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SILVER NANOPARTICLES – CAN THEY RULE OUT THE ANTIBIOTIC RESISTANCE IN AQUATIC PATHOGENS

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Abstract

Nanotechnology in the recent years has been a highly promising field of research and hence has occupied center stage in the modern era of scientific research. Various types of nanoparticles like magnetic-nanoparticle, gold-nano particles and Silver nano particles have been increasingly used in medicine particularly in prevention and treatment of diseases in the recent years. The use of Silver nano particles is also on the rise. Although several reports point to the advantages of silver nano particles, there are studies on the other hand demonstrate that silver nanoparticles are toxic to human health. In order to address this toxicity issue, the need of the hour is to identify methods to prepare silver nano particles with no toxicity. Moving in this direction, studies are being focused to exploit various plant materials and products for the biosynthesis of harmless or nontoxic silver nano particles that is termed as green technology. This review focuses on the importance of silver, nano silver and the green synthesis of silver nanoparticles and their applications particularly in combating the aquatic pathogens.

Key words: Magnetic nano particles, Silver nano particles, Green technology, aquatic pathogens.

Introduction:

Aquaculture, most importantly fisheries in India is an important economic activity and a flourishing sector with varied resources and potentials. Fisheries together with agriculture represent a vital sector. However, the major problem faced by the aquaculture industry worldwide is diseases caused due to various biological and non-biological agents. Among the groups of microorganisms bacterial infections are considered the major cause of mortality in

aquaculture leading to the loss of millions of dollars annually (Grisez et.al. 1995). Some of the bacterial pathogens include *Shigella flexneri*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus myxofaciens*, *Vibrio sp*., *Bacillus cereus*, *Staphylococcus aureus*, *Styphylococcus epidermis*, *Streptococcus pyogens* and *Clostridium species.* The list provided here is just a mention of few of the several pathogens.

Use of antibiotics in aquaculture:

In aquaculture, many strategies have been used to control these bacterial diseases and more solutions are still being investigated (Schneider, Steslow et al. 1991; Sugita H 1992). A fast and common solution to control bacterial diseases is through the use of antibiotics. In aquaculture, particularly shrimp hatcheries antibiotics have been applied to the shrimp feed and water in large quantities primarily to treat and prevent diseases in farmed shrimps. Orfloxacin, oxytetracycline, enrofloxacin, ciprofloxacin, chloramphenicol, erythromycin, furaxolidone. nifurpirinol, oxolinic acid, ormetoprim, rifampicin, trimethoprim and various sulfonamides are commonly used antibiotics in aquaculture (Gräslund 2001).

Multiple antibiotic resistance:

However, use of antibiotics has its downfalls-as the overuse and misuse of antibiotics can cause bacteria to become resistant. Of particular concern is the indiscriminate use of antibiotics leading to the development of Multiple Antibiotic Resistant (MAR) pathogenic bacteria in shrimps and humans (Zanetti S 2001). It has also been demonstrated that bacteria can transfer their resistance genes to human pathogens, thus endangering human health. In addition to the resistance problem, residual antibiotics can also have adverse effects on the organism's welfare and on the environment (Clatworthy, Pierson et al. 2007; Das, Ward et al. 2008). Hence, there is an immediate requirement for the development of novel methods to control and prevent infectious diseases in aquaculture also combating the drug resistant bacteria. One major alternative method being explored in recent times is the application of nanotechnology as an antimicrobial drug in aquaculture.

Nanotechnology:

The field of nanotechnology is one of the upcoming areas of research in the modern field of material science and is rapidly expanding into various areas, such as health, food, feed, environmental aspects and agricultural practices (Roszek et al. 2005). Nanoscale materials are structures ranging from 1 to 100 nm, as defined in the chemistry context, which

have contributed to the development of nanoscience and nanotechnology at an exponential rate in recent years. Nanomaterials by virtue of their physico-chemical properties have emerged as promising antimicrobial agents. These nanoparticles can adopt various mechanisms like inhibition of the synthesis of functional biomolecules or impeding normal cellular activities to kill pathogens (Rai and Bai. 2011). Various metals that are being used to prepare nanoparticles include Gold, Silver, Zinc, Copper, Aluminium and Iron.

