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Establishment of Biotechnology unit in Tribhuvan University:

A Conceptual Approach*

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ABSTRACT

Tribhuvan university can play a major role in the development of biotechnology as a full fledged science in Nepal. To institutionalise teaching and research in biotechnology, an interdepartmental Biotechnology Unit in the university is proposed. A complete set of facility for biotechnology laboratory for interdepartmental teaching and research is suggested. A wide range of courses in different fields of biotechnology is also proposed.

The decade of 1980s was marked by a rapid upsurge of biotechnological innovations in industry as well as in agriculture. Biotechnology has shown enormous promises to fulfill humanity's most basic needs - increasing food and energy production, solving environmental pollution, improving health care, etc. Many types of the vaccines, antibiotics and medicinal products, food additives, disease free and high yielding plants etc. are already produced routinely by applying recently developed technology.

Biotechnology has emerged as the technology for the third world. The applied biotechnologies are simple and inexpensive. It does not require long term capital and intellectual investments. Most importantly, it can be easily adapted for the use of small scale producers with semiskilled manpower. Therefore, biotechnology unfolds an opportunity to poor countries in eradicating many scourges and economic problems.

Laboratories of Nepal engaged in Biotechnological works:

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1. **Tribhuvan University and its affiliated Teaching and Research Institutions;**

Some experimental works on plant tissue culture are carried out in the Central Department of Botany, Kirtipur for M.Sc level thesis. The results of the works are frequently brought out in national and international meetings and conferences.

A group of teachers of Amrit Campus have organized a research laboratory of biotechnology in which some research projects sponsored by the Research division of Tribhuvan University are being conducted.

2. **The National Herbarium and Plant Research Laboratory, Godawari:**

Active research works on plant tissue culture are being pursued in this laboratory since several years; and in vitro propagation methods several plants have been suggested which includes potato, citrus plants, Brassica oleracea, Cymbidium sp., Ficus sp., Musa sp., etc. Another culture has been successful for Allium fistulum. Reports of research works are presented in national and international journals and scientific meetings.

3. **National Potato Development Programme Laboratory, Khumaltar:**

The well equipped laboratory supported by joint Swiss/HMG project is engaged in production of virus-free potato seeds tubers. The sample tubers have been distributed to the farmers. Thus the project is helping farmers to increase yield and improve quality of potato by the application of biotechnology.

4. **Central Plant Pathology Division, NARC, Khumaltar:**

Cultivation technology of mushrooms, Plerotus sp., Agaricus sp. has been developed in the laboratory. Some farmers are employing these methods to grow mushrooms and getting profit in their business.

5. **Veterinary Hospital, Tripureswor, Kathmandu:**

The veterinary hospital is producing first generation vaccines against some viral diseases of animals like rabies, foot-and-mouth disease. Application of biotechnology based on animal cell culture can easily be pursued for the production of more reliable and efficient second generation vaccines against human viral diseases.

6. **Research Laboratory of RONAST:**

Biotechnology related research works pursued in the research laboratories of RONAST are screening of microflora of lelguminous plants and Pinus sp.,

production of virus-free citrus plants buy grafting method. Organism bank consisting about 50 species of pathogenic bacteria has been maintained in the RONAST lab.

7. Private Sectors:

In recent years some plant tissue culture laboratories have been established in Kathmandu which are engaging in micropropagation of orchids and fruit trees.

Necessity of Biotechnology Unit in the University:

Hence, there are already some biotechnological activities in Nepal. However, the application of developed biotechnology is still at low profile in all sectors and play an insignificant role in the national economy. This is due to lack of definite national program and strategy for the rapid development of biotechnology. Major attempt should be directed towards exploring the potential areas of biotechnology, developing technology, and promoting capital investments on them.

In this regard the university should play a leading role. The university is the "think tank" of the nation. The university should incite technological innovations and development and should play a vital role in formulation of national plans and policies. Moreover, the teaching institutions of university are directly involved in the training technical man power for the nation. Needless to say, the technological expertise of the graduates should meet the requirement of the nation which now faces the wave of biotechnological revolution.

