



### भाभा परमाणु अनुसंधान केंद्र BHABHA ATOMIC RESEARCH CENTRE

स्वर्ण जयंती वर्ष GOLDEN JUBILEE YEAR 2006-2007

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#### FOREWORD

Bhabha Atomic Research Centre (BARC) is celebrating its golden jubilee year during 2006-07. On 20<sup>th</sup> January, 1956, Pandit Jawaharlal Nehru formally inaugurated the Atomic Energy Establishment Trombay (AEET), which was renamed as Bhabha Atomic Research Centre (BARC) on January 22, 1967. As a premier R&D centre of the Department of Atomic Energy (DAE), BARC has a mandate to provide R&D support to the nuclear power programme, to pursue all activities related to nuclear fuel cycle, to operate research reactors for supporting neutron beam research and supplying radioisotopes for various applications, to conduct frontline basic research in physical, chemical, biological and engineering sciences all of which lead towards improving quality of life of our people. The achievements BARC has made over the last 50 years are well known not only to the scientific community in the country but also to our people at large. Scientific achievements made by this premier research centre are well documented in various publications of DAE including a series named "BARC Highlights". During this golden jubilee year, we have made an effort to bring out some glimpses of recent research and development accomplishments in the form of 8 volumes, each highlighting the following areas:

- 1. Nuclear Fuel Cycle
- 2. Physical Sciences
- 3. Chemical Science and Engineering
- 4. Materials Science and Engineering
- 5. Life Sciences
- 6. Reactor Technology and Engineering
- 7. Electronics, Instrumentation and Computers
- 8. Environmental Science and Engineering

These volumes will showcase the latest work in the aforementioned areas and will demonstrate how each of these is directed towards achieving the overall goal of using nuclear energy for the benefit of our people.

Nuclear energy programme in India has now reached a level of maturity. Today, India is self-sufficient in building nuclear power stations of 540 MWe capacities and has gained mastery over the entire fuel cycle. We are at the threshold of entering the second stage of our nuclear power programme, in which a rapid growth in installed capacity is expected through the fast reactor programme. In the area of basic research in science and engineering, BARC has been maintaining a leading position both in national and international scenario. One of the strongest points of basic research in BARC, lies in its capability in building sophisticated research facilities in-house. The core competence of the scientists and engineers in our centre, covers a very wide range as is reflected in the 8 companion volumes being released on the occasion of the golden jubilee year.

The present volume, devoted to Life Sciences, highlights the achievements made during the last few years in basic and application oriented areas of Life Science. These cover studies on the effects of ionizing radiation on microbes, plants, animals and human beings at the cellular as well as molecular level, providing insights into mechanistic aspects of radio-sensitivity of living organisms and cells. Modern molecular biological approaches of proteomics and genomics are being used to explore new genes and proteins contributing to radiation response. High radio-resistance of the bacterium Deinococcus radiodurans, relative radio sensitivity of lymphocytes vis-à-vis tumor cells, intracellular signaling in radio-protective and cytotoxic pathways, immune response following exposure to low dose and low dose rate radiation and radiation-induced apoptosis are the key experimental systems being investigated. Image analysis software has been developed to score damage to cells at DNA and chromosomal level. The high level natural radiation areas off Kerala coast, offer a unique opportunity to evaluate the biological and health effects of continuous low dose radiation on human population. Cytogenetic monitoring, new born survey for congenital malformations and assessment of DNA

mutations in micro-satellites and mini-satellite loci are some of the important programmes undertaken in recent years. Biochemical assessment of oxidative damage to crucial biomolecules (such as lipids, proteins and DNA) and its modulation by natural products of plant origin (or their derivatives) are being intensively investigated. Three dimensional structure and structure activity relationships of several proteins have been deciphered using X-rays. The newly emerging concept of supramolecular organisation is being evaluated in terms of multi-protein complexes involved in photosynthesis, DNA repair and recombination.

A major program of societal importance is the radiation-induced mutation and plant breeding for crop improvement. Development of high yielding varieties of oil seeds and pulses has made a very strong impact at the national level. Twenty seven Trombay varieties of oil seeds, pulses, rice and jute have been notified/released for commercial cultivation, which makes BARC one of the leading institutions in agricultural research. Molecular (DNA) markers are being identified for some of these varieties and also for disease-resistant traits in wheat, mungbean and rice. Micro-propagation protocols have been developed for several elite varieties of banana, pineapple and sugarcane, some of which have been transferred to user agencies. Cloning foreign genes in banana has been achieved with a view to develop disease resistance and edible vaccines. Several approaches to bio-remediation of heavy metals including those in the nuclear waste are being developed which include biosorption, hairy roots and genetically engineered microbes. Solid waste management, especially of kitchen waste and other biodegradable vegetable, hospital and abattoir waste received a boost, with the development of the Nisargruna biogas plants. This has caught the attention of urban planners and environment-conscious public institutions. Radiation processing technology is being used to strengthen food security, improving food safety and promoting international trade by overcoming quarantine barriers. An onion-potato irradiator "KRUSHAK' for techno-commercial use, has been established at Nasik and several other food irradiation plants are coming up in different parts of the country.

BARC was the first to set up nuclear medicine related activities in the country. The installation of the country's first medical cyclotron and PET camera at Radiation Medicine Centre, have provided opportunities for investigations of cancer, epilepsy and other neurological and cardiological pathologies. The development and fabrication of the first indigenous Co-60 based teletherapy machine, Bhabhatron at BARC and its installation and commissioning at Advanced Centre for Treatment, Research and Education in Cancer, Tata Memorial Centre, Kharghar, marked a salutary recent development. Combined with the development and deployment of brachytherapy sources and continued R&D efforts in development of radiopharmaceuticals, isotope application programmes are poised for substantial growth in the near future.

Director

PREFACE

It has been a matter of privilege and pleasure for me to compile the highlights of research and development in the area of Life Sciences being carried out at BARC. Life science related research commenced in BARC right at its inception, over 50 years ago. Initially, it was exploratory in nature and largely addressed three major aspects of relevance to nuclear science and technology. The first and foremost were the studies on health-related cytogenetic and biochemical effects of ionizing radiations, including aspects of radiotherapy, radiosensitization and radioprotection. The second point of attention dealt with radiation-induced mutagenesis and crop improvement, including related phenomena of photosynthesis and nitrogen fixation and management of soil fertility and pesticide residues. The third intensive effort focused on human nutrition and aimed at utilizing radiation for hygienisation and preservation of food and encompassed analysis of nutritional and organoleptic qualities and biosafety of irradiated food and food products. In the subsequent years, new activities added on to these core activities, enhancing the scope and sharpening the focus of investigations.

Today the R&D activities in Life Sciences at BARC span a very wide range encompassing almost all thrust areas of contemporary biological research. The advent of recombinant DNA technology and availability of multidisciplinary expertise at BARC have imparted a clear modern biology orientation. On one hand we have frontline basic research in the areas of stress biology, immunology, photosynthesis, radiation biology and human genetics, published in international and national journals of high impact and repute. But at the other end of the spectrum one also finds cutting edge technologies of genomics, proteomics and transgenosis being combined with conventional cytogenetics, plant breeding, food microbiology or radiation biology to yield technologies and products like new crop varieties, longer shelf-life foods or edible vaccines and protocols to increase the efficacy of cancer radiotherapy. In addition to the excellence and relevance to the DAE research programmes, the third guiding principle of life science research at BARC is societal benefit. Establishment of an onion potato irradiator at Nasik, a PET scan facility for diagnosis at RMC, Parel and of several Nisarg-runa biogas plants in and around Mumbai for disposal of biowaste in recent years, all exemplify this.

This compilation is not meant to be a comprehensive review of all aspects of life science research, but merely provides glimpses of some of the significant and exciting things that happened in this area in the last 3-4 years. The material included herein was carefully chosen, to represent *recent exciting results with perceivable impact*. The introduction to each chapter provides some details about the division where the particular activity is being pursued. This is followed by presentation of activities in a way, that gives the reader the salient findings and the most important publications arising from the work. I hope the style of presentation encourages readers to go through all the relevant publications, which are listed towards the end.

Needless to say that an effort of this magnitude could not have been be completed without the cooperation and help of my peers and colleagues. I wish to thank Dr. S. Banerjee, Director, BARC for clearly defining the scope of the "highlights". I take this opportunity to thank all my colleagues in the MBD, NA&BTD, FTD, RB&HSD, LLRSS, RMC, WSCD, DRH&R of BARC who enthusiastically contributed to the scientific content and promptly responded to my queries. Help from all the heads of divisions in the Bioscience Group and Heads of RMC and WSCD in selecting the material to be highlighted is gratefully acknowledged. I thank Dr. K. B. Sainis, Director, Bio-medical Group for his valuable suggestions and Dr. Vijai kumar, Associate Director, Knowledge Management Group and Head, Scientific Information Resource Division for his timely help in various ways. Special thanks are due to Mrs. Shobhana Kutty (MBD) for meticulously typing out the list of publications.

