

## 9. International Collaboration

Biotechnology developments are very rapid world over. Through international collaborations, advanced research, technology development and training of human resource have been of significant benefits to this country.

### 9.1. BILATERAL COLLABORATIONS

#### **Indo German:**

Three ongoing projects one each at Guru Nanak Dev University, Amritsar; Drosophila Stock Center, Indore and College of Fisheries, Mangalore are progressing well. Achievements in individual projects are as under:

**Indo-German Cooperation in the field of human genome research and medical genetics at Guru Nanak Dev University (GNDU), Amritsar and Institute of Human Genetics, Berlin, Germany:** The research on various families of congenital cataract have resulted in localization of the gene for cerulean cataract with sutural opacities in one family of a 5-generation, congenital cataract family with more than 30 affected individuals. One hundred and nine cases have been investigated for genetic studies including 16 cases of retinitis, pigmentosa; 65 cases of congenital cataract, eight cases of Marfan's Syndrome; 14 cases of high myopia and six cases of other eye disorders. The detailed medical history of all the patients was recorded and blood samples of 130 patients of the above - mentioned categories have been collected and stored for DNA analysis.

A geneticist from GNDU was trained for three months at Microsatellite Center, Institute of Human genetics, Humboldt University, Berlin in linkage analysis for various gene localization studies. Three scientists from Germany visited the center and helped in conducting the joint training programmes.

**Growth control, cell shape and tumorigenesis studies at Devi Ahilya Vishva Vidhyalaya, Indore and German Cancer Research Center, Heidelberg:** A novel Drosophila/drosophilal tumor suppressor gene has been identified which displayed lethality and tumorous growth of the imaginal disc. The genetic locus has been characterized. Molecular cloning of the locus has been done in Germany, which regulates important signaling pathways in development. Collaborations also included identification of new members of the genetic circuitry, which partake in the lgl mediated tumor suppression. Experimental studies are being carried on to screen for new second site mutations, which enhance or suppress the lgl mutant phenotype.

**Rapid detection of seafood associated pathogens using molecular techniques at College of Fisheries, Mangalore and University of Wurzburg, Germany:** Rapid detection and confirmation of *Vibrio parahaemolyticus* by polymerase chain reaction has been attempted. Presence of tdh gene encoding Thermostable Direct Hemolysin (TDH) in the isolate from shrimp and another from pond sediment indicated the presence of tdh gene, a major virulence factor of *V. parahaemolyticus*.

Work has been initiated on development and evaluation of PCR based methods for rapid detection of Salmonella contamination of seafood. Oligonucleotide primers were synthesized based on the nucleotide sequence of a number of species and these

primers are currently being evaluated for rapid detection of Salmonella species in seafood.

The Second meeting of the Indo-German Committee on Science and technology identified biotechnology as an important emerging area of cooperation between the two countries. To step up the level of cooperation it was agreed to enter into Special Arrangement for Cooperation in Biotechnology between GBF and DBT. The thrust areas have been identified and a draft agreement is already exchanged.

#### *I. Indo-Sri Lanka*

Two ongoing projects; one on malaria at MRC, Delhi and the other multi-institutional project on plant transformation (millets) at JNU, ICGEB, UDSC, New Delhi and NBRI, Lucknow are progressing well. A joint workshop on Agriculture was held in Colombo during October 27-29, 1999. A four member Indian delegation attended the workshop. A workshop on medicinal plants and environment will be held at NII, New Delhi during February 2000. Achievements in individual ongoing projects are as under:

**Non-Cellulosic polysaccharides of lesser known-millets and grain legumes and their physiological effects - A Multicentric Study at JNU; ICGEB; Delhi University (South Campus); NBRI, Lucknow and CISIR, Colombo:**  $\alpha$ -1, 3 glucan had been identified as the important principle for hypocholesterolemic and hypoglycemic effects of the traditionally used millets and grain legumes. Study of mung bean, especially sprouted mung bean (72 h germinated) brought down the blood glucose level in the alloxan induced diabetic rats. Studies have also been carried out on effect of different phytohormones on the biological activity of glucan synthase in order to study the regulation of glucan by phytochromones.