Among the above metals, silver is gaining increased importance in the recent times as it provides superior material properties and functional versatility apart from being cheaper. Silver is naturally occurring precious material most often as a mineral ore in association with other elements and has the highest electrical and thermal conductivity of all metals and has the lowest contact resistance (Nordberg and Gerhardsson.1988). Reports indicate that metallic silver appear to exhibit minimal health hazards while few reports suggest soluble silver get readily absorbed and may cause potential adverse effects (Drake and Hazelwood. 2005).

Silver nanoparticles:

Silver has been largely used as a disinfectant in food and water. Further, Silver, Since ancient times, has been implicated in Ayurvedic medicine for microbial inhibition owing to its antimicrobial property and minimal toxic effects (Jung et al. 2008). Over the years, Application of Nano-silver has been shown to be an effective agent against a broad spectrum of Gram negative and Gram-positive bacteria (Burrell et al. 1999), including antibiotic-resistant strains (Wright et al. 2002; Percival et al. 2007). Further these particles have been reported to provide effective treatment against fungal as well as the viral diseases (Kim et al. 2007; Sun et al. 2005). Hence, formulated nanoparticles are incorporated in various nano-gels, creams and lotions to control microbial pathogens (Rai et al. 2009). The current statistics demonstrate that the various nano particles being used today kill more than 650 pathogens.

Green technology:

The green synthesis is a concept that is introduced to define the method used in synthesis, which is favoured over solvent medium. This is because green synthesis is environmentally friendly and contains a reducing agent that is benign to the environment. Besides, it also utilizes a non-toxic stabilizer in forming silver nanoparticles (AgNPs). Preparation of nanoparticles by green synthesis using the natural products obtained from the

plants and their by-products is of considerable interest since plant material seems to be the best candidate for large scale bio-synthesis of nano particles. Nanoparticles produced by plants are more stable, and the rate of synthesis is faster than that in the case of other organisms.

Several plants have been successfully used for efficient and rapid extracellular synthesis of silver nanoparticles. Different plant products like leaf extracts of Geranium (*Pelargonium graveolens*) (Shanker et al., 2003), Lemongrass (*Cymbopogonflexuosus*) (Shankar et al., 2005), *Cinnamommum camphora* (Huang et.al.,2007), Neem (*Azadirachtaindica*) (Shanker et.al., 2004), Aloe vera (Chandran et.al.,2006), Tamarind (*Tamarindus indica*) (Amkamwar et.al.,2005a) and fruit extract of *Emblica officinalis* (Amkamwar et.al.,2005b) have been successfully prepared and characterized. Yet there are several more to be added in this list.

Silver nanoparticles combating aquatic pathogens:

Aeromonas hydrophila is a heterotrophic, Gram negative bacterium which is primary or secondary cause of ulcers, fin rot, tail rot, and hemorrhagic septicaemia in fish. The treatments for this infection are only restricted to some antibiotics. The antimicrobial efficacy of AgNPs using *Cedrus deodar* leaf extract elicited antimicrobial activity against this major fish pathogen. *Aeromonas hydrophila* generates hope for its possible application as a disinfectant or antimicrobial agent for better fish health management (Mahanty. 2013). Antibacterial and antifungal activities of the native and chemically modified extracts from neem seeds, seed-hulls and antibacterial activity of synthesized AgNPs showed effective inhibitory activity against water borne pathogens like *Escherichia coli* and *Vibrio cholera* (Wafaa et al. 2007).

