Practical Biotechnology

Principles and Protocols

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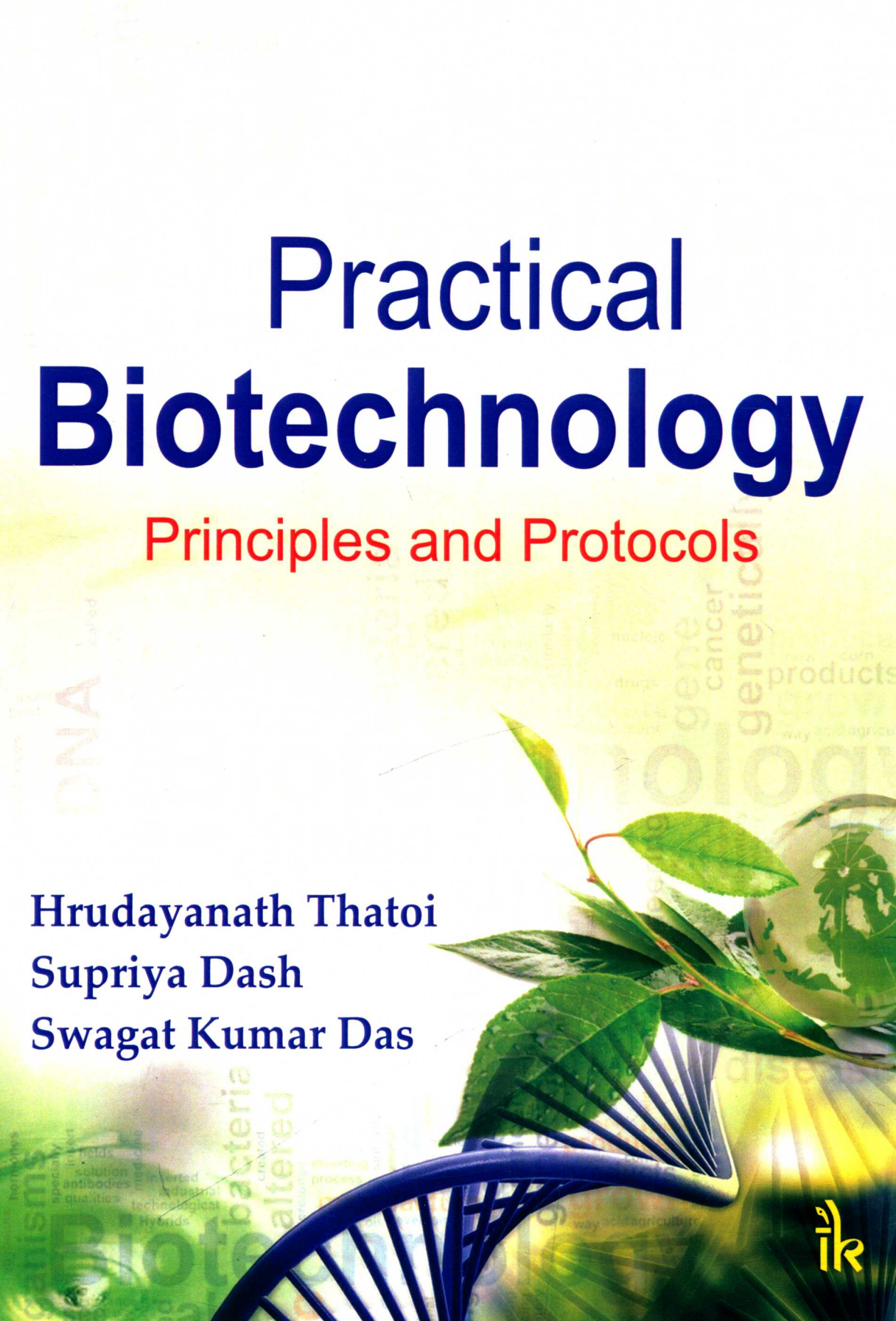

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Preface

In its simplest definition, biotechnology is the technology based on biology. Biotechnology harnesses cellular and molecular processes to develop technologies and products that improve our lives and the health of our planet. We have used the biological processes of microorganisms to make useful food products, such as bread and cheese, and to preserve dairy products. Modern biotechnology provides breakthrough products and technologies to combat debilitating and rare diseases, reduce our environmental footprint, feed the hungry, use less and cleaner energy, and have safer, cleaner and more efficient industrial manufacturing processes.