Shree Kumar Apte



#### LIFE SCIENCES

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#### 1. STRESS RESPONSE, DNA DAMAGE AND REPAIR, AND MICROBIAL RADIORESISTANCE

#### INTRODUCTION

The growth, metabolism and development of all life-forms, bacteria, plants and animals, are finely controlled by environmental stresses. The cellular response to these stresses originates from the interaction of environmental stimuli with cellular machinery and range from irreversible damage and cessation of growth to differentiation, stress-related gene expression and tolerance to a given stress. Of particular interest to life scientists in MBD at BARC are the agriculturally important environmental stressors, which limit crop productivity, and the ionizing radiations, on account of their relevance and immense importance to various DAE programs.

Photosynthetic nitrogen-fixing cyanobacteria offer an appropriate model system for analysis of stress tolerance in crop plants and are being used in MBD to elucidate mechanisms of tolerance to soil salinity, drought, nutrient deficiencies, heat, cold and pesticides. Salient findings in recent years include mechanisms underlying superior cyanobacterial thermotolerance, novel stimulons induced by potassium deficiency and signal transduction involved in potassium deficiency sensing.

DNA damage is the most significant adverse effect of ionizing radiations. However, repair of DNA damage is crucial to cell survival not only during post-irradiation recovery but also during normal growth of living cells. This important phenomenon is being investigated by MBD scientists in different biological systems. Some of the recent achievements include cloning of UV repair genes from bacteria and a recombinase from rice, and development of methods for accurate quantitation of DNA damage in animal cells.

The bacterium *Deinococcus radiodurans* exhibits phenomenal resistance to ionizing radiations and is being intensely investigated for mechanisms underlying such extreme radioresistance. Recent exciting findings from MBD include a novel protein recycling phenomenon accompanying post-irradiation recovery of this microbe from exposure to <sup>60</sup>Co gamma rays and discovery of a novel antioxidant PQQ, offering considerable protection against oxidative stress, from this organism.

In the FTD in BARC, a typically eukaryotic phenomenon of programmed cell death or apoptosis has been demonstrated in a bacterium *Xanthomonas* during nutritional stress.

#### 1.1 MOLECULAR BASIS OF MICROBIAL RESPONSE TO AGRICULTURALLY IMPORTANT STRESSES

Photosynthetic,  $N_2$ -fixing cyanobacteria, evolved > 3 billion years ago and are believed to be phylogenetically related to plant chloroplasts. *Anabaena* strains are filamentous forms, which possess special cells, called heterocysts and spores. When exposed to light absorbed by the blue pigment phycocyanin, heterocysts do not exhibit much red fluorescence, typical of photosynthesizing vegetative cells. Cyanobacteria are easy to culture in fermentors and are naturally abundant in tropical paddy fields.



Anabaena strains form very appropriate model systems for studying microbial/plant responses to environmental stresses. A major effort in the Molecular Biology Division (MBD) is aimed at understanding the molecular basis of cyanobacterial stress tolerance and exploiting it for development of suitable biotechnologies.

Earlier work in the MBD identified the basis of cyanobacterial tolerance to salinity/osmotic stresses (Apte, 2001) and their effects on nitrogen fixation (Fernandes & Apte, 2001). Current work focuses on responses of *Anabaena* strains to prolonged heat stress and K<sup>+</sup> deficiency. Aspects being investigated include the basic stress response, cloning and characterization and regulation of relevant genes, and their contribution to tolerance.

#### • Superior thermotolerance of nitrogenfixing *Anabaena* strains

Heat-shock response (HSR), has been explored in N2-fixing Anabaena strains.



*Anabaena* was found to exhibit superior thermotolerance than *E. coli* both in terms of survival and recovery from prolonged heat stress. This ability was correlated with the expression and accumulation of selected Hsp60 family heat-shock proteins.



Two genes of this family, *groESL* and *cpn60* have been cloned (GenBank Accessions AF324500 & AY32892) and are being characterized for their role in thermotolerance of *Anabaena* strains.

Rajaram, Ballal, Apte, Wiegert and Schumann, Biochim. Biophys. Acta (Gene Struct. Funct.) 1519: 143-146, 2001. Rajaram and Apte, Arch. Microbiol. 179: 423-429, 2003.

### • A novel potassium deficiency induced stimulon in *Anabaena*

Potassium starvation causes impairments in photosynthesis and nitrogen fixation and arrests growth of *Anabaen*a strains. Potassium also significantly influences gene expression in *Anabaena*.



Autoradiography of [<sup>35</sup>S]methionine radiolabeled proteins from potassium deficient or potassium supplemented Anabaena torulosa cultures.

A potassium deficiency-induced novel *stimulon* of several genes, was discovered recently in *Anabaena*, and also shown to be found in the enteric bacterium, *E. coli*.





A *kdp* operon, encoding a membrane-bound K<sup>+</sup> dependent ATPase (KdpATPase) was found to be part of this stimulon in *Anabaena*. It expressed strictly under K<sup>+</sup> deficiency and was not influenced by salinity or osmotic stresses.

Alahari, Ballal and Apte, J. Bacteriol.183: 5778-5781, 2001. Alahari and Apte, J. Biosci. 29: 153-161, 2004.

## • How does *Anabaena* sense potassium deficiency?

In bacteria,  $K^+$  deficiency is sensed by a 900 amino acid long trans-membrane protein, KdpD, which interacts with a cytosolic transcription factor, KdpE, to express KdpATPase from *kdp* operon.



Two *kdp* operons, one containing and the other lacking a truncated *kdpD* gene, were cloned from *Anabaena*.



*Anabaena* possesses a naturally short *kdpD* gene and lacks the *kdpE* gene. Using Northern blotting and hybridisation and RT-PCR techniques, only the *kdp2* operon was found to express in *Anabaena*, under potassium deficiency.



Northern blot analysis of kdp 1 and kdp 1 expression in Anabaena



To understand how this truncated KdpD may regulate *kdp* operon expression, the *Anabaena kdpD* gene was fused with the C-terminal half of the *E. coli kdpD* gene and transformed into *E. coli*. A chimeric protein AnacoliKdpD, expressed in *E. coli* from this construct, complemented the growth of an *E. coli kdpD* mutant, and also expressed the *kdp* operon. However, compared to *E. coli* KdpD, the AnacoliKdpD showed much less induction with salinity stress.

The role of this protein in regulating *kdp* expression in the cyanobacterium *Anabaena* is currently under investigation, using site-directed mutagenesis approach.



Ballal, Heermann, Jung, Gassel, Apte and Altendorf, Arch Microbiol. 178: 141-148, 2002.

Ballal, Bramkamp, Rajaram, Zimmann, Apte and Altendorf, J. Bacteriol. 187: 4921-4927, 2005.

Ballal and Apte, Appl. Env. Microbiol. 71: 5297-5303,2005.

#### Stress-related signal transduction in Trichoderma

A new programme was recently initiated in the Nuclear Agriculture and Biotechnology Division on *Trichoderma*, a fungus with biotechnological potential as a biocontrol agent for plant diseases. The project aims to clone, characterise and mutate genes involved in stress tolerance, secondary metabolism and reproduction in this fungus, to understandthe molecular basis of biocontrol of fungal/bacterial pathogens.



As a first step, a MAP-kinase and two G-protein-encoding genes, involved in the stress-related signal transduction have been cloned and characterized.



Mukherjee, Latha, Hadar and Horwitz, Eukaryotic Cell 2: 446-455, 2003.

Mukherjee, Latha, Hadar and Horwitz, Appl. Env. Microbiol. 70: 542-549, 2004.

#### 1.2 DNA DAMAGE AND REPAIR IN LIVING ORGANISMS

Damage to DNA occurs in living cells, both during normal growth and development as also during exposure to stress conditions. If left un-repaired, the DNA damage can be fatal or inherited as mutations, and may affect the subsequent progeny. Repair of DNA damage is therefore a function vital to all living cells. Current interests include cloning and characterization of DNA repair genes from bacteria and plants, and development of methods for the quantitation of DNA damage in animal cells.

#### Repair of UV damaged DNA in haemophilus Influenzae Rd

*Haemophilus influenzae* Rd lacks the photo reactivation repair of UV-damaged DNA and depends largely on its nucleotide excision repair (NER), wherein the *uvrA*, *B*, *C* genes play important roles. To understand structure-function relationship in the UvrA protein, the wild type *uvrA* and a *uvrA* $\Delta$ *C44* mutant lacking the C-terminal 44 amino acids have been cloned, expressed and purified.

