellular Biology
Plant Biology	Genome Studies
Structural Biology	Microbiology Virology Protein Structure and Function Molecular Pathology Molecular Immunology

The Centre as a whole is headed by a Director, Professor Arturo Falaschi. The head of the New Delhi component is Professsor K. K. Tewari and the head of the Trieste component Prof. Francisco Baralle.

The following points may be mentioned in regard to the Centre:

(a) Political commitment;

(b) Scientific support;

(c) Long-term funding;

(d) Network of affiliated centres;

(e) International cooperation;

(f) Cooperation with industry; and

(g) Spin-offs of the Centre.

(a) Political commitment

Though the establishment of a centre as an intergovernmental organization with specific statutes and the signature and ratification of them by member governments has been a time-consuming process, it has had the effect of generating, and in fact was meant to generate, political will for international cooperation and for long-term support to such cooperation. The benefits of such political commitment have on

the whole been more substantial than the time being taken by the Centre to gain an autonomous identity. Adequate contributions could be also expected from Member States in a sustained manner only through such political commitment.

(b) Scientific support

The Centre from the beginning has had the benefit of support from high-level scientists starting with scientists like Dr. Saran Narang of the National Research Council of Canada, Prof. Anada Chakrabarty of the University of Illinois, Prof. Ray Wu of the University of Cornell, Prof. Heden of Sweden and several others. The Centre has a Panel of Scientific Advisers with the following membership:

A. Chakrabarty, W. J. Rutter, P. Chambon, J. Salk, R. Haselkorn, M.S. Swaminathan, A. Kornberg, C.C. Tan, J. Lederberg, R. Wu, S. Narang, F.B. Zapata.

Currently, Professor Cavalli-Sforza is the Chairman of the Panel.

In addition to the Panel, whose support has been very valuable, the process of building up of the Centre and its operation has attracted willing cooperation from many scientists and this is a major asset to the Centre. The work programme of the Centre has been reviewed by the Panel and found to be quite satisfactory. It may be noted in this connection that nearly 50 scientific papers in important scientific journals have been published by the Centre's scientists over the past three years. The Committee on Biotechnology (COBIOTECH) of the International Council of Scientific Unions (ICSU) has sought and obtained affiliation with the ICGEB. With a view to attracting senior scientists to come and work with the Centre, selected senior scientists could be given contracts for five years at a time. This is perhaps a unique feature in international projects.

(c) Long-term funding

In order to attract high-level scientists and also to give a table image to the Centre, it was decided to have a five-year programme with firm funding commitments. It was also decided that such a programme would be rolled every year by another year so that the five-year perspective is always kept, and funds are available for five years at any given time. The first five-year programme was for the period 1 July 1989 to 30 June 1994; and the second from 1 July 1990 up to 30 June 1995. The latter five-year programme is for an amount of over $55 million which has been firmly secured. The major contributor is the Government of Italy which has passed a law to pay regular annual contributions to the ICGEB. The Government of India also provides annual contributions for the operation of the New Delhi component. Both the host countries, the Government of Italy, through the Research Area of Trieste, and India have generously provided land and buildings and also equipment. Other members have started making voluntary contributions which are received into a multi-donor trust fund opened by UNIDO.

The adoption of the principle of a rolling five-year programme is a unique one in multilateral institutions and this has had a salutary effect on the Centres' operations and on the enthusiasm of the scientists working there.

(d) Network of affiliated centres

An integral part of the concept of the Centre is the Network of Affiliated Centres. These are national centres which through affiliation to the ICGEB maintain close cooperation with it. They could also conduct training programmes with partial funding by the Centre and also receive research grants awarded on the basis of peer review. The Affiliated Centres are now 15 in number, the countries involved being: Algeria, Argentina, Brazil, Bulgaria, Chile, China, Cuba, Egypt, Greece, Hungary, Mexico, Nigeria, Tunisia, Venezuela, Yugoslavia.

The Regional Network for Biotechnology in Latin America has also sought and obtained an affiliation with the ICGEB. The concept of affiliation has enabled the activities of the Centre and of international cooperation in biotechnology being broad-based over a wide range of countries, and also provides an opportunity for the Centre to have a closer appreciation of the work being carried out in national laboratories.

(e) International cooperation

The interest in cooperating with the Centre has been increasing continuously, particularly after adoption of the concept of the five-year programme. The Rockefeller Foundation, UNEP and WHO have given grants to the Centre. In addition, UNEP and WHO sponsored joint scientific workshops. Several non-member countries are considering joining the Centre. The Centre participates actively in international conferences and in international research programmes. The existence of the Centre also makes it a natural focal point for discussion of international biotechnology issues such as biotechnology safety and intellectual property rights relating to biotechnology. These are areas to which the Preparatory Committee on the Establishment of the Centre (which is an intergovernmental committee composed of all State signatories to the Statutes) has been giving attention.

(f) Cooperation with industry

The Centre seeks to cooperate with industry in its member countries. It will pursue an active licensing policy. Measures for this purpose are being worked out. In the meantime, an Indian company, Messrs. Wockhardt Ltd., has concluded an agreement by which it will give a research grant up to Rs. 50 million in return for an exclusive licence in India and a non-exclusive licence elsewhere for selected products which may emerge from some of the Centre's research activities. Similar cooperation with industry in other Member States is a subject receiving the serious attention of the Centre.

(g) Spin-off of the Centre

The establishment of the Centre has alerted policy-makers in many developing countries to the importance of biotechnology. A major spin-off of the promotion of the Centre has been the interest in individual developing countries to establish biotechnology programmes and institutions. This has happened in several member countries which have approached UNIDO for assistance and help in the matter. This process is continuing.

Regional network for biotechnology in Latin America

UNIDO with the financial assistance from the United Nations Fund for Science and Technology and UNDP initiated a preparatory phase of biotechnology activities in Latin America with the objective to promote industrial development in the region. An expert group visited the region and after extensive consultations with representatives of industry, universities and the public sector recommended establishment of a Regional Biotechnology Programme which tranformed into regional network for biotechnology in Latin America. The network envisaged improvement of information bases, identification of priority research areas, coordination of research efforts in the region and strengthening the link between the scientific and public sectors and offering advice to Governments, institutes and industrial organizations on aspects relative to the development and application of biotechnology and genetic engineering. In addition, UNIDO implemented technical assistance programmes at national and regional levels. Among these are establishment of the Biotechnology and Genetic Engineering Centre at Cuba; production of vaccines relevant to human health in Mexico and Venezuela; leaching of minerals by biotechnological methods at Chile; production of alcohol by conversion of wastes from the sugar industry; design of a national biotechnology programme in Peru; and organization of a regional technical meeting on the production of blood derivatives.

Under the network, programme assistance nas also been rendered to Argentina, Costa Rica, Cuba and Venezuela in formulating national and regional policies on biotechnology; to study the impact of copyrights on biotechnology industry in Latin America and organize a meeting on the subject in Venezuela and to conduct a course on industrial biotechnology in Chile.

The Industrial Operations Technology Division also implements several technical cooperation projects in biotechnology, including pharmaceuticals and vaccines.

Code on biosafety

To facilitate the safe application of biotechnology on the one hand and the production and trade in biotechnology products on the other, and since regulations are lacking at the international level and in many countries at the national level, UNIDO has felt the need for a "Code of Conduct" for release of genetically-modified organisms (GMOs) into the environment. A certain measure of international understanding in relation to safety in the laboratory and in manufacturing could be said to exist but the subject of release into the environment required special attention. Hence, as part of the work of the informal UNIDO/UNEP/WHO/FAO Working Group on Biosafety, UNIDO has taken the initiative to formulate a Voluntary International Code of Conduct on release of GMOs into the environment. This has been done through two expert group meetings as a result of which a draft code has emerged. It is being submitted to the Preparatory Committee of the UN Conference on Environment and Development and to other forums.

Basically, the Code attempts to lay down the minimum commonly accepted principles in regard to the subject. It draws on existing directives and regulations and does not intend to develop new concepts. It stipulates general principles, the obligations of Governments and the obligations of the proposer and the researcher. It has been framed in such a way that more specific guidelines could be built up on it for specific products or applications. Cooperation of the ICGEB, and OECD and several experts has been enlisted by UNIDO for the preparation of the Code.

Alongside of the Code, the experts have felt the need for an information-cum-advisory service which could serve countries and enterprises on request. Interested countries could benefit from information and advice for framing new regulations or in any particular case of release of an organism into the environment. The structure of such an advisory service is under elaboration by UNIDO.

It may be noted that the Code of Conduct as worked out may not always provide an adequate framework to assess the risks posed by organisms not indigenous to the introduction site. It also does not deal with issues related to the contained industrial applications of GMOs. Similar effort to internationalize principles and codes of practice covering these categories is under consideration.

It is expected that the promotion and monitoring of the Code will be done by UNIDO. It is also intended that regional meetings will be held to further promote the Code and also suggest any regional variations or adaptations that may be necessary.

In relation to the Code, UNIDO is in constant touch with the Secretariat of the UN Conference for Environment and Development. It also cooperates with UNEP in consideration of issues relating to biological diversity. Both these agencies have also stressed the role of the ICGEB in the area of biotechnology in relevant documents.

Bioremediation

UNIDO has initiated a bioremediation and oil recovery programme (BIOROR programme) taking into account the recent damage to environment through oil spills and also the need to maximize oil recovery. The programme includes, *inter alia*, demonstration projects for oil companies in developing countries so that they could see directly the benefits of bioremediation and microbial enhanced recovery of oil. The programme also includes assistance, on request, in building up a core of biotechnological capabilities in oil companies which would help them in dealing with the recovery by themselves or in using outside help effectively.

Fermented foods

The application of biotechnology to food processing would be typically a case of blending of modern and traditional technologies. UNIDO has in the past commissioned studies on fermented foods in Africa. An expert group meeting on the application of biotechnology to food processing in Africa is scheduled to be held in December, 1991 in Nigeria. In addition, a project implemented by UNIDO in the Republic of Korea on lactic acid fermentation is of particular interest. The project enabled the development of a protein-rich traditional beverage involving the fermentation of soya beans and rice. The project has enabled a

greater concentration of protein in the beverage and also the imparting of an agreeable flavour to the beverage. The beverage is called "risogurt". It is expected to be taken up for commercial production by a company in the Republic of Korea. The technology involved in such lactic acid fermentation is also sought to be passed on to other developing countries through a training programme which is scheduled to start on 1 October 1991. UNIDO expects to give greater attention to these types of projects in future in Asia, Africa and Latin America.

The Monitor

The Genetic Engineering and Biotechnology Monitor, started in 1982, has become a highly popular quarterly bulletin on developments in the field of biotechnology and genetic engineering including scientific as well as commercial developments. The Monitor also includes commissioned articles from time to time. Professionals, both in developed and developing countries, find that it helps them in increasing current awareness without having to go through scores of journals.

In future, UNIDO intends to move beyond capability-building activities to commercialization of biotechnology, enterprise-level cooperation and investment promotion. One of the actions taken in this aspect is to convene an expert group meeting on commercialization of biotechnology which will be held in October this year. The field of action required in biotechnology is vast. Considerable promotional work still needs to be done. The International Centre for Genetic Engineering and Biotechnology will continue to be a major vehicle for increasing awareness and strengthening the capabilities of developing countries' scientists in this field.

B. RESEARCH

Research on human genes

Growth factor

A neurotrophic protein produced and released by glial cells in the brain has been found to have a new activity: it is a growth factor for glial cells. The preparation of biologically active recombinant S100ß, expressed in *E. coli* from a synthetic gene for use as a growth factor, has been developed. S100ß would be useful in studying fundamental cellular processes such as mitosis, differentiation, development and growth. Because the human S100ß gene is located in the Down syndrome region of chromosome 21 and because S100ß protein is found in abnormally high levels in certain neurodegenerative diseases and tumours, the technology may be useful in conjunction with these diseases. Patent applied for. (Licence available.) Further details from: Ref. VU#9031, Jacqueline B. Shrago, Office of Technology Transfer, 405 Kirkland Hall, Vanderbilt University, Nashville, Tennessee 37240. Tel.: (615)322-7056 (Source: International New Product Newsletter, September 1991)

Research on animal genes

Gene-spliced tumour cells immunize mice against cancer

A vaccine based on genetically engineered tumour cells can seek out and destroy small cancers in mice. This finding, by researchers in the United States, raises fresh hopes that human tumours may one day be treated in a similar way, although there is no immediate prospect of a "cancer vaccine".

Drew Pardoll and his colleages at Johns Hopkins University in Baltimore took cells from kidney tumours in mice. They inserted into the cells a stretch of genetic material that encodes interleukin-4, a substance secreted by immune cells. IL-4, a member of the group of "cell messengers" known as cytokines, helps to stimulate the production of "killer" T-cells that attack tumour cells.

When the team injected the cells back into the mice, the animals produced a general immune response that rejected the cells at the injection site. More importantly, however, most of the mice also produced specific killer T-cells that sought out and destroyed existing small tumours elsewhere in the body. These tumours had already begun to invade their surrounding tissue.

For the first time, scientists have shown that it is possible to use cytokines to trigger a specific immune response to tumours without producing a general, toxic effect. In the past, doctors have tried to stimulate the immune response to cancer in animals and people by giving them a systemic injection of cytokines in large doses, but the results have been disappointing, and the large doses have often stimulated a "sledgehammer" response that destroys innocent cells too.

The team believes that IL-4 works by helping to "present" proteins from the tumour to the immune system in a more effective way. As a result, they believe, the immune system recognizes the tumour proteins as being foreign and attacks them. More experiments are under way to test this.

Pardoll stresses that there is no immediate prospect of a "cancer vaccine" using this approach. He doubts that genetically engineered cells could destroy large tumours in animals or people. Instead, he says, the aim would be to protect patients whose main tumour had been surgically removed from any recurrence or spread of the cancer cells. (Source: New Scientist, 9 November 1991)

Gene transplants to zap sap-suckers

Genetic engineers have discovered a natural protein with the potential to kill aphids, plant hoppers, whiteflies and other insects that damage plants by sucking their sap. The researchers have also identified the gene in snowdrop flowers (*Galanthus nivalis*) which produces the protein. They now plan to transplant it into commercial crops and so give them in-built protection against sap-suckers.

Vaughn Hilder of the Agricultural Genetic Company in Cambridge and Angharad Gatehouse of the Department of Biological Sciences at the University of Durham worked together to identify the protein. They found that the protein derived from snowdrops, a lectin similar to many compounds found in garlic, killed sap-sucking insects called brown plant hoppers (*Nilaparvata lugens*). These insects damage rice plantations in Japan and South-East Asia.

The company has successfully implanted the gene into tobacco and lettuce using an infectious bacterium called *Agrobacterium tumefaciens*. This bacterium acts as a "Trojan Horse", transferring the foreign gene into its new host. Their aim now is to find a similar bacterium which transplants the gene more specifically, ensuring that the protein is only produced in the phloem of plants, the tubes that exude sap.

