

## **BIOTECHNOLOGY CAPACITY OF LDCs IN THE ASIAN PACIFIC RIM**

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Biotechnology is comprised of a continuum of technologies ranging from long-established and widely applied technologies to more recent recombinant DNA (rDNA) techniques. Several of the technologies are reviewed in this paper, along with specific applications in Indonesia, Thailand, Malaysia, China, Vietnam, The Philippines, and India. I conclude that there is substantial variation among less developed countries (LDCs) in terms of their capacity to develop and use products from biotechnology.

*Key words:* biotechnology, recombinant DNA (r-DNA); Asian Pacific countries; LDCs.

**B**iotechnology can be defined as the integrated use of molecular genetics, biochemistry, microbiology and process technology employing micro-organisms, parts of micro-organisms, genes, cells, and tissues of higher organisms to supply goods and services. Biotechnology is comprised of a continuum of technologies ranging from long-established and widely-applied technologies to more recent recombinant DNA (rDNA) technologies of micro-organisms, plants, and animals. The major techniques of agricultural biotechnology include fermentation, microbial inoculation of plants, plant cell and tissue culture, enzyme technology, embryo transfer, protoplast fusion, hybridoma or monoclonal antibody technology, and rDNA technologies. Many of the more traditional biotechnologies are widely used in developing countries, or have significant potential use. I review such technologies below.

### **Biotechnologies In Developing Countries**

#### Fermentation Technology

For thousands of years humankind has been taking advantage of the activities of micro-organisms to produce foodstuffs and drinks without understanding the microbial processes (fermentation). It was not until the end of the nineteenth century that the first attempts were made to standardize the fermentation processes in order to obtain products of uniform quality. Fermentation is used in a number of different ways: (1) for the production of cell matter or biomass (e.g., bakers yeast, single cell protein); (2) for the production of cell components (e.g., enzymes, nucleic acids); (3) for the production of metabolites, including both primary metabolites (e.g., ethanol and lactic acid, and secondary metabolites (e.g., antibiotics); (4) for catalysis of specific single-substrate conversions (e.g., glucose to fructose, penicillin to 6-amino-penicillanic acid and stereospecific synthesis of fine chemicals (e.g., pharmaceuticals); and (5) for catalysis of multiple-substrate conversions (e.g., biological waste treatment).

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### Application of Fermentation Technology in Developing Countries

Fermentation is the most mature area of biotechnology. It is a relatively proven technology that is amenable to immediate application in developing countries. Equipment requirements for medium- and large-scale production are a fermentor, sterilization equipment, sensors, instrumentation, and computers. With traditional fermentation no control equipment is used; instead it involves the work of highly experienced craftsmen or technicians who judge the state of fermentation and control the effective course of the process. Progress made recently in scaling down the equipment used in some processes, as well as the transfer of know-how, can compensate for the high capital cost of many fermentation processes. The purity of the chemicals need not be very high, depending on the process. Facilities for alcoholic fermentation and biogas production are neither sophisticated nor expensive. However, the production of single cell proteins, amino acids and secondary metabolites is more complicated; the initial capital investments are quite high and, once established, high-quality technical expertise and support facilities are required to maintain production

Fermentation is a widely practiced traditional means of food and beverage production in developing countries. A broad range of fermentation processes are employed in home production and production in small and medium-scale enterprises. In a considerable number of these processes, local raw materials are used. Domestic small and medium-scale industries apply techniques which have already been available, in some cases, for centuries. For example, the beer and wine industry in many developing countries relies on fermentation techniques, as does the processing of cassava roots and the production of tempehs (compact cake made from soybeans, groundnuts or coconuts). In India and China, millions of biogas digesters are in service. Biogas production has the advantage that it can replace firewood, thus, contributing to the struggle against deforestation and desertification. More recent developments in fermentation technology in developing countries include: the large-scale production of ethanol as a liquid fuel in Brazil and India; the production of Single Cell Proteins (SCP) from agricultural raw materials in Cuba; and the production of amino acids (lysine production) and antibiotics (kanamycin) in Thailand.

