

## 4. BIOTECH PRODUCT, PROCESS DEVELOPMENT AND TECHNOLOGY TRANSFER

### 4.1. TECHNOLOGY TRANSFER & DEMONSTRATION

**Micropropagation Technology Park:** The Two Micropropagation Technology Parks (MTPs) established by DBT at NCL, Pune and TERI, New Delhi provided an effective platform for interaction between the Academics and the Industry. The MTPs have continued production of forest trees through tissue culture and research efforts have concentrated on development of tissue culture protocols for economically important species that are either difficult to propagate or show marked variability.

#### (i) MTP – NCL, Pune

**Large scale production and R&D:** Large-scale production continued for forestry and horticulture species. Teak, Eucalyptus and Bamboo were produced from material collected from the new elite clones identified by the Forest Department. Field trials have been conducted, 10 lakh plants have been field planted at 100 locations over 1000 ha.

The protocols for Horticultural Crops were suitably modified to make the process cost effective. The protocols for Banana, Turmeric, Sugarcane have been modified and a single step procedure developed to get multiplication, elongation and rooting simultaneously in the same medium. In the case of Banana, sturdy rosettes have been formed during initiation phase, which increased the survival of plants in the greenhouse. For easy transportation of Turmeric plants, in vitro rhizome formation has been achieved.

Extensive R&D efforts are continuing for development and refinement with an aim to get cost effective and commercially viable protocols of commercially important plants of interest to the Industry – *Salvadora*, *Casuarina*, *Centelle asiatica*, Phragmites and Potato microtuber.

**Transfer of Technology / Training:** The technology for Teak has been successfully transferred to the International Plant Laboratories, UK. The transfer package includes transfer of know-how and hands on training. This is the first reverse transfer of Tissue Culture technology from a developing country to a developed country. The Technology for Teak and Bamboo has also been transferred to Cadila Pharmaceuticals, Ahmedabad. The package includes, transfer of know-how, hands on training and consultancy for successful production and adoption of the technology by the Industry.

Training has also been imparted to Scientific and Technical personnel from various Universities, private companies and Government organisations. In addition the NCL-MTP is working closely with various State Horticulture / Forestry Departments, Agricultural Universities and Private Industries for establishing Tissue Culture Laboratories on a turnkey basis. Consultancy services are being provided for setting up of the tissue culture laboratories and Greenhouse, training to the scientific and technical personnel, and transfer of protocols / technology packages. Contract production of plants on a large scale has also been taken up in addition to contract research for the Industry / User Agencies. Over 2.00 lakh plants of Bamboo, Teak and Banana have been supplied to the State Forest Departments and Private Industries.

(ii) **MTP – TERI, New Delhi**

**Large scale production and R&D:** Large scale production continued of forest tree species and Horticultural Crops –Anogeissus sp, Dendrocalamus strictus, Eucalyptus tereticornis, Populus deltoides, Paulownia sp, Strawberry, Banana, Sugarcane and floricultural crops. A total of 10 lakh plants were produced and distributed for field trials during the year. The forest trees are being evaluated in mainly 12 different states over an area of 2500 ha. Field evaluation results indicate that the tissue culture raised plants have a better root system and perform better than the conventionally raised plants. The plants are also being used extensively as agroforestry species intercropped with other crops.

The Horticultural crops are also in high demand. A cost benefit analysis conducted for Strawberry, indicates that the tissue culture raised plants give a net profit Rs. 2.15 lakhs / acre. In the case of Sugarcane also an average increase in yield of 1.7 time was reported. Sugar content was also reportedly increased by 1%. The tissue culture technology is being used for bulking up seed material for faster multiplication of newly released varieties. More than 2 lakh microtubers have been field planted. The technology has proved to be superior and cost-effective.

The Research Unit at the MTP is involved in developing and refining protocols for a large number of species of economic and commercial interest.

**Technology Transfer and Training:** The MTP has successfully transferred the technology for 3 species – Eucalyptus, Populus and Sugarcane to Cadila Pharmaceuticals. The package includes know-how, a complete hands on training guide for the scientists and technicians. A short term training programme was held for scientists, officials of State Departments, representatives from Industries and students. The main objective of the training was to popularise the technology and to impart training on protocol development and commercialisation. In addition the MTP is also undertaking contract production and research for industries / user agencies.

**Table I. Number of plants of forest species despatched for field trials**

<b>Species</b>	<b>Number of Plants Despatched</b>
Delhi	38750
Gujarat	6500
Haryana	442269
Himachal Pradesh	1000
Madhya Pradesh	38455
Punjab	6665
Rajasthan	356221
Uttar Pradesh	365780
Andhra Pradesh	200
Assam	415
Orissa	1220
Tamil Nadu	2100
West Bengal	525
<b>Total</b>	<b>1260100</b>

**Table 2: Total area covered under forest trees**

Species	Spacing	Area covered (ha)
<i>Anogeissus pendula</i>	3m x 3m	388.07
<i>Anogeissus latifolia</i>	3m x 3m	97.06
<i>Eucalyptus spp.</i>	3m x 2m	536.9
<i>Populus deltoides</i>	3m x 5m	1039.46
<i>Dendrocalamus strictus</i>	5m x 5m	161.36
<b>Total</b>		<b>2222.85</b>

iii) **Satellite Centre at North-East:** In an effort to promote the micropropagation technology in the North-Eastern region, a Satellite Centre has been established at Guwahati. The main objective is to make available large quantities of superior planting material of forest trees / horticultural crops. Protocol refinement is being done for species of commercial importance. Interactions have been held with the Industry and User Agencies of the region and an effort is being made to cater to the requirements.