AGC is hoping to forge partnerships with other companies to commercialize the discovery. It is also seeking a deal with the International Rice Research Institute in the Philippines to make the technology available cheaply - or even free of charge - to nations that could not afford to pay a market price for it. (Source: New Scientist, 14 December 1991)

Research on viral genes

Octameric peptide elicits HIV antibodies

A new type of experimental AIDS vaccine has stimulated, in guinea pigs, a higher level of antibodies to several different strains of human immunodeficiency virus (HIV-1) than have other sera tested so far. The new approach involves a synthetic, branched immunogen - a globular, arboreal cluster containing eight copies of a 33-amino-acid sequence found in the principal neutralizing determinant (PND). The PND is a loop-shaped structure on the virus's envelope that contains a short, conserved amino acid sequence flanked by longer sequences that vary from strain to strain. These sequences are an attractive antibody target when the goal is to elicit antibodies to all the important strains. Chemist-immunologist Chang Yi Wang of United Biomedical Inc. of Lake Success, New York, and her colleagues there and in California have shown that their octameric peptide can elicit an "extraordinarily high" level of neutralizing antibodies, according to co-worker Alan M. Walfield. Moreover, the antibody levels were sustained in the test animals over a three-and-a-half-year period. A cocktail of octamers representing six different HIV strains served to accelerate and broaden the response to several strains. Although the results are encouraging, they have

yet to be duplicated in chimpanzees. The researchers also would need to show such a vaccine can protect against infection by HIV. (Reprinted with permission from Chemical and Engineering News, 14 October 1991, p. 24. Copyright (1991) American Chemical Society)

How HIV avoids detection in the body

The human immunodeficiency virus can elude its greatest enemies in the body, the "killer" T-cells of the immune system. Researchers in Oxford have found that mutations in the gene encoding the virus's core protein change precise stretches of animo acids that the T-cells recognize. Once changed, these stretches can become unrecognizable to the cells so that they do not challenge HIV.

The finding is the strongest evidence yet for something that scientists have long suspected but have been unable to show until now. HIV is unlike most infectious agents because, although it triggers an immune response, the virus persists and overwhelms the body. For several years, researchers have thought this might be partly due to viral mutations.

The immune response has two "arms" - the production of antibodies and the response of specific T-cells. There are two types of T-cells, the "helper" cells, which the virus infects, and "killer" cells, known more accurately as cytolytic T-lymphocytes, CTLs. Although antibodies are important in attacking HIV, most scientists believe that CTLs are the most effective response to it. If an effective vaccine can be developed, it will probably need to stimulate CTLs. (Extracted from New Scientist, 14 December 1991)

Research on bacterial genes

Shortening the odds against evolutionary change

The latest challenge to orthodox evolutionary theory comes from a genetic phenomenon which flouts all the rules of molecular biology. A study by Barry Hall of the University of Rochester, New York, shows that the bacterium *Escherichia coli* has a hitherto unrecognized talent, the ability to manipulate the rate at which favourable combinations of "chance" mutations appear in its genes (Proceedings of the National Academy of Science, Vol. 88, p. 5882). The discovery provides yet more evidence for the controversial idea that bacteria can mutate in a seemingly non-random fashion.

Hall's study broaches one of the most vexing questions of all in evolutionary biology: how adaptive changes that depend on many chance mutations ever arise. Most evolutionary innovations require not one but a series of mutations, all in different genes. And that poses a problem. For if, individually, each mutation offers little or no survival advantage. then natural selection can act strongly only on organisms which have acquired the full set. Assuming each mutation is random, such organisms should be freakishly rare. The odds against the adaptation will be overwhelmingly high, almost as if there is a barrier between the species and the adaptation.

The conventional view is that species can climb such barriers, but only slowly. Either each mutational step is marginally beneficial (for example, a light-sensitive organ, while obviously not as useful as an eye, may be better than nothing), or random genetic drift, acting over vast tracts of time, eventually produces organisms with all the necessary mutations.

Hall's study throws up a radical alternative. It suggests *E. coli* is somehow able to leap the barriers to certain adaptations.

The idea is based on the beaviour of a strain of *E. coli* with defects in two genes. The genes, known as *trpA* and *trpB*, encode enzymes needed to break down the amino acid tryptophan; without them *E. coli* cannot grow in a tryptophan-based medium.

To solve this growth problem, the bacterium needs two specific mutations, one to remedy each of the two defects. When Hall measured the frequency of the mutations in laboratory cultures, he found they cropped up as a pair 100 million times more often than expected - as if the bacterium had some way of directing the mutations instead of leaving them to chance.

The finding comes three years after John Cairns and his colleagues at Harvard University first mooted the "heresy" of directed

mutagenesis, and is certain to fuel the ongoing controversy. Cairns published further evidence for the phenomenon in the journal Genetics.

Many biologists, Hall included, have attempted to resolve the conflict by arguing that, despite appearances, the mutations are in fact the result of random genetic events.

According to Hall, however, none of the explanations suggested can account for his latest results. There are two main stumbling blocks, he says. First, the mutations in the *trpA* and *trpB* genes appear to occur simultaneously, rather than sequentially as theories based on random events would predict. Secondly, the beneficial mutations arise from highly specific genetic changes. Hall found no changes in the DNA sequences of the two genes save those responsible for the beneficial mutations themselves. Most random mechanisms would generate a spread of genetic changes.

Another orthodox explanation is that some chance physiological event somehow acts as a trigger, opening the way to mutations in the *trpA* and *trpB* genes. Once again, says Hall, his results rule this out. He concludes instead that bacteria must have some "mechanism of specifically increasing the rate of advantageous multiple mutations".

But what this mechanism is is anyone's guess. Hall is working on the assumption that the special adaptive mechanism comes into play only when the bacterium is highly stressed. An important clue, he says, is that the mutations arise only after the bacterium has been starved for about 20 days. (Source: New Scientist, 21 September 1991)

IL-1 enhances bacterial growth

Mammalian Interleukin-1 enhances bacterial growth, according to the findings of a research team at Tufts University, New England Medical Center Hospitals in Boston, United States. Reporting in Science, the scientists found IL-1 enhanced the growth of virulent but not avirulent strains of *Escherichia coli*. The significance of the findings for the progression of infections still remains unclear although it does seem possible that IL-1 produced *in vivo* in response to inflammation or bacterial infection could in fact exacerbate the problem. (Source: European Chemical News, 9 December 1991)

Research instrumentation

Chromosome "paints" developed

The Lawrence Livermore National Laboratory has developed special fluorescent dyes, called whole chromosome paints, that can detect chromosomal abnormalities that often indicate cancer or leukaemia.

The technology is based on the ability of fluorescent dyes to light up entire chromosomes within cells. It enables researchers to monitor genetic changes and determine whether cells are normal or cancerous.

The technology stems from the Human Genome Program, a joint effort by the Department of Energy and the National Institutes of Health. (Source: Chemical Marketing Reporter, 28 October 1991)

Immunotherapeutic for drug detoxification

The tricyclic group of antidepressant drugs are commonly prescribed both as anti-depressants and sedatives. Overdose is relatively frequent and has been estimated to be the leading cause of death from intentional overdose in the United States. Toxic effects include cardiac arrythmias, convulsions, coma. There is a clear clinical need for an effective treatment for the removal of toxic levels of drugs from vital organs.

BioResearch Ireland has developed an antibody-based therapeutic which shows excellent results in tricyclic salvage from tissue in an experimental model. Partners and potential licensees are now being sought to further develop this product.

For further information contact: Dr. Enda Kenny, BioResearch Ireland, EOLAS, Glasnevin, Dublin 9. Tel.: 353-1-370177. Fax. 353-1-370176. (Source: Irish Biotech News, December 1991)

Assay for determination of susceptibility of tumours to anti-cancer drugs

An *in vitro* tissue culture assay has been developed which can be used to determine the

susceptibility to anti-cancer drugs of tumour cells from individual patients. What is offered is a method for culturing tumour tissues excised from patients, for exposure of these cultures to anti-cancer drugs for measurement of the growth of cultured tissues, and for determination of the drug most likely to be effective in *in vivo* treatment of the tumour.

BioResearch Ireland are seeking licensees for the method. Details from Dr. Jim Ryan, Marketing Manager, BioResearch Ireland, EOLAS, Dublin 9. Tel.: (01) 370177. Fax: (01) 370176. (Source: Irish Biotech News, December 1991)

Miscellaneous

Peroxidase

A peroxidase stable at pH2 and 45°C will survive conditions found in field sites requiring bioremediation. Used as a label in consumer test kits and fieldable assay packets, the neutral pH active form requires no refrigeration, a distinct advantage over the relatively labile horseradish peroxidase. Other applications can be found in the food industry and biosensors. (Licence available.) Further details from Kenneth Runnion, President, JK Research, 210 South Wallace, Bozeman, Montana 59715-4857. Tel.: (406) 586-8744. (Source: International New Product Newsletter, September 1991)

C. APPLICATIONS

Pharmaceutical and medical applications

Gene therapy rids mice of cancer

Gene therapy has been used for the first time to eliminate an existing cancer in mice by inducing their immune systems to destroy tumour cells, a result that offers hope of a similar treatment for human cancers. Until now most studies have sought to immunize or vaccinate animals against developing subsequent cancers. Paul T. Golumbek and Drew M. Pardoll of Johns Hopkins University and co-workers took tumour cells from a mouse kidney cancer and genetically engineered them to secrete large amounts of interleukin-4 (IL-4). Injection of the engineered cells back into the mice caused them to develop an immune response to the cancer that destroyed their tumours. A major advantage of the technique is its ability to destroy cancer cells not only at the tumour site but also at remote sites in the body, without damage to normal cells. Normally, when T-cells in the immune system detect the presence of foreign substances they release lymphokines like IL-4 that activate the rest of the immune system. However, cancer cells do not trigger this T-cell response. Secretion of IL-4 by the engineered cancer cells apparently alerts the immune system that the cancer tissue is foreign, bypassing the inactive T-cells. In a related study begun last month, NIH researchers are attempting to elicit cancer immunity in humans by modifying live tumour cells to produce tumour necrosis factor. (Reprinted with permission from Chemical & Engineering News, 4 November 1991, p. 29. Copyright (1991) American Chemical Society)

Anticancer drug teaches unruly cells to toe the line

A new drug developed in the US "educates" cancer cells to behave like mature cells, rather than ones which proliferate wildly. It therefore operates quite differently to other cancer drugs, which attempt to kill unruly cells.

The compound was developed by researchers at Columbia University and the Memorial Sloan-Kettering Cancer Center in New York. They presented their results at a meeting of the American Chemical Society in New York.

The new drug is based on an earlier drug called HMBA. Clinical trials with HMBA began seven years ago, but because large doses have to be used - several grams each day - treatment is expensive and bothersome for patients. The new drug is a thousand times more powerful than HMBA, and produces a similar effect with a dose of only a few milligrams a day.

Both HMBA and the new drug appear to bind to protein kinase C, a key enzyme in all cells. They activate the enzyme, probably causing it to attach phosphorus groups to the cells. It is this that causes the cells to stop dividing wildly and "exhibit more mature behaviour". The new drug binds to the

enzyme at two sites, wheras HMBA binds at one site only. The drug has no name. The researchers have not even published a full description of it, because a patent on it is still pending.

In test-tube experiments, the new drug has stopped many types of cancers spreading, including cancers of the ovaries, brain, breast and colon, as well as leukaemia. At the moment, the researchers are testing it on mice. So far, the only serious side-effect is a reduced production of white blood cells, which recovered however when the drug's dose was reduced. (Extracted from New Scientist, 7 September 1991)

Leukaemia patient receive first bone marrow transplant with rDNA cells

An 11-year-old leukaemia patient at St. Jude Children's Research Hospital in Memphis, Tennessee, is the first patient to receive an autologous bone marrow transplant (ABMT) with genetically engineered cells. His own marrow had been removed during a period of remission, modified by retroviral vectors supplied by Genetic Therapy, Inc., Gaithersburg, Maryland, and stored. Although ABMT is an effective treatment initially, patients often relapse. After this patient received intensive drug and radiation treatment, Malcolm K. Brenner, director of the hospital's bone marrow transplant programme, reintroduced the victim's own cells, but now carrying a recombinant neomycin-resistance marker.

Use of such non-therapeutic genetic markers permits physicians to track the transplanted cells, or see where new cancerous cells arise. By following the marked cells, researchers can determine whether relapses result from ineffective drug and radiation treatment or from undetected cancer cells in the transplanted bone marrow. (Source: McGraw Hill's Biotechnology Newswatch, 16 September 1991)

AIDS antisense survives *in vivo*

Hybridon, Inc., of Worcester, Massachussetts, reported that when its researchers injected an anti-AIDS, antisense sequence into a laboratory animal, the compound survived many hours. Such oligonucleotides were believed to be quickly broken down in the body by enzymes, therefore the feasibility of using these compounds as therapeutics was problematic. The research was published in the Proceedings of the National Academy of Sciences for September. In previous studies, the company had demonstrated that HIV was inhibited *in vitro* by synthetic, antisense, nucleic-acid sequences. Hybridon plans to file an Investigational New Drug (IND) application in the coming months to conduct human clinical trials of this compound, which was chemically modified to increase its longevity in the body. (Source: McGraw Hill's Biotechnology Newswatch, 16 September 1991)

Monkey tests force rethink on AIDS vaccine

The search for an AIDS vaccine was thrown into disarray with the disclosure of "stunning" findings from experiments on monkeys carried out by Britain's Medical Research Council. The results undermine earlier studies on monkeys, which many considered crucial steps towards the development of a human vaccine. Some researchers now think they should completely revise their approach to understanding the way the body protects itself against retroviruses, the family that includes HIV.

Scientists have always assumed that the immune system protects itself against HIV by making antibodies to the virus's proteins. Theoretically, these antibodies should stop the virus entering cells. But the new results challenge these assumptions. In essence, they suggest that, however the vaccines protect monkeys, it may not be by stimulating the production of antibodies against viral proteins. Instead, the vaccines seem to rely on stimulating antibodies to proteins from human cells. These cells are used in the preparation of the vaccines.

Whatever the findings mean, they come at an awkward time for the MRC team. There is serious disquiet among many British AIDS scientists about what they see as an overemphasis on the study of monkeys in the MRC's AIDS programme.

Like many of the studies so far, the British experiment involved macaques. These animals

have their own equivalent of HIV, a virus called simian immunodeficiency virus (SIV), which causes an AIDS-like disease. Because of this, many groups have believed that macaques are a good model for developing AIDS vaccines. About 100 macaques in the US and Britian have now been successfully immunized against live SIV by vaccines based on preparations of whole, killed virus.