Therefore, Tribhuvan University should organise Biotechnology Unit which should bear mandate to lead biotechnology research and development in Nepal. The unit should entail interdepartmental status. It should provide teaching and research facilities for the biotechnology related subjects in the affiliated departments. Research work of projects and thesis will be conducted in the laboratories of the unit.

The organization of the Biotechnology Unit will be as followings: (Fig. 1)

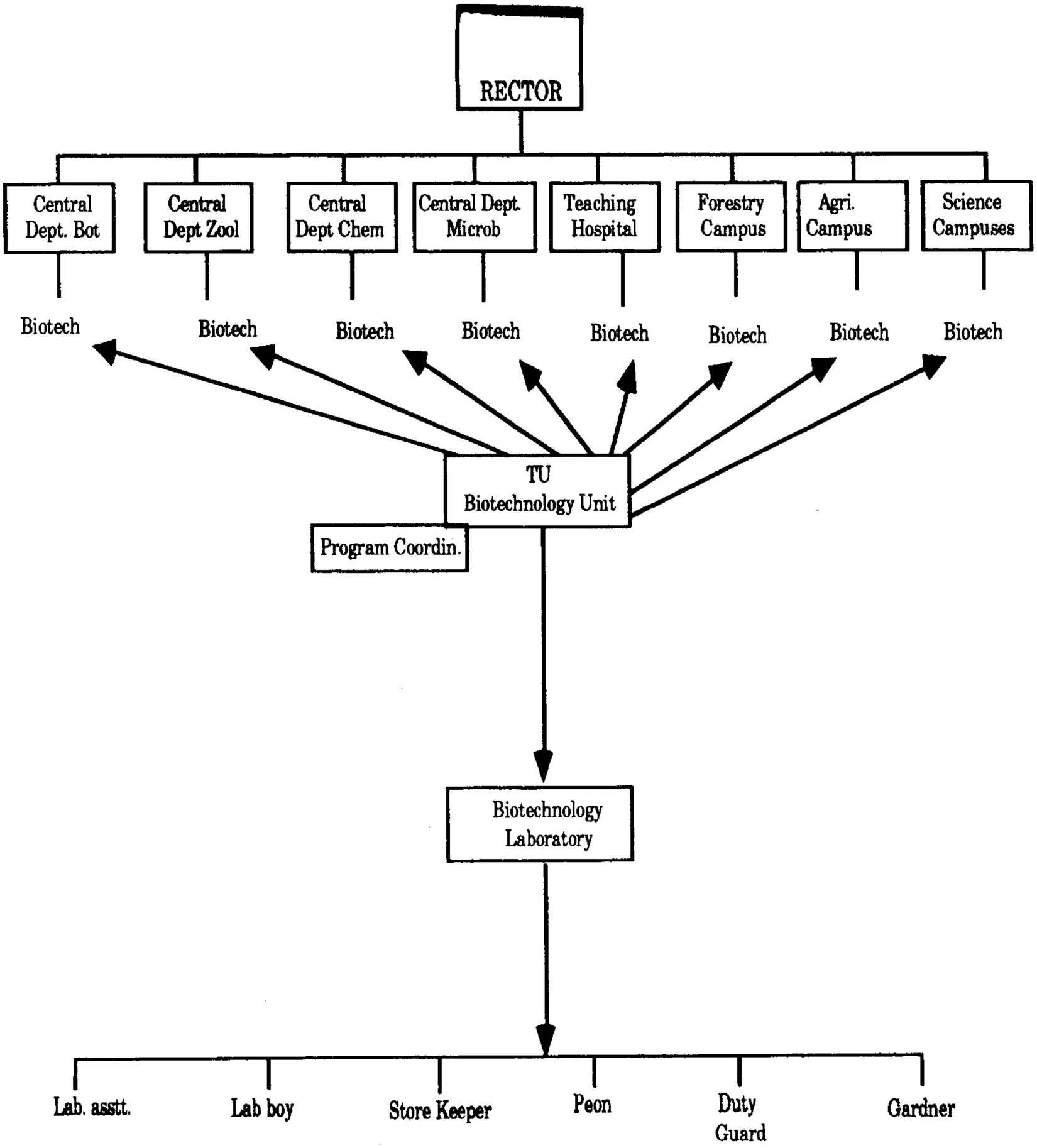

Fig. 1

The teachers of different departments may be affiliated with the Biotechnology Unit in two ways: first, By teaching biotechnology courses and second, by conducting biotechnological research works. Their teaching duties will be determined by the respective departments. The affiliated department will nominate one member to the Biotechnology Unit. Those member should invariably

come from those which are involving in teaching biotechnology courses or undertaking biotechnology or biotechnology related researches.