The department also organised a National Workshop on Commercialisation of Plant Tissue Culture, which was attended Scientists from Research Institutes, Industries, Universities and Policy makers. Specific issues, which need to be tackled in order to promote commercialisation, were highlighted during the discussion and the gap areas identified both in form of research efforts and policy issue. The Department is now addressing these issues in consultation with other related Ministries / Departments.

#### 4.2. TISSUE CULTURE LARGE CARDAMOM - PRODUCT PLAN

The project envisages evaluation of the performance of tissue culture plantlets of large cardamom (*Amomum subulatum*) vis-à-vis open pollinated seedlings in planters' field in Sikkim (52.5 ha) and West Bengal (17.5 ha). An area of 12.25 ha in 49 units was field planted during 1998 planting season. During the 1999 season, an area of 28.00 ha covering 112 units was planted. Comparative study of growth performance revealed that plants propagated through tissue culture performed better than those through open pollinated seedlings. The growth performance of tissue culture plants vis-à-vis open pollinated seedlings is given below in the table. About 60,000 tissue culture plants and 15000 open pollinated seedlings are maintained in the nursery for hardening and subsequent field planting during the next planting season in 2000.

#### Growth performance of tissue cultured plants (TC) vis-à-vis open pollinated seedlings (OP) of large cardamom

Year of Planting	Months after planting	Average No. of tillers		Average No. of tillers		Average No. of tillers	
		TC	OP	TC	OP	TC	OP
1998	9	6.0	3.6	8.9	7.1	73.8	61.3
1999	3	3.1	2.5	6.6	5.9	60.0	32.0

### 4.3. TISSUE CULTURE VANILLA – PRODUCT PLAN

The demonstration project on evaluation of the performance of tissue cultured plantlets of vanilla (*Vanilla planifolia*) vis-à-vis stem cuttings in planters field over an area of 150 ha in the states of Kerala (60 ha), Karnataka (60 ha) and Tamil Nadu (30 ha) continued. An area of 89.75 ha during the last three planting seasons 1996(5.50 ha), 1997 (33.75 ha) and 1998 (50.50 ha) was planted. During the 1999 season an area of 15.25 ha covering 61 units was planted in three states. The details of state-wise coverage from 1996 to 1999 planting season are given below in the table. Though the total area envisaged under the project was 150 ha, later due to short supply of planting material by the biotech firms, it was revised to 105 ha covering three states by the Project Monitoring and Management Committee (PMMC). So far 1,47,000 tissue cultured plantlets and 34,125 stem cuttings were supplied to farmers under the project and areas of 105 ha covering 420 units were planted.

The growth data were collected from all the demonstration plots. In general, plants raised from stem cuttings exhibited a better growth over the tissue-cultured plantlets. However, if the height of tissue cultured plantlets used is same as that of stem cuttings this difference is not pronounced and in some of the zones growth of tissue cultured plantlets was better. In most of the plots planted during 1996 season, flowering was recorded from a few vines raised from stem cuttings. Among the tissue culture plots, flowering was recorded only at Myladumpara zone in Kerala where longer plantlets were used. The fruits (beans) sets were normal in all the cases.

Monthly rainfall data has also been collected from all the zones and it will be utilised to study the influence of rainfall on growth of vanilla. Fourteen training programmes were conducted during the year in which 375 farmers participated. Besides, beneficiaries of Wynad and Idukki zones were taken to various vanillaries for field exposure and practical demonstration of cultural practices to be followed.

#### Details of field transfer of tissue cultured vanilla plants during 1996, 1997, 1998 and 1999 planting seasons.

State	Zone	1996	1997	1998	1999	
Total		season	season	season	season	
		Area(ha)				
<b>KERALA</b>						
	Trivandrum	1.50	4.25	4.00	0.25	10.00
	Idukki	2.00	6.00	3.50	3.25	14.75
	Ernakulam	--	4.50	7.25	2.00	13.75
	Wynad	0.50	3.75	14.25	6.00	24.50
	Sub total	4.00	18.50	29.00	11.50	63.00

I.