The MRC researchers gave four macaques a vaccine based on cultured human T-cells that had been infected with SIV and then inactivated. When they gave these monkeys live virus, three out of four were protected. But the shock came from four other monkeys. The researchers gave these animals uninfected human cells of the same type as those used to create the vaccine. These cells had never seen SIV. To the team's amazement, when they gave the animals live SIV, two of them were protected.

Next, the researchers examined the animals' antibodies. The protected animals had high levels of antibodies to proteins that came not from SIV, but from the human T-cells. Unprotected animals had only a tenth of the level of these "anticell" antibodies. This is the first time researchers have been able to link the level of an antibody with the degree of protection in macaques.

The team also studied antibodies from macaques used in previous vaccine experiments. They found the same pattern of immune response in animals that had received a vaccine based on killed SIV alone, without human cells. But the vaccine had been cultured in human cells. Researchers know that proteins from the cells can become incorporated into the virus.

Scientists have based most of their optimism about AIDS vaccines on studies in macaques. About five chimpanzees have also been protected from HIV infection. However, a large number of chimpanzee experiments have failed.

About a dozen potential AIDS vaccines are being tested in people to check safety. No one is ready to test whether a vaccine protects against infections. (Extracted from New Scientist, 21 September 1991)

Green light for ddI

After an expedited review of just six months, the US Food and Drug Administration (FDA) gave the New York-based drug company Bristol-Myers Squibb approval to market its antiretroviral drug Videx (also known as dideoxyinosine or ddI) for the treatment of AIDS. The drug, approved initially for narrow use, can be used to treat both adult and paediatric AIDS patients who are intolerant to AZT, the only other approved drug for the treatment for HIV infection.

Under an expanded access programme and through clinical trials, the company estimates that more than 30,000 patients have already received Videx therapy. After a review of preliminary phase I trial data, the drug was approved on the basis of ddI's effect on increasing the patient's CD4 helper cell count, a surrogate marker for determining clinical effectiveness. (Source: Nature, Vol. 353, 17 October 1991)

Treating AIDS with worts

The first clinical trial of a new anti-AIDS drug derived from the plant genus *Hypericum* (better known as Saint-John's Wort) has been started by Fred T. Valentine and Howard Hochster, researchers at New York University Medical Center. The trial will involve about two dozen patients, and is designed to test the safety of the drug. Laboratory tests suggest it will be a useful adjunct to available therapies.

Preclinical research at NYU has shown that the drug, hypericin, can prevent uninfected T-cells from being infected with the AIDS virus in cell culture. Hypericin is a virucidal agent, meaning it can precisely target new virus particles and prevent them from infecting other cells. Since hypericin does not appear to affect reverse transcriptase, and animal tests show that it has low toxicity at therapeutic doses, researchers hope it will not only work on its own, but also have a synergistic effect when taken with either of those drugs.

Hypericin was originally synthesized at the Weizmann Institute of Science in Rehovot,

Israel. It is being manufactured by VIMRx Pharmaceuticals, Inc. of Stamford, Connecticut. (Source: Science, Vol. 254, p. 522, 25 October 1991)

AIDS drug trials

A new drug for AIDS has begun trials in patients in Australia, Italy and the Netherlands. Nevirapene, developed by Boehringer Ingelheim, blocks the replication of HIV by interfering with the action of the virus's enzyme, reverse transcriptase.

Nevirapene - formerly known simply as BI-RG 587 - has already been through tests in the laboratory and in animals. The new trials will assess the drug's safety and how well it works in humans.

Nevirapene is a member of a group of compounds related to the benzodiazepines, the class of tranquillizers that includes Valium. But unlike the benzodiazepines, it does not have a tranquillizing effect.

The trials will compare the effect of Nevirapene with zidovudine on people who are HIV-positive and progressing towards AIDS. One third will receive the new drug alone, one-third zidovudine alone, and the remainder will receive a combination of both. (Source: New Scientist, 23 November 1991)

The hunt for an animal model

Progress towards a therapy for cystic fibrosis has long been hampered by the lack of a biological bridge between the test tube and patient - that is, an animal model for the disease.

But not for much longer. Several teams of researchers are on the verge of creating genetically engineered mice that carry genetic defects similar to those which cause cystic fibrosis in humans. The mice should be a boon to researchers trying to test new drugs and evaluate the potential efficacy of gene therapy.

Animal models already exist for many human hereditary diseases, including Alzheimer's disease, type I diabetes, hypertension and Duchenne muscular dystrophy. Most of these, however, are not genetically engineered; rather they are the result of careful breeding of natural strains of mice or rats.

The failure to find a comparable strain of mouse for cystic fibrosis has encouraged researchers to create one artifically. The favoured approach is to "knock out" the mouse equivalent of the gene which when faulty in humans causes cystic fibrosis. Recent studies by two veteran cystic fibrosis researchers, Bob Williamson, of St. Mary's Hospital in London, and Art Beaudet at the Baylor College of Medicine in Houston, Texas, show that the mouse carries a gene similar to the CFTR gene in humans. The key problem has been how to disable it. A relatively new and powerful technique called homologous gene targeting may hold the answer.

Homologous gene targeting was developed in the mid-1980s by Martin Evans at the University of Cambridge. It works by replacing a healthy version of a gene with a copy engineered to carry a disabling flaw, such as the most common of the cystic fibrosis mutations.

At an international meeting on cystic fibrosis held this summer in Williamsburg, Virginia, two groups reported that they had succeeded in knocking out the cystic fibrosis gene in embryonic stem cells from mice. It should now be only a matter of months before these groups and others are able to breed a mouse containing two copies of the faulty gene, giving researchers the animal model for cystic fibrosis they so desperately seek. (Extracted from New Scientist, 7 December 1991)

Treatment hope for muscular dystrophy

Injection of the normal dystrophin gene into the muscles of dystrophin-deficient mice restores a small amount of dystrophin production in muscle fibres, a result that opens the possibility for gene therapy of muscular dystrophy. This is the conclusion of a study conducted by Jon A. Wolff and co-workers at the Department of Paediatrics and Medical Genetics of the University of Wisconsin, Madison; Kay E. Davies and associates at John Radcliffe Hospital, Oxford, UK; and another group at Guy's Hospital, London. The mice used in the study are an animal model for

Duchenne's muscular dystrophy, a disease that causes progressive degeneration of skeletal and cardiac muscles and premature death. The researchers found that injecting the mice with plasmids containing complementary DNAs for the dystrophin gene led to expression of the protein in 1 per cent of muscle fibres. Indications are that the effect is long lasting, with stable expression of introduced genes continuing for several months. However, "the efficiency of this gene-transfer technique needs to be increased before it can be used clinically", the researchers say. (Reprinted with permission from Chemical & Engineering News, 2 September 1991, p. 30. Copyright (1991) American Chemical Society)

DNase offers a potential therapy for chronic lung disease

For victims of chronic lung diseases, such as cystic fibrosis and chronic bronchitis, keeping the lungs clear and free of mucus is a constant, often life-threatening, problem. No fully effective treatment exists for either the inherited cystic fibrosis or the smoking-related chronic bronchitis.

Now Genentech is looking at DNase, a naturally occurring enzyme whose function is to dissolve DNA. Produced by recombinant DNA technology, DNase was first cloned and isolated by the company's researchers - and offers a promising means of dissolving mucus. Clinical trials show that when inhaled recombinant DNase appears to reduce the thickness of infected lung secretions, which consist largely of DNA excreted as a by-product of inflammatory cells. Genentech has now received Orphan Drug designation for DNase to treat cystic fibrosis.

Clinical trials are under way in the US. Phase I trials have already been completed and have shown the drug to be well tolerated by patients. Phase II trials to study effectiveness and optimum dosage are also under way. Phase III trials needed for FDA approval are scheduled to begin later this year.

Phase II trials will also begin in Europe. The market for DNase is thought likely to be bigger in Europe than in the US. As with most new products currently in the development pipeline, Genentech has retained European rights for DNase. Details from: Genentech Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080, USA or on Tel.: +1(415)266-1000. (Source: Biotechnology Bulletin, Vol. 10, No. 9, October 1991)

Clearance for cholesterol treatment

Gene therapy for treating patients prone to developing very high levels of cholesterol has been recommended for approval by the US National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC). The committee also approved gene therapy tests on cancer patients by a research team at NIH, but rejected a proposal from the University of Rochester, New York, to develop a combination genetic and immune-based strategy to combat cancer.

RAC chairman, Gerard McGarrity, calls the committee's approval of the University of Michigan's cholesterol proposal a "milestone" because it is the first attempt to use genetic engineering to correct an inherited disease. It is also the first time a gene therapy rather than a gene marking procedure will be tried somewhere other than NIH.

James Wilson and his colleagues in Michigan are studying patients with a severe disorder of lipid metabolism called familial hypercholestero- laemia. This disorder, caused by a missing or defective version of a critical cell receptor, leads to greatly accelerated deposition of cholesterol in the circulation - a prelude to heart disease and, often, early death.

The Michigan team plans to insert the gene for the missing receptor into liver cells that have been removed surgically from patients and cultured. These altered cells will then be re-implanted to gain some control over cholesterol metabolism. (Source: Chemistry & Industry, 4 November 1991)

Clinical trials for new drug

ImmunoGen (Cambridge, Massachusetts) reports its European partner Roussel Uclaf (Paris) has begun clinical trials of Oncolysin B, a treatment for B-cell leukaemia and lymphoma. The drug - a monoclonal antibody linked to a proprietary form of a plant toxin called ricin - is in Phase II clinical trials in the US. Roussel Uclaf gained exclusive European

marketing rights to the drug in a 1990 deal with ImmunoGen. (Source: Chemical Week, 25 September 1991)

Oriental Yeast to market human tissue test kit

Oriental Yeast Co. Ltd., Tokyo, plans to begin marketing "Skin2", a human tissue kit for *in vitro* screening of cosmetics, drugs and chemicals. Skin2 consists of a 24-well plate with each of the wells containing 1.2 sq. cm. of living human three-dimensional tissue. The tissue is for use as a replacement for the ocular and dermal tissue of laboratory animals used in the conventional Draize irritation and toxicity tests. The test kit gives nine documented endpoints to determine the mechanisms of toxicity. These include neutral red, MTT uptake, PGE2, LDH, NAGS, IL-6 and glucose utilization. Skin2 will be priced at 190,000 yen/set and Oriental Yeast predicts annual sales of 100 million yen in the first year of marketing. (Source: McGraw Hill's Biotechnology Newswatch, 2 December 1991)

Relief for cat persons?

As the term "cat person" suggests, many people find cats irresistible. But along with feline charms come some irritating - even dangerous - forms of pathology, including allergic responses and the sweaty, achey condition known as "cat scratch fever".

But two separate research groups reported advances that may reduce the perils posed by *Felis domesticus*. Jay P. Morgenstern of the ImmunLogic Pharmaceutical Corp. in Cambridge, Massachusetts, and colleagues have cloned the major allergen produced by cats, called Fel dl. Cats' skins are coated with the protein, which bears some resemblance to rabbit uteroglobulin - believed to limit maternal immune response during embryo implantation. So far, researchers have not discovered the allergen's biological function. They suggest that if further studies show it can modulate immune processes, better management of cat-allergic patients may be down the road.

Meanwhile a team at the Centers for Disease Control in Atlanta has, for the first time, characterized the bacterium that causes cat scratch fever. The malady, which strikes about 6,000 people a year, causes fever, swollen lymph glands, anorexia and general malaise, and can last as long as a couple of months. The organism, *Afipia felis*, is a resistant one, and has been difficult to keep in laboratory culture, but the team has devised a means for doing so and efforts are now being directed at finding a suitable antibiotic. (Source: Science, Vol. 254, 8 November 1991, p. 797)

Throwing light on arthritis

For the first time scientists can look inside living cells, and find out what has gone wrong with them, without having to kill them first. Anthony Campbell, professor of medical biochemistry at the University of Wales College of Medicine in Cardiff, is using luminous marine organisms to light up cell chemistry.

Campbell has been looking at cells from the joints of rheumatoid arthritis sufferers using bioluminescence from the common piddock. The cells' normal job is to kill invading bacteria in the body using "bleach". Unfortunately, in rheumatoid arthritis, the cells infiltrate the joints. Campbell's aim is to find out what triggers these cells to release bleach and inflame the joints.

The release of this bleach can be measured in the test tube by using a luminous protein isolated from the piddock. This bivalve lives inside a hole in a rock and squirts a luminous cloud when disturbed. One of the chemicals which sparks off this light emission is bleach.

Using another luminescent protein obelin, isolated from the luminous jelly fish *Obelia*, Campbell has found that calcium is the key signal for bleach release. "Simply adding calcium to the obelin triggers a flash of light."

In arthritis sufferers, this calcium release is abnormal, Campbell has discovered. To find out how the calcium works, a new family of biolumines- cent indicators have been genetically engineered.

Bioluminescent proteins from a jelly fish and the firefly have been isolated and engineered. When the engineered protein is injected into living cells, the cells make the protein light up. Abnormalities in the cells can

then be identified by looking at the characteristics and location of the light emitted.

So far, researchers have only worked on two marine systems. However, in deep sea virtually every organism is bioluminescent. These organisms offer exciting potential for new proteins which will light up other chemical features of cells. (Source: Chemistry & Industry, 16 September 1991)

Cal Tech's molecular imprints

In research that could eventually lead to production of synthetic antibodies, scientists at California Institute of Technology (Pasadena) report a synthesis technique to make metal-complexing polymers that are highly selective in recognizing protein-like molecules. Based on the observation that the amino acid histidine has a high affinity for metal ions, the scientists mixed together monomers containing copper ions attached through iminodiacetate groups with bisimidazole molecules - used to mimic proteins - that have histidine-like imidazole groups. After polymerization, a solid polymer formed that bore the imprint of the target molecule and the copper ions in the precise position of the imidazole groups. An initial application could involve selective separations of small molecules, according to the Cal Tech scientsts. (Source: Chemicalweek, 18 September 1991)

"Foolproof" DNA fingerprints within grasp

A radical change in the technique of genetic fingerprinting is about to revolutionize forensic science. The development, which makes it possible to express an individual's genetic information in digital code, means DNA fingerprints will be many times more accurate than before and easier to compare.

Alec Jeffreys, the British scientist from the University of Leicester who invented DNA fingerprinting in 1984, has now updated the technique to produce "a totally different way of tackling the problem". He and his colleagues published the method in Nature but warn, however, that the technique needs further testing before it is ready for use in the courts.

The discovery will intensify the debate about whether Britain and other countries should have giant databases containing genetic "signatures" of millions of people.

The new method, which should be virtually foolproof, would make such databases workable.

Instead of comparing the length of a specific minisatellite small stretches of DNA) from two individuals, the new technique compares the variations in the minisatellite's DNA sequence.