With the ultimate aim of analyzing and engineering contents of non-cellulosic polysaccharides, a regeneration protocol for rapid multiplication and transformation of millets is being developed at the University of Delhi (South Campus), New Delhi. One of the genes involved in glucan synthesis has been cloned and sequenced. Evaluation of various promoters for millets by biolistics and genetic transformation of rice by glucan synthase gene have been initiated.

The NBRI center has initiated experiments for cloning the genes for the key enzymes in the pathway of  $\alpha$ -glucan metabolism with a long term objective of engineering the pathway genetically for enhancing steady state content of  $\alpha$ -glucan in seeds.

#### **Indo-Sweden**

Two International Workshops were held one each at CFTRI, Mysore from November 15-17, 1999 and at NCL, Pune from November 17-19, 1999. Four ongoing projects one each at CFTRI, Mysore, NCL, Pune; PROMED, Chennai and NEERI, Nagpur are progressing well, as described below.

**Cultivation of Micro-organisms and Production of Lactic Acid by Fermentation at Central Food Technological Research Institute, Mysore and University of Lund, Sweden:** Experimental investigations were carried out on optimization of process conditions for lactic acid from glucose in the flasks as well as laboratory fermenters and the technology has been developed for lactic acid and calcium lactate. A memorandum of understanding was signed with a Secunderabad based company for the transfer of technology. The process was demonstrated to the company at

200-liter fermentor scale. The technology is under implementation by the company with advisory assistance from CFTRI team involved in the project.

A microprocessor based biosensor for monitoring lactic acid in food and fermentation processes has been developed. Industry has shown keen interest in this biosensor device and technology transfer possibilities. Flow injection analysis system for on-line estimation of lactic acid is also developed. The flow injection system is further modified to monitor simultaneously the concentration of lactic acid and glucose during fermentation.

An international workshop on Lactic Acid Bacteria (LAB) organised by CFTRI, Mysore and Biotechnology Department, Lund University, Sweden at Mysore had participation of active researchers, policy makers and representatives from industries from eleven countries viz. Argentina, Belgium, China, Japan, Indonesia, Nigeria, Sri Lanka, Thailand, Zimbabwe, Sweden and India. Important recommendations included establishment of a regional culture collection and training center, networking between various laboratories with respect to R&D on LAB, IPR issues related to LAB and emphasis on R&D efforts in the developing countries with a global synergy on LAB.

#### **INDO-US**

Under the Joint Programme on Contraception and Reproductive Health Research, six proposals have been approved by both sides for implementation. A sub-committee will consider another two projects on social and behavioral research on Indian side. These projects are approved for implementation by US side. A call for proposals has been made to solicit new proposals. The next Joint Working Group meeting on Contraception and Reproductive Health Research will be held in DBT during February 14-16, 2000. A Joint Workshop on Methodologies on Clinical Trials Research was held at IRR, Mumbai during October 29-November 5, 1999. Four ongoing projects with CONRAD one at NII and 3 at IRR, Mumbai, are progressing well. Significant achievements in individual projects are as under:

#### **Indo-US Vaccine Action Programme :**

The programme is under implementation since 1987 with the objective of promoting focussed and applied research for development of new and improved vaccines, immunodiagnosics and other biologicals by bringing together leading Indian and US Scientists. The programme has now entered into its third phase.

Twenty-six projects have been implemented so far in the areas of viral hepatitis (B,C and E), typhoid, polio, E.coli, rotaviral diarrhoea, acute respiratory infections, respiratory syncytial virus infection, tuberculosis leishmaniasis etc. Scientific exchange visits have taken place. Two hundred and thirty one Indian scientists have received training in technologies related to development of vaccines and immunodiagnosics in US laboratories.

A two day workshop on Group 'A' Streptococcus was organised in April, 1999, in New Delhi, for development and use of vaccine in primary prevention of rheumatic fever/rheumatic heart disease in India.