#### II. KARNATAKA

	Chickmagalore	1.50	3.75	4.50	0.50	10.25
	Coorg	--	1.50	2.75	0.25	4.25

Mangalore	--	2.25	2.75	1.25	6.25
Sirsi	--	2.50	4.00	0.50	7.00
Sub total	1.50	10.00	14.00	2.50	28.00
<b>III. TAMIL NADU</b>					
Kanyakumari	--	5.25	5.00	1.25	11.50
Nilgiris	--	--	2.50	--	2.50
Sub total	--	5.25	7.50	1.25	14.00
<b>Grand Total</b>	<b>5.50</b>	<b>33.75</b>	<b>50.50</b>	<b>15.25</b>	<b>105.00</b>

#### 4.4. MICROBIAL AND INDUSTRIAL BIOTECHNOLOGY

In the current year there are 25 on-going projects and five projects have been completed some of which have provided important leads for further investigations.

**Biotransformation, Biocatalysts and Biodegradation:** At IISc, Bangalore *Nocardia* spp. was shown to bring about novel and useful transformation of tetranortriterpenes, such as conversion of azadirachtin into 3-deacetylazadirachtin, 1-detigloy-3-deacetylazadirachtin-1-en-3-one and 1-detigloy-1-3-deacetyl-11, 19-oxa-11-oxo-azadirachtin-1-en-3-one. This is the first report on the transformation of azadirachtin using biocatalysts; the latter two metabolites were hitherto unknown. Biotransformation of a monoterpene ketone, R-(+)-pulegone was studied using a fungal strain, *Mucor piriformis*. On the basis of the identification of the metabolites, pathways for the biotransformation of R-(+)-pulegone have been proposed.

Polymer matrices of controlled size and porosity having functional epoxy groups have been made at NCL, Pune. The spherical beaded copolymers were synthesised by suspension polymerisation. A number of probable affinity ligand matrices for penicillinase and penicillin G acylase were synthesised.

Scientists at IMTECH, Chandigarh are working with several nitroaromatics-degrading organisms. One of the organism *Burkholderia cepacia* RKJ200 has been shown to utilise p-nitrophenol (PNP) as sole carbon and energy sources. Another organism *Arthrobacter protophormiae* RKJ100, isolated by selective enrichment, was capable of utilising o-nitrobenzoic acid derivatives (ONB) as sole carbon, nitrogen and energy sources. A plasmid of approximately 65-kb was found to be responsible for harbouring genes for ONB degradation in this organism. Plasmid library was obtained after cloning the plasmid DNA fragments present in *A. protophormiae* in to *E. coli* JM 109 using abroad-host-range vector pLAFR3 by conventional molecular biology techniques.

**Process optimisation, fermentation and microbial strain improvement for antibiotics:** New high performance cephalosporin antibiotics are very useful antibacterial agents in clinical practice. Wider antibacterial spectrum and low toxicity have enabled cephalosporins to dominate market share in the oral as well as parenteral antibiotics market. At IMTECH, Chandigarh experiments were carried out in order to develop methods for the strain improvement and for obtaining *Trigonopsis variabilis* cells with high D-amino acid oxidase activity. An efficient method for permeablising cell of *T. variabilis* has been standardised.

Production of cephamycin C antibiotic by *Streptomyces clavuligerus* is highly oxygen dependent process. To meet this demand, attempts were made to improve the

utilisation of the available oxygen more efficiently by expressing heterologous bacterial (*Vitreoscilla* sp.) haemoglobin (VHb) protein gene in Cephamycin C producer strain of *S. clavuligerus*. *Vitreoscilla* is a strict aerobe but it grows in oxygen poor environment because of the unique nature of haemoglobin, which is produced, in such hypoxic conditions. The haemoglobin gene along with its promoter was subjected to nucleotide sequencing. Understanding the difference in DNA sequence of the new isolate, upon detailed comparison with the reported sequence, revealed certain important scientific information which may have great value in understanding the evolutionary relationship among Haemoglobins and the naturally occurring oxygen depleted environment.

*Amycolatopsis mediterranei* is a Gram positive bacterium, which produces rifamycins. Rifampicin, a rifamycin derivative is in extensive use for the treatment of tuberculosis (TB) and leprosy. Resistant *Mycobacterium tuberculosis* strains are increasingly being found leading to an alarming resurgence of the disease, making TB the worlds number one killer as an infectious disease. One alternative to overcome this problem is the generation of novel antibiotics or analogous of rifamycin by manipulating rifamycin biosynthetic gene cluster through combinatorial approaches. As a prerequisite to this, a project at Delhi University, Department of Zoology is working on: cloning and characterisation all the genes for rifamycin biosynthesis in two strains of *A. mediterranei*; developing a series of cloning vectors and suitable methods for introducing DNA into *A. mediterranei* and demonstrating a system for gene or domain replacement. In addition, a series of cloning vectors for *A. mediterranei* strains have been developed. One of the cloning vectors pRL 60 contains three marker genes i.e. genes which confer resistance to kanamycin and erythromycin and  $\alpha$ - amylase genes makes it easier to select the transformants directly on a medium containing 1% starch and erythromycin and exposing the plates to iodine vapours. The availability of these cloning vectors have now made it possible to carry out genetic manipulations of different strains of *Amycolatopsis*.