In a double strand of DNA, each gene comes in two alternative forms, or alleles - one from the mother and one from the father. In a stretch of repeats, there are three possible states for each pair of alleles. For example, if one allele is "red" and the other "blue", then each position can either have two red, two blue or one of each. If each of these combinations is given a number, 1, 2 and 3 respectively, the whole repeat region can be expressed digitally, in ternary code.

Not only does this system allow instant comparison between individuals and between laboratories; it also checks its own errors. It also dramatically increases the number of genes that can be compared, from about 50, to 100 million.

The system has so far been tested only on broken-down DNA and so needs further testing on realistic samples, such as old bloodstains. Jeffreys stresses that the technique should not be rushed from the laboratory to criminal proceedings. (Source: New Scientist, 23 November 1991)

Livestock applications

Eurogen cattle tech to market

Anglo-Italian biotechnology firm, Eurogen, has announced it will commercialize its *in vitro* embryo-transfer technology in cattle by the end of 1991.

Eurogen was established in 1990 by Italian RDS Agrobiotec and Animal Biotechnology Cambridge (ABC) specifically to commercialize the technology marketed under the name Mastercalf for the transfer of beef and dairy embryos of high genetic value.

RDS Agrobiotec is a subsidiary of the industrial financial corporation Raggio di Sole Finanziara, which specializes in developing agro-industrial applications of biotechnology.

The first production laboratory to implant the Italian genes into embryos on a commercial scale is to be established just outside Milan, an ABC statement says.

ABC also announced that with the development of freezing technology that enhances the pregnancy rates with frozen embryos to that of fresh embryos, it has now been able to commence an operation in Scotland which enables farmers within a 100-mile radius of its base in Aberdeen to order frozen beef embryos the day their cows come on heat and for them to be delivered and implanted within seven days.

The Scotcalf scheme is operated by Scottish Beef Developments, owned jointly by ABC, the Scottish Agricultural College and Buchan Meat Producers, which is a farmer-owned cooperative. (Source: European Chemical News, 11 November 1991)

Vaccination tests in Africa pre-assessed

The US Agency for International Development (USAID) has asked a panel of experts to review the proposed test of a genetically engineered rinderpest vaccine. The development of the vaccine has been sponsored by USAID. It is supposed to protect cattle against rinderpest (cattle plague), a highly contagious viral disease causing 90 per cent mortality in ruminants. The vaccine consists of a *vaccinia* virus implanted with one or two genes from the rinderpest virus.

After having been used against smallpox the *vaccinia* virus was discontinued as a vaccine for humans because of the severe, sometimes fatal, reactions it had caused at the rate of one or two per million vaccinations. It can be transmitted by skin contact between people and from animals, though rarely. Moreover, it has been established that people with deficient immune systems are especially susceptible to the disease caused by *vaccinia*.

USAID proposed to test the newly developed rinderpest vaccine in central Africa. Cattle-raising nomadic peoples in Africa are hit most severely by rinderpest as their livelihood depends on the animals' health, but some of the areas in Africa where rinderpest is endemic also have a high incidence of AIDS (Acquired Immune Deficiency Syndrome). People suffering from AIDS would be particularly susceptible to the *vaccinia*-caused disease. Deaths of AIDS patients caused by *vaccinia* have been reported. It would be risky for a HIV positive person to work with the vaccinated cattle.

USAID has arranged for a thorough pre-assessment - according to the present state of scientific knowledge - of the vaccine's effect on the people as well as on populations of wild animals that might get in contact with vaccinated cattle. (Source: African Diversity, No. 6, October 1991)

Agricultural applications

Du Pont transfers fungal resistance to tobacco

Researchers at Du Pont have developed transgenic tobacco and canola plants with enhanced resistance to fungal attack. The plants contain a gene transferred from bean plants, which promotes the production of chitinase, an enzyme that hydrolyses chitin found in fungi cell walls.

Plants can respond to attack by fungal pathogens in a number of different ways, including plant cell wall fortification to restrict entry, production of phenolic toxins and the induction and accumulation of proteinase inhibitors and lytic enzymes such as chitinase. The scale of protection is limited by the speed of the defence responses and the coordination between them.

Du Pont's research target is to cut the time taken by the plants to produce and accumulate chitinase. Transgenic tobacco and canola plants containing the bean chitinase genes and the cauliflower mosaic virus 35S promoter have been produced. Both sets of modified plants showed greater resistance to attack from *Rhizoctonia solani*, an important fungal pathogen, which has been linked with canola yield losses of 23-53 per cent.

However, while effective against *R. solani*, the enhanced chitinase expression alone may not be sufficient to give protection against a wide range of chitinous fungal pathogens. Tobacco plants containing increased tobacco chitinase levels were found to be only slightly more resistant than control plants to attack by *Cercospora nicotinae*.

However, Du Pont researchers believe that the chitinase-induced delay in appearance of symptoms and the curbing of disease severity caused by *R. solani* may enable young seedlings to survive the critical period during strand establishment in the field when they are most susceptible to attack by soil-borne pathogens. (Source: European Chemical News, 9 December 1991)

Novel biopesticide developed

An Indian biologist in the United States has inserted a scorpion gene into a virus to create a genetically-engineered version of the virus that quickly kills crop-destroying insects. Working with his American colleagues, Dr. V. P. Choudary, director of the antibody engineering laboratory at the University of California, Davis, sewed up the scorpion toxin gene in the genetic structure of the butterfly virus.

The engineered viruses are able to kill pests three times as fast as non-engineered viruses, said Dr. Choudary, till recently a professor of biotechnology at the Jawaharlal Nehru University, New Delhi. Following refinements and successful field trials, the biopesticides are now ready to tackle pests damaging major crops including cotton, cabbage, tobacco and tomato in India and elsewhere, he said. Dr. Choudary said the development of this novel biopesticide had generated considerable enthusiasm in financial circles about commercial potential and its impact on crop growth. (Source: Chemical Business, 5-19 October 1991)

Fast rice outpaces drought

The New Delhi-based Indian Agricultural Research Institute (IARI) is testing a new variety of "instant rice" that can be harvested within an incredible 65 days of sowing.

Called *pusa jaldi dhan* ("quick rice" in Hindi), it is not only the world's fastest-growing rice variety, but is also resistant to pests and can withstand long periods of drought.

The more common varieties of rice seedlings need up to 140 days and abundant water to grow, but IARI scientists claim the new strain only needs three days of rain to give a reasonable harvest.

The "green revolution" that boosted rice yields in the 1960s were confined to irrigated areas. Rain-fed lowlands and hill terraces which produce one fourth of Asia's rice were largely bypassed by the new breeds.

In an effort to improve crops that poorer farmers can afford to produce, research organizations like IARI, with help from the United Nations Development Programme (UNDP) and the Food and Agriculture Organization (FAO), have turned their attention to breeding these new varieties of rice.

On-farm trials of *pusa* rice in Orissa, central Madhya Pradesh state and parts of the eastern state of West Bengal have reportedly been successful. "The new rice varieties offer tremendous potential for other developing countries prone to crop damage by drought and floods", says S. N. Chakrabarti, who spent 10 years designing and breeding the super-fast rice at IARI.

Besides being drought-resistant, Chakrabarti says his quick rice would also be ideal for flood-prone areas.

Agronomists say the new rice can also be planted with other cash crops. In eastern India, thousands of acres of jute fields lie fallow between September and November. *Pusa* could be profitably grown in the region during those three months.

Unlike the "green revolution" rice varieties, the new hybrid does not need large doses of costly chemical fertilizers and pesticides. It produces 0.8 tons of rice per acre,

compared to the present 0.13 tons per acre yield in rain-fed farms.

An estimated 70 per cent of India's 420 million acres of paddy fields are rain-fed. But most of the annual rice production of 70 million tons is grown on artificially irrigated fields.

Indian planners warn the country will have to raise rain-fed rice production to be able to feed its population, which is expected to reach one billion by the turn of the century. United Nations estimates show that India needs to increase its rice production to 105 million tons by then.

To meet the challenge, FAO has been providing technical help to the Rice Development Directorate in the southern Indian city of Hyderabad. Indian scientists are visiting rice institutes in the Philippines and China, where scientists are trying to develop a new generation of genetically- engineered "super-rice". (Source: Development Forum, September/October 1991)

Genetic gun makes rice growers' day

A genetic gun that shoots foreign genes into plant cells may soon provide farmers in devloping countries with cheap, genetically engineered rice with built-in resistance to pests that devastate their plantations. Scientists in Wisconsin have used this technique for the first time to alter the genetic constitution of Indica rice varieties. These account for four fifths of global rice production and provide the staple food for at least two billion people.

The scientists at Wisconsin are negotiating with the Rockerfeller Foundation to work out a way to provide the varieties cheaply - perhaps even free - to the countries that need them most. They hope to make money by selling transformed commercial varieties in the United States and Japan.

In the future, they hope to make Indica varieties resistant to widespread pests. These include tungro virus - common in South-East Asia, India and China - which completely destroys crops. Another candidate is a beetle called the rice water weaver, which damages yields by burrowing into and colonizing the roots.

Paul Christou and colleagues at Agracetus, a company based at Middleton in Wisconsin, report that genetic engineers have already perfected methods to transform commercial Japonica varieties of rice grown in the US and Japan.

Indica rice proved more stubborn than Japonica, and researchers found it impossible to grow new Indica rice plants from treated material. Christou and colleagues tried a new approach that had already worked in 1988 with soya.

The process, known as electric discharge particle acceleration or "biolistics", involves firing the foreign DNA like a bullet into immature embryos that still retain their outer skin, or scutellum. The technique is a refinement of a system pioneered by John Sanford of Cornell University. (Source: New Scientist, 2 November 1991)

Food containers that stop the rot at sea

Exotic fruits, flowers and vegetables could soon be commonplace in every corner shop, thanks to new techniques for keeping them fresh in transit. Shipping companies usually send high-value perishables - such as mangoes, kiwi fruits and cut flowers - by air over long distances. But the two new systems developed in Britain and the US can now preserve the cargo long enough to allow companies to send it by sea or land, at about half the cost of air freight.

The key to both systems is to halt or retard the ripening process by altering the atmospheric conditions in large refrigerated containers. Such controlled atmosphere systems are widely used in warehouses but this is the first time they have been used in transit.

Fruits, vegetables and flowers ripen and rot in atmospheres that are rich in oxygen, and both systems are based on methods of flushing it out. Air contains approximately 78 per cent nitrogen, 21 per cent oxygen and 1 per cent other gases.

Now BOC of Guildford in Surrey has unveiled a sophisticated system which tailors the atmosphere to the needs of the particular cargo.

It controls independently the levels of carbon dioxide, water vapour, oxygen and ethylene. All four gases contribute to ripening, but ethylene is particularly important. Fruit and vegetables produce it when they respire and ethylene concentrations as low as a few parts per million accelerate the ripening process.

Researchers at BOC and at the shipping agency Freshtainer of Austria found that different conditions suit different cargoes. Cut carnations, for example, prefer an atmosphere of 2 per cent carbon dioxide, 5 per cent oxygen and 93 per cent nitrogen, together with a temperature of 0.5°C and a relative humidity of 92 per cent. Mangoes, by contrast, survive best without ripening at 10°C in an atmosphere comprising 10 per cent carbon dioxide, 5 per cent oxygen and 85 per cent nitrogen, with a relative humidity of 90 per cent. Peter Sadkowski, manager of operations and technology at BOC, says that BOC has worked out the optimum conditions for more than 100 different products.

The system relies on four pairs of tubes containing molecular sieves called zeolites that can selectively adsorb gases. Each pair is geared to filtering out one of the four gases crucial to the ripening process. How much gas each pair of sieves filters out depends on the prevailing temperature and pressure, and these are controlled by computer. The filters come in pairs, so that as one is adsorbing, the other can refresh itself by discharging to the atmosphere outside the storage container.

Air Products of Allentown, Pennsylvania, has also developed a system that relies on a canister of delicate membrane fibres which filter out oxygen. Passing air through the membrane filter increases nitrogen content to 98 per cent. A computer controls temperature and humidity. The fibres were developed by Akzo of Arnhem in the Netherlands. (Source: New Scientist, 5 October 1991)

Sweet news for papaya lovers

Commercial production of papayas could boom thanks to a sweeter, larger, faster-growing version of the fruit developed at the San Antonio Botanical Center. The "San Antonio Sweet" and "San Antonio Early" were created when a plant-breeding specialist crossed two different strains of papayas - the "Mexican Yellow" and the "Hawaiian Solo". These super-sweet papayas can produce fruit within a year or less - and the "Sweet" variety can grow as large as 20 pounds. City officials are in the process of obtaining a plant patent from the US Department of Agriculture. For more information contact: Robin Magers or Donna Butler at Dublin-McCarter & Associates, Tel.: 512-227-0221. (Source: BioBytes, San Antonio Biotechnology News & Information, December 1991)

Ethylene fuels fruit ripening

Ethylene production is the driving force behind the fruit ripening process and not just one of the outcomes, according to scientists working at the Plant Gene Expression Centre at the University of California at Berkeley.

They observed that in tomatoes, ripening was preceded by a rapid burst of respiration and an increase in the production of ethylene. To prove the cause-effect relationship, the research team, headed by Paul Oeller, treated tomato fruits with antisense RNA molecules that blocked the production of an enzyme, ACC synthase, that produces 1-aminocylcopropane-1-carboxylic acid (ACC), a precursor to ethylene.

The procedure was effective in preventing ripening and when the fruits were exogenously treated with ethylene or propylene the inhibitory effect was reversed. The actual mode of action of ethylene as a plant hormone is still not fully understood, but the researchers believe that the use of antisense RNA for ACC synthase could prevent commercial losses due to the over-ripening of fruits and vegetables. (Source: European Chemical News, 9 December 1991)

Food and food-processing applications

The call of the mushroom

On 16 October 1990, the market price the picker was making in western Washington State for matsuke, the pine mushroom, exceeded even the grandest expectations. Snug buttons, with no presenting veils, brought $22 a pound; buttons with unbroken veils, $12 a pound. A single picker could clear as much as

$600 in a single afternoon. Wild mushrooms are big business.

The actual forays are carried out by freelancers who gather and sell to buyers (there are around 200 in the Pacific Northwest, working for one of four companies), who in turn sell to processors, who, twice removed, weigh, clean and sort the wild harvest, and then rinse and pack the fungi in drums for shipment abroad.

So great has been the commerical lure of mushroom picking that Crater Lake National Park, whose regulations outlaw such picking, found buyers sitting in cars at the entrances with wads of six or seven thousand dollars in their pockets. Park officials hired undercover agents to control the looting.

Last year the state parks in California, where picking for personal consumption has been a tradition, moved to ban all picking, commercial and personal. Washington's Edible Mushroom Task Group called on the state to regulate harvesting as a cottage industry, urged scientific research into impacts on the ecology and sought to turn the tide of picking to non-mycorrhizal species. In the meantime, national forests, where most of the picking took place, issued all-you-can-gather permits for as little as a signature and $25.