From among the members of Biotechnoloyg Unit one program coordinator will be appointed by the Rector's office. The program coordinator would have teaching duty in his department but this load will be light. He will draw some allowance for his extra service.

The members of the Biotechnology Unit will met at the request from the affiliated department or the member or called by the program coordinator with appropriate agenda. Additional responsibility of the program coordinator will be to administer the Unit, to draft annual budget and to execute other responsibilities entrusted to him by the Unit. He will also be responsible for the maintaining good condition of the equipments of the laboratory.

Objectives of the Biotechnology Unit:

1. To provide facility for conducting theoretical and practical classes on biotechnological subjects of different departments.
2. To coordinate the different faculties to formulate special courses on biotechnology, pertaining to special papers in different affiliated departments.
3. To maintain laboratories and to conduct researches on plant tissue culture, animal and plant cell culture, pathology and agronomical studies, plant natural product analysis and extraction, and other chemistry and biology based biotechnological works.
4. To coordinate with different biotechnology related HMG departments and research projects to identify potential areas in which the Biotechnology Unit should emphasize its teaching and research activities so that its graduates will be usefully recruited in the development of national economy.
5. To scale up the application of mature biotechnologies at the farmers level and industrial level. Towards this pursuit facility will be necessary to be created for undertaking bench scale and pilot scale projects.
6. To collaborate with different international research institutes or universities for the purpose of importing useful technology.
7. To run joint research projects in collaboration with HMG departments and international institutions:
8. To organize seminar, workshops and conferences on biotechnology:

To sum up, the idea behind creating interdepartmental unit is mainly due to two reasons: first, to coordinate application oriented technologies of different faculties for the purpose of generating appropriate biotechnology which are economically feasible for the implementation and secondly, to avoid redundancy of the same types of laboratory facility and teaching courses of biotechnology in different departments.

Potential areas of Research and Teaching Activities in the Biotechnology Unit of Tribhuvan University

Plant Biotechnology

Plant Tissue and Cell Culture:

This is a very prospective area of plant biotechnology in Nepal. Plant tissue culture technology has the following implications:

i) To propagate rapidly economically valuable plants which poorly multiply by conventional methods. List of such plants include banana, bamboo, sugar cane, orchids, hybrid ornamental plants, endangered medicinal plants, etc.

ii) To produce virus free stocks of potato, citrus plants etc. by meristem culture.

iii) To produce natural products of plants from callus and cell culture, particularly of those plants which are listed as endangered.

iv) To maintain germ plasm bank of endangered plants by the method of cryopreservation of tissues and cells.

Some of these research works can be done in collaboration with the National Herbarium and Plant Research Laboratory, Godawari.

v) To produce disease resistant, cold resistant, draught resistant and high yielding varieties of potato, wheat, rice, etc. by using the technology of another culture, protoplast culture, protoplast fusion, and wide crossing.

Microbial Biotechnology

i) Bioconversion of agricultural and industrial waste into fertilizers, animal feeds, energy (gases) and other useful products like vitamins, aminoacids, corticosteroids, etc.

ii) Screening superior starter organisms for improving traditional food processing methods like, alcoholic beverages, vinegar, cheese, pickle, mushroom cultivation etc. There are also scopes for increasing quality of

microbiologically produced foods and beverages by improving production methods.

iii). Production of antibiotics like penicillin and streptomycin. Works on the production of new types of antibiotics can be done in collaboration with pharmaceutical companies.