### Microbial Inoculation of Plants

Microbial inoculant technology involves the selection and multiplication of plant-beneficial micro-organisms, such as those used for improved plant nutrition (biofertilizers), and for improved biological control of pests, weeds, and diseases (biological control agents). The concept of microbial inoculation of plants goes back almost one hundred years and has been commercialized over the last decade. Despite this long history, the global market for microbial fertilizers and pesticides has been insignificant when compared to the corresponding markets for the equivalent chemical pesticides or transgenic products.

### Biofertilizers

Many Soil micro-organisms may enhance the nutrient uptake of plants. Those microorganisms which have a direct beneficial effect on the plant may have considerable potential as biofertilizers. Three groups of plant-beneficial micro-organisms can be distinguished: nitrogen-fixing micro-organisms, mycorrhiza fungi and plant growth-promoting rhizobacteria.

- *Biological nitrogen fixation (BNF)*. In modern agriculture, the natural processes for replenishing nitrogen used up by crops are too slow to sustain the productivity needed. The shortfall is made up by chemical fertilizers, prepared industrially by taking nitrogen from the atmosphere. Some

micro-organisms are also capable of fixing atmospheric nitrogen, for example, the blue-green algae (cyanobacteria), the soil bacteria *Azotobacter*, *Klebsiella*, *Bradyrhizobium*, *Rhizobium*, and Actinomycetes. Plants cannot use nitrogen from the atmosphere. Nitrogen-fixing bacteria possess the enzyme nitrogenase, which converts atmospheric nitrogen to ammonia. To fuel the process bacteria need energy. Some, such as the cyanobacteria, use solar energy by combining BNF with photosynthesis. Others, such as the soil bacteria *Azotobacter* and *Klebsiella*, use organic food. Yet another group of bacteria, notably the *Rhizobia*, enter into symbiotic relationships with plants; the bacteria receive some products of the plants' photosynthesis and, in return, donate ammonia to the plant, which the plant uses for synthesizing proteins.

- *Mycorrhiza associations.* Mycorrhiza associations can be described as the symbioses between certain fungi, particularly Vesicular-Arbuscular (VA)-mycorrhizas, and the roots of vascular plants (e.g., trees, shrubs and certain plants like wheat, sorghum, cassava, soya, tea, and coffee). A VA-mycorrhizal fungus is unspecific, i.e., it can infect a very wide range of host plants; and it is assumed that more than 90% of all vascular plants are capable of forming mycorrhiza associations. In many circumstances, mycorrhizal infection can greatly increase the rate of uptake of nutrients, particularly phosphorus and nitrogen, from deficient soils. Vesicular-Arbuscular mycorrhizas are known to solubilize iron phosphate in low fertility soils. In addition, they may mobilize other scarce elements like copper, zinc, and possibly iron.
- *Plant growth-promoting rhizobacteria.* The impact of rhizobacteria (those members of the total rhizosphere bacteria that are able to colonize roots) on plant growth may be neutral, deleterious or beneficial. The term plant growth-promoting rhizobacteria (PGPR) was given to beneficial rhizobacteria in 1978. Most PGPR are fluorescent *Pseudomonas* or strains of *Bacillus subtilis*. Plant growth promoting rhizobacteria can be inoculated on to some crops and can subsequently improve growth. The beneficial effects of PGPR fall into two categories: growth promotion and plant disease suppression. Growth promotion is evidenced by increases in seedling emergence, vigour, seedling weight, root system development, and yield. The different mechanisms of PGPR which enhance growth are not yet well understood. Some PGPR strains are supposed to increase plant growth by positively interacting with various plant-symbiotic micro-organisms, such as *Rhizobium*, *Bradyrhizobium*, *Frankia* and mycorrhizal fungi. For example, several *Pseudomonas* and *Bacillus* sp. are capable of enhancing modulation. There are also PGPR which produce plant growth hormones in culture, but there is no actual proof of such activity under normal field conditions. Some PGPR strains do not promote growth per se, but the inoculated bacteria are controlling minor root pathogens which are not producing enough obvious symptoms to be noticed, but which hinder the complete expression of the plant's potential.

### Biological control agents

Certain micro-organisms are natural pesticides, fungicides, bactericides, and herbicides. Over one hundred bacteria, fungi and viruses that infect insects have been described. About 60 years ago the basic ideas on the use of added inoculants in biological control were established, but were for a long time overshadowed by the development of chemical pesticides, fungicides, and herbicides.