Purification of the wild type and the mutant UvrA proteins revealed that the UvrA $\Delta$ C44 protein possessed the expected size truncation and showed negligible DNA-binding and ATPase activities.





*In cell* complementation studies with the cloned *uvrA* of *H. influenzae* in to the *uvrA* mutant of *E. coli* (AB1886), indicated close similarity in the NER mechanism in both the bacteria.



While the wild type full length UvrA could complement *E. coli* mutant *in toto*, the deletion mutant could only partially restore UV resistance, revealing the importance of the C-terminal of the UvrA protein for its function. Cloning of the *uvrB* and *uvrC* genes of *H. influenzae* is in progress.

Studies are also in progress to understand the role of DNA repair genes, if any, in the UV sensitivity of the photosystems in the cyanbacterium *Anacystis nidulans* strain R2. Attempts are also on to isolate a DNA repair complex from the highly radioresistant bacterium *Deinococcus radiodurans*.

Kulkarni, Khalap and Joshi, Protein Expr. Purif. 37: 462-467, 2003. Kulkarni, Khalap and Joshi, Ind. J. Exp. Biol., 2005

### • Cloning and characterization of the *DMC1* gene from rice

The *DMC1* gene is a major homologous recombination gene, expressed during prophase I of meiosis in eukaryotic cells. Two *DMC1* genes, located within a region duplicated on chromosomes 11 and 12, were found in rice. A cloned cDNA corresponding to *OsDMC1* from rice anthers was over-expressed, purified (from *E. coll*) to homogeneity, and characterized.



The *DMC1* protein exhibited binding towards single and double stranded DNA (ssDNA and dsDNA). The binding to either DNA showed no ATP dependence but required high Mg<sup>2+</sup> in presence of ATP.



The rice DMC1 also exhibited an ATPase activity dependent on ssDNA but insensitive to dsDNA. OsDmc1-ssDNA complex mediated renaturation of homologous complementary strands as well as assimilation of single strands into homologous super coiled duplexes leading to D-loop formation. The D-loop formation was lowered by excess of OsDmc1 protein. *The DMC1* protein showed a ssDNA dependent ATPase activity.



The properties of the protein reflect the classical hallmarks of a recombinase. The study also represents the first biochemical characterization of a plant Dmc1 protein.

Metkar, Sainis and Mahajan, Curr. Sci. 87: 353-357, 2004. Kant, Rao and Sainis, Plant Molec. Biol. 57: 1-11, 2005.

## • Comet assay for estimation of cellular DNA damage

Single Cell Gel Electrophoresis (SCGE) or Comet assay has been standardised and a Software SCGE-Pro developed by the RB&HSD in collaboration with Computer Division, BARC, for the measurement of DNA damage in cells.



The method is being used in measurement of DNA damage (single or double strand breaks, base damage) and apoptosis induced by ionizing radiation or other stresses. The technique is useful in evaluation of genotoxic effects and cellular susceptibity to cancer, and molecular epidemiology of human populations.

Chaubey, Bhilwade, Rajagopalan and Bannur, Mutation Res. 490:187-197, 2001.

#### 1.3 EXTREME RADIORESISTANCE OF DEINOCOCCUS RADIODURANS

The Gram-positive bacterium *Deinococcus radiodurans* exhibits phenomemal DNA damage repair proficiency and survives very high (up to 15-30 kGy) doses of ionizing radiations. A new research programme, initiated In the Molecular Biology Division three years ago, examines novel mechanisms underlying the extreme radioresistance of this microbe.

## • Stress proteomics during post irradiation recovery

Identity/function of nearly one-third of deinococcal genome is not known. To assess the contribution of these genes in radioresistance, if any, a proteomic approach was undertaken.

During post-irradiation recovery of cells from exposure to 6 kGy of  ${}^{60}Co-\gamma$ -rays, several radiation responsive genes and proteins were identified using 2-D electrophoretic resolution followed by N-terminal amino acid sequencing of proteins. These included major chaperones, few TCA cycle enzymes, membrane-bound peptide and amino acid transporters, oxidative stress alleviating enzymes and a handful of "unknown" or "hypothetical" genes.



A major new discovery was of the phenomenon of protein recycling, i.e. destruction and re-synthesis of radiation-damaged proteins, before resumption of growth during post-irradiation recovery.



Prominent recycled proteins include the chaperones. Proteases, specifically induced for such recycling, and their target proteins are currently under investigation.

Joshi, Schmid, Apte and Altendorf, Biochem. Biophys. Res. Commun. 320: 1112-1117, 2004.

## • PQQ - a major antioxidant in *Deinococcus* radiodurans

*Deinococcus* genome contains a *pqqE* gene (which synthesizes pyrroloquinoline quinone or PQQ) but lacks a *gcd* gene encoding glucose dehydrogenase, for which PQQ acts as a cofactor. *E. coli* possesses a *gcd* but lacks *pqqE* gene. Transgenic *E. coli* cells expressing the deinococcal PQQ synthase (pETpqq clone), showed superior oxidative stress tolerance.



*E. coli* cells carrying deinococcal *pqqE* also displayed enhanced levels of catalase and superoxide dismutase (SOD) activities, suggesting that PQQ acted as a signaling molecule to induce oxidative stress alleviating mechanisms.



Pulse radiolysis experiments revealed that PQQ *per se* acts as a scavenger of reactive oxygen species. PQQ reacted with ROS forming long-lived adducts, thereby preventing free radical triggered cellular damage and protecting cells.



*In vitro* PQQ suppressed DNA damage [nicking and conversion of closed circular (CC) to open circular (OC) DNA, shown above and protein (carbonylation) damage, caused by oxidative stress.



*E. coli* cells transformed with deinococcal pqqE exhibited a small increase in their tolerance to  $^{60}$ Co,  $\gamma$ -rays.



PQQ is, thus, likely to contribute to deinococcal oxidative stress tolerance and radioresistance, both as a ROS scavenger and as a signaling molecule switching on the expression and/or activity of catalase and SOD enzymes.

Khairnar, Misra and Apte, Biochem. Biophys. Res. Commun. 312: 303-308, 2003.

Misra, Khairnar, Barik, Priyadarshini, Hari Mohan and Apte, FEBS Lett. 578: 26-30, 2004.

#### 1.4 PROGRAMMED CELL DEATH IN XANTHOMONAS

*Xanthomonas campestris pv. glycines* (XcgAM2), the etiological agent of bacterial pustule disease of soybean, was found to exhibit post-exponential programmed cell death (PCD) in LB medium. Interestingly, this PCD was not displayed in starch medium, but was noticed only in LB medium.



XcgAM2 colony morphology



The PCD was found to be associated with the synthesis of an endogenous enzyme similar to human caspase-3, a known executioner of apoptosis in eukaryotes. The caspase-3 like protein was detected in all the *Xanthomonas* strains tested. The *Xanthomonas* caspase was detected in Xcg cells growing in LB medium but not when growing in starch medium.



The protein was detectable during exponential phase of growth up to a period of 32h but not beyond. Addition of starch to LB cultures of Xcg was found to arrest the onset of the PCD and also terminated the synthesis of the caspase-like protein.

The cells undergoing PCD also displayed the other markers of eukaryotic apoptosis, such as binding of annexin V to plasma membrane of cells undergoing PCD, and the presence of nicked DNA in culture supernatant as evidenced by the TUNEL assay. MNNG mutagenesis of XcgAM2 yielded mutants, which failed to exhibit PCD, but retained the other characteristics of XcgAM2 including the amylase activity, pigment production, SDS-PAGE protein profile and the two indigenous plasmids. The caspase like protein, in these mutants, though synthesized, was inactive.



Soyabean leaf showing chlorosis upon inoculation with XcgAM2

Gautam and Sharma, Mol. Microbiol. 44: 393-401, 2001. Gautam and Sharma, J. Gen. Appl. Microbiol. 48: 67-76, 2002.



#### 2. RADIATION BIOLOGY AND HUMAN HEALTH

#### INTRODUCTION

Radiation biology research at BARC started right at its inception. The earlier work explored effects of ionizing and non-ionising radiation on a variety of biological phenomena and their modulation by environment in bacteria, plants and animals, both *in vivo* and *in vitro* as also during storage. Today the emphasis in Radiation Biology & Health Sciences Division (RB&HSD) is on the specific nature of radiation damage in targets such as biological membrane or immune system, mechanisms underlying radioresistance of cancers, screening for radioprotectors and antioxidants, and developing methods to increase the efficacy of cancer radiotherapy.

Molecular changes in response to exposure to ionizing radiations are being examined in biological membranes from whole animals or in cell lines and even in liposomes, using several biochemical and biophysical tools and techniques. Immuno-modulatory properties of low doses of radiation and compounds such as chlorophyllin have been elucidated. An interesting offshoot of such research is the isolation of an anticancer immunosuppressive bacterium from shrimp by scientists in the Food Technology Division (FTD) and RB&HSD.