Animal Cell Culture Biotechnology

1. Monolayer culture of fibroblast and epithelial cells sreves as excellent materials for teaching cell biology. Students can study different cell organelles with the help of simple microtechniques.

i) Production of second generation vaccines against some human and animal diseases based on animal cell culture techniques is foreseeable possibility in Nepal. List of such diseases includes human viral diseases like rabies, tetanus, hepatitis B, polio and animal viral diseases like foot-and-mouth disease, gumboro disease, etc. Works in these areas can be pursued in collaboration with teaching hospital (TU) and veterinary hospital, Tripureswor Kathmandu.

ii) Production of antibodies for diagnostic tools such as ELISA test, Western blot etc. and for therapeutic uses. These application entail the necessity of more advanced technologies such as cell fusion, recombinant DNA technology and hybridoma cloning. Even though at present, these high technology areas have not yielded results which can easily be implemented in agriculture and industry, they promise spectacular results in near future. Therefore, it is imperative that we keep our scientific capability abreast of the developments in these areas. Moreover, the basic technologies of these fields have already found straightforward use in breeding works and in rapid and accurate diagnosis of diseases.

iii) Cultured animal cells can be directly used for testing toxicity and carcenogenicity of various chemical substances and drugs which are used as food, food additives, cosmetics, medicines or which come in human contact in various ways.

FINANCIAL MATTERS:

Biotechnology unit will be an integral part of Tribhuvan University the basic expenditure of the unit will be borne by the University. Following will be the categories of the expenditure :1. Construction of Laboratory space, class rooms, seminar room, store, office room, greenhouse, computer room etc. 2. Purchase of equipments, glasswares, chemicals. 3. Salary of supporting staffs and allowances. 4. Running cost. 5. Maintenance cost. 6. Miscellaneous.

When the researchers and the Biotechnology Unit will have research projects, funds for expanding laboratory facilities, some portion of the running cost and maintenance cost will be covered by the research grants. Only subsistence financial support from the university will be enough to run the Unit.

The researchers will have full freedom to design and conduct experimental works and to formulate budget. But the Biotechnology unit can set a policy regarding how much percentage of the project's budget should be allocated for the purchase of equipments and chemicals. The researcher should abide by the policy. This is necessary to replace the spent chemicals, glasswares, and damaged equipments and to run the laboratory on self sustainable basis. The equipments and chemicals which are procured for the project works obtained by the name of the laboratory will be the sole property of the Laboratory after the research work has been concluded.

Equipments and Chemicals necessary for setting up

Biotechnology Laboratory

Equipments

Laminar flow hood, Culture cabinet, Refrigerator, Hot-air oven, Autoclave, Temperature regulated incubator, pH meter, Research microscope, Research microscope with Photographic facility, Phase-contrast accessories, Invered microscope, Dissecting microscope, Distillation unit, Electric balance up to 0.1 mg, Centrifuge, Ultracentrifuge, UV sterilization unit, Magnetic strirrer and stirring bars, Micropipettes of various capacities and tips, Test tube shaker, Test tube stands, Glass-ware dryer, Fox-Rosentyle cavity slide micrometer, Millipore filter set (steel) and membrances, 0.2-0.22 mkm, Peristaltic pump, Nylon filter, Gyratory shaker with regulator, Paraffin film, Silicon 200 fluid C,Plastic culture tubes, Haemocytometer, Surgical box, Forceps, Scissors, Resin corsk for mattress, karrels and ampoule bottles, Closures for culture tubes, Mixer, Thermometer,

Liquid nitrogen container, Vessel for cryopreservation, Porcelain jar, Aluminum foil, Air conditioner and AC electricity generator.

Chemicals for plant cell and tissue culture:

Calcium nitrate, Ferrous sulphate, Magensium sulphate, Boric acid, Zinc sulphate, Potassium iodite, Copper sulphate, Sodium molybdate, Cobalt chloride, Potassium sulphate, Hydrochloric acid, Sulphuric acid, Ammonium sulphate, Potassium chloride, Sodium phosphate, Hydrogen peroxide, Sodium hypochloride, Mercuric chloride, Activated charcoal, Teepol, Triton X100, Sodium hydroxide, Potassium hydroxide, Ethyl alcohol, absolute, Ethyl alcohol, rectified Soium-EDTA, Sucrose, Potassium hydrogen phosphate, Potassium phosphate,Potassium nitrate, Potassium dichromate, Ammonium nitrate, Calcium chloride, Agar, different types, Myoinositol, Pyridoxin, Nicotinic acid, Thiamine, Glutamin, Cassein hydrolysate, Biotin, Glycine.