Silver nanoparticles using the leaf extract of *Acalypha indica* exhibited significant antibacterial activity against water borne pathogens such as *Escherichia coli* and *Vibrio cholerae*. In addition, respiratory characteristics and membrane dynamics of the cells were studied to validate the antimicrobial activity of synthesized AgNPs (Krishnaraj. 2009). The studies of Umashankari et al. (2012) reveal that the biosynthesized AgNPs using leaf bud extract of Rhizophora mucronata were found to be equally potent to that of the synthetic antibiotics. These biosynthesized AgNPs showed high antimicrobial activity against marine ornamental fish pathogens such as *Proteus species, Pseudomonas florescence* and *Flavobacterium species*, isolated from an infected fish, *Dascyllus trimaculatus* (Umashankari et al. 2012).

The therapeutic effects of the AgNPs prepared from the wild tea leaf extracts were tested against *Vibrio* species which is an opportunistic aquatic pathogen that attacks the shrimp cultures. Employing the spread plate method Vaseeharan et al. (2010) have demonstrated a significant and dose dependent inhibitory effect on the colony forming units of *Vibrio harveyi.* Further it has been showed that the inhibition of the growth curve of pathogenic bacterium, *Vibrio harveyi* is directly proportional to the concentration of AgNPs used (Pal et al. 2007).

Other applications of nanoparticles in aquaculture:

Apart from possessing enhanced therapeutic activity, the AgNPs could be used in the development of aquatic nano-bio sensors for detecting fish and other aquatic pathogens, disease diagnosis, smart and effective drug delivery, for improving the fish growth, food packaging, to maintain the quality of water through filtration and remediation, waste water treatment, management of aquatic environment.

Conclusions:

Overall, various types of biosynthesized AgNPs have multiple applications in the fisheries and aquaculture. The developments in this area of research provide us new tools and techniques for aquaculture, fish genetics and for the improvement of aquatic health. The antimicrobial properties of various nanosilver based herbal formulations could be exploited to reduce the accumulation of bacteria and to reduce their effects in the aquaculture system. This technology provides us a promising alternative approach for controlling diseases caused by various aquatic pathogens. Thus the biosynthesized AgNPs may in future be included among the potential biological disease controlling agent in aquatic pathogens. Although several reports have demonstrated the beneficial effects with respect to controlling various pathogens one has to understand the impact and toxicity level of AgNPs on soil microbial community and plants. Hence there is an immediate requirement from the scientific community for a complete and comprehensive understanding of the toxicity of the particle if any, their interactions within the ecosystem and ultimately the fate of the biosynthesized AgNPs so that this powerful technology could be adapted for the welfare of the human and animal welfare as well in the environmental conservation.

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PHYTOCOMPOUNDS

Sources and Bioactivities





Mallappa Kumara Swamy
Gudepalya Renukaiah Rudramurthy

PHYTOCOMPOUNDS

Sources and Bioactivities

About the Book

This book provides a systematically reviewed up-to-date information on several aspects of bioactive phytocompounds, their natural resources and production. Several classes of phytocompounds, such as phenolics, flavonoids, alkaloids, glycosides, etc., and their prospective biomedical applications are discussed along with their pharmacological importance. Phytocompounds and their curative properties against various human diseases, including cancer, diabetes, atherosclerosis, neurological diseases, skin diseases, other microbial infections, rheumatic pains, fever, and many more are discussed. Further, the occurrence of less explored medicinal plants and their pharmacologically active phytocompounds have been emphasized in this book. This information will be very useful for the scientific community to further examine for many other unknown compounds and their potential pharmacological benefits in detail. Topics covered in this book include medicinal plants ethnopharmacology, phytochemistry, extraction methods, challenges in medicinal plants cultivation, use of biotechnological approaches, toxicological effects, clinical studies, mode of action, targeted therapy, newly identified potential phytocompounds, novel drug discovery approaches, and drug-delivery strategies including computational approaches and nanobiotechnology are discussed in detail. Overall, this book will be a valuable resource for researchers to work towards identifying and characterizing new phytocompounds possessing bioactivity from a diversified flora, and to enable the discovery of novel therapeutic leads in the near future against various human ailments. Besides this, the book will produce a good information for the students, teachers, scientists, and research professionals involved in the drug discovery research.