Thirteenth meeting of Joint Working Group (JWG) of Indo-US Vaccine Action Programme (VAP) was organised during October 28-29, 1999 in DBT. In addition to reviewing the progress made under various ongoing projects and completed projects, other major issues such as initiating Phase-I clinical trial in India for the candidate vaccine strains developed for rotaviral diarrhoea (I16E and I321) under the programme were also discussed.

Prior to Joint Working Group meeting a two day joint Workshop on "Novel Vaccines Technologies", was organised during October 26-27, 1999 at NII, New Delhi. The workshop discussed on recent developments in vaccinology such as DNA based, recombinant DNA, edible vaccines and regulatory aspects.

### **Rotaviral Diarrhoea**

At AIIMS neonatal prototype rotavirus strain I16E has been fully characterised as a candidate vaccine. The nucleotide sequence analysis of VP<sub>4</sub>, VP<sub>7</sub>, NSP<sub>1</sub>, NSP<sub>3</sub> and NSP<sub>4</sub> of two unusual rota virus isolates MP409 & MP480 obtained from children suffering from acute diarrhoea revealed that these strains are closer to G8 type bovine A<sub>5</sub> strain previously isolated from calf with diarrhoea in Thailand.

The IISc. candidate vaccine strain of rotavirus (I321) is capable of replication in cattle which implies the possible transmission of this strain from cattle to humans. The children positive for strain I321 during neonatal period had significantly fewer number of rotavirus diarrhoeal episodes, thus demonstrating protective effects of neonatal infection by I321 against subsequent rotavirus diarrhoeas.

### **Leishmaniasis**

The leishmaniasis project focussed on two putative genes, ORF-F and ORF-G. The ORF-F protein was found to be differentially expressed during parasite life cycle. The predicted amino acid sequence has no sequence homology to any known protein in the databases nor does it contains any informative motifs. Thus it is a novel and specific leishmania protein and may represent a potential target for novel chemotherapeutic agent. Recombinant ORF-F protein also appears to provide the basis for a simple, sensitive and specific ELISA test system for L.donovani infection. A correlation exists between increased copy number of ORF-F and ORF-G genes and the infectivity and survival of strains of L.donovani in macrophages, suggest that these genes are related to virulence of L.donovani. Immunization with recombinant antigens ORF-F, ORF-G or ORF-F + ORF-G conferred partial protection to Balb/c mice against L.donovani infection.

A total of 189 isolates of leishmania were analyzed using lipophosphoglycan specific monoclonal antibody, CATAE for L.donovani and IH2-H8 for L.tropica, amplification of k DNA and isoenzymes analysis indicated that all the 159 isolates studied to be L.donovani. The IL-12 appears to play an important role in the regulation

of the cellular immune responses in Indian visceral leishmaniasis cases. The antibodies to crude soluble antigen of *L.donovani* did not predict the relapse of disease after an unsuccessful first treatment while fall in antibodies to recombinant K39 predicted the relapse of disease.

Studies were undertaken on identification and characterisation of genes with stage specific expression in *L.donovani* from Indian Kala-azar patients at the Institute of Pathology, New Delhi. Two fragments (of sizes 400bp and 1200bp) were identified as unique to Indian isolates of *L.donovani*.

**Epididymal Sperm Maturation Antigen: Molecular Cloning and Evaluation of Antifertility Effect of Epididymal 26kDa Glycoprotein at Institute for Research in Reproduction, Mumbai:** A 26kDa epididymal protein has been identified using a monoclonal antibody. This has been immunochemically characterized and found to have an antifertility effect both in male and female mice.

The protein showed cross reactivity with the proteins present in ovine epididymal fluid. Protein has been purified on anion exchange chromatography resulting reduction in the number of steps of purification to improve the yields of 26kDa protein. The N-terminal analysis of the purified protein is in progress. This purified preparation has been used to immunize two male rabbits to raise polyclonal antibodies for further studies.