**Bioremediation, Biohydrometallurgy and Biobleaching:** Demonstration, protection and restoration of manganese and coal mine spoil and fly ash dumps has helped in the reclamation sites of mine and fly ash dumps by biotechnological interventions showing growth of trees on such dumps in Maharashtra and Karnataka. At present fuel and woody tree species have been used for cultivation, the follow up studies would continue beyond the project completion period. The survival rates of various plant species are under monitoring.

At IICB, Calcutta Studies on mesophilic vs. thermophilic leaching of some ores revealed a better extraction by the latter than the former. Initially attempts were made to isolate such microbes (both heterotrophs and iron and iron/or sulphur oxidising autotrophs) from of a few hot springs and soil samples from adjoining areas; thereafter, soil and water samples from mining sites were tested. Though in few cases some initial growth was observed only in the heterotrophic medium, these cultures did not grow on sub-culturing. Leaching experiments were carried out using three-thermoacidophilic archaeal strain procured from DSM (Germany). The strains used were (i) *Acidianus brierleyi* DSM 1651, (ii) *Sulfolobus metallicus* DSM 6482, and (iii) *Metallasphaera sedula* DSM 5348. It was observed that among the archeal strains only *A. brierleyi* could leach copper from the ground ores.

Leather industry is a highly polluting industry generating enormous amounts of liquid as well as solid wastes. The liquid effluents containing large quantities of sulphide, lime and chromium (150-350ppm) are being discharged in to rivers and streams and solid wastes are dumped as landfills leading to land and water pollution. In a co-ordinated project between NCL Pune and CLRI Chennai, studies with chromium tolerant alkalophilic/alkalotolerant Actinomycetes secreting alkaline protease active in presence of chromium ions showed that they could tolerate and also grow in tannery effluents. Chrome bearing effluent from tannery contain different species of chromium (+3) differing in charge as well as in the degree of polymerisation. Studies were also carried out for reduction of COD and chromium uptake from tannery effluents with pregrown biomass of Actinomycetes strains. Studies on effect of time on COD reduction and chromium uptake indicated that chromium uptake was fast but COD reduction was slow. Effect of biomass concentration on chromium uptake and COD reduction from tannery effluents were also studied.

**Microbial Biodiversity:** For collection, identification, indexing and preservation of the microbial germplasm of the country for national use, 14 projects were sanctioned. Studies have been undertaken on the microbial diversity of north-western Himalayas, Central Himalayan region, marine ecosystem, crude oil spill degrading microorganisms, arid zones and saline lakes, cold habitats, hot springs, food ecosystems and microbial biodiversity of indigenous Actinomycetes, entomopathogenic, keratinolytic and higher fungi germplams. An Expert group will monitor the progress.

**Biosynthesis of value added Industrial products:** The commercial significance of the amino acid L-Phenylalanine (L-Phe) has increased with the advent of the dipeptide sweetener Aspartame. The enzymes can be obtained from plants and microbes, especially red yeasts. CB Patel Research Centre, Mumbai has initiated thorough investigation of this process in order to establish an indigenous method for synthesis of L-Phe using PAL from Red yeast.

Gellan, the water-soluble polysaccharide, has commercially attractive food, analytical and biomedical applications. At BISR, Jaipur, using *Sphingomonas paucimobilis* in a sucrose and corn steep liquor media, gellan was produced in high concentration. Rheological studies of the solvent precipitated native and deacetylated gellan showed thinning properties. Comparison of HPLC chromatograms of acid hydrolysed gellan showed similar profiles as that of commercial gellan gum.

Two strains namely *Bacillus firmus* (culture A) and *Klebsiella pneumoniae* were isolated from soil at NCL, Pune. Enzyme studies were conducted to optimise the products of cyclodextrines where the desired ratios of alpha, beta and gamma forms were maintained.

At NCL, Pune, using a microbial strain designated as RL4, manifesting nitrite hydrolase activity, acrylonitrile was converted into acrylamide. The enzyme was characterised in terms of temperature optima, heat stability, pH optima, pH stability and storage stability.

At RRL Jorhat seventy nine strains of bacteria were isolated from the samples collected from sewage and garden soil and out of these twenty two were found to be polyhydroxybutyrate (PHB) producers. Among these organisms five strains were screened

for higher PHB production and identified as *Bacillus mycoides*, *B. badius*, *B. cereus*, *B. brevis* and *Pseudomonas oleovorans*. *B. mycoides* was found to be the best and was employed in further studies. DSC (Differential Scanning Chromatography) and molecular weight determination studied the biopolymer thus obtained for their physical properties, such as melting point and glass transition temperature by GPC (Gel Permeation Chromatography).

**Mathematical modelling and simulation studies:** In many fermentation processes it is difficult to monitor on-line all the relevant variables. Mathematical modelling and use of fuzzy logic of neural networks offer convenient methods to predict and optimise the performance and control of fermentation. At IMTECH, Chandigarh a project was successfully completed using the techniques of Neural networks. Modelling and simulation on fluidisation bed bioreactors using such technique is in progress at Osmania University, Hyderabad.