Hormones:

IAA	BAP
2,4-D	Zeatin
IBA	GA
Kinetin	NAA

Enzymes & Chemicals for Protoplast isolation:

Cellulase R10 (Onozuka)
Cellulase (Driselase)
Hemicellulase (Rhozyme HP 150)
Macerage
D-Sorbitol
D-Mannitol
Macerozyme R10
Pectinase
D-Ribose
D-Xylose
K-Dextran sulphate
Agarose

Stains:

Gimsa
Acetocarmine
Fuchsin
Haemtoxylin
Trepan blue
Erythrocine B
Safranin
Phenol red indicator

Glasswares:

(Borosilicate quality, Croning or Pyrex)
Beakers
Kolb vessels

Conical flasks

Mattress

Measuring cylinders
Petri-dishes
Vials with cap
Pipettes
Karrels

Pasture pipettes
Falcon petri dishes
Culture tubes

Media and other solutions for animal cell culture:

Hanks solution
Earl solution
Medium 199
Eagle Medium in MEM
Dulbecco's Modified Eagles medium
RPMI 1629
RPMI 1640
Bovine serum
Calf serum

Trypsin
Versin
Penicillin
Streptomycin
Gentamycin
DMSO
Bovine serum albumin
Collagen
Polyethylene glycols
MESP
PIPES
PIPES

Audiovisual aids

hotography printing and developing facility

Personal computer set with printer and acessories

BASIC BIOTECHNOLOGY COURSES OF STUDY

The Biotechnology Unit envisage a long-range objective to introduce biotechnology courses in the postgraduate level. In the initial periods, Biotechnology special papers would be offered by separate central departments. After few years exercises, the Biotechnology Unit would be elevated to the rank of central department by pooling course works of the different departments.

The following are the possible areas which could be included in the postgraduate biotechnology courses.

MICROBIAL BIOTECHNOLOGY

1. Differences in cellular structures of prokaryotic and eukaryotic forms of microorganisms.
2. Structure and life cycle of economically important microorganisms. Phase virus, Yeast, Agrobacterium, nitrogen fixing bacteria, Penicillium, Puccinia, malaria parasite, Escherichia coli.
3. Microbial Physiology. Range of nutritional requirements of microorganisms, Aerobic and anerobic bacteria, Free living, heterotrohic, pathological and thermophilic bacteria. Metabolic pathways of biosynthesis of important substances.
4. Important Microbial Processes:
   a) Nitrogen fixing bacteria. Symbiotic and free living nif bacteria, agronomic importance of nif bacteria, structural and physiological association of Symbiotic nif bacteria, pathway of nitrogen fixation, nif gene.

      Scopes of application of biotechnology with nif bacteria. Nitrogen fixing bacteria inoculation (free living, root nodule and rhizosphere associated), genetic engineering of root nodule bacteria, rhizosphere associated bacteria of non-leguminous plants.

   b) Decaying bacteria. Process of preparation of biofertilizer and biogas, Scopes of improvements, roles of different types of microorganisms in biogeochemical cycles of important elements.

   c) Traditional Microbial Biotechnology. Alcohol fermentation, food preservation, dairy.

   d) Industrial Microbial Biotechnology.

Potable alcohol. Fermenting organism, Substrate and method of production of various types of alcoholic beverages.

Other alcohols, Organic acids and Vitamins. Fermenting organism, Substrate and method of production.

Amino acids. Fermenting organism of different types of amino acids, Substrate and method of production of various types of amino acids.

Single Cell Protein (SCP). Different processes for different types of microorganisms and utilization of different types of substrates (byproducts and wastes of agriculture and industry).

Antibiotics. Fermenting organism - Substrate and method of production of penicillin and other antibiotics like streptomycin, tetracyclins, erythromycins, etc.

Steroids. Fermenting organism, substrates and methods of production of different types of steroids.