Currently the most widely used bacterium in insect control (90% of all microbial insecticides) is *Bacillus thuringiensis* (Bt), an aerobic spore-former, which produces a proteinaceous crystal toxic against many insect species, including gypsy moths, inchworms, hornworms, and cabbage looper. The toxin is very selective, safe, and biodegradable. Ingestion of the toxin by a susceptible insect leads to death within a few days. *Bacillus thuringiensis* was first isolated in 1911 and has been commercially produced since the 1960s. The only other significant bacterium for insect control is

*Bacillus papilliae*. It is used to control the larval stages of the Japanese Beetle in ornamental turf (applied as spore dust). Insect viruses have also been applied to control cotton bollworm, pine caterpillars, and spruce sawfly. Fungi can also be used but these control agents are not yet widely commercialized.

Besides insect control, micro-organisms are to a minor extent applied as herbicides. The oldest example of a microbial herbicide is the fungus *Collectotrichum gloeosporioides* which is used in rice and soybean. Microbial inoculants are also applied in the control of plant diseases caused by root pathogens. A commercially produced inoculant is the fungus *Trichoderma* which is used against the Dutch elm disease. Another promising antagonist is the PGPR, *Pseudomonas*. Biological control via PGPR is usually directed toward minor root pathogens (fungi and bacteria). These PGPR are supposed to suppress plant disease by producing siderophores (chelating compounds with a special affinity for iron) which reduce the availability of iron for deleterious rhizobacteria or fungi, or by producing toxic compounds like antibiotics and HCN which are active against deleterious rhizobacteria.

Techniques for the production of biological control agents are being actively developed. The production of agents is, however, a long process and needs research and development time and money. Most of the microbial inoculants which are now commercial or near-commercial have been worked on with varying intensity for about 20 years. A disadvantage of microbial inoculants is their sensitivity to environmental changes in the field. Microbial inoculants are alive and growing and, hence, more variable and more subject to environmental effects than chemicals. Furthermore, the sort of organisms to investigate or to search for is difficult to decide upon. The organism should be suitable to the environment, competitive and effective. A strategy to enhance biocontrol agents is to combine them with other control methods, such as the use of chemicals. Current research on biological control agents is concentrated on minimizing the cost of production of the micro-organisms and searching for effective means for dispersing the microorganisms.

#### Application of Microbial Inoculants in Developing Countries

The prospects for improved agriculture, by the use of microbial inoculants as biofertilizers or biological control agents are particularly good in less intensive, low-input agricultural systems. Hence, in developing countries microbial inoculation of plants could be of great importance. The advantages are better yields, lower costs, and reduced dependence on chemicals. The production of microbial inoculants is not very difficult; unsophisticated fermentors of modest volume can be used to produce significant quantities of inoculants. More problematic is the selection of effective strains that show consistent benefits, i.e., maintain viability and sustain biological activity. Quality control of the inoculants is very important. Rapid assays for biological activity (growth promotion or biological control) are, therefore, required for use during product development and manufacture. Extensive regional trials must be conducted with the product prototype; the environmental limits on biological activity must be determined, and the survival and dispersal of the inocula in the environment must be monitored. Attention should be given to the delivery system to allow application by small-scale farmers.

#### Plant Cell and Tissue Culture

Plant tissue culture is a technique that has been known for 30 years. For many plant species it is technically possible to regenerate a whole plant from a single cell. A single plant cell can be derived from a leaf, root, anther, protoplast or meristem, and can be grown in a test tube filled with appropriate culture medium under sterile conditions. The ultimate goal is to regenerate whole plants

that can then be transferred to soil. In certain important cereal grains, like wheat, oats, and barley, however, it has been difficult to regenerate the plant.