**Industrially important microbial enzymes:** As a result of extensive screening of novel Actinomycetes and fungi, NCL had isolated fungal cultures that produces high yields of alkaline protease in short fermentation cycles. The fungal origin of the enzyme offers a distinct advantage over the currently employed bacterial enzymes in terms of ease of downstream processing. Evaluation of the enzyme conducted at CLRI, Chennai had demonstrated that the enzyme holds great promise for application in leather industries.

It was earlier reported by Osmania University, Hyderabad that endogenous conversion of the protoxin to toxin in *Bacillus thuringiensis* sub sp. *Kurstaki* is mediated by an endogenous protease. *Bacillus thuringiensis* var. *tenebrionis* produces a 73 kDa protoxin and a protease of 69-kDa molecular weight is expressed at stage II of sporulation, which involved in the conversion of this protoxin to toxin. This enzyme has been purified and found to be a metalloprotease.

Microorganisms producing thermostable lipases have been isolated from various sources. In order to understand the structure function relationship of the enzyme, studies were undertaken with the objectives of characterisation of microorganism producing thermostable lipase with respect to enzyme production under various environmental conditions; enhancement of lipase yields by strain mutation; isolation and purification of thermostable lipase; physico-chemical characterisation of thermostable lipase; amino acid analysis and protein sequencing; positional and substrate specificities of thermostable lipase, and resolution studies. The Scientists from University of Delhi South Campus, RRL Jammu, Panjab University, Chandigarh, NIO Goa and IIT Delhi are engaged in the development of strains and production of large-scale lipases for industrial use.

The scientists at UDSC, Delhi have successfully patented a process for the production of thermostable alpha-amylase for the use of bakery industry.

The scientists at University of Delhi South Campus, Tata Energy Research Institute, Guru Nanak Dev University, Amritsar, National Chemical Laboratory, Pune and Regional Research Laboratory, Jorhat are engaged in the perfection of the production technique for xylanase enzyme from various extremophilic organisms. A brain storming session on the extremophiles gave avenue for the generation of co-ordinated projects on the available leads for the industrial enzymes.

#### **4.5. FOOD BIOTECHNOLOGY**

**Low cost nutritious food supplements:** Through a joint project on the Development of low cost nutritious food supplement(s) using biotechnological approaches at Central Food Technological Research Institute, Mysore; National Institute of Nutrition, Hyderabad and National Dairy Development Board, Anand, efforts are underway for the commissioning of pilot plants for large-scale production of a few low cost nutrient food supplements namely (a) soft chikki (b) suruchi meetha (c) nutro-crispo, sweet and salty. Production methods for all these have been standardised in small scale; the products have also been validated for their organoleptic and nutritional properties. Various states have been approached to consider introduction of the above products in their 'mid day meal programme', for school going children where high incidence of malnutrition is observed.

**Development of Diagnostic kits:** The department took an initiative for generating R & D projects, by convening a Brain Storming session for the Development of Molecular Techniques/Gene probes/ Immunological Assays for the Rapid Detection of Food Borne Pathogens. The aim of the project at *Department of Microbiology, All India Institute of Medical Sciences, New Delhi* is to develop PCR based techniques to detect common food borne pathogens like *Salmonella sp.*, *Campylobacter*, *Vibrio*, *E. histolytica* and *Rotavirus*. During the year, the standardisation of PCR method was achieved for all the above using the standard strains. In case of *Salmonella sp.*, the primers designed were also used to determine the specificity using the strains obtained from other parts of the country. The spiked milk samples were used to determine the sensitivity of the method and it was possible to achieve the detection of 100-200 cells. PCR for detection of Rotavirus in faeces and food was standardised by removing inhibitory substances. RT-PCR in faecal samples was also developed for detecting calcivirus and was used to study the epidemic strains during the year.

At Industrial Toxicological Research Centre, Lucknow specific primers to detect *Salmonella* and *Shigella spp.* in contaminated food materials using PCR techniques were developed. The PCR product could detect upto single cell either alive or dead (non-cultivable). RT-PCR is being performed to delineate between the presence of dead and alive cells.

At Central Food Technological Research Institute, Mysore, a PCR-based assay has been developed by using primers for Phospholipase (PL-1), haemolysins (Ha-1 and Ha-2) and toxin (Bce T) for the detection of isolates of *Bacillus cereus* in pure culture system as well as directly in traditional foods. In all 26 native isolates of *B. cereus* were obtained, which were characterised. Of these, 9 isolates exhibited haemolysin activity in gel diffusion assay, which appear to have relationship with enterotoxin producing ability of the strains. All the native isolates of *B. cereus* reacted positively in PCR assay with PL-1 primer but a varied pattern was observed with haemolysin and toxin primers. The study showed the potential of PCR assay for the detection of native isolates of *B. cereus* in pure culture system as well as direct detection in food samples.