Increasing radioresistance of cancers is a serious cause of concern for radiotherapy. Recent research in RB&HSD at BARC has traced such radioresistance to enhanced antioxidant status in these cancers and some of the signaling markers for radioresistance have been identified. Screening of compounds of plant origin for anticancer, antioxidant or radioprotective properties has led to identification of eugenol, troxerutin as potential candidates. Attempts have been made to enhance radiotherapy efficiency by using a combination of irradiation and electroporation protocols.

High background radiation from monazite sands in Kerala provides a natural laboratory for examining the health effects of low doses of ionizing radiations. A detailed analysis of congenital malformations, cytogenetic abnormalities and heritable DNA mutations in the population residing in these areas is currently underway in the Low Level Radiation Studies Section (LLRSS) at BARC, and has not revealed any ill effects on health or dose-effect relationships, so far.

#### 2.1 EFFECTS OF HIGH LEVEL NATURAL RADIATION ON HUMAN POPULATION OF KERALA COAST

Naturally occurring high background radiation areas (monazite sands) in Kerala provide unique opportunities to investigate health effects of low level radiation directly on human populations living in the region.

- Nature's own laboratory
- Thickly populated
- Inhabited for generations
- Opportunity to study low level radiation effects directly on humans
- Exposure at all stages of human development
- Wide range of radiation Levels (<1.0 to 35mGy/yr)</li>
- Ideal for dose response studies

An ongoing study compares congenital malformations in new-borns, cytogenetic abnormalities and heritable DNA mutations in populations residing in the high level natural radiation areas (HLNRA) and normal level natural radiation areas (NLNRA).



Down syndrome, Congenital Malformation and Stillbirths A comparison of Delhi, Mumbai, Baroda & Kollam populations Study area n CM% DS SB% Delhi\* 23.367 1.46 1 in 1235 3.1 Mumbai\* 42,304 2.3 1 in 1510 2.6 Baroda\* 31.775 2.05 1 in 962 4.0

2.03

1 in 1471

0.5

n-Sample size, CM-Congenital malformation

92.689

Kollam, Kerala

DS - Down syndrome, SB - Still Birth

Incidence of malformations, down symdrome and stillbirths observed in Kollam is comparable to the values reported from other part of India



any difference between HLNR and NLNR areas



The overall frequency of chromosomal aberrations was comparable among newborns from HLNRA & NLNRA. No

significant increase in chromosomal aberrations / anomalies with respect to increase in background radiation levels were observed.

The investigations carried out so far (chromosomal aberrations, karyotype abnormalities and congenital malformations) have not indicated any significant difference between the control populations and those exposed to natural high background radiation. The ongoing health audit survey and house-to-house radiation dose monitoring, will help in identifying families exposed to different doses of radiation. Further screening of such families for mutations in cellular/mitochondrial DNA and microarray analysis (for gene expression profiles) would enable better assessment of the dose-effects relationships, if any.

Thampi et al, In: High Levels of Natural Radiation and Radon Areas: Radiation Dose and Health Effects. International Congress Series, Vol. 1276, pp. 8 -12, February, 2005.



### 2.2 RADIATION, IMMUNO-MODULATION AND IMMUNO-SUPPRESSION

#### Low dose radiation-induced immunomodulation

Low doses of ionizing radiation (LDR) have been reported to alter immune responses, which play a major role in the defense against infection and cancer. Exposure of 4 week old C57BL/6 mice to a fractionated (4 cGy/ day: 5 days/wk) whole body gamma radiation dose of 20 cGy, enhanced (60%) the response of their spleen cells to the mitogen concanavalin A *in vitro*, compared to that of cells of sham irradiated mice **(Shankar et al, Immunol. letters 68; 237-245. 1999)**. Likewise, cell mediated responses such as mixed lymphocyte reaction and T cell cytotoxicity were also enhanced but delayed type hypersensitivity response to mycobacteria and dinitroflurobenzene were suppressed.







Expression of proteins associated with activation of cell cycle was evaluated by flow cytometry. In the con A stimulated cells of C57BL/6 mice, the expression of CD69, Cyclin D1, proliferating cell nuclear antigen (PCNA) and Cyclin A was enhanced.



In C57BL/6 mice less apoptosis was seen in LDR exposed con A stimulated cells as compared to their sham-irradiated controls. This correlated with the reduction in expression of p53, caspase 3 activity and upregulation of anti-apoptotic Bcl-2 protein and mitochondrial stability in 20 cGy irradiated C57BL/6 mice. The data suggest that expression of CD69 and PCNA proteins could serve as potential biomarkers for LDR exposure.

Shankar and Sainis, J. Env. Pathol. Toxicol. Oncol. 24:33-43, 2005.

#### Chlorophyllin : Antioxidant activity & immunomodulation

Chlorophyllin (CHL), a water-soluble mixture of sodium-copper salts of chlorophyll used as a coloring agent, is a potent antimutagenic and anticarcinogenic agent *in vitro* and *in vivo*. Its chemo-preventive actionhas recently been shown in a Chinese population at high risk of aflatoxin B1 (a liver carcinogen).



Using dihydrodichlorofluoreseindiacetae dye ( $H_2$ DCFDA) and flow cytometry, CHL was shown to inhibit radiation-induced generation of intracellular reactive oxygen species (ROS) and confer protection against radiation-induced apoptosis in spleen cells.

Administration of CHL to mice prevented apoptosis and led to a systemic expansion in the number of leukocytes, resulting in splenomegaly and lymphoadenopathy within 48h.



CHL treated mice exhibited enhanced phagocytosis (innate immunity) and enhanced antibody (B cell) and delayed type hypersensitivity.

The available data suggest that CHLOROPHYLLIN may thus have potential application as an adjuvant for enhancement of specific immune responses as well as in radioprotection.

Kumar, Devasagayam, Bhushan and Verma, Free Radic. Res. 35: 563-574, 2001.

Kumar, Shankar and Sainis, Biochim. et Biophys. Acta 1672: 100-111, 2004.

#### An immuno-suppressive anti-ancer bacterium from shrimp

*Serratia marcescens* strain ost3, isolated from shrimp, was found to elaborate a new pigment – a tripyrinol compound similar to prodiogisin or MAMPDM.





Administration of the purified compound as well as radiation attenuated bacterium showed potent immunosuppressive and anticancer activities in treated mice.



Pandey, Chander and Sainis, Int. Immunopharmacol. 3: 159-167, 2003.

#### 2.3 RADIORESISTANCE OF CANCERS

#### Oxidative stress and radioresistance of tumor cells

The biological effects of ionizing radiation are mediated through the generation of highly reactive oxygen species (ROS), such as hydroxyl radical, superoxide anion, peroxyl radical and hydrogen peroxide. Damage caused by ROS to critical cellular targes, if not repaired by the cellular machinery, can result in cell death or apoptosis. Normal, healthy cells and tumor cells usually display differential sensitivity to radiation-induced damage.

Using two different lymphoid tumor cell lines (EL-4 and P 388) and histocompatibility matched lymphocytes from spleens of



C57BL/6 and BALB/c mice and the dye H<sub>2</sub>DCFDA or dihydrodichlorofluoreseindiacetae, ROS levels were found to be low in tumor cells compared to their normal lymphocyte counterparts, by flow cytometry.

Tumor cells contained higher amounts of antioxidant enzymes and consequently higher levels of total antioxidants compared to normal lymphocytes. This resulted in the differences in the



magnitude of downstream events such as reduction in mitochondrial membrane potential, increase in cytosolic calcium and nuclear apoptotic changes. Such changes were evident in lymphocytes but not in tumor cells. Tumor cells did not show apoptosis following radiation exposure (upto 10 Gy) but instead, showed arrest in cell cycle at G2/M stage. Thus, the intrinsic radioresistance of tumor cells can be attributed to better ROS scavenging ability and down-regulation of subsequent apoptotic events.

Shankar, Kumar and Sainis, Radiat. Res. 160 : 478-487, 2003.

#### Signaling markers in radioresistance of cancers

Following radiotherapy of cancers, a few surviving cells become radioresistant. Such increase in the radioresistance is a serious cause of concern for the subsequent radiotherapy. The activation of some anti-apoptotic signaling factors like PKC, NF-kB, PI-3 kinase, Ras and MAP kinase has been implicated in this phenomenon. To achieve maximum tumour cell killing during radiotherapy, certain pharmacological inhibitors of the signaling factors are being examined.

Studies carried out in the Radiation Biology and Health Sciences Division have monitored the levels of an important signaling factor PKC in the cytosolic and particulate fractions of *ex vivo* and *in vivo* irradiated lymphocytes at various doses. The data suggest that activation profile of signaling factors depends upon the micro-environment of the cell. Therefore, the results obtained *in vitro* should not be extrapolated to *in vivo* conditions, especially if the data are to be clinically deployed.