#### *Application of Tissue Culture Techniques in Developing Countries*

Plant tissue culture is a straightforward technique and many developing countries have already mastered it. Its application only requires a sterile workplace, nursery, and green house, and trained manpower. Tissue culture is, however, labor intensive, time consuming, and consequently costly. There have not been very convincing instances where tissue culture plants have been shown to be an improvement on seeds. Seeds remain cheaper and easier to handle. However, if a particularly promising elite plant exists, it would be worthwhile to multiply it by tissue culture so as not to lose the superior genetic combination. Plants important to developing countries that have been grown in tissue culture are oil palm, plantain, pine, fir, banana, date, eggplant, ginseng, jojoba, pineapple, rubber tree, cassava, yam, sweet potato, and tomato.

#### *Enzyme Technology*

Enzyme technology is concerned with production, purification, and immobilization of enzymes and their subsequent application. Enzymes are proteins that catalyze chemical reactions while remaining unchanged upon the reaction's completion. They are highly specific, non-toxic and biodegradable, and operate in conditions of mild pH (4-8), relatively low temperature (10-80 degrees centigrade) and at normal atmospheric pressure.

All enzymes currently in use are obtained from living sources, usually from microbes, but sometimes from animal organs or plant tissues. The utilization of enzymes has been improved considerably since the development of enzyme immobilization techniques. Immobilized systems make the enzyme re-usable and open up the way to continuous processing.

Today, enzymes have five distinct areas of application: (1) as scientific research tools; (2) in cosmetics; (3) for diagnostic purposes; (4) in therapeutic treatment; and (5) for use in industry. The first important product produced with the aid of enzyme technology is high fructose corn from maize.

Enzymes may be produced at relatively low costs in virtually unlimited quantities. Their application makes savings possible in fixed capital costs as enzyme-catalyzed processes operate under milder conditions of pH, temperature, and pressure than their chemical counterparts

#### *Application of Enzyme Technology in Developing Countries*

Enzyme technology (production, purification and immobilization of enzymes) is a complicated technology and most developing countries are not yet capable of producing high quality enzymes. Only more advanced developing countries like Thailand, India, Brazil, Cuba, and Mexico are active in the field of enzyme technology.

#### *Embryo Technology*

Over the last 30–40 years, the techniques for recovery, storage and implantation of animal embryos have been perfected. This technique is already applied in cattle, sheep and goat breeding. The principal benefit of embryo transfer is the ability to produce more offspring from a female animal than would be possible with normal reproduction. For example, each cow would normally give birth to about four calves in an average lifetime. With embryo transfer, this could be increased to at least

25 calves. This could be used to enhance the rapid expansion of rare genetic stock (e.g., a new breed). Embryo transfer can also reduce costs of imported cattle because importing an animal as an embryo is much cheaper. Moreover, by raising these animals in their new home country they are better able to adapt to local environmental conditions.

#### *Application of Embryo Transfer in Developing Countries*

The costs involved with embryo transfer are quite high. Field application of embryo transfer demands proper methods for cryo-preservation so that embryos collected in an institutional farm can be taken to distant places in liquid nitrogen. Costs per embryo transfer are prohibitively high for most developing countries.

The overriding conclusion is that developing countries could benefit substantially from implementation of long-established methods of biotechnology. Experience with such methods can also provide a platform for more advanced r-DNA expertise to be developed. In what follows, I briefly review the state of such biotechnology. Platforms in selected LDCs, mainly in Asia and the Pacific Rim countries, are discussed.

### **The State Of Biotechnology In Developing Countries**

#### Indonesia

As in most developing countries, plant and microbial biotechnologies are more strongly developed than animal biotechnologies in Indonesia. Plant tissue culture and micropropagation techniques are well established in several laboratories, and large-scale commercial production of planting material has been achieved for oil palms. Similar techniques are used to eradicate viruses. The Indonesian government encourages links between industry and government research institutions, and draws the country's private sector into the biotechnology arena. Research programs to develop commercial products are underway, as with the work at the Research Institute for Oil Palm at Marihat.

Industry is sponsoring research, either in collaboration with public research institutions, or as a fee-for-service approach. Twenty Indonesian universities have been linked to research projects with the Agency for Agricultural Research and Development. Conservation, collection, utilization, and protection of plant and animal genetic resources have also been given due importance.