Biotechnology is an intensive practical oriented subject. It requires laboratories with sophisticated instruments and modern facilities. It has made tremendous progress in the recent past with great scope for application in all fields of science. There has been rapid development of new methodologies and techniques in molecular biology and allied subjects that have been frequently used in the study and research in biotechnology. New techniques have been regularly introduced with greater sensitivity and improved methodologies have been developed with greater accuracy. Developments in the field of genetic engineering, in particular, have contributed a wide range of new techniques. It is essential to make the students and researchers familiar with the basic techniques used in biotechnology for enhancing their theoretical knowledge and practical skills to become a biotechnologist. Keeping these in view, this book is designed the application of biological sciences in engineering with theoretical and practical aspects of biotechnology. This book comprises questions and answers on all aspects of biotechnology. For this a number of techniques have been selected and presented in a simple way with theoretical background, principles, materials required, protocol, troubleshooting, and review questions pertaining to the experiment in order to make the students understand intricacies of the techniques and follow a healthy laboratory practice for successfully conducting the experiments. This book is designed as an introduction to the various tools of molecular biology. It introduces all the basic methods of molecular biology including cloning, PCR, Southern (DNA) blotting, Northern (RNA) blotting, Western blotting, DNA sequencing, oligo-directed mutagenesis, and protein expression.

We hope this book would be of use to all those engaged in biotechnology teaching and research.

Authors

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Authors

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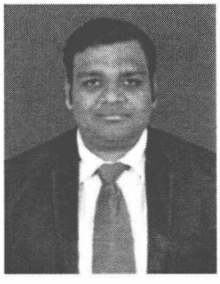
Dr. H.N. Thatoi is Professor and Head, Department of Biotechnology, North Orissa University, Baripada, Odisha. Dr. Thatoi completed his P.G. M.Phil. and Ph.D. from Utkal University. He has also served as Reader and Head of Dept. of Biotechnology, College of Engineering and Technology, a constituent college of Biju Patnaik University of Technology, Rourkela during 2009-2015. Dr. Thatoi has 12 years of teaching and 20 years of research experience in the field of Microbiology and Biotechnology. His areas of teaching and research include Microbial Diversity, Molecular Biology, Genetic Engineering, Environmental and Industrial Biotechnology. He has implemented many research projects funded by DST, Government of Odisha, UGC-DAE, Govt. of India and state forest department, Government of Odisha. Dr. Thatoi has co-authored, two reference books "Cultivation of Medicinal Plants" published by Forest Department Govt. of Odisha, and "Medicinal Plants: Ethnomedicine and Biotechnology" published by Biotech Books, New Delhi and a textbook on "Microbiology and Immunology" published by India Tech, New Delhi meant for postgraduation students of Life Sciences and Biotechnology. Besides, Dr. Thatoi has also edited three books, namely, 'Microbial Biotechnology' 'Microbial Biotechnology: Methods and Applications' and Advances in Biotechnology. He has also edited one proceedings on "Mangrove Conservation and Restoration" published by M.S. Swaminathan Research Foundation, Chennai. He is a member of various scientific societies and is also reviewer and editor of national and internationals journals. He has guided 10 PhDs till date and 8 are in progress. Besides, he has also guided 4 students for their M.Tech., dissertation and many students for their B.Tech and M.Sc. dissertations. To his credit, Dr. Thatoi has published more than 156 research papers in national and international journals, book chapters and proceedings of seminars.

Dr. Supriya Dash, is a faculty in the Department of Biotechnology at College of Engineering and Technology, a constituent college of Biju Patnaik University of Technology, Rourkela, Odisha. She carried out her research work from Central Institute of Freshwater Aquaculture (CIFA), Bhubaneswar, a premiere institute of ICAR as a research fellow. She obtained her PhD from Utkal University in the year 2008 on the topic 'Fish Immunology'. She teaches various subjects such as Immunology and Immunotechnology, Cell Biology and Genetics, Animal and Stem Cell Technology, Genomics and Proteomics etc. in B.Tech. and M.Tech. level. She has more than 7 years of teaching

experience in the field of Biotechnology. She has more than 20 research publications in various reputed peer reviewed international and national journals. She has done extensive work in the field of fish immunology. She has supervised many students in B.Tech. and M.Sc. level. She has also one patent to her credit on 'Male specific protein in fish'. She also attended many training programs on molecular biology and immunology organized by different institutes and also worked as trainer for DNA Fingerprinting and Molecular biology training organized by CET, BPUT.