But how endangered is the mushroom? Rumour abounds about mushroomers over-collecting their culinary doles and making them scarce, but the scientific evidence of the impact of over-harvesting is scanty, even non-existent. Most knowledgeable experts agree that over-picking could drive a species to the brink of extinction, whether the exploited species be a fish, a bird or a showy orchid. But a mushroom? How would anybody know?

Mushrooms have always been so abundant that we take them for granted, and even doubt their essential roles in ecology until the past two decades, when the natures of mycorrhizal relationships were revealed.

Most of the definitive research has been done in the old-growth Douglas fir forests. Every hectare of forest soil contains as much as 4,200 kilograms of fungal mycelium, dry weight. The mycorrhizae, also known as the "root-mushrooms", sheathe the feeder roots of Douglas fir and service the ecosystem by returning the biomass to the soil, accounting for 50 per cent of the annual accrual. Some 42 per cent of the nitrogen captured and put into the soil is done by these mycorrhizae. But this is only a beginning, because mycorrhizae move phosphate ions around which hardly dissolve in the water, and they buffer roots against extremes of drought, mositure, pH and temperature, reducing the pressure of stresses that ordinarily do a plant in. Those mushrooms are so indispensable that plants depend on them for health, and seedlings of any plant fare poorly without sufficient inoculation with their favourite mycorrhizal spoor.

It is only logical to conclude that what we do to satisfy our craving for mushrooms might rebound on the health of forests and the essential balance of nature. In a world of vanishing life and fading greenery, such a scenario is terribly possible - probable against a formidable background of shrinking habitats.

In 1986 the US Department of Agriculture and the Oregon Mycology Society pooled efforts in a 10-year study of 10 plots on undisturbed Mount Hood to detect what the effects of picking were on "subsequent fruiting" and on "mycorrhizal partners", mainly timber trees. Though the study is not completed, the early verdict of these researchers is that "chanterelles would rather be cut than pulled".

Surely the pressure of picking mushrooms has the potential to reduce the release of spores and result in fewer and fewer mushrooms and fewer partners - like the Douglas fir, which entertains chanterelles as mycorrhizal host, but the real impacts are not scientifically known. Clear-cutting, slashburning, pesticides, and air pollutants - all have shown the ability to annihilate mycorrhizae from the soil, but decision-makers draw a blank in scientific mushroom management. With a runaway market propelling the extravagant exploitations, acting quickly and urgently to protect the mycorrhizosphere, the world's umbilici, is an ever more crucial endeavour. (Extracted from an article by Bud Hoekstra (Eureka, California), published in New Scientist, 7 December 1991)

Energy and environmental applications

Getting bugs to remove heavy metals from bulk wastes

Scientists at TNO Environmental and Energy Research (The Netherlands) have recently confirmed the feasibility of using micro-organisms to remove heavy metals from waste streams and are now studying the possibility of recovering such contaminants in a collaborative project with other European research laboratories. Funding for the new project is being given by the EC and a number of major companies.

The prospect of removing pollutants with micro-organisms has aroused significant interest in the scientific world. The main advantage of microbiological methods compared with conventional technology is that they are relatively simple to use and can be applied on a large scale at low cost. In their recent feasibility study, the TNO scientists showed that, given the right conditions, the micro-organisms *Thiobacillus ferrooxidans* could be used to produce sulphuric acid, which reduced the concentrations of various heavy metal components contained in the waste stream.

The results obtained in the laboratory were particularly encouraging and bode well for the treatment of ash, polluted soil and industrial, dredging and waste water sludges containing heavy metals. The tests showed that the removal efficiency of *Thiobacillus ferrooxidans* is such that the residual concentration of heavy metals in contaminated soil can be reduced to the Category B value stipulated by the Dutch Ministry of Housing, Physical Planning and the Environment, while the concentration of heavy metals in treated waste can be reduced below the level at which a chemical waste designation would be required.

In view of the fact that over 100 million tons of waste are produced in the Netherlands each year - much of which contains heavy metals - getting the bugs to clean up part of this waste mountain might not be a bad thing after all. (Source: TNO Newsletter, No. 38, September 1991)

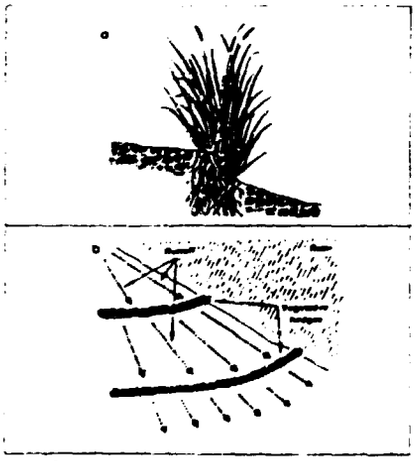
Miracle grass takes root

Vetiver grass is today's best plant for preventing soil and moisture losses on rainfed arable lands. It is cheap and proven effective under diverse field conditions. Low establishment costs and minimal maintenance make vetiver grass especially useful to subsistence farmers in developing countries.

The vetiver technology for soil conservation has already been tested in many countries throughout the world from India to Fiji.

Unlike man-made structures that cost a lot to build, vetiver grass is a simple technology requiring no engineering experts. Planted as hedgerows, a barrier against erosion becomes effective in a few years, and the farmer can do it all himself with little or no help. And best of all, it is a natural solution that becomes a permanent part of the landscape.

When planted as a vegetative contour hedge, the densely tufted vetiver grass (*Vetiveria zizanioides*) blocks the erosive force of runoff. By slowing down runoff, the hedge can filter out silt that has already begun to settle. Silt then collects behind the vetiver hedges, and over the years it slowly builds into a natural terrace, anchored by dense spongy vetiver roots as long as 3 metres.



Vetivergrass catches silt to form a natural terrace (a), then spreads out surface runoff (b).

In addition to saving soil, water is also held up by the hedge and has more time to seep into the ground, thus reducing runoff and increasing water retention. Water slowly passing through the hedge then spreads over a larger area and increases plant available moisture.

To be effective, vetiver hedges must be planted along the contour, or "averaged" contour in rough terrain. Depending on growing conditions and spacing, a line of vetiver slips will take 1 to 4 years to close into a dense barrier. Slips should be planted at the beginning of the rainy season with a spacing of no more than 20 cm.

A vertical interval of 2 metres between hedges should be adequate. Although vetiver hedges require little maintenance once established, pruning will promote tillering and reduce time to hedge closure. Low pruning will eliminate any light competition between hedge and crop. Otherwise, vetiver hedgerows do not compete with crops and, when planted in only 50 cm strips, take little land out of production.

Vetiver grass has many good traits:

- Wide temperature range (-9°C to 45°C);
- pH tolerant (4.5 to 10.5);
- Low seed viability prevents spreading;
- Fire resistant;
- Unpalatable to livestock;
- Not susceptible to diseases, insects, or rodents;
- Vertical roots do not compete with crops;
- Long-lived perennial plants up to 100 years;
- Source of mulch and thatch.

Some potential problem areas include saline, cold and drought tolerance, because their limits have not yet been established. This is not necessarily unfavourable. For example, vetiver grass has been successful from 600 to 6,000 mm of rainfall and has survived dry seasons of 10 months.

It has also been successful from sea level to 2,600 m above MSL. However, there are local markets for the aromatic oil distilled from vetiver roots, and this has led to the uprooting of plants in a few areas.

Vetiveria zizanioides has been found in over 70 countries world-wide. The best way to find plant material is to track it down in your own country. If vetiver does not grow in your area, look for an herbarium by contacting universities, agriculture departments, or botanical gardens.

They should be able to tell you where to find vetiver if it has been reported in your country. If not, try the Vetiver Information Network at the World Bank in Washington, D.C., or the Royal Botanical Gardens, Kew Richmond, Surrey, TW9 3AB, England.

After finding plant material, propagate vetiver grass by using root divisions or slips. Establishing a vetiver nursery or hedge from seed is too risky due to low seed viability and peculiar germination requirements.

Vetiver contour hedges for soil conservation provide a cheap alternative to physical structures like bench terraces, embankments, and their waterways. They require no engineering technology or equipment, a lot less labour to establish, and will not wash out in heavy rains. At one tenth to one hundredth of the cost, vetiver hedges take less land out of production to do the same job.

Farmers with plant material can install their own vetiver hedges at no cost. After the hedge becomes permanent in a few years, there is no maintenance. When combined with contour farming, the vetiver hedge increases moisture conservation, leading to yields more than 50 per cent better than traditional cultivation.

Copies of the third edition of this useful booklet on vetiver grass may be ordered from:

R. G. Grimshaw, Chief, Agricultural Division Technical Dept., Asia Region
World Bank, 1818 H Street, N.W.
Washington, DC 20433
USA

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Growing plants in salty soils

Along the dunes where water laps the coast near Bhavnagar, India, something curious is taking place. Farming. Seedlings of Bajra - a small, round grain - are being planted in the sand and nurtured with seawater. The yields are promising, with up to one-and-a-half tons harvested per hectare (2.5 acres).

Bajra, a type of millet, is one of a number of plants that grow in salty soils and water. In a new report, the Washington D.C.-based National Research Council identifies hundreds of such salt-tolerant species and halophytes - plants that grow well in saline soils and water - that could contribute significantly to the developing world's supply of food, fibre, fuel and fodder.

With the availability of salt water, salt-tolerant plants in many third world regions could "improve food or fuel supplies, increase employment, help stem desertification, and contribute to soil reclamation", the study panel said.

Salt-tolerant plants may hold promise for areas where farmland has been salinized by poor irrigation practices, for land that overlies reservoirs of brackish water, or for coastal desert regions. The report concludes that "the combination of sand, salt water, sun and salt-tolerant plants presents a valuable opportunity for many countries".

While salinity spells disaster for many popular crops - beans and lettuce, for example - halophytes flourish under such conditions. Their ability to do so can be vital in combating poverty and malnutrition, since millions of hectares of land in developing countries suffer from high salt content, making them unsuitable for cultivating many crops.

Funded by the United States Agency for International Development (USAID), the report was prepared as a resource for scientists in developing nations where growing conditions may be less than optimal.

Although many halophytes are unfamiliar (Distichlis, Salicornia and Sporobolus, for example), other common crops, including asparagus, and some types of wheat, cotton, barley and rice, can be bred or selected for salt tolerance.

A number of halophytes were harvested by ancient cultures but never gained wide acceptance. A sea grass known as *Zostera marina*, for instance, played an important role in the diet of the Seri Indians of the southwestern United States. They pounded the seeds into flour for bread. Similarly, the Cocopa Indians harvested Palmer's saltgrass from tidal flats at the head of the California Gulf. Its seed, about the same size as wheat, also was ground into flour.

The report, according to panel member James O'Leary, is the broadest publication produced on the subject of halophytes, the result of a four-year study in more than 30 countries by some 100 contributors. O'Leary is a plant physiologist at the Environmental Research Laboratory in Tuscon, Arizona, with more than a dozen years of research in saline agriculture.

O'Leary cautioned, however, that "using seawater to irrigate brings with it its own set of problems. You continuously have to flush out the soil so that salt doesn't build up. Then, as a result of the flushing, you have to replace important nutrients. It can get complicated and labour-intensive".

Still, saline agriculture is meeting with marked success in a number of countries, including Israel and Pakistan. Israeli farmers are successfully growing tomatoes and cotton irrigated with saline water. In Pakistan, kallar grass is grown in waterlogged, high-saline soils, harvested, then fed to sheep, goats, buffalo and cattle.

In Mexico, farmers are cultivating and irrigating with seawater an experimental plot of 20 hectares of salicornia, a succulent plant that produces a safflower-type oil. Harvests produce some 20 tons of plant material per hectare. The oils seeds comprise about two tons of the total; some of the straw from the plant has been used as animal fodder.

Halophytes are ubiquitous with a multitude of uses. Some are grasses; others fruit trees - the quandong, for example, grows throughout Australia's arid inland and produces cherry-like fruit, popular in jams and pies. Other halophytes boast medicinal properties. A variety of periwinkle, found in the coastal sands of India, contains compounds in its roots that are used to treat leukemia, and its leaves contain alkaloids reported to lower blood pressure.

A climbing vine, *Derris trifoliata*, grows profusely in mangrove forests and along muddy shores from East Africa to India and through Malaysia to Polynesia. Its leaves contain rotenone, a chemical that is extracted and used to eliminate predators and competitors in freshwater and brackish ponds where crustaceans and finfish are cultivated.

While the report offers a long list of halophytes and their potential uses, it makes clear that much research remains to be done before many of the species can be domesticated. "Undomesticated salt-tolerant plants usually have poor agronomic qualities, such as wide variations in germination and maturation", it says. "Most important", the report stresses, "salt-tolerant plants should not be cultivated as a substitute for good agricultural practice, nor should they be used as a palliative for improper irrigation. They should be introduced only when and where conventional crops cannot be grown".

An improvement of genetic traits in halophytes is critical if such plants are ever to become truly useful to many people. "Seed from a wild tomato found on the seashore of the Galapagos Islands produced tomatoes that were small and bitter", according to the report. "When this species was crossed with a commercial tomato cultivar, flavourful fruit and the size and colour of cherry tomatoes were obtained in 70 per cent seawater".

In addition, advances in plant biotechnology could prove helpful in making conventional crops more tolerant of stress caused by salinity.

Interdisciplinary communication among plant ecologists, plant physiologists, plant breeders, soil scientists, and agricultural engineers is critical to successful research, the report maintains. To facilitate such interaction, the report provides a detailed bibliography.

The panel was chaired by J.R. Goodin, Texas Tech University, Lubbock. The National Research Council is the principal operating agency of the National Academies of Sciences and Engineering.

The report, Saline Agriculture: Salt-Tolerant Plants for Developing Countries, is available for $15.00 (pre-paid) from the National Academy Press, 2101 Constitution Avenue, N.W., Washington D.C. 20418, USA. (Source: Agricultural Information Development Bulletin)

D. PATENTS AND INTELLECTUAL PROPERTY ISSUES

Will third world lose out if crop genes are patented?

Directors of the international agricultural research centres (IARCs), trustees of the world's most valuable collections of crop genes,

are discussing whether they should establish intellectual property rights over the material stored in the centres' gene banks. The issue has divided the directors, attending their annual meeting in Washington. At issue is whether intellectual property rights would help or hinder third world farmers and consumers.

Lukas Brader, chairman of the centres' subcommittee on intellectual property rights, says that patents could protect third world genes from misappropriation by industry. "Patents, were they to be established in the IARCs, would never be to make money for individual centres. Any income would be channelled back to third world countries, for example, via the UN International Fund for Plant Genetic Resources", Brader says.