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Drug Discovery Approaches and the Role of Plant Products in Treating Neuronal Disorders

Pichili Vijaya Bhaskar Reddy[1]*, Chadipiralla Kiranmai[3] and Pankaj Kalita[1,2]

ABSTRACT

Phytotherapy plays a key role in traditional medicine system in the management of diseases. Traditional medication practices have been serving as an effective alternative source of medicine among many societies in spite of the availability of well-established drug therapy. Natural products have been playing vital role in the traditional treatment as well as in medication system for hundreds of years, in parallel with the development of the pharmaceutical industry. These natural products contain complicated mixtures of organic chemicals, which may include fatty acids, sterols, alkaloids, flavonoids, glycosides, saponins, tannins, terpenes and so forth. Use of medicinal plants against neurological disorders is an age old practice. The traditional system of utilizing medicinal plants is effectively applied to improve the brain function. The pharmacogenic plant extracts interact with the targeted signaling pathways affecting the pharmacology and thereby potentially playing a role in human disease and treatment. Neural drugs work by balancing of particular chemicals (neurotransmitters) or by selective enhancement of cerebral blood flow, cerebral oxygen usage, metabolic rate

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A Glimpse of Biodiversity in India

Dipak Konwar • Pankaj Kalita

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The book is a collection of articles written by a group of eminent scientist working in the field of Biodiversity of N E India. The book covers the topics starting from concept on biodiversity to microbial, floral and faunal diversity of N E India including their conservation strategies. Some of the articles included are pioneers of botanical exploration, algal diversity, medicinal and aromatic plants diversity, pteridophytes, fish diversity, reptiles, birds and diversity of primates. The last chapter is on ecosystem diversity of the area.

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1 Concept of Biodiversity

Pichili Vijaya Bhaskar Reddy
Chadipiralla Kiranmai
Pankaj Kalita

Introduction

Diversity among the biological environment is meant by the word biodiversity; contracting the phrase biological diversity. It is defined as the measurement of total association of genes, species and ecosystem exploring the richness and variety of life. Biodiversity of a location is the measure of variety and richness of different organisms and biological environment in the selected area.

Levels of biodiversity

The term biodiversity is described at three levels: Genetic diversity, Species diversity and Ecosystem diversity. All these three levels are interrelated. However, they are significantly distinct enough to be studied as three separate entities. Although few of the researchers are of the opinion that there exist more diverse levels of biodiversity, these three levels are a good number to work with and discuss.

Genetic diversity: Genetic diversity refers to the variation of genes within a species. This diversity is due to the different gene combinations possible in the genetic set up of an organism that gives its specific characteristics. Genetic variation is extremely important to the survival of species. Genetic variability, that is majorly responsible for these different traits, interact with local environmental conditions to determine the extent to which populations can adapt to environmental changes and survive when exposure to new conditions or diseases. It enables a population to adapt to its environment and to respond to

About the Editors

Dr Vandana Rai received her education at the universities in Lucknow (B. Sc) and Allahabad (M.Sc and D. Phil.) excelling in all the courses of study. Her research interest ranges from Cell Biology, Genetics, Molecular Biology and Human Molecular Genetics; Her contributions on Human Molecular Genetics are well recognized. She joined Veer Bahadur Singh Purvanchal University in 2000, and has involved in teaching and research since then. She is having over seventeen years of teaching experience at post graduate level. She has been guiding post graduate (M Sc) and doctoral (Ph D.) students since 2005. She has published over 6 dozen research articles, more than 12 chapters and edited 1 book. She has successfully completed five research projects funded by UGC, DST, DBT and CSIR. Dr Rai has been actively engaged in curriculum development of Biotechnology courses for postgraduate students and pharmacy courses for under graduate students. She has attended more than 30 international and national conferences, seminars and presented her research. She has also organized more than 10 various seminars, workshops, training programmes and genetic awareness programes. She is member of Editorial Board and reviewers of several journals like: International Journal of Life Science and Pharma Review, Asian Journal of Medical Sciences , American Journal of Biomedical Research, Molecular Biology Reports, Genetic Testing and Molecular Biomarkers, Indian Journal of Medical Research, Meta Gene, Neuroscience Letter, PLoS One, Journal of Assisted Reproduction and Genetics and The Journal of Obstetrics and Gynecology etc. Dr Vandana Rai is life member of Indian Academy of Neurosciences (IAN), Biotech Research Society of India (BRSI) and Indian Society of Human Genetics (ISHG) etc.