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CHAPTER

General Laboratory Principles

BIOTECHNOLOGY AND ITS POTENTIAL

Biotechnology is defined as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for their specific uses". The term biotechnology is of recent origin. However, it has flourished since prehistoric times when the primitive human race learned to modify native plants into improved food crops through artificial selection and hybridization. The discovery of fermentation of fruit juices fermented into wine, or conversion of milk into cheese or yogurt, and solution of malt and hops into beer was the beginning of biotechnology. Such processes are based on natural capabilities of microorganisms and are commonly considered *old biotechnology*. Biotechnology was revolutionized when scientists learned the techniques of isolating a desirable genes virtually from any living organism and inserting them into virtually any other organism. This process allowed man to modify microorganisms and other organisms to create in them highly valuable, novel and naturally non-existing capabilities. All these genetic manipulations become possible due to the discovery of so-called recombinant DNA technology or genetic engineering technology. Thus, crop varieties and animal breeds with entirely new and highly useful traits were created with the help of genetic engineering, which marked the development of *modern biotechnology*. Biotechnology is a unique and potential technology, since it helps humans in many ways. The ultimate aim of biotechnological research is the development of commercial products/services which generally cannot be achieved by conventional methods. Today, the biotechnology industry has grown and expanded to affect us on a day-to-day basis. Some of the notable achievements of modern biotechnology are outlined below.

- **More than 130 biotechnology drugs and vaccines are** approved by the US Food and Drug Administration (USFDA) for use by people. **More than 350 biotech drug products and vaccines are currently in clinical trials** targeting more than **200 diseases**, including cancers, Alzheimer's disease, heart disease, diabetes, multiple sclerosis, AIDS, arthritis, etc.
- Biotechnological applications have vital role in the development of **hundreds of medical diagnostic tests** that detect diseases early for their successful treatment. Some of the biotechnology based diagnostic products include detection kits for AIDS, hepatitis B, pregnancy, malaria, blood glucose level, etc.

- With the use of biotechnological techniques, high yielding and more nutritious crop varieties can be produced. Transgenic plants have been established in many crop varieties which include features such as insect resistance, virus resistance, herbicide resistance, high yield, and stress tolerance. Besides, biopesticides, biofertilisers and other agricultural products are also used to improve food supply and reduce dependence on conventional chemical fertilizers and pesticides. The massive production of improved crop varieties is achieved through *in vitro* tissue culture techniques.
- **Environmental biotechnology** makes use of natural as well as genetically engineered microorganisms for efficient sewage treatment, bioremediation of oil spills, xenobiotics and effluents.
- Industrial biotechnology processes make use of microorganisms for the production of commercial products. It includes the practice of using cells of microorganisms, or components of cells like enzymes, to generate industrially useful products in sectors such as chemicals, food and feed, detergents, paper and pulp, pharmaceuticals, textiles and biofuels, etc. The biotechnological applications have led to **cleaner processes that produce less waste and use less energy and water.**
- **DNA fingerprinting** or DNA profiling is used in forensic analysis to determine parentage, identify criminals and investigation, anthropology and wildlife cases, etc.

The biotechnology industry has been growing steadily, and companies that produce products and services such as pharmaceuticals, preventive medicines, medical devices, laboratory tools and analysis, and gene based cancer therapies are increasing. In 1985, there were over 400 biotech companies in the United States and today there are 900 in the US alone and 1200 worldwide. This new discipline demands manpower with very high experience, expertise and skill for the development of biotechnology in all sectors.

Requirements of Biotechnology Laboratory

Laboratory is a room or space for testing, analysis, research, instruction, or similar activities that involve the use of chemicals and instruments. Biotechnology laboratories require adequate space and should be facilitated with 24-hr power and water supply. Generator or online UPS are essential to provide uninterrupted power supply for smooth functioning of equipment like PCR, HPLC, deep freezer, electrophoresis units, etc. Certain laboratories like molecular biology, fermentation technology, bioinformatics, etc., require air conditioning facilities. High quality furniture and cleanable surface are the basic requirements of a laboratory. Besides, these laboratories must be dust and contamination free with proper arrangement for waste disposal of different categories. Design and arrangement of laboratories is also important. It should be made specifically for the purpose for which the laboratory is to be used. For carrying out wet laboratory experiments, a laboratory is usually provided with demonstration table, student working table, fume cupboards, balance room, exhaust fans, reagent shelves, first aid box, fire extinguisher, sinks and water taps, gas taps and disposable containers. Clean environment is also essential for conducting biotechnology practicals. Besides theoretical knowledge, a person working in the laboratory should have undergone adequate training on practical aspects.