Patents could also help IARC researchers to bargain with industry for transfer of technology. At present IARC scientists visiting private laboratories stand to lose rights over any part of their work that turns out to have commercial significance.

The arguments have been roundly criticized by Genetic Resources Action International (GRAIN), a non-governmental organization campaigning on behalf of third world farmers. GRAIN holds that patents would hamper free exchange of genetic resources, which are essential for rapid progress in plant breeding.

The IARCs are intergovernmental organizations set up to benefit countries of the South. They have long been criticized for their technological approach to solving the problems of the poor, as in the green revolution. Crop varieties that produced higher yields benefited not poor farmers but those wealthy enough to afford the fertilizers, pesticides and machinery that the higher yields depended on. GRAIN argues that by jumping on the biotechnology bandwagon the IARCs move further away from the needs of their beneficiaries.

The centres are dominated by Northern researchers and are under Northern control. Who owns the genes in their banks is still unclear. These worries threaten the already delicate relationship that some IARCs have with the governments of some countries. Any move towards patenting is seen as a move that strengthens the influence of the North over organizations whose only concern should be how best to serve the interests of the South. "It will create a lot more distrust than cooperation", says Renee Vellve of GRAIN. (Source: New Scientist, 2 November 1991)

Onco-mouse can be patented, says EPO

Despite its 1989 decision that animals could not be patented, the European Patent Office (EPO) has decided that the Harvard University "onco-mouse" can be patented. The onco-mouse, developed by Philip Leder and Timothy Stewart of Harvard University, is made susceptible to tumours by inserting an oncogene at the embryo stage.

This latest decision could lead to a considerable number of patent applications covering genetically altered animals, but it relates exclusively to the onco-mouse, rather than to transgenic animals in general. Although the onco-mouse has already been patented in the US, in 1988, the EPO argued that the 1962 European Patent Convention prohibits the patenting of animals. Du Pont, which now owns rights to the US onco-mouse patent, had appealed the 1989 decision.

The EPO justified the decision on the basis that the onco-mouse's role in cancer research is "of paramount importance for the welfare of mankind". Animal rights groups protested the decision, which is expected to fuel the controversy surrounding the proposed EEC Directive covering the patenting of life forms. (Source: Biotechnology Bulletin, Vol. 10, No. 10, November 1991)

A battle as old as the nation

Behind the current gene patent controversy lies a debate that has raged since Thomas Jefferson introduced the first US intellectual property laws nearly 200 years ago: When are patents good for industry and investment, and when do they just get in the way?

That question is especially contentious in research, where patent procedures often clash with ideals of free exchange of scientific data. US patent applications are by law secret and can take years to be approved; during that period applicants may refuse to publish or

share the data for fear of losing all rights if the patent is not granted.

Until a 1980 Supreme Court decision allowing the patenting of oil-eating bacteria, the United States took the moral high ground: it put all government research (such as that done by the National Institutes of Health (NIH) and the laboratories of the Department of Energy) in the public domain. This was despite some very good evidence that the policy was doing the US taxpayer few favours - a study conducted in the 1960s found that no government invention to date had ever been commercialized. During the Second World War, the Government itself had to develop penicillin to combat infections in troops because no company would invest the money developing an antibiotic it could not own.

Universities also wrestle with balancing science and commerce. At a technology transfer meeting at NIH last week, Hoffman-LaRoche patent attorney George Gould noted that until the 1970s Harvard University had a policy of never seeking patents for the inventions of its scientists.

Although most US research universities now have technology transfer offices to encourage patents, a new spectre - product liability - has arrived to cloud the issue. Reid Adler, NIH's technology transfer director, reported that increasingly aggressive litigation to recover damages in medical-product liability cases is starting to raise fears throughout the patent chain. If a university licenses its technology to a small company to manufacture a product that eventually becomes the subject of a lawsuit, a plaintiff may choose to sue the university as well as the company. In the face of such prospects, some universities are starting to reconsider the benefits of technology transfer, especially to start-up companies. (Source: Nature, Vol. 354, 21 November 1991)

More questions than answers

Seeking to interject a little reason and experience into what has become a heated international fracas over the patenting of human genes, the National Institutes of Health (NIH) has asked a dozen patent and technology-transfer experts to argue the issues in public. Fourteen hours of debate later, the experts appeared to have vindicated NIH for launching the controversy, even if they were no closer to agreeing whether it is desirable, or even possible, to patent the genetic sequences under question.

If there were still doubt that the question of whether unidentified gene fragments should be patentable has become the most contentious scientific debate of the day, the NIH meeting should have banished it.

Since news emerged that NIH had quietly filed a patent application for some 350 cDNA fragments of unknown function and physical significance, the agency has been accused of breaking the international Genome Project's code of free data exchange, as well as of undermining US biotechnology industry by attempting to put frivolous licensing hurdles in the path of commercialization. Although most of the industry represenatives and lawyers who spoke out at the meeting thought that patents on such gene fragments would do industry more harm than good, they also noted that much the same had been said of the first attempts to patent recombinant DNA technology in the 1970s; now those patents are considered the foundations of the biotechnology industry.

Continuing controversy on this issue may not bode well for future harmony in the genome project, but it does appear to take the immediate pressure off NIH. When news of NIH's application first broke last month, many researchers blamed both the agency and the NIH scientist who discovered the genes, for filing the application in the first place. Now that industry and legal experts have turned out to be divided on the issue themselves, most of the participants agreed that NIH had been right all along in taking the safe route: given the uncertainty in the viability and implications of gene patents, letting the sequences appear in the public domain without patent protection would have been tantamount to legal malpractice.

Given the lack of consensus on the merits of gene patents - or even agreement on the application of standard patent law to this case - some lawyers at the conference argued for special patent office treatment for gene patent

applications until the issue is settled.
Lawyers at the conference noted that the US Congress has recently raised another issue that appears to bear on the controversy: that of the extent to which scientists are entitled to use patented materials in the course of their research, without paying royalities. Long-standing patent law allows for free use if the researcher has only "philosophical interest" in the patented material. But in a bill introduced in 1990, the House Judiciary Committee questioned the meaning of that wording in modern laboratories, where researchers are encouraged to turn every invention into a marketable product. (Source: Nature, Vol. 354, 21 November 1991)

Revision of patent law in Brazil and Mexico

A new industrial property code bill before the Brazilian Congress is expected to come into force in the relatively near future. Major changes include an increase in the patent term from 15 to 20 years from the date of filing, and the removal of restrictions on patentability of inventions relating to chemicals, pharmaceuticals and foodstuffs. This relaxation of restrictions will apply to all pending applications, and will bring Brazil into line with the European Patent Convention. In addition, a grace period of one year following publication by the inventor will be adopted.

A new law for the development and protection of industrial property has been passed by the Mexican Senate, and is expected to be approved shortly by the Chamber of Representatives. The patent term will be 20 years from the filing date, with a possible extension of three years for pharmaceutical products provided that certain working conditions are satisfied. Applications will be published 18 months from the priority date. The type of invention which is patentable has been greatly broadened, but the following will not be patentable:

(a) Micro-organisms *per se;*

(b) Biological processes for plant and animal breeding;

(c) Genetic processes which simply consist of selecting or isolating available biological material and multiplying it under natural conditions;

(d) Plant and animal species and strains;

(e) Naturally-occurring biological material;

(f) Inventions relating to living human material;

(g) Surgical, therapeutic and diagnostic methods applicable to the human or animal body.

The new law will provide "pipe-line protection" for inventions for which an application has been filed in any PCT country before the date of enactment of the new law, provided that the Mexican application is filed within one year of enactment of the new law and there has been no commercial exploitation or importation in Mexico. (Source: Australian Biotechnology, Vol. 1, No. 2, October 1991)

E. BIO-INFORMATICS

Microbiology testing: An analysis of the US market 1990-1993

Theta Market Report No. 164, published in September 1991.

Dramatic changes are impacting the market for microbiology testing. This report covers what the results of these changes have been, and how they will affect the future of testing. Theta found that hospitals will remain the major market for microbiology testing, growing 11 per cent per year.

The lines between traditional microbiology and chemistry are being blurred. New technologies, such as immunoassay and DNA probes, are now being applied to infectious disease testing. Infectious disease testing encompasses microbiology, virology, serology, and blood banking. New tests which cut across the "-ology" lines are bringing new competitors into the field.

This report covers topics such as:

- Why Roche bought part of Cetus, and what to expect from that.
- Why DNA probes remain in the promising category.
- What is going on with traditional microbiology companies.
- Winners and losers in the emerging market for new tests.
- Rapid ID tests and automated microbiology - where do they fit?
- Why infectious disease testing in the physician's office looks promising.

Sections of this report include:

- Infectious diseases - incidence and growth. Importance of STD.
- Products - Product categories, by size, growth, and significance.
- Markets - Market segments, size and growth, hospital, private lab and physician's office markets.
- Companies - Market shares, profiles of leading competitors.
- Appendix - Company directory, analysis of leading infectious diseases and the role of testing.

Subscribers to this report will understand where the market for infectious diseases has been, where it is today, and what may be expected over the next three years. It will enable them to position themselves and their products properly to take advantage of growth in selected segments.

Information developed for this report came from published market surveys, databases, company literature, product brochures, end-user comments, and opinions and information secured from marketing personnel at leading companies in the field. Projections were developed, based on past trends, coupled with interpretations of how strategic changes will impact these trends in the future. The result is a report which seeks to provide a comprehensive picture of the market, with enough detail to support these observations. Further details available from Ms. Phyllis Klaben, Theta Building, Middlefield, CT 06455 (USA). Tel.: (203) 349-1054. Fax: (203) 349-1227.

The Biotechnoligists by Stephanie Jones

"The Biotechnologists" is a lively new study of entrepreneurship in a high-risk - but potentially very profitable and certainly extremely exciting - new field of commercial scientific endeavour.

Biotechnology gripped the public imagination in the USA in the late 1970s and early 1980s, and crossed the Atlantic to take off in a big way in the UK and Europe, notably Belgium and France. Although there are many science-orientated studies of biotechnology and genetic engineering, there has been no previous attempt to consider the problems and challenges of managing a biotechnology enterprise.

The book features extensive profiles of leading biotech entrepreneurs and the companies they founded and/or are now managing, including: in the USA, Jim Vincent (Biogen), Hubert Schoemaker (Centocor), George Rathmann (Amgen), Richard Laster (DNA Plant Technology), Sandford Smith (Repligen) and Gabriel Schmergel (Genetics Institute); and in Europe, Gerard Fairclough (Celltech), Walter de Logi (Plant Genetic Systems) and Keith McCullagh (British Bio-technology); and many biotech venture capitalists.

"The Biotechnoligists" (£25.00, ISBN 0-333-55021-8) is available from Belinda Holdsworth or Za Bushell, Macmillan Press, Houndmills, Basingstoke, Hampshire, RG21 2XS, UK. Tel.: (0256) 29242; Fax: (0256) 810526.

Cassava and biotechnology

The proceedings of a March 1990 workshop on cassava and biotechnology, organized by the Dutch Government's Directorate General for International Cooperation, are now available. Details from: Directorate General for International Cooperation, Section DTO/SO, Ministry of

Foreign Affairs, P.O. Box 20061, 2500 EB The Hague, The Netherlands or Tel.: +31 70 348 43 79; Fax: +31 70 348 48 48.

Biotechnology demystified

The latest in a long line of publications aiming to demystify biotechnology - and explain its myriad existing and potential applications - has been published by the Economist Intelligence Unit. Priced at £345.00, Biotechnology in Industry, Healthcare and the Environment is available from: Economist Intelligence Unit, 40 Duke Street, London W1A 1DW. (Source: Biotechnology Bulletin, Vol. 10, No. 10, November 1991)

The UK Biotechnology Handbook '91-'92

Essential for anyone active in biotechnology, the UK Biotechnology Handbook '91-'92 provides up-to-date information on over 675 organizations involved in British biotechnology.

Produced by BioCommerce Data in collaboration with the BioIndustry Association (BIA), the publication covers such areas as agriculture, cell culture, diagnostics, equipment, genetic engineering, pharmaceuticals, reagents and waste treatment. Details of the Handbook, priced at £95.00/$175.00 from: BioCommerce Data Ltd., Prudential Buildings, 95 High Street, Slough, Berkshire SL1 1DH or Tel.: 0753 511777. Fax: 0753 512239. (Source: Biotechnology Bulletin, Vol. 10, No. 10, November 1991)

Biotechnology Worldwide

Did you know that the centrepiece of Iceland's biotechnology programme is a project to make practical use of the heat-loving bacteria that live in the country's geysers? Or that the Date Palm Research Centre of King Faisal University is a jewel in the crown of Saudi Arabia's biotechnology programme? If you did not, but you would like to know more about what each country is up to in biotechnology, you might find invaluable a reference book called Biotechnology Worldwide (CPL, 330 pages $60 paperback). It is published by the International Scientific Committee for Biotechnology, which is affiliated to the International Council of Scientific Unions.

From Nepal and the Netherlands to Venezuela and Viet Nam, you can find out a country's policy for controlling and exploiting biotechnology, the administration responsible for it, the status of intellectual property rights and the laws that govern activities in bioscience. The handbook also lists those areas of biotechnology on which individual countries have focused, the breadth of domestic genetic resources, and the prospects for international collaboration and future growth. Most importantly, perhaps, it provides a barometer of public perception of biotechnology. In some countries - Nepal and Sudan for example - hardly anyone has heard of biotechnology.

Jim Coombs and Peter Campbell, the authors of the guide, are to be congratulated on gathering so much valuable information together in one place. (Source: New Scientist, 5 October 1991)

The strength and weaknesses of the Japanese innovation system in biotechnology

The Institute for Japanese-European Technology Studies paper No. 3 by Martin Fransman, of the Institute for Japanese-European Technology Studies, University of Edinburgh and Shoko Tanaka, Cornell University pays particular attention to the three high-profile cooperative Japanese research programmes:

(i) The "NEXT Generation Base Technologies" Programme;

(ii) The Protein Engineering Research Institute (PERI);

(iii) The Exploratory Research for Advanced Technology (ERATO) programme.

The distinctiveness of Japanese Government industry relations in biotechnology is highlighted.

The 37-page paper is available from the University of Edinburgh at UK£4.50 plus UK£1.50 postage on Fax (44) 31 668 3094. (Source: EBIS, No. 5, October 1991)

US Japanese Technology and Evaluation Centre takes measure of Japanese biotechnology

For those following biotechnology developments in Japan another study has been made showing that the Japanese are getting better at exploiting biotechnology and turning it into products. The study team headed by Daniel Wang of MIT's Chemical Engineering Department pays particular attention to Japanese R&D programmes and their dependence on foreign scientists.