Dr Pradeep Kumar has done his M Sc (Biotechnology) from University of Roorkee, Roorkee and Ph.D. in Biotechnology from VBS Purvanchal University, Jaunpur. His research interest ranges from Bioinformatics to Human Molecular Genetics. He joined Veer Bahadur Singh Purvanchal University in 2000 and has involved in Teaching and Research since then. He has published over 3 dozen research articles and several book chapters. He has presented his research work in several international and national conferences. He has successfully completed two UGC funded research projects. He is member of Editorial Board and reviewers of several Journals. Dr Pradeep Kumar is life member of Biotech Research Society of India (BRSI), Indian National Science Congress, Indian Society for Technical Education and Indian Society of Human Genetics (ISHG).

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7

Commercial Micro Propagation and Challenges in Plant Tissue Culture

Kiranmai Chadipiralla*, Pachipala Gayathri*, Uday Sankar Allam* and P.V.B. Reddy**

INTRODUCTION

Plant tissue culture is widely used to produce clones of a plant in a method known as **Micro propagation**, a technique that helps in rapid multiplication of stock material thereby generating a large number of progeny plantlets. Commercial tissue culture industry is in existence globally since the last 25 years. India has started plant tissue culture industry about 15 years back and is revolutionizing the commercial agriculture sector by enabling mass propagation of quality of planting material. Plant tissue culture is a collection of techniques which are used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of particular composition. Tissue culture techniques are important to obtain disease free plants, ranging through molecular genetics, recombinant DNA studies, genome characterization, gene-transfer techniques, aseptic growth of cells, tissues and organ etc., Worldwide there have been numerous efforts to effectively commercialize the large scale propagation of plants by micro propagation and some of these efforts have been more successful. Micropropagation is one of the most commercially exploited areas of plant tissue culture. Micropropagation is used to multiply desired plants such as those that have been genetically improved commercial plants or bred through conventional plant breeding methods. Micropropagation is extremely useful to provide the sufficient quantity/ number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to reproduction. The advances in the technology during the last 30 years have made it possible to efficiently regenerate plantlets from callus and explants from all types of plants.

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** Department of Life Science & Bioinformatics, Assam University, Diphu Campus, Diphu, Assam-782460