Good Laboratory Practices (GLP)

Work in a biotechnology laboratory involves hazardous chemicals, carcinogens, inflammable solvents, and radioactive compounds, live and infectious microorganisms. Personnel working in the laboratory should follow safety measures to prevent accidental damage to the workers and spread of contamination. Each laboratory should develop or adopt a biosafety operation manual that identifies type of the hazards that may be encountered, and specific practices or procedures should be adopted to minimize or eliminate exposures to these hazards. Scientists trained and knowledgeable in appropriate laboratory techniques and experienced in handling infectious agents must be given responsibility for conducting work with any infectious agent/material. The individuals working in the laboratory should consult with biosafety or other health and safety professionals regarding risk assessment. Besides the laboratory must be supplemented by appropriate facility design and engineering features, safety equipment and management practices. For proper documentation purpose, lab notebook is a must and should be used regularly.

Safety Guidelines

Biotechnology laboratories are equipped with supplies and equipment that may pose a threat if used carelessly. So, it is important that one should learn how to handle the equipment properly. There are special approaches and precautions that must be taken while working in any biotechnological laboratory. It is often the responsibility of the laboratory technician to make sure that safety rules are followed. Working in the laboratory requires strict adherence to prescribed rules for personal and environmental safety. The individual must thoroughly understand the safety measures and accordingly perform laboratory experiments with maximum care. This includes procedures for safe handling and storage of hazardous chemicals and biologicals. The safety issues may be divided into several types as discussed below:

- General laboratory safety
- Chemical safety
- Physical safety
- Biological safety
- Radiation safety
- Animal safety

1.1 SAFETY RULES

General Laboratory Safety

All biotechnology students must understand laboratory safety and emergency procedures prior to their first laboratory session. It is expected that each student should apply his/her commonsense and work with responsibility while attending practical classes. In case of difficulty, the students should seek advice from lab instructor or teaching assistant for handling a particular situation. Safety training and/or information should be provided by a faculty member or staff member at the beginning of a new assignment. It is advised that while working in the laboratory, students should adhere to the following do's and don'ts for safe execution of practicals.

Do's

1. Laboratory can be a dangerous place to work and all users need to be aware of the potential hazards and safety measures to be followed.
2. Upon entering the laboratory, place lab coat, books and other belongings in specified locations.
3. If you have long hair or loose clothes, make sure it is tied back or confined.
4. Read labels carefully prior to use.
5. Wash your hands with liquid detergent and dry them with paper towels upon entering and prior to leaving the laboratory.
6. Wear a lab coat or apron while working in laboratory to protect clothing from contamination or accidental discoloration by staining solutions.
7. Wear gloves, safety glasses or face shields when working with hazardous materials and/or toxic agent. Wear goggles for protection from UV light.
8. Carry chemicals and glassware in tray or specific containers.
9. Closed-toe shoes are required in laboratory sessions which can protect your foot from chemicals and microbial contamination.
10. Coats should be hung in the hall or placed in a locker.
11. Speak quietly and avoid unnecessary movements around the laboratory to prevent distractions that may cause accidents.
12. Notify your instructor immediately after any injury, fire, explosion, or spill.
13. Dispose of waste and broken glassware in proper containers.
14. Clean up spills immediately.
15. Keep your hands away from your face, eyes, mouth, and body while using chemicals.
16. Keep the working area clear of all materials except those essential for your work.
17. Always use a pipetting device to dispense chemicals or microbial contaminated liquids.
18. Discard solids into the appropriate waste containers. If you have any question regarding disposal method of any material in the lab, be sure to ask the instructor or technical assistant for instructions.
19. Leave stock solutions at the stock table with proper labelling. Bring test tubes or beakers to the shelf for transferring chemicals and carrying them to your desk.
20. At the beginning and termination of each laboratory session, wipe bench tops with a disinfectant solution provided by the instructor.
21. Turn off all heating apparatus, gas valves, and water taps when lab session is out or when not in use.

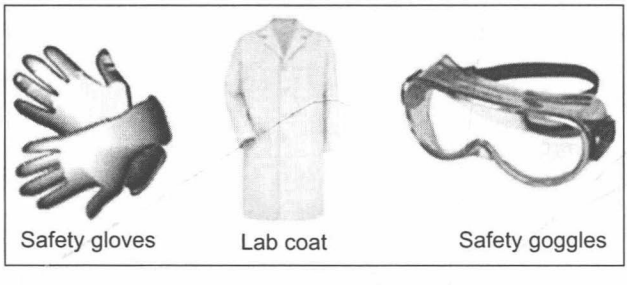

Figure 1.1 Lab safety items for personal protection