**Development of an *in vitro* screening system for evaluating the potencies of progestins developed for therapeutic and contraceptive applications at Institute for Research in Reproduction, Mumbai:** The specific objective of this project is to develop progesterone receptor / reporter gene system using mammalian cells to evolve a series of potency estimations for progestins. Studies have been carried out with progesterone responsive reporter gene construct pUGCAT, for dose related expression in culture and CAT activity. Work is continuing to standardize progesterone dose response curve for CAT expression by transfected cells. This novel approach would give an improved bioassay progesterone response because it is an estrogen independent system that can measure the intrinsic progestational response of compounds. It also eliminates the use of animals, which will be a major contribution towards global attempt to conserve the Bio-diversity.

**Evaluation of anti-fertility effect of FSHBI at Institute for Research in Reproduction, Mumbai:** FSH binding to ovarian granulosa cells showed peak on reversed phase high performance liquid chromatography (RP-HPLC). It revealed suppression of ovulation in mice. The peptide has been injected in marmosets (new world monkeys) during the follicular phase to study its antifertility effect. Further, to understand the mechanism of signal transduction the effect of this peptide on progesterone (P4) production by granulosa cells *in vitro* was studied. The results demonstrate that FSHBI acts by regulating P4 production from granulosa cells *in vitro*. This action may be responsible for suppressing ovulation and impairing fertility in mice and monkeys, as progesterone is known to be essential for ovulation.

**Recombinant Non-Human Primate Zona Pellucida Glycoproteins: Role in Fertility and Efficacy to Regulate Fertility at National Institute of Immunology, New Delhi:** To minimize the extent of premature termination of translocation products, expression of recombinant bonnet monkey zona pellucida glycoprotein – 1 (r-bmZP1) was optimized. A group of female bonnet monkeys (n=5), immunized with glycoprotein conjugated to diphtheria toxoid (DT) were monitored for antibody titers, menstrual cycles, progesterone profiles and status of fertility. The immunized animals generated antibodies against r-bmZP1 and also DT. Cumulative data from mating of the five immunized animals showed protection for 16 ovulatory cycles. The immunized animals are being followed up for the contraceptive efficacy and reversal of the fertility, subsequent to the decline in antibody titers.

To design synthetic peptide based immunocontraceptive vaccine, the B-cell epitope recognized by monoclonal antibodies generated against r-bmZP1 and capable of inhibiting binding of human spermatozoa to the human oocytes, was mapped to the amino acid.

#### **INDO-UK**

A project by Dr. Sher Ali, NII is being funded by Department of Science & Technology under Indo-UK collaboration in Science and Technology. A proposal by NCCS, Pune titled “Detection of cross contamination of cell lines using heteroduplex analysis using mitochondrial DNA as a marker” has also been approved..

**Indo-France:** Under the DBT-CNRS agreement a joint project on Silk Worm genome made very good progress and other projects in the area of genetics of epilepsy and bioinformatics are under finalisation. Further, it was agreed to establish an Indo-French Joint research Laboratory in the area of molecular pathogenesis and Immunology at National Institute of Immunology, New Delhi. In a separate arrangement with University of Picarde, France, a Joint Workshop was held in December 1999 to identify areas of co-operation. Seven specific areas related to expression of recombinant proteins in plants, design and large-scale bio-reactions for secondary metabolic production and downstream processing were identified for joint projects and technology transfer.

**Indo-Israel:** Six joint projects have been finalised in the area of human genome research.

**Indo-Japan:** A joint committee meeting was held in August 1999 and specific areas such as marine biotechnology, seri-biotechnology, food biotechnology with special reference to health food and biotechnology of medicinal plants have been identified for joint R&D projects and workshops.

**Indo-Polish:** The Joint project on immunomodulator is progressing well with leads for joint patenting and publications. A joint seminar on phytomedicine is scheduled in early 2000.

**Indo-Russia:** The first meeting of the Sub-Working Group on Biotechnology under the over all umbrella of ILTP was held in December 1999 and several areas of cooperation in medical biotechnology, agricultural biotechnology and bio-remediation were identified. A proposal to establish Indo-Russian Centre in Biotechnology is being considered.

BIBCOL has made good progress in collaboration with Russian side to achieve their targets of the production of the OPV. .