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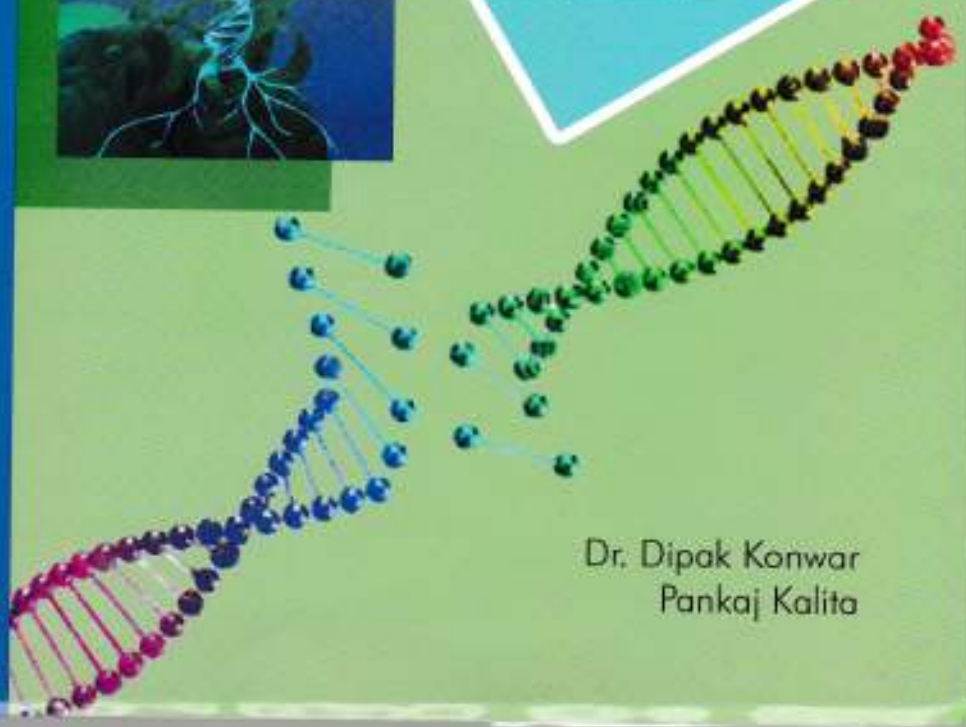

Dr. Dipak Konwar
Pankaj Kalita

Pub Kamrup College, Kamrup, Assam, India was established in 1972, initially with Arts Stream by local people led by doyens of the greater Baihata Chariali area. The College is affiliated to the Gauhati University (affiliation attained on 24th November, 1973). The Science Stream was introduced in the College on 21st August, 1986 which was brought under the GIA System on January 11, 1996. Besides regular Major courses in Humanities and Science subjects our College offers self-finance courses in BBA, BCA, PGDCA, - and side by side under the new UGC scheme entitled as 'Add- on- Course" MLT and TTM has been introduced from the session 2014-2015. It is also a matter of encouragement that our college is one of the six selected colleges from Assam recently (2014) to have the grant to start vocational degree courses under B.Voc. programme of UGC.

Institutional Level Biotech Hub (IBH), Pub Kamrup College was established in the year 2011, with full financial support from the Department of Biotechnology, Govt. of India. The Hub aims to provide facilities for research, development and training in the area of plant tissue culture, biochemical studies of protein, histochemical and histopathological studies of animal tissues, enzyme purification and separation and identification of different plant constituents. It is noteworthy that our College feels honoured being recognised under the 'Star College Scheme' in 2012 by the Department of Biotechnology, Govt. of India.

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Pankaj Kalita

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I

An Introduction to Microscope

Uday Sankar Allam
Chadipiralla Kiranmai
P. Vijaya Bhaskar Reddy

Many of the interesting features of biological systems are too small to be seen with the naked eye. Microscope (from the ancient Greek: *mikrós*, "small" and *skopeîn*, "to look" or "see") is one of the most important optical instruments used in microbiology for observing microorganisms. Microscopes are instruments designed to produce magnified visual or photographic images of small objects. It will have a single or series of lenses which are used to magnify miniature objects (<0.10 mm). Microscopy is the use of a microscope or investigation by a microscope. The microscope must accomplish three tasks: produce a magnified image of the specimen, separate the details in the image and render the details visible to the human eye or camera.