Varadkar, Krishna and Verma, **Rad. Res.** 159:453-457, 2003. Varadkar and Krishna, **J. Rad. Res.** 45: 127-134, 2004.

#### Modulation of signaling factors involved in cell survival

Curcumin, rutin and nicotinamide, administered prior to 3 Gy <sup>60</sup>Co γ-ray exposure of mice, were found to effectively inhibit expression of anti-apoptotic factors P44/42 MAPK and NF-kB.



Mitra and Krishna, J. Radiat. Res. 45: 491-495, 2004.

#### 2.4 RADIOPROTECTORS, ANTI-OXIDANTS AND ANTI-CANCER COMPOUNDS FROM PLANTS

One of the major challenges in radiation biology is to protect life from deleterious effects of ionizing radiation. Several plant compounds, possessing high antioxidant activity have been investigated.

#### Eugenol

Eugenol (4-allyl-2-methoxy phenol) is an active principle of Indian medicinal plants, clove (*Syzygium aromaticum*) and tulsi (*Oscimum sanctum*). It has nonmutagenic antioxidant and noncarcinogenic properties



#### Troxerutin

Troxerutin, a flavonoid derivative, used for the treatment of vascular system disorders, showed radioprotective properties in cells and in animal systems. Both the pre and post-irradiation administration of troxerutin (0.25% for 7 days) enhanced the survival of mice exposed to 8 Gy of  $\gamma$ -rays.



Maurya, Salvi and Nair, J. Rad. Res. 45: 221-228, 2004.

Mishra, J. Env. Pathol. Toxicol. Oncol. 23: 61-66, 2004.

Pandey and Mishra, J. Env. Pathol, Toxicol. Oncol. 23: 179-183, 2004.

#### 2.5 LIPOSOMES FOR ANALYSIS OF OXIDATIVE DAMAGE TO MEMBRANE AND TARGETED DRUG DELIVERY

Liposomes are artificially prepared microspheres consisting of concentric bilayers of phospholipids separated by aqueous compartments. Liposome vesicles offer a convenient and suitable membrane mimicking system to investgate the molecular mechanisms of damage caused by ionizing radiation. Radiation induced oxidative damage in liposomal membranes was investigated.



Release of pre-encapsulated fluorescent probe from liposomes reflected their permeability after  $\gamma$ -irradiation and incubation for 7 h. The fluidity of the membrane, measured in terms of fluorescence polarization of membrane labeled with DPH fluorescent probe, was altered in response to  $\gamma$ -ray dose and dose rate [100 Gy/min (**1**) and 7.9 Gy/min (**•**)].

Tocopherol is a major constituent of Vit E - a lipid soluble antioxidant located in the bilayer core of liposomal membranes. Irradiated liposomes showed dose dependent membrane lipid peroxidation, measured in terms of production of malonaldehyde (MDA) as its by-product. Membrane damage significantly decreased in the presence of membrane specific antioxidant, tocopherol. The protective effect of tocopherol was found diminishing with the doses of radiation exposure. [%: 0 (1) and 3.8 (•)].

To achieve targeted delivery of anticancer drugs, temperature sensitive liposomes (TSL) were designed using DPPC, DPPE and cholesterol (in a 1:0.1:0.1 ratio), loaded with



doxorubicin and then tested in tumor bearing animals. Delivery of this anticancer drug produced remarkable tumor growth regression in tumor transplanted mice in combination with local hyperthermia (data not shown). In the same way, liposome encapsulated diospyrin was also found to be more effective than free diosporin in causing tumor growth delay in transplanted Ehrlich Ascites Tumor in mice.



Marathe and Mishra, Radiat. Res. 157: 685-692, 2002.

Pandey and Mishra, J. Biochem. Mol. Biol. Biophys. 6: 267-272, 2002.

#### 2.6 BIOMEDICAL APPLICATIONS OF IONISING RADIATION AND ELECTROPORATION

#### Development of a Cell Electroporator

Exposure of living cells to high intensity, short duration (ms) electrical pulses (few kV/cm), popularly called electroporation, induces transient increase in the permeability of plasma membrane and allows entry of exogenous molecules e.g. drugs, DNA etc. into the cells. A Cell Electroporator was indigenously designed and developed in the Radiation Biology and Health Sciences Division. It offers a versatile tool to achieve increased cytotoxic effects on tumors by combination of electric pulse, ionizing radiation and anticancer drugs *in vitro* as well as *in vivo*.



Electropermeabilization of thyroid tumor cells delivered greater amount of radiolabeled <sup>125</sup>I-thyroxin into tumor cells. Electroradioiodine therapy (ERT) may prove valuable in thyroid cancer patients for treating non-iodine concentrating skull metastases, using surface electrodes, or for treating nodular lung metastases, using needle arrays.

Gopal, Narkar, Mishra, Samuel and Nair, Appl. Radi. Isotopes 59: 305-310, 2003.

#### Enhancing tumor cytotoxicity by radiation and electroporation

A special electrode was developed for using electroporation technique in an *in vivo* tumor model. Fibrosarcoma transplanted tumor on the hind leg of mouse was electroporated in combination with gamma radiation treatment.





Results showed that electroporation and radiation caused markedly enhanced tumor regression compared with either of the treatment modality alone, thus demonstrating the potential for application of combined treatment modality in clinics for effective treatment of cancer.



#### 3. CROP IMPROVEMENT PROGRAMS AND PLANT PRODUCTIVITY

#### INTRODUCTION

Crop improvement using radiation induced mutagenesis and conventional plant breeding has been an ongoing successful R&D activity at Nuclear Agriculture & Biotechnology Division for the last 4 decades. To date, 25 varieties in different crops have been released in the country through these efforts at BARC. Major target crops include cereals, pulses, oil seeds, jute and others. While yield has been the most desirable trait, in recent years efforts have also been focused on improvement of quality, disease resistance and early maturity. Some of the successful varieties developed are being deployed with the help of several agricultural universities, ICAR, seed corporations and progressive farmers. The varieties are also being tried out in non-traditional niches.

Agriculture is becoming increasingly stressful. Use of pesticides and fertilizers while alleviating some of the environmental stressors such as nutritional deficiencies or insect pests, make their own adverse contribution as serious environmental pollutants. Management of pesticide residues is a major research programme in the NA&BTD, with an emphasis on the rice ecosystem.

Plant productivity is synonymous with photosynthesis. Enhancing photosynthesis is a logical means of increasing crop productivity and involves a thorough understanding of the basic process of photosynthesis. The functional organization of photosynthesis and the structure-function relationships involved therein form an important area of basic reseach in the MBD.

#### 3.1 OIL-SEEDS : GROUNDNUT, MUSTARD, SUNFLOWER

#### Groundnut

Groundnut is a major target crop of oilseed improvement programme in the Nuclear Agriculture & Biotechnology Division. BARC has till now contributed ten Trombay Groundnut (TG) varieties to the national agriculture. Two more varieties (TG 37A and TPG 41) were released and four new genotypes (TG 18 AM, TGE 1, Small leaf mutant and TAG 24M) were registered and deposited with the NBPGR in 2004.



Breeding for large seed size, stress tolerance and early maturity are the important ongoing R&D activities. Some of the successful varieties released earlier are being deployed with the help of a large number of agricultural universities, ICAR institutes, Seed Corporations, Oilseed Grower's Federation, State Agricultural Departments, progressive farmers etc.



Trombay groundnut varieties have made good impact in terms of yield, large area coverage, deployment in diverse cropping systems/non-traditional niches, and trading.

#### Indian Mustard TPM-1

A new yellow seed coat variety, Trombay Phule Mustard-1 (TPM-1), was recently developed in collaboration with Mahatma Phule Krishi Vidyapeeth, Rahuri. It possesses favorable characters like early maturity, high seed and oil yields, and field tolerance to powdery mildew disease.



It can cater to an increase in demand for mustard cultivation in Maharashtra, which is otherwise a non-traditional area for mustard.

#### Sunflower

Using induced mutagenesis, a high-yielding variety Trombay Akola Sunflower-82 (TAS-82) was developed. It possesses dark black seed coat with high seed and oil yields and performs better under low rainfall conditions, compared to other varieties. The variety has been identified by the RRC and RFRC of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, for release in Maharashtra's Vidarbha region.





A "fasciation" mutant, controlled by single recessive nuclear gene (denoted  $sf^{1}$ : sunflower fasciation one) has been obtained for the first time. It has narrow and large number of leaves (~120) with disrupted phyllotaxy, more leaf area (6850 cm<sup>2</sup>), and more fresh leaf biomass (203 g) than control plant. It's flower head looks like fusion of 3-4 flower heads without round boundaries for ray and disc florets. The mutant has been registered with the NBPGR, Delhi.