**Indo-Swiss:** The new phase of Indo-Swiss Collaboration in Biotechnology (ISCB) commenced with an initial planning phase. Through joint workshops specific areas of priority in Agricultural and Environmental Biotechnology have identified and prioritised. More than 100 joint projects were received, and screened. The first meeting of Joint Apex Committee (JAC) was held in September, 1999. The projects approved by JAC are being processed for networking and final implementation. The 2<sup>nd</sup> meeting was held on January 28, 2000 in which projects have been approved. New areas of rice and wheat have been included for developing joint projects.

**Indo-Tunisia:** Two projects each in the area of Somatic Embryogenesis and transformation of wheat and isolation and characterisation of novel BT genes from *Bacillus* strains of Tunisia have been sanctioned.

**Indo-ASEAN:** Two projects each in the area of Plant Biotechnology and Animal Biotechnology involving seven ASEAN countries has been approved for implementation. A concept paper on India-ASEAN Bioinformatic Network (IABN) was prepared and circulated among ten ASEAN member-countries for their inputs.

## **9.2. COLLABORATION AMONG THE SARRC COUNTRIES**

The proposal for establishment of institutional framework for cooperation in biotechnology was circulated among SARRC members of their comments. The same would be considered in the next meeting of the Technical committee on Science & Technology. In addition concept papers received from the member countries have been consolidated and the document has been submitted to SARRC Secretariat through MEA for consideration by the Expert Group.

## **9.3. G-15 ACTIVITIES**

Three national gene banks at CIMAP, Lucknow; TBGRI, Thiruvananthapuram and NBPGR, New Delhi initiated under the aegis of G-15 GEBMAP are now fully equipped with state-of-the art facilities for conservation of medicinal and aromatic plants. A fourth gene bank has been added at RRL, Jammu to cover Western Himalayan regions. The proceedings of the G-15 symposium on “State-of-the-art strategies and technologies for conservation of medicinal and aromatic plants” held at Kuala Lumpur during September, 1998 has been published by the FRIM, Kuala Lumpur, Malaysia. An inventory of important medicinal and aromatic plants of the Asian region has also been published.

## **9.4. Jute Biotechnology**

Under the Phase-1 of the UNDP-GOI supported project being implemented at 3 centres – IIT Kharagpur, M.S.University Baroda and Calcutta University a laboratory scale protocol for enzyme retting of jute fibre was developed. The process involves

retting of the fibre using an enzyme obtained from different microorganisms. A number of fungal and bacterial strains have been identified which secrete large quantities of retting enzymes. These organisms have been identified from the soil and water available in the natural retting environment and also from other sources. Both solid and liquid state fermentation protocols were standardised for optimal growth of these organisms in culture conditions. A laboratory level protocol was found to be suitable for both green and dried jute ribbons and the lab level results indicated that the fibre obtained after enzymatic retting was superior in quality without deterioration of the physical characteristics.

A field demonstration was conducted to test the efficacy of the protocol. The results of the demonstration trial indicated that further process optimisation was required in order to tackle the environmental conditions. The field trials also gave an insight into the type of infrastructure required both for large-scale enzyme production and the retting tanks. From the results obtained there was a clear understanding of the temperature conditions required for storage of both green and dried ribbons.

A second demonstration has also been conducted in 1999 alongwith National Centre for Jute Diversification, Calcutta. The results of this demonstration indicate that the enzyme could now be produced on a large scale at the farmers field. However there have been clear results which now indicate that the enzyme consortia to be prepared should take into account other related parameters such as maturity of the raw-material, temperature for storage, ambient temperature for retting and other environmental factors which effect the retting process.

Based on the results achieved during the two demonstration trials the scientists from the three institutes are now working on fine-tuning of the technology. A complete survey and collection has been done of the microorganisms from both soil and water available in the natural retting environment. Superior strains have been identified which yield higher quantities of enzymes. There is also an attempt to genetically manipulate these micro-organisms so that not only is there higher production of enzyme, but it is also possible for the enzyme to tackle different types of raw-material. There is a concerted effort to develop a user and environmental friendly enzyme, which can be conveniently and cost effectively produced by the farmers themselves. This process has enormous potential not only for reducing the duration of the retting process but also in reducing the cost.