A simple PCR protocol was developed at National Dairy Research Institute, Karnal for rapid detection of different of *E. coli*. By using this PCR assay, 50% of the raw milk samples indicated the presence of *E.coli*. However, the assay was not able to detect *E.coli* in pasteurised milk samples. A highly sensitive and specific multiplex PCR assay has been developed which can detect the presence of *E. coli* 0157: H7 directly from naturally contaminated raw milk samples within 10-12 hrs. This appears to be the first

report of its kind in India wherein *E. coli* 0157: H7 has been detected directly from raw milk samples. Introduction of such a rapid and multiplex PCR based assay in Quality Assurance and Food Laboratories in India for monitoring dairy foods for *E. coli* 0157: H7 on routine basis will not only ensure the safety and wholesomeness of these foods but will also boost the confidence of consumers.

**Probiotics/Babyfood supplements:** Preparation of Rota Colostrum in goats and its use as a baby food supplement is under execution at *National Institute of Virology, Pune and National Chemical Laboratory, Pune*. One of the major causes of mortality and morbidity among children below 2 years of age is rotaviral infection. Passive immunity through immune rota colostrum, fed to children has been considered a good alternative strategy to prevent rotavirus gastro-enteritis. Pregnant goats were immunised by using different schedules of immunisation, with major serotypes of rotavirus viz. G1, G2 and NIV isolates. The colostrum samples from 4-5 goats were tested for ELISA antibody and neutralising antibody against homologous and heterologous antibody. Colostrum from goats was spray dried into powder form and ELISA tested the dried powder for the presence of neutralising antibodies. If children less than 6 months are fed immune goat colostrum before the onset of winter, severe diarrhoea could be prevented. Moreover, if the colostrum is fed to new born children within 48 hours (before the gut closure), the rotavirus antibody from colostrum will be transported to blood. This strategy of immunising rotavirus serotype could lead to prevention of rotavirus diarrhoea.

**Animal/Poultry feed:** To enrich the nutritional value of Agro-Industrial by-products by enzyme supplementation in Poultry, the *University of Agricultural Sciences, Bungler* screened various agro-industrial by-products such as the residues of sunflower or groundnut extraction, deoiled rice bran, rapeseed extraction for the Non-starch polysaccharides (NSP), which are known for their anti-nutritive property. The ingredients screened except jowar and ragi contained moderate to high content of pentosans. Enzyme activity assay was done in four commercial enzyme mixtures and activity levels of different enzymes were observed. The two stage in-vitro NSP digestion assays showed that enzyme mixture with a good xylanase and cellulase activities significantly reduced the relative viscosity and increased the total sugars released from the residues of sunflower extraction, deoiled ricebran and broiler starter diet. Xylanase plus cellulase combination was effective in maize, sunflower extraction and Deoiled Rice Bran based diets, whereas xylanase plus cellulase plus pectinase combination gave the best results in maize, soybean, sunflower and deoiled ricebran based diets. In the first biological trial on broilers, the standard corn-soy diets was replaced with 0, 10 and 20% sunflower residues and supplemented with enzyme mixtures such as (A) (cellulase plus pectinase) and (B) (xylanase plus cellulase). The product reduced the viscosity of gut contents and improved feed conversion ratio. In the second biological trial on broilers, the economy diet was replaced with 0, 7.5 and 15% rice bran and supplemented with enzyme mixtures A and B above, which resulted in reduced viscosity of gut contents at all levels of inclusion and improved body weight of birds by 6.8 and 5.5% and feed conversion ratio by 5.78 and 2.3 % respectively. In another field trial conducted in broilers in Bangalore area with an enzyme mix comprising xylanase plus cellulase plus pectinase added to the broiler diet showed improved weight gain of 20 gm per bird and 50 gm of feed saving for every kg of

chicken produced with a revenue increase of 90 paise per bird. In the next field trial weight gain of 47 gm/bird and 22 gm feed saving for every kg of chicken was achieved with a revenue increase of Rs. 1.22 per bird.

**Fruit juice processing:** The department initiated suitable R & D projects in various institutions to de-bitter citrus juices and develop economic processes for various fruit juices. At Indian Institute Technology, New Delhi, production & application of microbial enzyme-Naringinase for Fruit Juice debittering was undertaken. After screening eight cultures of *Aspergillus/Penicillium spp.*, *Aspergillus niger* strain isolated from citrus peel was selected for further studies because it exhibited maximum extracellular target enzyme activity. Solid substrate fermentation was done and effect of moisture content and temperature on the production of enzyme by *A. niger* was studied. A two stage counter current process was then used to extract the fermented solids. Amount of naringinase was determined. Results of thin layer chromatography showed that the enzyme thus produced possessed both rhamnosidase and glucosidase activities. Naringinase was immobilised on DEAE-cellulose but it was observed that percentage hydrolysis of naringin in the kinnow juice was low.

At Defence Food Research Laboratory, Mysore **use of enzyme technology for fruit juice processing** is being undertaken. Screening of fruits and vegetables was carried out to detect concentration of endogenous enzymes. The activities of the pectinolytic as well as amylolytic and cellulolytic enzymes were estimated in commercial enzyme formulations. Standardisation of processing conditions was undertaken with the commercially formulated pectinex. Effect of endogenous enzymes and commercial enzyme formulation on physicochemical characteristics of clarified juices was studied and it was observed that: (i) in papaya, treatment of pulp with enzyme Pectinex resulted in 25% more yield; enzymatic treatment brought about extraction of 85-90% total carotenoids and 85-95% total sugars from pulp to juice. (ii) in guava, maximum increase in yield was 81.6%. (iii) in muskmelon, treatment of the pulp with pectinex resulted in maximum yield 78.4%. (iv) In mango, treatment of pulp with pectinex resulted in maximum yield 77%. and (v) in carrot, treatment of carrot pulp with Pectinex resulted in maximum yield 90.4%.