Jambhulkar, Curr. Sci. 83: 116, 2002. Jambhulkar, J. Genet. Breed. 56: 327-330, 2002.

#### 3.2 PULSE CROPS : SOYBEAN, COWPEA, PIGEONPEA BLACKGRAM AND MUNGBEAN

Pulse improvement efforts in the Nuclear Agriculture & Biotechnology Division have resulted in the release of 12 varieties of



grain legumes, in recent past. Two new varieties of soybean (TAMS 38) and mungbean (TM 99-37) were released recently. Three genotypes of cowpea and 1 of pigeonpea have also been deposited with the National Gene Bank, NBPGR, New Delhi.

#### Released variety

2004

95 days

#### Soybean TAMS 38

Year of release
Area of adaptation
Yield
Maturity
Special features

Vidarbha region of Maharashtra 1800-2200 kg/ha

Resistant to bacterial pustule, Myrothecium leaf spot Moderately resistant to pod blight, soyb ean m osaic virus, Rhizoct aerial blight and insects (stemfly & girdle beetle)



Molecular markers are being developed for the released varieties, and signature sequences are being identified for those under various stages of development for IPR-related issues.



Crop/Genotype	Salient features	National Identity No.
Pigeonpea TT2000-1	High yielding (38% higher over UPAS-120 during 3 years coordinated trials of ICAR). Resistant to Fusarium wilt, Early maturity (<150 days)	IC395310
Cowpea ECM9902 (Mutant of EC394736)	Determinate growth habit Large seed size Early maturity (65 days) High yield (800-1000 kg/ha)	IC395311
Cowpea TCM 77-4 (Mutant of V-130)	Dwarf and compact plant Large seed size Increased leaf area	IC296546
Cowpea TC99-1 (Selection from exotic cowpea EC394763)	Early maturity (65 days) High yield (>1000 kg/ha)	IC296441

Improvement in yield, earliness & disease resistance as also ideotype breeding are being attempted in five major grain legumes namely, mungbean, pigeonpea, blackgram, soybean, and cowpea.



#### 3.3 SUPRAMOLECULAR ORGANISATION IN **PHOTOSYNTHESIS**

Role of thylakoid membranes in the Supramolecular Organization of Calvin Cycle was studied in permeabilized Anacystis nidulans cells, by transmission electron microscopy. Sequential reactions of entire Calvin cycle could be detected in the cells, which had retained the internal organization of the thylakoid membranes (B) after permeabilization and were lost on disruption of this organization (C). Thus, integrity of thylakoid membranes may be essential for the organization and function of enzymes of Calvin cycle in vivo.





The arrows marked alphabetically, indicate interacting proteins. Double headed arrow shows co-purification of proteins, single headed arrow is directed to protein being purified (head) from the contaminant (tail).



ATP synthesis in light by complete electron transport activity in thylakoid membranes and subsequent use of ATP for CO<sub>2</sub> fixation by associated Calvin cycle enzymes. The 150K fraction of thylakoids showed significantly high rate of this synchronized activity, as represented schematically. The data suggest existence of a photosynthasome encompassing all photosynthetic functions.

Sainis, Dani and Dey, J. Plant Physiol. 160: 23-32, 2003.

Dani and Sainis, Biochim. Biophys. Acta 1969: 43-52,2005.

#### 3.4 PESTICIDE RESIDUES

A major research programme in NA&BTD deals with monitoring the distribution of pesticide residues in ecosystems, identification of bound residues, and their bioremediation. The prominent pesticides studied include HCH, DDT, carbofuran, carbaryl, chlorpyrifos, endosulfan, nitrofen, and glyfosate. Continuous flow methods or simulated rice ecosystem were devised to examine pesticide residues using radioisotopes and Lysimeters. Major findings from these studies show that (i) HCH and Carbofuran are not bioaccumulated, (ii) Clams degrade HCH,



DDT, DDE and Chlorpyrifos completely in 2-4 days, (iii) DDT and HCH are degraded faster in rice fields and (iv) Organic manure amendment enhances the degradation of pesticides.



Kale, Murthy and Raghu, Chemosphere 44: 893-895, 2001. Kale, Sherkhane and Murthy, Environ. Technol. 23: 1309-1311, 2002. Mehetre, Murthy and Kale, Soil Sediment Contam. 12: 1-6, 2003.

#### 4. NEW TECHNOLOGIES AND PRODUCTS

#### INTRODUCTION

The multidisciplinary expertise available at BARC makes it a fertile ground for development of new innovative technologies and products, both for research as well as applications in the areas of excellence and relevance to various programmes of the DAE. Fruitful interactions amongst physicists, chemists, biologists, and experts in computer hardware software and robotics has led to the creation of cutting edge technologies related to research and applications in life sciences, as also establishment of facilities of immense societal benefit, in recent years.

In the post-genomic era of biological research, high throughput transcriptome and proteome analyses are being highly sought after. The DRHR, Electronics and Computer Divisions and Molecular Biology Division in BARC took up the challenge to fabricate the first prototype DNA microarrayer indigenously. DRHR also fabricated and set up the first state-of-the-art indigenous teletherapy machine, BHABHATRON, at ACTREC, Kharghar, Navi Mumbai, for research and application in cancer radiotherapy.

Three new facilities of considerable societal impact were established in the last 4 years. These include: (a) the KRUSHAK irradiation facility for agricultural commodities, particularly onions and potato, at Lasalgaon, Nasik, (b) a series of NISARGRUNA plants in and around Mumbai for generation of bioenergy (biogas and electricity) through disposal of biodegradable waste, and (c) procurement and commissioning of the PET facility at RMC, Parel for efficient diagnosis and therapy of cancer and other common diseases.

New equipment, such as vibrothermal disinfestor and foldable solar heater for food preservation, have been commercialized through technology transfer. A new product, *banana juice*, developed by the FTD and NA&BTD has been patented and commercialized.

### 4.1 NEW EQUIPMENT FOR RESEARCH AND APPLICATIONS

 BARC develops a prototype DNA micro-arrayer

A device capable of arraying DNA fragments (probes) at defined addresses on solid surfaces has been developed by a joint effort between the DRHR and MBD, BARC. The system contains (i) quill type printing tips, which collect the probe samples by capillary action (ii) an ultrasonic cleaner for cleaning the tips (iii) a dry chamber for drying the tips, and (iv) a vacuum-based slide tray for clamping the glass slides. An XYZ system of stepper motors and ball screws (for X and Y axes) and lead screws (for Z axis) moves the printing head to different locations during operation.



A PC-based system controls the operation through graphical user interface based in WIN 32 application software. The software allows the user to select different configurations of well plates , printing heads, slides and different types of security features, automatic process execution as also manual operation of all the three axes. Various printing as well as hybridization parameters are being standardized using this prototype system.

The microarrayer is proposed to be used for analysis of stress-responsive gene expression in various research programs of relevance to various ongoing activities in life sciences.

#### BHABHATRON: a teletherapy machine for cancer cure

Teletherapy machines are used to eliminate or shrink localized cancers in human tissues. The first indigenous state-of-the-art teleptherapy machine, named "BHABHATRON", has been recently developed by the DRHR, BARC and installed at ACTREC, Kharghar, Navi Mumbai.

The machine incorporates latest concepts in safety controls and user interface. A fully closable collimeter for improved radiation safety is a unique feature of the machine. It contains a 250 RMM Cobalt-60 source and has minimum couch height and noise-free movements on par with equivalent imported machines. In addition, the machine has lower penumbra for better beam quality, total digital controls with self-calibration of motors and controls, single cable communication between machine and control console and total treatment data acquisition and data analysis.



BHABHATRON Teletherapy Machine

The cost of the machine is significantly lower than an imported machine. It is expected that the development of this machine would result in reduction in treatment cost of cancer patients.

#### A Vibrothemal Disinfestor for disinfestation of food grains

Based on the killing of insects in stored grains by heat, a vibro-thermal grain disinfestor has been constructed by the Food Technology Division and Division of Remote Handling & Robotics of BARC.



Foldable Solar Driers of various capacity (10, 25 and 100 kg) are available for the preparation of raisins, drying of grapes, jack fruit pulp, ginger, green pepper, and herbal medicines.

The know-how for the 25 kg capacity FSD has been transferred to 12 different parties. Of these, M/s. Gumpro Chem. at Mumbai has already started manufacturing the FSD from 2002.

The unique features of the equipment include :

- Each grain vibrates to provide quick and uniform heating
- Air surrounding the grains gets equilibrated with hot air
- Same hot air is being circulated
- Conveyor system enclosed in an insulated box, to minimize heat loss.

The technology has already been transferred to five different companies.