#### Thailand

The Thai government is attempting to provide the infrastructure to enable private companies to take a leading role in biotechnology R&D. Direct support from the United States Agency for International Development (USAID) is available for projects involving application of biotechnology to agriculture. Bio-industrial activity in Thailand includes the production of: amino acids (lysine for animal and poultry feed); organic acids (citric acid); modification of cassava starch; and propagation of commercial plants through tissue culture. Generally, there is promotion of investments in biotechnologies through various incentives, e.g., tax deductions, soft loans, reduced custom tariffs and special permits to foreigners. Within this environment, biotechnology in Thailand is poised for a leap because of presence of a large number of young scientists trained abroad and also because of the availability of high quality equipment.

### Malaysia

In Malaysia, the government has fought to increase R&D expenditures to 2% of Gross National Product (GNP) by the year 2000. The National Working Group on Biotechnology plays a pivotal role in research; funding; providing incentives to industries; monitoring worldwide developments in biotechnology and their relation to national needs; evaluation of trade implications of biotechnology; cooperation in R&D between research institutions and industry; devising mechanisms for funding research activities; and establishing guidelines on biosafety and ethical issues.

Commercialization of research is encouraged through tax exemptions for institutions. Research and development on plant and agricultural biotechnologies is carried out by large plantation companies. The private sector has so far restricted itself mainly to micropropagation for large-scale cloning of elite cultivars, and to areas of short-term pay off.

### China

China is one of the most advanced (developing) countries. In China, plant tissue culture has been widely used since 1970s (Pray, 1999). According to estimates, China's output of products derived from the new biotechnologies will constitute nearly 20% of all Asian biotechnology products by the year 2000. The Chinese government encourages innovation and creativity in new technologies as a general policy. China had a late start in advanced biotechnology research, and most projects are focussed mainly on the country's most pressing needs. It is expected that China will continue to apply traditional biotechnology techniques, such as plant tissue culture, alongside advanced genetic engineering, into the new millenium to meet the demands of rural development.

Many microspore-derived cultivars have been released in China to date, e.g., the first tobacco cultivar, rice, wheat and vegetable cultivars. In excess of forty microspore-derived plant species including: wheat, maize, hot pepper, and sugarbeet, have been obtained by Chinese scientists. *In vitro* tissue culture techniques are being used to produce virus-free grape varieties, triploid watermelons, and so on. Peaches, pears, litchis are being micropropogated. Bananas are being clonally multiplied. Virus-free potato, strawberry, garlic and asparagus seedlings are produced using meristem culture. A sugarcane cultivar with a high sucrose content has been mass-propogated and grown. Clones of rubber trees producing 20% more rubber than standard clones have been produced. Biotechnological applications in pisciculture and livestock husbandry are also taking place. According to estimates, more than sixty kinds of enzyme preparations have been produced totaling several thousand tonnes per year. Finally, China has also made significant strides in genetic engineering (Pray, 1999).

### Vietnam

The Vietnam government considers biotechnology crucial to the development of small-scale production units throughout the country, and as a means of increasing agricultural output. The public biotechnology research program focuses on a wide range of topics. A national program is being implemented by twenty research units in universities, institutes, and centers. Since government funds are not sufficient to meet the research needs, investment is concentrated in pilot-scale projects aimed at bridging the gap between basic research and applications.

The general policy approach has been to combine advanced and traditional biotechnologies to improve the latter, while focussing on small and medium-scale projects that can meet the needs of rural communities. For plant biotechnologies priority is given to crop improvement and reforestation. The public sector is introducing new crop varieties, developing virus free plantings of national and new breeds of cultivars. This work is carried out in national research institutes. The national tissue culture facilities engage in conservation of germplasm *in vitro*, exchange of germplasm within and outside Vietnam, and initial *in vitro* micropropogations. The private sector has developed farmer tissue culture facilities for: micropropogation of selected crop plantlets; soil bed multiplication by apical bud cuttings; potting of apical bud cuttings; potting of apical and axillary bud cuttings; and commercial production of plantlets. Vietnam encourages joint ventures between local and foreign corporations relying on the comparative advantages offered by skilled and inexpensive labor in Vietnam.

### The Philippines

In the case of the Philippines, almost 90% of R&D spending comes from the government. Some financial incentives are being set-up to encourage a private sector contribution. In general, private investment has largely concentrated on cassava production and the fermentation industries. Research laboratories have developed tissue culture techniques for disease elimination and micropropogation in ornamentals, such as orchids and Anthuriums, and in food crop species ranging from vegetables to plantation crops. Although many commercial laboratories carry out orchid tissue culture, very few of them work with plantation and food crop species.