The National Chemical laboratory, Pune has been able to develop a small ceramic membrane filtration assembly that can process 50 litres of fresh juice per day (at the rate of 5 litre/hour). The juice so obtained would be fresh and free from bacteria and other spoilage microorganisms. The shelf life under refrigerated conditions has been observed to be more than 3 months. The technology is ready for demonstration purpose.

**Edible Mushrooms:** Efforts are underway for Screening and identification of edible tribal mushroom species of Madhya Pradesh: Development of Database and protocols for their large-scale production at Rani Durgawati University, Jabalpur. Cultural studies were carried out on 166 mushroom species, which were collected, and the growth pattern for various genera was studied. A large number of *Russula* and *Lactarius* (threatened species) could be isolated in pure culture. Two genera viz. *Cantharellus* sp. and *Russula* sp. were nutritionally evaluated. Results showed that: crude protein, carbohydrate and energy value was more in *Cantharellus* sp. than in *Russula* sp. whereas content of crude fat, ash and crude fibre was higher in *Rusulla* sp. than in *Cantharellus* sp. Grains of sorghum were used for preparation of mother spawn and on complete colonisation, each grain was found impregnated with pure culture of mushroom, milky white in colour, soft consistency and pleasant colour. To check fruiting, compost was prepared

and filled in polythene bags. The bags were then inoculated with spawn and kept for complete spawn running. Development of protocols for large-scale production is under progress.

**Food Safety:** Studies on biodegradability of aflatoxin through biotechnological interventions are being carried out at University of Agricultural Sciences, Bangalore. A total of 175 samples comprising of oil cakes, animal and poultry feeds, cereal grains and pulses, petrified fruits and vegetables, milk based sweets, and farm soils were screened during the year and it was observed that oil cakes, poultry feed, milk based sweets, soil samples contained a high count of mould, while petrified vegetables and milk based sweets had high bacterial counts. Among 650 isolates of moulds, *Aspergillus flavus* was predominant followed by *Aspergillus niger*, *Fusarium* spp. and *Mucor* spp. A variety of bacterial groups could also be isolated and these included *Bacillus* spp., *Listeria* spp., *Mirococcus* spp. *Lactobacillus* spp. & *Leuconostoc* spp. Bacterial isolates belonging to different genera were screened for their ability to produce antagonistic property against *Aspergillus flavus* by cross streaking against isolates. Among the *Aspergillus flavus* isolates tested, 30% produced B1, B2 and G1 types of aflatoxin, 20% produced B1 and B2 while 12% produced B1 type only.

A major program on Biotechnology Research for food safety is under its fifth year of execution at *Central Food Technological Research Institute, Mysore*, which is jointly supported by Ministry of Food Processing Industries. Apart from undertaking studies on safety analysis of genetically engineered plants of food value; development of ELISA based detection kit for pesticides of importance in foods; development of rapid detection techniques for Aflatoxin B1 based on ELISA; development of a biosensor for the detection of organophosphorous (OP) pesticide; biomarkers of toxicity, a new initiative to develop a rapid immunological (ELISA) diagnostic test is underway to detect argimone contamination in edible oils.

**Strengthening of skills for food biotech research:** The department visualised the need of manpower with requisite training of biotechnology for food processing industries, and accordingly identified various institutions spread across the country for **Strengthening of Food Biotechnology research and training**. The programmes were supported at seven institutions offering postgraduate degrees in the area of food science/ technology and allied areas. The institutions are to execute research in identified areas such as applications of enzymes in food processing; bioconversion of foods; lactic acid bacteria; fermented foods; traditionally fermented foods of north eastern regions and solid state fermentation. All these areas identified areas will be of relevance to Indian food processing industries and it is expected that over a period of three to five years adequate trained manpower will be available. The institutions executing this programme are Harcourt Butler Technological Institute, Kanpur; Jawahar Lal Nehru Krishi Vishwavidyalaya, Jabalpur; Jadavpur University, Calcutta; Sardar Patel University, Vallabhvidhya Nagar; Guwahati University, Guwahati; Laxminarayan Institute of Technology, Nagpur and Central Food Technological Research Institute, Mysore.

#### 4.6. BIOPRODUCTS

The department has initiated a number of co-ordinated projects for the development of Bioproducts. In association with Department of Electronics two projects are being undertaken. The development of electroporation equipment is reaching a standardisation stage. The transducers developed in this project are under test for finalisation of protocols necessary for the development of electroporation equipment.