5. Pathology

a) Some important plant diseases. Viral diseases (Leaf roll, potato x and y, cirtrus greening) - Bacterial diseases (Agrobacterium) - Fungal diseases (root rot, smut, rust etc.)

b) Different modes of disease resistance in plants (necrosis, phytoaglutinins, phytoalexins, phytotoxins etc.). Special emphasis on the application of biotechnological methods for improving disease resistance in plants.

c) Some important animal diseases. Viral diseases (flue, Hepatitis, AIDS, Rabies), Bacterial diseases (Tetanus), Protozoan diseases (Malaria, Amoebiasis).

d) Principles of immunity in animal (Humoral and Cellular basis of immunity), Chemotheraputic methods (Antibiotics, Interferon, Interleukin), Imunotherapeutic methods (Monoclonal and Polyclonal antibodies).

B. PLANT BIOTECHNOLOGY

1. Scopes and fields of Plant Biotechnology. Somatic tissue culture (Micropropagation, callus culture, somatic embryogenesis), Gametic tissue culture (Anther culture, Ovary culture, Pollen culture), Suspension culture,Protoplast culture, Genetic manipulation of protoplast, Cryopreservation.

2. Basic techniques of somatic tissue culture. Laboratory requirements, Preparations of glasswares, Preparation of media, Explants and their sterilization, Different nutritional and hormonal requirements for different modes of in vitro culture (callus induction, embryogenesis, organogenesis,

subculturing, rooting), Acclimatization, Field evaluation, Success and scopes of somatic tissue culture.

3. Basic techniques of suspension cell culture and immobilized cell culture.
4. Basic Techniques of Anther culture. Stage of anther development, Nutritional and hormonal requirements for different phases of anrogenesis (Callus induction, Embryogenesis, Morphogenesis), Pollen culture and Ovary culture, Success and scopes of gametic tissue culture.
5. Protoplast culture. Basic techniques of protoplast isolation and culture (from leaf mesophyll cells, from suspension cells, from young in vitro plants).
6. Protoplast fusion and micromanipulation; Basic method of protoplast fusion using PEG and electrofusion, Micropipetting of nuclei and other cell organelles, chromosomes, Selection of transformed clones or somatic hybrids.
7. Cryopreservation and gene bank. Slow growing culture with growth retardants or low temperature, Deep freezing cells or callus.

C. ANIMAL CELL BIOTECHNOLOGY

1. In vitro culture of animal cells and animal organs (Historical account and developments)
2. Nutritional requirements of animal cell culture. Different types of media, Role of different components of serum, Growth factors, Serum-free media, Environmental factors, Growth dynamics of cell culture.
3. Types of cell culture. Monolayer culture, Suspension culture, Immobilized cell culture.
4. Cell lines. Primary cell line, Secondary cell line, Transformed cell line, Epithelial, Endothelial and fibroblast cell lines.
5. Cell fusion and cell fragment fusion. Fusion with PEG, Electrofusion, Fusion involving cytoplast and karyoplast, Micromanipulation of cells.
6. Microbial contamination of cultures and prevention.
7. Use of animal cell culture for test of toxicity and carcenogenicity of chemical substances or drugs.

8. Methods of studying cell culture, Chromosome studies, Isoenzyme analysis, Luminescence microscopy of living cells, Flow cytometry.

D. MOLECULAR GENETICS

1. Basic tools of molecular genetics. Ultracentrifugation, Different types of chromatography (paper, TLC, PAGE, Southern blot, Norther blot, Western blot), Light Microscopy, Electron microscopy Autoradiography, X-ray crystallography, immunobloting.
2. Structure of DNA and DNA duplication
3. Protein synthesis, concept of gene, mechanism of regulating gene activity.
4. Recombination of DNA in vivo. Mutation, Sexual reproduction, Mobile genes, Lysogenic cycle of virus.
5. Recombination of DNA in vitro (Genetic engineering). Restriction Endonuclease, Ligase, Reverse Transcriptase, Terminal Transferases Cloning vehicles (plasmids, viral DNA), DNA sequencing, Chemical synthesis of genes, Screening methods.

Scopes of recombinant DNA technology. Transgenic plants and animals, Salinity and draught resistant plants, Monoclonal antibodies, Transfer of nif gene to rhizosphere bacteria of cereal plants, Increasing protein or aminoacid in cereals, pollution control, Enzymes confactors and hormones for clinical uses.

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