1.1 History

About 1590, two Dutch spectacle makers, Zaccharias Janssen and his son Hans, while experimenting with several lenses

III

Basic Principles and Applications of Centrifugation

P. Vijaya Bhaskar Reddy
NL Raju
Uday Sankar Allam
Chadipiralla Kiranmai

A centrifuge is an important device that is used to separate or concentrate materials suspended in a liquid medium and this process of separation is termed as centrifugation. A centrifuge uses centrifugal force (gravitational force) to isolate suspended particles from their surrounding medium on either a batch or a continuous flow basis. Applications for centrifugation are many and may include sedimentation of cells and viruses, separation of subcellular organelles and isolation of macromolecules such as DNA, RNA, proteins or lipids. Centrifugation is one of the most important and widely applied research techniques in biochemistry, cellular and molecular biology and in medicine. It separates particles from suspensions or even macromolecules from solutions according to their size, shape and density by subjecting the dispersed systems to artificially induced

IV

Spectrophotometry and Colorimetry: Principles and Applications

Chadipiralla Kiranmai
Uday Sankar Allam
NL Raju
P. Vijaya Bhaskar Reddy

The term spectroscopy is derived from Latin where *"Spectrum"* means light and *"Scopy"* means "to look at or to study". Light is of two kinds. i. Polychromatic light (light of various colors or wavelengths), and ii. Monochromatic light (light of single color or wavelength). Spectrophotometry is an analytical technique used for identification and quantification of a compound. In spectrophotometry, monochromatic light is used. Spectrophotometry is based on a simple principle that every object or particle of matter absorbs light of a particular wavelength and is a specific characteristic of that particle. When we plot the amount of light absorbed by the compound at different wavelengths we get the spectrum of the compound. Every compound has a property of absorbing maximal light at a particular wavelength. Such absorption is significantly reduced or becomes zero above or below

V

Polymerase Chain Reaction (PCR): Its Variants and Applications

N. L. Raju
P. Vijaya Bhaskar Reddy
Chadipiralla Kiranmai

In 1983, a new technique was conceived by Kary Mullis of Cetus Corporation that has become widely used to amplify specific DNA fragments without the need for bacterial cells. This technique is known as polymerase chain reaction (PCR). Earlier, cloning of DNA fragment was made by growing up bacterial cultures and extracting and purifying the DNA. As the name PCR indicates, DNA polymerase is used to amplify DNA using a pre-existing DNA molecule as template and each new DNA molecule synthesized becomes a template for generating more, thus creating a chain reaction. Of all the technical advances in modern molecular biology, the polymerase chain reaction (PCR) is one of the most useful technique. In particular, PCR has revolutionized and speeded up the whole area of recombinant DNA technology. PCR allows the rapid generation of large amounts of specific DNA sequences that are easier to purify

and less damaged. There
for a multitude of different
to make more copies of re
amounts of any particular
generate microgram qua
sequencing.

The technique emplo
Taq polymerase that w
aquaticus, a bacterium
temperatures above 90°
1983, he used normal D
needed to separate DNA
he had to add a fresh dos
Luckily, heat resistant DN
aquaticus just a year or
to the reaction mixture a
the heating steps. The
temperature to make ne

5.1 Principle and proc

PCR is used to amp
target DNA). Most PCR
of upto ~10 kilo base p
for amplification of frag

A basic PCR set up
that include:

i) DNA template
amplified.

ii) Two primers th
ends of each of the sen

iii) Taq polymera
temperature optimum

iv) Deoxynucleotid
from which the DNA p

X

Theoretical and Technical Aspects of Microtomy

Pankaj Kalita
Rituparna Sarma
P. Vijaya Bhaskar Reddy

Microtomy is a technique used for making thin sections of organic tissue including bones, teeth etc. into very minutely thickened size and attached to a surface for further microscopic examination. Since 1875, the development of this technique is facilitating the researchers to observe the minute anatomy of the biological samples. The basic instrument used in microtomy is the microtome. A microtome is a tool used to engrave extremely thin slices of biological materials. The earliest form of microtome was the freehand sectioning of quite thin and translucent slices from fresh or fixed material using a sharp razor. On the contrary, modern microtomes are precise instruments designed to cut uniformly thin sections of a variety of materials for detailed microscopic examination.

The microtome consists of a fixed blade and a mobile arm, which anticipates upward and downward movement, so that it falls on the blade and gets cut.