The technology for the disinfection of hospital waste using microwave is under constant monitoring for the large-scale use in hospital waste management. The sample preparation, the test schedule and the procedure for validation of results would be finalised soon. Department of Biochemistry at University of Delhi South Campus has developed a whole blood based naked eye visible agglutination assay for the differential detection and screening of antibodies to HIV-1 and HIV-2. The reagents comprise a set of recombinant molecules each containing a monovalent fragment of an anti-human RBC monoclonal antibody (Mab) fused to a protein antigen specific to HIV-1 or HIV-2. A drop of the reagents when mixed with a drop of whole blood from an infected individual results in visible agglutination (clumping of RBCs) within 2 minutes indicating the presence of antibodies to HIV-1 and/or HIV-2, whereas an uninfected blood shows no agglutination. This project on the optimisation of production of HIV-1 and HIV-2 reagents was also partly funded by Cadila Pharmaceuticals Limited, Ahmedabad besides the DBT (which provides the major share of money as grant).

At Shriram Institute for Industrial Research, New Delhi, development of Plasticwares for biotechnology research has been carried out. Specific grade of polypropylene (PP) was used for the production of Cryogenic vial, Centrifuge tube and Micropipette tips; a few additives had been identified and incorporated into the base polymer to make it gamma radiation sterilizable and for better contact clarity. Incorporating suitable nucleating agent to make it gamma radiation sterilizable also modified indigenously available grade of polypropylene. Suitable grade of polystyrene was identified and modified for production of gamma radiation sterilizable tissue culture tubes and plates including ELISA plates. Polystyrene was modified by incorporating suitable hydrophilic coating on to the surface for better attachment and growth of cell and to enhance wettability. In an independent study carried out on the developed tissue culture plates, it was revealed that they are comparable with the standard imported ones.

#### **4.7. BIOSAFETY ISSUES**

Several public debates were organised by universities, institutions and NGOs; various biosafety issues were discussed and clarified. The public concerns were given great importance and all experiments authorised by the government adequately took care of containing the risks. Up to the end of the year more than seventy-five limited field experiments were authorised; the results are going to be consolidated. Initial results on transgenic crops indicate substantial agronomic benefits. The Review Committee on Genetic Manipulation (RCGM) (Annexure-IV) reviewed all the high-risk experiments conducted in the country and had convened three meetings during the year. Substantial information on two transgenic crops has been compiled based on scientific experiments conducted in Indian environment, and information on eight other crops is being generated. The Monitoring-cum-Evaluation Committee (MEC) (Annexure-V) constituted by the RCGM visited the experimental sites to ensure compliance of experimental conditions within the biosafety protocol of the government. MEC has the best expertise, as the members are eminent biotechnologists, agricultural scientists and senior science managers. The RCGM had also reviewed the data generated in the country on several recombinant drugs during the year to find their suitability for intended use, which included insulin, erythropoietin, and interleukin.

#### **4.8. Biotechnology Patent Facilitating Cell**

The Department of Biotechnology created an independent single window Biotechnology Patent Facilitating Cell (BPFC) on 15<sup>th</sup> July 1999, with the objectives of creating awareness and understanding among biologists and biotechnologists, relating to patents by arranging workshops, seminars, conference, etc., at all levels; introducing patent information as a vital input in the process of promotion of R&D programmes in biotechnology and biology; providing patenting facilities to biologists and biotechnologists in the country for filing Indian and foreign patents on a sustained basis; keeping a watch on development in the area of IPR and make important issues known to policy makers, bio-scientists, biotech industry, etc. The activities of the cell involve:

**National Roving Seminars on Patenting in Biotechnology:** In order to create awareness among the scientists, BPFC conducted 4 National Roving Seminars on “Patenting in Biotechnology” at four different locations in the country namely Birla Institute of Scientific Research, Jaipur; Central Institute of Aromatic and Medicinal plants, Lucknow; Rajiv Gandhi centre for Biotechnology, Trivandrum; and Department of Biotechnology, Guwahati university, Guwahati. The basic objective of these seminars was to stir the scientists, make them aware about the increasing strategic importance of the IPR rights, especially the patents, and to bring out important issues relating to the patents in the area of biotechnology.

**Patent Applications:** During the year 1999-2000, Patent Screening Committee (PSC) has been reconstituted as Biotechnology Patent Screening Committee (BPSC) under the purview of BPFC, in the Department. This has been done to systematize and streamline the patent facilitation in India and abroad for inventions resulting from the DBT sponsored projects. Since its inception, BPSC has considered 13 new patent applications, including 5 patent applications from DBT projects in CSIR laboratories. Thirty patent applications are under different stages of processing. So far the department has received 61 patent applications.

**Publications:** In order to give wide publicity and provide access to information on various aspects related to patents and to enable as an easy way for searching the patent information, BPFC has brought out two Publications viz. biotechnology Patent Facilitating Cell – the flier describes the background, objectives, facilities, service and conditions, patent search services and requirements for filing the patents and patenting in Biotechnology: Patent Information on Internet – The publication describes different issues related to patenting in biotechnology and the various sites related to patents, patent information and patent search and various other aspects of patenting.