Report available from JTEC's director, Paul Herer, National Science Foundation, 1800 J Street S.W., Washington, DC 20550. Tel.: (1)202 357 9774. (Source: EBIS, No. 5, October 1991)

The International Biotechnology Directory 1992 by J. Coombs and Y. R. Alston

Macmillan Publishers have announced the publication of The International Biotechnology Directory 1992, the established reference source for the biotechnology industry. The 1992 edition has been fully revised and updated to provide the most comprehensive and current information available on organizations involved in the industry world-wide.

As the need for information on this industry increases so does the need for accurate reference sources. This directory provides access to detailed information on more than 10,000 companies, organizations, institutes, university and government departments. In addition it provides a Buyers Guide with over 2,000 categories of products and services. With detailed information on organizations in 22 countries, the coverage is totally international.

This new 1992 edition is split into three sections for ease of accessibility:

Part I consists of international organizations and information services, listed alphabetically with full contact details. This includes databases, journals and newsletters.

Part II comprises companies, university and government departments, institutes and societies, providing contact details and product information.

Part III is a Buyers Guide to Products and Services, a unique feature of such a directory, with companies listing their location as well as products and services to ensure that entries can be easily located.

The International Biotechnology Directory 1992 is available from Globe Book Services Ltd., Macmillan Press, Stockton House, 1 Melbourne Place, London WC2B 4LF. Tel.: 071-379 4687. For further information please contact Carla Jones.

BAP (Biotechnology Action Programme) 1985-1990 final report makes important contribution to understanding biotechnology risks

This final report on BAP projects funded 1985-1990 in two parts includes two important reviews: "The release of genetically modified micro-organisms in the environment", by J. Davies, and "The release of transgenic plants into the environment", by W. De Greef. The results of BAP projects on risk assessment are described in the areas of: microbial containment, depollution bacteria, plant interacting microorganisms, transgenic plants and genetically engineered viruses.

The report (in English) is available on request from Dr. I. Economidis, DGXII. Fax: (23) 22355365. (Source: EBIS, No. 5, October 1991)

Progress with CEFIC recommendations on bioinformatics

The European Chemical Industry Federation (CEFIC) recently published the outcome of a study, co-financed by the Commission of the European Communities, in which strong recommendations for a European biotechnology information infrastructure strategy were made. In May 1991 the Commission, through its high-level Biotechnology Coordination Committee (BCC), endorsed the recommendations in general and an ad hoc inter-service group discussed the various ways and means of implementing these. Several important recommendations will be implemented under the Community's R&D Framework programmes.

Probably the most crucial one is the Management Study for the possible establishment of a European Nucleotide Sequence Centre (ENSC). This centre should guarantee a future for the European nucleotide sequence database, currently based at the European Molecular Biology Laboratory (the EMBL Data Library), as well as its international collaboration. An organizational blueprint and implementation plan for the establishment of an ENSC is needed and a management study is expected to start before the end of the year. The results of the study will make an input to the discussions on the next Community R&D Framework programme. (Source: EBIS, No. 5, October 1991)

Biotechnology - A strategy for industrial strength

The UK's Biotechnology, Joint Advisory Board (BJAB) which advises the Department of Trade and Industry (DTI) and the Research Councils (AFRC, NERC and SERC) has published a strategy document for biotechnology in the UK. The report makes recommendations on how the science base can better respond to the broad innovation requirements of industry through specific areas of future research, technology transfer, training, public perception and balanced regulations.

Details: DTI Biotechnology Unit, Laboratory of the Government Chemist, Queens Road, Teddington, Middlesex TW11 OLY. Tel.: (44) 819437381. (Source: EBIS, No. 5, October 1991)

Nature Genetics

Nature, the international journal of science, plans to publish a supplementary journal, Nature Genetics, beginning in April 1992. The new journal is intended to cater for the publication of the substantial volume of research expected to flow from present interest in the better understanding of the human genome.

To that end, Nature Genetics will consider for publication research articles in human genetics proper, both clinical and otherwise, studies of the genomes of other organisms likely to have a bearing on the human genome and studies in human genetics involving a degree of detail that could not be accommodated in Nature on editorial grounds, perhaps because they include detailed genetic maps or nucleotide sequences. In particular, the new journal will include research papers longer than are normally included in Nature.

Nature Genetics will aim at the highest editorial standards, but its editorial management will be separate from that of Nature except in one respect: the authors of articles submitted to Nature but not accepted for publication for lack of space will be offered the opportunity of publication in Nature Genetics, often without further recourse to referees. It is expected that most submissions will be directly to Nature Genetics.

Nature Genetics will be based in Washington; the Editor is Dr. Kevin Davies, previously a member of Nature's biology staff.

Subscription details will be published at a later stage. (Source: Nature, Vol. 354, 5 December 1991)

African Diversity

African Diversity is published three times a year under the authority of the African Committee for Plant Genetic Resources (ACPGR) through an enabling grant from the African "Seeds of Survival" Programme supported by a consortium of Canadian NGOs led by USC Canada. African Diversity is intended to inform scientists, policy-makers, non-governmental organizations and other concerned members of Africa's agricultural community of recent and significant developments in the fields of plant genetic resources, biotechnologies and biological diversity. The material in African Diversity is prepared by the editorial staff and does not necessarily represent the views of the African Committee for Plant Genetic Resources. Available from: RAFI - Austrian Office, c/o IIZ, Wipplingerstr. 32, A-1010 Vienna, Austria, at an annual subscription fee of US$9.00.

AT-Source

The quarterly magazine AT-Source publishes articles in the field of technology and development in order to help local organizations in the third world.

The March 1991 issue contains a mini-special on vegetable garden programmes. The following subjects are dealt with: preconditions for vegetable gardening, nutritional advantages of vegetables and fruits, extension and practical solutions for input problems.

Also in this issue a mini-special on mobility aids: conditions for success, about orthopaedic shoes and about the production process of wheel-chairs. Also single articles, including one on wooden ox-cart bearings and one on a wind pump. The whole is completed with a list of references and recommended literature. AT-Source is published both in English and French. A subscription (NGL.35.00/year for 4 issues) and single issues can be obtained from: AT-Source, P.O. Box 41, 6700 AA Wageningen, The Netherlands. (Source: BioTechnology and Development, No. 8, September 1991)

Bioresource Technology

Bioresource Technology is a new journal formed by merging Biological Wastes and Biomass. The main focus of the new publication is on the exploitation of low-cost and waste biological materials. The journal appears quarterly and the annual subscription is £450. Details from: Bioresource Technology, Elsevier Science Publishers, Crown House, Linton Road, Barking, Essex IG11 8JU or Tel.: 081 594 7272. Fax: 081 594 5942.

Biomass and Bioenergy

A new international journal, Biomass and Bioenergy focuses on the biological resources, chemical and biological processes, and the use of biomass products as renewable sources of energy, food and materials. Details of the publication, 1992 subscription (12 issues) priced at £295, from: Pergammon Press plc, Headington Hill Hall, Oxford OX3 0BW.

ATCC Catalogue on Filamentous Fungi

The American Type Culture Collection (ATCC) has published the 18th edition of its Catalogue of Filamentous Fungi. Over 19,000 fungal strains available from ATCC are listed in the 667-page catalogue. Media formulae and an index to culture applications are included.

A new feature of the catalogue is the index of strain designations, which lists cultures according to the designations assigned by other laboratories and culture collections. For example, by looking up the culture designation CBS 104.45 in the strain index, the corresponding ATCC strain (*Aspergillus clavatus* ATCC 9600) can be determined. Details from: ATCC, 12301 Parklawn Drive, Rockville, Maryland 20852-1776, USA or Tel.: +1 (301) 881-2600. Fax: +1 (301) 770-2587. (Source: Biotechnology Bulletin, Vol. 10, No. 10, November 1991)

Databases on animal science and forestry

SilverPlatter Information will release CD-ROM databases on animal science, animal production, forestry, and drugs. The VETCD database encompasses the area of veterinary science, and includes records in mycology, helminthology, protozoology, and applied entomology. The BEASTCD database encompasses animal production, and includes records on animal nutrition, animal breeding, and dairy science and technology. The TREECD database features all the abstracts and citations published in Forestry Abstracts. The RINGDOC on CD-ROM database encompasses the field of drugs, and initially will feature just the unified database, which covers 1/83 to the present. (Source: Online, September 1991)

Genome databases

The Human Genome Project will generate more data than any single project to date in biology. A major product of this 15-year, $3 billion effort will be either one huge database or perhaps a set of linked databases that will list the location of every one of the genome's 100,000 or so genes. The database(s) will also contain information on thousands of other landmarks along the chromosomes, and ultimately, the entire nucleotide sequence of the human genome and of several experimental animals.

Since the Genome Project began several years ago, a plethora of databases have been developed or are in the works. They range from the massive Genome Data Base at Johns Hopkins University, the central repository of all gene mapping information, to small

databases focusing on single chromosomes or organisms. Some are publicly available, other are essentially private electronic lab notebooks. Still others limit access to a consortium of researchers working on, say, a single human chromosome. An increasing number incorporate sophisticated search and analytical software, while others operate as little more than data lists.

As a service to their readers, Science, in consultation with numerous experts in the field, has compiled a list of some key genome-related databases. That is not limited to map and sequence databases but also included the tools investigators use to interpret and elucidate genetic data, such as protein sequence and protein structure databases. Because a major goal of the Genome Project is to map and sequence the genomes of several experimental animals, including *E. coli*, yeast, fruit fly, nematode, and mouse, they have listed the available databases for those organisms as well. Also included are several databases that are still under development, including some ambitious efforts that go beyond data compilation to create what are being called electronic research communities, enabling many users, rather than just one or a few curators, to add or edit the data and tag it as raw or confirmed.

Given the speed with which new databases are appearing, or existing ones are metamorphosing, the list cannot possibly be inclusive. For the most up-to-date information on the databases listed here, and on those unable to be included, please consult either the Listing of Molecular Biology (LIMB) databases or the Directory of Biotechnology Information Resources (DBIR).

Contact: Christian Burks, LIMB Los Alamos National Laboratory, T-10, MS K 710, Los Alamos, New Mexico 87545, USA; Phone: 505-667-6683; Fax: 505-665-3493; E-mail: limb@genome@lanl.gov.

Contact: DBIR, NLM Specialized Info. Services Div., 8600 Rockville Pike, Bethesda, Maryland 20894, USA; Phone: 301-496-6531 or 496-1131. (Source: Science, Vol. 254, pp. 201-207, 11 October 1991)

F. MICROBIAL ENHANCED OIL RECOVERY

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Introduction

Energy in all its forms is the motive sustenance of modern, industrialized societies. Its use and availability determines social stability, economic viability, and power in terms of competing international economies and geopolitical affairs. The majority of the world's energy come from non-renewable fossil resources, with these resources and the energy derived from them representing the lifeblood of economies world-wide. Currently, crude oil reserves are estimated in excess of one trillion barrels of oil world-wide, with more than one-half of this fossil resource located in the Middle East.

Conventional oil production technologies recover only approximately one-third of the original known in-place oil following water flooding. Technologies to produce this remaining oil offer enormous economic potential through the development of new and cost-effective methods. For example, the total original in-place oil for the United States is estimated around 488 billion barrels of which 133 billion barrels have been produced with 28-30 billion barrels considered as recoverable reserves. The more than 300 billion barrels not recoverable by conventional technologies represent the target of all enhanced oil recovery (EOR) research and development programs. Currently, EOR represents a relatively small percentage of total oil production (approximately 5 per cent and 18 per cent for the United States and Canada, respectively), with some analysts predicting that within 20 years 33 per cent of total oil production will be through EOR technologies.

Microbial enhanced oil recovery (MEOR) has been a technology of interest for many years, with focused programmatic development by the U.S. Department of Energy

beginning the late seventies and early eighties. The first international conference on MEOR occurred in 1982, the latest in 1990, where researchers discussed the latest information and advances. MEOR has progressed from a position of questionable efficacy to a status where well-documented field studies indicate beneficial effects for "new" oil production from the application of MEOR concepts and principles. A number of excellent reviews and treatises have appeared recently which serve to discuss the status of MEOR as well as address the advantages and limitations of this technology [1, 2, 3, 4].

Microbial enhanced oil recovery is the application of microorganisms and the exploitation of their metabolic processes to increase the production of oil from reservoirs of marginal productivity. MEOR appears more feasible to tertiary oil recovery, although field situations are possible where the introduction of MEOR during secondary treatment is warranted for extended oil production.

Oil production and MEOR

Conventional oil production occurs in three stages: primary, secondary, and tertiary. Primary production is the result of natural internal reservoir pressures which exist within the formation. When this internal driving force diminishes to a point where oil production declines, then secondary treatment follows in the form of water flooding. Water flooding continues until the ratio of oil produced to water injected yields more water than oil and the economics of the treatment become non-sustaining in terms of oil production. At this point, tertiary methods are introduced to enhance further oil production. Tertiary EOR technologies include: (1) surfactant/polymer flooding; (2) solvent flooding; (3) miscible gas flooding; (4) *in situ* emulsification generating oil-in-water and water-in-oil emulsions; (5) steam flooding; and (6) *in situ* combustion, all designed for the microscopic displacement of trapped oil from the reservoir. Factors governing the microscopic displacement of oil include the geometry of the pore network within the formation, fluid-fluid properties, fluid-solid properties, pressure gradients and gravity, interphase mass transfer, interface aging effects, wettability changes, and emulsification [5]. All EOR procedures have shortcomings and constraints, basically relating to cost, amount of new oil recovered, and suitability of complex reservoirs for treatment. The efficiency of oil recovery from a reservoir is determined by various properties such as porosity, permeability, nature of rock surface, fluid properties relating to the bulk viscosity of the trapped oil, and the interfacial tension that exists between the oil, water, and rock matrix within the formation. It is known that low oil viscosity and low oil-water interfacial tensions promote efficient displacement [6, 7, 8]. An understanding of the physics of multiphasic fluid flow through porous media provides the basis for explaining how these various reservoir parameters influence oil production. The mobilization of oil trapped in rock matrices is determined by the relative magnitude of viscous forces allowing fluid flow and the capillary forces which inhibit oil movement in water-wet formations. A dimensionless parameter known as the capillary number, N_{Ca}, represents the ratio of viscous to capillary forces on oil trapped in pores [9]. Under reservoir conditions a N_{Ca} value of 10^{-2} corresponds to interfacial tension values in the order of 10^{-2} to 10^{-4} mN/m. Such ultralow interfacial tension values are achieved with synthetic surfactants and biosurfactants [10]. The introduction of surface active agents into oil-bearing formations has the potential for greatly increasing oil production when the surface properties of these agents are compatible to the physical and chemical properties of the reservoir such as brine concentration, pH, temperature, oil type and other critical factors. Do microorganisms and their associated metabolic processes have the potential to play a meaningful role as supplementary technologies in EOR programs?