#### A foldable solar food drier

An efficient Foldable Solar Dryer (FSD) has been developed for substantial weight reduction of food items. For example, 100 kg of grapes can be converted to 25 kg of raisin quickly. It is hygienic, has no adverse effect on environment and prevents infestation by microorganisms, insects, and pests, thereby reducing spoilages.

#### 4.2 NEW FACILITIES CREATED

Three new facilities were established in the last 3-4 years. These cater to the needs of food preservation(KRUSHAK), biogas from waste (NISARGRUNA) and cancer diagnosis (PET).



 A low dose gamma radiation processing plant for onion, and other agricultural commodities KRUSHAK (Krushi Utpadan Sanrakshan Kendra), a technology demonstration plant for processing onion and other agricultural commodities with low doses of <sup>60</sup>Co gamma radiation, became operational at Lasalgaon (near Nasik).

KRUSHAK	Krushi Utpadan Sanrakshan Kendra		
Purpose of the facility	radiation processing of onion and other low dose requiring agricultural commodities		
Date of Commissioning	October, 2002		
Declared operational	August, 2003		
Class of Irradiator (AERB SS-6, 1993)	Panoramic wet storage Type IV – cobalt 60 4 pass 2 sided continuous irradiation		
Source distribution	3 tier source rack (total slots = 96)		
Designed capacity	300 kCi (Current loading – 40 kCi)		
Storage of source	Under DM water pool – 3x1.6 x6 M(d) = 30 kL		
Biological shield	1.7 M thick (inner wall 1 M, outer wall 0.7 M separated by labyrinth 1.02 M wide		
Dimensions of Product box	850 X 545 X 1350 (H) mm		
Dimensions of Carrier box	945 X 600 X 1598 (H) mm		
Product box capacity	200 kg (5 bags - each of 40 kg)		
Conveyer speed (Min: Max)	0.095 : 0.95 M/m		
Irradiation path length in cell area	22 M		
Designed throughput at 30 Gray	10 T / h (Current throughput – 3 T / h)		
Product movement	Overhead monorail with ground support guide rails		
Power requirement	100 kW		
Product handling	Manual / auto		



Containers used for irradiation



Storage of irradiated gunny bags



Irradiated onions in the market

In 2004, the facility processed nearly 300 tons of onions, spices, potatoes, mangoes, raisins and protein hydrolysates, and the trend is growing.

#### Bioenergy and Nisargruna

A biogas plant to process solid biodegradable waste, from kitchens, gardens and vegetable markets, was developed with following improvements:

- 1. Conversion of waste to slurry for faster microbial degradation
- **2**. Biphasic separation
- **3**. Use of aerobic thermophile in the predigestor
- **4.** Solar energy to maintain high temperature
- **5.** Recycling of water.





Named NISARGRUNA (paying back nature's debt), the plant reduces pollution and is a step towards the zero garbage dream. It comprehensively disposes biological waste. The two byproducts (methane + manure) recycle nutrients and make the project self-sustainable. Weed-free manure obtained from such waste has high nitrogen content and acts as an excellent soil conditioner. Technology of biphasic biomethanation has high potential of solving the solid waste management problems of



**BARC Hospital Site** 



Shatabdi Hospital, Govandi (MCGM)



INS Kunjali, Colaba

the urban areas and provides organic manure and bio-gas as a fuel. Six plants have so far been constructed in Mumbai. Ministry of Non-Conventional Energy Sources, New Delhi and Solid Waste Management Cell, Maharashtra Government are working in close collaboration for deployment of the technology and several MoUs have been signed for its implementation. The technology has also been transferred to 16 private parties.

Kale, Bioenergy News. 8: 22-26, 2004.

#### Positron Emission Tomography (PET) established at RMC, Parel

A Positron Emission Tomography (PET) facility (Cyclotron PET trace from General Electric) was established at the Radiation Medicine Centre, Mumbai on October 31, 2002. The facility is meant for the diagnosis of cancer and other human diseases and metabolic disorders, primarily using incorporation of radiolabeled metabolites in to the human body followed by detection of the radiotracer molecule using a PET scanner.



The major effort, so far, has been on cyclotron-based production of <sup>18</sup>F labeled deoxyglucose (<sup>18</sup>FDG), an analogue of glucose. This molecule is taken up and incorporated at much higher levels in cancer cells than normal cells but is not metabolized, thereby facilitating its concentration and detection using the PET scanner. This depends on five glucose transporters (GLUT 1-5), which express at high levels in cancer cells.

The facility has been successfully used for (a) diagnosis of common as well as some uncommon cancers, and (b) for monitoring their response to therapeutic treatment.





Gastrointestinal stromal tumor (GIST) GIST is a rare type of sarcoma affecting stomach and intestine. Compared to CT scan, the FDG-PET can detect the response to the drug, Glivec, within 1 day of treatment.

PET scans are useful in effectively determining the extent of degeneration in the case of bone tumors, or the extent of metastases in the case of ovarian cancers.



PET scans are most useful in the case of cancers, which cannot be detected by CT scan, such as Non-iodine concentrating thyroid cancers. PET scan has also substantially strengthened basic research on glucose transporters of mammalian cells, especially in cancers.



The PET scans also play a vital role in down-staging of cancers where CT scans are not so effective. Shown below is an example of lung cancer, wherein Staging changes from N3 in CT to N0 in PET thus radically altering the treatment.



Role of PET: lung cancer mediastinal staging In addition to the left lung nodule, CT scan shows several lymph nodes in the mediastinum (white arrows). In contrast, the FDG-PET shows only the lung mass and no nodal uptake (black arrows).

However, it is not in cancer alone that FDG PET has been used at the RMC. It has also been used to good effect in cardiac disease to detect viable myocardium following an ischemic insult, and in neuro-psychiatric disorders, such as schizophrenia, Obsessive Compulsive Disorder, Alzheimer, Drug addiction and epilepsy. The RMC is a premier center for nuclear medicine teaching and practice in the country, and also a referral center for the IAEA and the WHO. With the introduction of PET at RMC-BARC, India has moved up yet another notch in achieving parity with diagnostic centers the world over. The facility is expected to result in a far superior management of cancer in years to come.

Alzheimer's Disease Reduced uptake (hypometabolism) in the posterior parieto-temporal complex alongwith preservarion of function in the sensorimotor comples and basal ganglia are typical symptoms of dementia in Alzheimer's disease.

Basu and Nair, Clin. Nucl. Med. 29: 626-628, 2004.

Basu, Nair, Awasare, Tiwari, Asopa and Nair, Br. J. Radiol. 916: 347-61, 2004.

Nair, Basu and Pakhale, Br. J. Radiol. 77: 63-67, 2004.

Basu and Nair, Eur. J. Nucl. Med. Mol. Imaging 32: 384, 2005.

Basu and Nair, Indian J. Cancer 42: 60-62, 2005.

Nair and Basu, Clin. Nucl. Med. 30: 289-290, 2005.

#### 4.3 BANANA JUICE

A lab-scale process has been developed at BARC for extraction of clear Banana Juice, a potential beverage, from the pulp of ripe banana [Patent No. 189999 (336/Mum/01)]. The bio-process achieves a juice yield of around 55% (w/w) while the remaining pulp can be converted into fine banana powder, a by-product which can be used as a substitute for flour in cakes and biscuits. The know-how for this technology has been transferred to M/s ITEMATRIX Co. Ltd., Bangkok on 7.12.2004. Indian entrepreneurs have shown interest in this technology.



#### **BANANA JUICE PROCESSIGN**

**Characteristic features:** 

- The banana juice extraction process-commercial variety 'Harichal' (550-600 ml juices/Kg pulp).
- Residual pulp retained aroma of banana-ripe banana powder : could be used as supplement in confectionaries.

$\succ$	Analysis : banana	juice	
	Sugar content	1	20-22%
	Solid content	:	22-25%
	Specific gravity	:	1.1 to 1.2
	рН	:	4.5 to 4.8
	Potassium level	:	120 mg / 100 ml

#### 5. UPCOMING BIOTECHNOLOGIES

#### INTRODUCTION

The advent of more incisive biophysical, biochemical and molecular biology tools and techniques has facilitated new explorations and has also imparted a cutting edge to the conventional methods and technologies. Synergistic interactions among life science researchers across the various divisions in BARC, have led to some promising technologies in the area of agricultural biotechnology, food technology and microbial technology.

Crop improvement programmes are being strengthened by development of molecular markers and image analysis techniques at Molecular Biology Division (MBD) and NA&BTD. These are aimed at varietal indentification and quality and disease resistance in target crops. Plant tissue culture based micropropagation techniques have been perfected for fruit crops like banana and pineapple. Transgenic plant technology has been developed and used for production of edible vaccines and disease-resistant plants.

Radiation-based hygienisation technologies have been developed in FTD for a variety of food items from salads to meat products. Radiation-induced enhancement of aroma, and isolation of antioxidants from a variety of spices and vegetables and meat wastes hold promise. Use of poultry and fish waste for making protein hydrolysates and proteases also seems quite attractive.