### India

India has focused on both agricultural and medicinal applications of biotechnology, and has developed significant infrastructure both in terms of production and advanced methods. Plant applications include: biological control of plant pests, diseases and weeds; biofertilizers; application of tissue culture to trees and woody species; bioprospecting; medicinal and aromatic plants; seribiotechnology; biodiversity conservation and environment. In addition, an emphasis has been place on rice, especially Basmati rice, mustard, wheat, chickpea, potato, vegetables, banana, oil palm, and coconut. Specific applications are discussed in more detail below. Animal biotechnology focuses on aquaculture and marine biotechnology.

India maintains several plant tissue repositories (or gene banks): the Microbial Type Culture Collection (MTCC) at IMTECH, Chandigarh; the National Center for Conservation and Utilization of Blue-Green Algae (NCCUBGA), at the Indian Agriculture Research Institute, New Delhi; the National Facility for Marine Cynobacterial Germplasm Collection, Bharathidasan University, Tiruchirapalli; the National Facility for Plant Tissue Repository (NFPTCR), IARI Campus, New Delhi; and the Repository on Medicinal and Aromatic Plant Materials at CIMAP, Lucknow.

Medical applications of biotechnology focus on human genetics and genome analysis; and vaccine R&D. Two centers of excellence related to medical applications have been established in India – The Infectious Diseases Center and the Drug and Molecular Design Center.

There is a nation wide bioinformation system in India. Major databanks, such as the Animal Virus Information System, have been developed. Software tools for sequence analysis are made available at low cost. India is also the nodal country for providing training and coordination of the Information Network for agricultural scientists in eight countries in South East Asia.

The most important biotechnology research areas are identified by the Department of Biotechnology, at the Ministry of Science and Technology. The bulk of research funds are provided by the government. Venture capital firms have begun to explore prospects in biotechnology but nothing concrete has emerged yet.

India has endeavored to further evaluate and develop short duration mustard lines; combining oil and meal qualities with enhanced yields. Efforts are also underway to improve hybrids and semi-dwarf high yielding somaclonal variants of *Basmati-370* rice. *B. juncea* has been genetically engineered for low erucic acid content; and to change its lipid metabolism. Transgenic tomatoes and banana have been developed with slow ripening properties; with novel gene to delay fruit ripening and senescence.

India is using molecular breeding and DNA fingerprinting in several plant applications. Molecular mapping is being used to identify and segregate rust resistance in recombinant inbred lines of mustard. Molecular mapping of the white rust resistance gene AC2 is also being undertaken; as well as the molecular mapping of a gene for the yellow seed coat color in mustard. Molecular mapping is also being used to map the loci affecting the cooking quality traits in rice and identify rice hybrids. DNA fingerprinting is being used in different crop varieties (brassica, wheat, rice).

India is also focusing on identifying and isolating novel genes from microorganisms with useful agronomic properties. For example, India is engaged in isolating insecticidal genes, such as *Bacillus thuringiensis*, and the larvicidal effects of their toxin proteins. Restriction mapping of cloned DNA is being used to isolate and characterize the protease inhibitor gene from cowpea, and a meristem-specific promoter from sorghum. In addition, India has developed several transgenic crops – Bt-transgenic pigeonpea, Bt-transgenic brinjal, and Bt-transgenic tomato. Open field trials of Bt-tomato, Bt-transgenic cauliflower and cabbage have been conducted. India has also generated transgenic *indica* rice, stacked with insect and disease resistance traits; and has transformed *B. juncea* to include an antifungal gene. Regeneration and transformation studies are being conducted with chickpea and *Brassica aleracea*.

## Conclusions

There is substantial variation among LDCs in terms of their capacity to develop and use products from biotechnologies. As I have argued here, traditional biotechnologies like fermentation and tissue culture can yield significant benefits to LDCs, and provide a technological platform for the development of model biotechnologies. As such, they should be looked at as complementing the more advanced biotechnologies being pursued by developed countries today.

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