Microbiology and EOR

In view of the constraints and restraints associated with oil-bearing formations, what are the principles and concepts of MEOR that offer the promise of potentially new technologies for the microscopic displacement of trapped oil from a multitude of formations worldwide. The MEOR strategies currently emphasized are

1. Injection of microorganisms along with appropriate nutrients to support microbial growth for the production of metabolic products considered

beneficial to trapped oil displacement. Metabolic products considered of value are: acids (acetic, propionic, and butyric acids) for reservoir rock modification, improved formation porosity and permeability, and carbonate rock dissolution; biomass accumulation for selective and nonselective plugging of channels and fractures, wetting of rock surfaces, oil emulsification through bacterial adherance; gas production (CO_2, CH_4, H_2) for reservoir repressurization, oil swelling, and viscosity reduction; solvents production (ethanol, propanols, butanols, acetone) for oil solubilization; *in situ* production of biosurfactants for lowering of interfacial tension and emulsification; and biopolymer production for plugging and mobility control.

2. The above-ground production of specific bioproducts (biosurfactants and biopolymers) and the subsequent application of these bioproducts as chemical enhanced oil recovery agents (CEOR), a technology not dissimilar to synthetic surfactant flooding programs under development for years. The central question is whether biosurfactants exhibit performance advantages in the reservoir over synthetic surfactants.

Reservoir microbiology

Studies concerning the microbiology of oil-bearing formations indicate the presence of a heterogeneous microflora consisting of aerobic, facultative, and anaerobic microorganisms. The taxonomic classification and cataloguing of their physiological and biochemical properties remains to be accomplished. However, the realization has surfaced that oil reservoirs and, in general, deep subsurface environments are microbiologically-rich, metabolically-active ecosystems [2, 11]. The source of this indigenous microflora is undetermined, with possible point source contamination from surface origins or, alternatively, of subterranean origin. The nature and quality of crude oil is highly variable, representing varying states of chemical maturation and alteration. Microbial degradation represents a potential major destructive phenomena affecting the quality of crude oil. The injection of microorganisms and/or nutrients to stimulate the growth of nascent microbial populations within the reservoir could result in the biotransformation of good oil to oil of poorer quality. The simpler hydrocarbons (paraffins, isoparaffins, simple 1, 2, and 3 ring aromatic hydrocarbons) are rapidly metabolized by microorganisms [10]. Insights into the biodegradation of the heavy fractions of crude oil (the asphaltenes and resins) are essentially nonexistent, being largely considered biologically recalcitrant structures. Crude oil biodegradation, therefore, results in the disappearance of the light fractions of crude oil, a concentration of the heavy ends, an increased viscosity and density, and the enrichment of nitrogen, sulfur, and oxygen heteroatoms, yielding an oil of poorer quality than the original oil. Crude oils being susceptible to significant biochemical alterations, biodegradation appears to represent one mechanism whereby vast heavy oil deposits are formed worldwide. It has generally been assumed that hydrocarbon oxidations occurs only in the presence of molecular oxygen. Recent studies have established irrefutably that anaerobic microorganisms can and do metabolize simple paraffinic (dodecane to eicosane) and aromatic hydrocarbons (benzene, toluene, xylene) [12, 13, 14]. These findings place new perspectives on the long term effects of injecting microorganisms and nutrients into oil-bearing formations and the short term gains to be realized from the application of such MEOR technologies. The introduction of microorganisms and nutrients can possibly result in the accelerated downgrading of oil quality.

Reservoirs and MEOR

The physical and chemical characteristics of oil reservoirs do present restrictions on the applications of MEOR processes. Collation of these physico-chemical properties has been assembled for specific areas of the United States which reportedly support MEOR options [2, 15]. These citations provide the first comprehensive analysis of the limitations and potential applications of MEOR in select reservoirs. The factors identified in oil reservoirs for consideration of implementing

MEOR options were porosity, permeability, pH, salinity, API gravity of the trapped oil, and temperature. Porosity being a measure of the total pore volume and permeability a measure of the ability of a porous matrix to transmit fluids, relate to the ability of microorganisms to penetrate the formation. The pore entrance size becomes an important factor affecting the ability of bacteria to be transported through consolidated rock, due to the fact that the size of bacteria fall into the range of pore entrance sizes, causing the restriction of bacterial transport through the porous media. Jenneman *et al* [16] reports the injection of *Pseudomonas* species into Berea sandstone cores did not decrease permeability significantly; whereas, a 100 per cent reduction of permeability resulted from the injection of a *Bacillus* species [17]. Therefore, filtration of bacteria from the injection fluid by the rock matrix restricts the penetration of bacteria into deeper regions of the formation, limiting the effects of any MEOR process. Approaches to counteracting this problem has been the injection of spores and ultramicrobacteria (UMB). UMB are bacteria which have much smaller diameters resulting from nutrient starvation [18] and appear to transport readily through porous media. The study of UMB for EOR processes has demonstrated their ability to penetrate throughout sandstone cores without significantly reducing injectivity or permeability [19, 20]. Accordingly, UMB appears to offer an attractive solution for the transport and dispersion of bacteria throughout the outer regions and depths of oil-bearing formations. It has also been observed that a greater depth of bacterial penetration occurs when the bacteria are allowed to grow through the porous media by the intermittent injection of nutrients [21].

Temperature and pressure will increase as the depth increases, averaging 1-2°C per 100 ft and 0.43-1.0 psig per foot [2, 22]. Average reservoir temperatures range from 49-89°C, with the temperature-depth profile restricting microbial growth in reservoirs at depths of less than 3,500 meters(<100°C). In general, most bacteria will be restricted to reservoirs with depths less than 2500 meters (<85°C). Pressure considerations seem of less importance in that temperature appears the most important parameter in determining any MEOR process. Salinity and pH in oil reservoirs vary from 1 per cent to 10 per cent and from pH 3.0 to 9.9 [2, 22]. Thus salt tolerance is required of microorganisms used for MEOR as well as the ability to grow over wide pH ranges. Many microorganisms adapt easily to higher salt concentrations, although most microorganisms grown optimally between pH 6.0-8.0. Exceptions do exist, however, in the acidophilic, alkanophilic and halophilic groups of microorganisms.

The type of oil present in a reservoir is an additional factor of importance. The toxicity of light volatile oil fractions to microorganisms and the density of heavy oils are generally considered unfavorable for the application of MEOR processes.

Accordingly, based on the limitations imposed by the reservoir, it is possible to identify certain conditions which appear to be most favorable for the application of MEOR processes. These reservoir conditions are: less than 10 per cent brine, pH 4-9, permeability greater than 75 mD, API gravity of the oil above 18, and reservoir temperatures less than 75°C [2, 22]. Permeability and temperature appear to be the most restrictive parameters for MEOR processes.

MEOR field tests

Hitzman has reviewed the status of over 300 MEOR field tests conducted since 1953 [23], concluding that positive MEOR responses occurred in several reservoirs, that *in situ* microbial growth does result in chemical and petrophysical changes within the reservoir, and that the oil reservoir is not a totally biologically restrictive environment for microorganisms. Numerous field trials conducted in eastern Europe encompassed a wide range of reservoir parameters: temperatures ranging from 22°C to 97°C; depths from 50 to 1,500 meters; porosities of 11 per cent to 36 per cent; permeabilities from 10 mD to 8100 mD, oil types from asphaltenic to light paraffinic oils; and treatment of limestone and sandstone formations [23]. Inocula generally consisted of mixed cultures (aerobic, facultative anaerobes, and anaerobic microorganisms) with molasses as the carbon source. Successes were highly variable with incremental oil production ranging from 0 per cent to 200 per cent after treatment. The classical studies conducted in 1953 involved the injection of *Clostridium acetobutylicum* and

molasses into a loosely consolidated sand of high permeability. Significant changes began three months after the initial injection with incremental oil production increasing over 200 per cent per month concomitant with large quantities of organic acids, CO_2, and CH_4 [24]. Recent studies of MEOR processes involve oil displacement from Berea sandstone cores injected with *Bacillus lichenformis* and a *Clostridium* species, resulting in alterations of the rock wettability, increased permeability, and oil displacement [25]. An investigation of oil displacement from cores using organisms isolated from a proposed field site involving high salinities resulted in gas formation and incremental oil production [26]. The results were interpreted as resulting in a shift in the capillary number of the system as a function of gas formation in the pore spaces, reduction of interfacial tension, and plugging by the mixed culture. The Mink Unit field study of a microbially-augmented water flood conducted over 2.5 years involving the injection of a mixed culture consisting of 4 microorganisms resulted in a 13 per cent increase in incremental oil without loss of injectivity or plugging [27]. The organisms traveled 100 meters to the production well through a 90 mD permeability formation. A successful MEOR process in Australia reported a 40 per cent increase in incremental oil. An improved water-to-oil ratio, reduction of the interfacial tension, microbial growth, and repressurization were noted in a 260 mD sandstone formation at 76°C containing a light paraffin oil [28]. Successful MEOR field tests in Germany employed thermophilic halophiles growing on molasses [29]. Incremental oil production was observed in dolomitic formations at 15 per cent salinity and 55°C. Gas formation was identified as the key mechanism for oil release. Interestingly, filtered culture broths injected into the formation released more oil. Oil release was attributed to the dissolution of carbonate rock by organic acids improving permeability and to gas and solvent effects acting to reduce oil viscosity, repressurization and modification of rock wettability. A comprehensive review by Tanner *et al.* [3] discusses MEOR options in carbonate reservoirs, citing advantages such as acid production for the solublization of carbonate rock to CO_2, solvent production for miscible flooding, and biosurfactant production for altering matrix wettability and reduction of interfacial tension. Computer simulation of MEOR processes is a new developing tool for exploring various MEOR options [30, 31]. Further information is required, however, on the biological component of the models to obtain greater reliability and precision of computer-generated predictions.

Biopolymer and biosurfactant applications in MEOR

Selective plugging of high permeability zones by *in situ* biopolymer production has received interest as a potentially useful MEOR technology for improving sweep efficiencies of lower permability zones. A field test designed for selective plugging and control of water flow in a Canadian heavy oil formation employed *Leuconostoc mesenteroides*, an extracellular slime producer [32, 33]. The organism was injected in a nutrient free medium, followed by the injection of molasses to induce *in situ* biopolymer production. Although the experiment failed to effectively block water flow, the organisms were transported more than 1 km into the formation, where they multiplied and produced biopolymer. *Pseudomonas* species and *Klebsiella pneumoniae* were injected as nutrient starved ultramicrobacteria into sand packs and revitalized by nutrient stimulation. These laboratory-based studies demonstrated uniform penetration and plugging of the sand pack by the bacteria and the ability of such systems to selectively plug high permeability zones having varying permeability characteristics [34, 35]. A number of Gram-positive bacteria have been isolated from oil brines which grow anaerobically at temperatures up to 50°C and produce extracellular biopolymers [36]. The injection of these bacteria into the reservoir may not be required due to their existing presence as indigenous microflora. Nutrient stimulation may possibly induce biopolymer formation and desired plugging effects. The selective plugging of high permeability zones by *in situ* biopolymer production appears to offer potential opportunities for highly successful MEOR applications in improving sweep efficiencies and incremental oil recovery.

The use of synthetic surfactants in EOR technologies has been and continues to be an active area of development. The application of biosurfactants to EOR scenarios as CEOR agents is not a well-studied technology. The basic question concerning biosurfactants is

whether they have surface active performance properties equal to existing synthetic surfactants systems used in EOR processes. A number of biosurfactant systems have been described in the literature and their properties and applications reviewed [37]. Many MEOR studies allude to and implicate the production of biosurfactants by diverse anaerobic microorganisms within the reservoir, mainly to explain some of the effects and mechanisms involved in oil displacement. However, little information is presented on chemical and physical properties of these biosurfactants produced by the anaerobic microflora. An exception is the extracellular synthesis of a cyclic lipopeptide by *B. lichenformis* JF-2, a facultative anaerobe [38]. Although biosurfactants are not currently in use or considered as CEOR agents by the oil industry, potential applications are indicated for these surface active bioproducts in chemical flooding, *in situ* emulsification and deemulsification, viscosity reduction of heavy crude oils, and rock wetting properties. There are currently no examples of successful field applications of biosurfactants as CEOR agents. An extensive study has, however, developed a number of extracellular biosurfactant systems with physical properties commensurate to the effective displacement of oil [W.R. Finnerty, unpublished data]. These extracellular biosurfactants have been demonstrated to effect 60 per cent to 80 per cent microscopic oil displacement from sandstone cores containing a targeted oil. The physical properties supporting the application of these bioproducts as performance-effective CEOR agents are ultralow interfacial tension values (0.001 to 0.00007 mN/m, low critical micelle concentrations (100 to 300 micrograms/ ml), performance-effectiveness in 1 per cent to 12 per cent brine, broad pH range (pH 5.0-10.0), stable temperature profiles exceeding 200°C, surface activity insensitive to divalent cations, low adsorption to rock matrices with greater than 95 per cent recovery in produced fluids, and are produced from cheap substrates. The success of these products in oil displacement is the targeting of biosurfactant surface properties to the oil type and reservoir characteristics. A matching of each biosurfactant to the oil type and the reservoir is a prerequisite for successful oil recovery. Also, the use of the spent culture broth as the surface active solution, following removal of microorganisms, has impacted significantly on the cost-effectiveness of the technology, since there are no requirements for costly downstream product recovery. This surface active culture broth is formulated with respect to pH, salinity, and viscosity to match the physico-chemical properties of the oil and the reservoir. In many cases, culture broth dilutions ranging from 1:1,000 to 1:10,000 are possible, without the loss of performance effectiveness.

Conclusions

MEOR represents a new and innovative technology which has progressed to a status of potential significance for recovering known in-place oil. The sophistication of MEOR studies has steadily advanced where controlled experimentation is demonstrating the effectiveness of MEOR options to stimulate and enhance oil recovery. A need continues for more and improved laboratory and field studies to better understand how microorganisms release oil. Adequate documentation exists that microorganisms can be injected into reservoirs, it is possible to stimulate their *in situ* growth and production of bioproducts considered of value to oil release, and such MEOR treatments do not uniformly result in such known deleterious effects as well souring or plugging. Most MEOR field studies to date have been limited to marginal reservoirs, to minimize possible economic losses. Future field studies will hopefully involve more favorable reservoir systems for treatment, rather than worst case formations. Many of the MEOR programs have been at or near the limits of biological applicability in terms of formation permeability, salinity, and temperature [39]. Future experimentation will be required to assess the long term effects of MEOR packages on the *in situ* biodegradation of the lighter hydrocarbon fractions by anaerobic microorganisms. The rate and magnitude of alkane and simple aromatic hydrocarbon biodegradation is unknown, including biotransformations of the heavy fractions of crude oils. The future interface between the oil industry and the applications of MEOR programs appears most favorable and complementary in terms of developing performance- and cost-effective strategies for increased oil production. It may well develop that MEOR packages will become the only economically feasible option for viable EOR technologies in the near to mid term future.

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