An upcoming technology of considerable relevance and important to DAE is the use of microbes for bioremediation of nuclear waste. This technology, being developed in the MBD, NA&BTD and WSCD involves the use of either efficient native microbes or microgranules prepared thereform, or genetic engineering of microbes with desirable genes to achieve biosorption/bioprecipitation of uranium and other heavy metals or biodegradation of organic solvents like TBP.

Genetic engineering is also being employed for construction of transgenic cyanobacterial biofertilisers under a DBT-sponsored research project in the MBD.

#### 5.1 AGRICULTURAL BIOTECHNOLOGY

Major research activities in agricultural biotechnology are carried out in the MBD and NA&BTD. These relate to the development of molecular markers for breeding and identification of crop varieties and micro-propagation and construction of transgenic plants.

#### Molecular markers for crops

Different types of DNA and protein-based molecular markers are being developed for application in breeding programmes of target crops. The major mandate in the Nuclear Agriculture & Biotechnology Division is to distinguish crop varieties (Blackgram) developed by BARC and identification of disease-infested plant material (Rice Seeds), using RAPD, ISSR and AFLP derived DNA markers.



Specificity PCR of various fungal species with m/23 & rDNA primers assay

In the Molecular Biology Division, the emphasis is on genome analysis in mungbean and development of markers for stem rust in wheat crops.



Saini, Reddy and Jawali, Indian J. Biotechnol. 3: 511-518, 2004.

Nalini, Bhagwat and Jawali, Cereal Res. Commun., 33 : 439-446, 2005.

#### Protein markers for bread quality

Wheat genetics is an important research activity in the NA & BTD. End use of wheat depends upon the protein (gluten) quality and quantity. Varieties with strong dough are good for *Bread*-making while medium and weak dough are suitable for *Chapati* and *Biscuit* making, respectively.



Gluten strength is significantly influenced by the high molecular weight (HMW) subunits of glutenin proteins. Modification of subunit composition has been used for improvement of bread making quality.



Other major interests in wheat include development of molecular markers for rust resistance. Current work also focuses on thermotolerance in this important crop.

Bhagwat, Das and Rao, Trop. Agri. Res. Extension 4 : 83-89, 2001. Sharma, Rao , Bhagwat and Bapat M.M. J. Food Sci. Technol. 41:160-164, 2004.

#### Image analysis for varietal identification in wheat

Wheat grain morphometry and computer-based image analysis are being developed and evaluated as tools for varietal identification in wheat.

A Comprehensive Image Processing Software (CIPS) has been developed for morphometric analysis of wheat grains. Using this imaging system, shape variation, based on grain morphology, was quantified in fifteen Indian wheat varieties, as well as among the genetically related wheat selections.



Euclidean distances calculated using differences in means could serve as a basis of distinguishing between these genetically closely related samples.

Shouche, Rastogi, Bhagwat and Sainis, Computers & Electronics in Agriculture. 33: 55-76, 2001.

#### Micropropagation of fruit crops

Micropropagation of fruit crops is a major research programme in the Nuclear Agriculture and Biotechnology Division.

Protocols for micropropagation and somatic embryogenesis of several fruits, such as banana, sugarcane, pineapple and grapes have been established in recent years.

#### **MICROPROPAGATION OF FRUIT CROPS**



#### SOMATIC EMBRYOGENESIS IN BANANA VARIETIES RASTHALI AND RAJELI







#### SOMATIC EMBRYOGENESIS IN SUGARCANE (var. CoC-671 and CoC 86032)







#### SOMATIC EMBRYOGENESIS IN PINEAPPLE VARIETY QUEEN







The technology for production of banana has been transferred to MSSC, Akola and KKVK, Pondicherry. A banana tissue culture laboratory was also established at the Anandwan Agriculture College, Warora.

#### Transgenic plants : disease resistance and edible vaccines

Constructing plants resistant to diseases and capable of producing edible vaccines are the major objectives of the programme on development of transgenic plants.

MAGAININ, a 23-amino acid long alpha helical peptide found in *Xenopus laevis*, is known to disrupt membrane integrity and kill microbes. A *msi168* gene encoding a modified magainin was constructed and transformed into pineapple and banana.



Pineapple transformed with msi168 in tissue culture



Transformed, hardened plants



Transgenic Banana Plants (T-168)



Transformed plants expressing maiganin (T) were resistant to *Fusarium oxysporum cubense* compared to control plants (C).

Transgenic potato plants carrying the "potato virus Y" coat protein gene were developed and shown to exhibit resistance to the potato virus-Y infection.



Genetic transformation of banana for expression of hepatitis-B surface antigen (HBsAg) in fruits has been achieved in the NA&BTD. Characterisation of the plant derived HbsAg and development of an edible vaccine is in progress in collaboration with Shantha Biotechnics Pvt. Ltd., Hyderabad.



Edible Vaccine from Banana

Chakrabarti, Ganapathi, Mukherjee and Bapat, Planta 216: 587-596, 2003.

Sunilkumar, Ganapathi, Revathi, Prasad and Bapat, Protein Expr. Purif. 32: 10-17, 2003.

#### 5.2 FOOD TECHNOLOGY

In food science and technology, the main emphasis has been on hygienisation of minimally processed foods and increase in the shelf life and quality of foods using radiation. Isolation of novel antioxidants from plants and preparation of useful food products from fish and chicken waste has also been successful.

#### Radiation hygienisation of food with (<sup>60</sup>Co γ-rays)

Consumption of minimally processed fruits and vegetables, including sprouts is on the rise in India. Market survey of these products in Mumbai, and of >95% poultry samples from Mumbai and Pune revealed poor microbiological quality and even the presence of pathogenic organisms.





PCR-based rapid detection methods have been developed to detect *Salmonella* in such samples.



Radiation processing (2 kGy dose of  ${}^{60}$ Co  $\gamma$ -rays) eliminated most of the pathogenic microbes, improved microbial quality and shelf stability of these products while retaining the nutritional and organoleptic qualities of food.



Bandekar, Dhokane, Shashidhar, Hajare, Ghadge, Kamat and Sharma, J. Food Sci. Technol. 42: 99-101, 2005.

#### Radiation-induced enhancement of aroma

Radiation processing was found to help quality enhancement of monsooned coffee, a speciality coffee of India. Release of isoeugenol and 4-vinyl guaiacol from their glucosidic precursors were demonstrated to be responsible for the unique aroma of this speciality coffee. Radiation processing resulted in increased rate of monsooning. Similarly, in nutmeg the major aroma glycosides of p-cymene 7-ol, alpha terpeniol, isoeugenol and methyl eugenol were detected.





A dose-dependent break down of nutmeg glycosides was noted and that resulted in enhanced aroma due to release of aromatic compounds from the glycosidic bond. In pomegranate the major aroma glycosides of alpha terpeniol, phenyl ethanol, and methyl benzaldehyde were detected. A novel indole derivative with a characteristic aroma of mogra flower has also been identified.

Variyar, Ahmad, Bhat, Niaz and Sharma, J. Agric. Food Chem. 52: 7945-7950, 2003.

#### Natural antioxidants from spices, and vegetable and meat waste

Novel phenolic compounds from green pepper [3,4-dihydroxy phenyl ethanol glucoside and 3,4-dihydroxy 6-(N-ethyl amino) benzamide] and lignans from mace, having high antioxidant activity, were isolated and identified for the first time. Some new antioxidants were also isolated from turmeric and showed the following structures:





Irradiation can accelerate lipid peroxidation and other oxidative changes that influence the sensory quality of meat. Natural antioxidants are receiving increased attention for their potential effects in the prevention of chronic and degenerative diseases such as cancer, cardiovascular diseases as well as aging.

Novel natural antioxidants have been isolated from food wastes, such as potato peel (PPE), mint, natural and irradiated chitosan,

and shrimp waste (BHT) and their efficacy demonstrated in minimizing oxidative rancidity of radiation-processed meat.



Niaz, Variyar, Gholap and Sharma, J. Agric. Food Chem., 51: 6502-6504, 2003.

Kanatt, Chander and Sharma, J. Agric. Food Chem. 53: 1499-1504, 2005.

Kanatt, Chander and Sharma, J. Food Sci. Technol. 39: 997-1003, 2004.

#### Shelf-stable/chilled ready-to-eat (RTE) meat products

There has been a surge in demand for processed convenient meat products such as kababs, tikkas, lollipops, fingers, patties, sausages and chicken meat products, in urban Indian markets.

"Fresh-like-chilled-meats" can considerably save energy costs and are preferred by customers due to their better texture. However, their short shelf-life (3-4 days) limits their marketability. There are also serious concerns about their microbiological safety.

The Food Technology Division, in BARC has standardized procedures for several chilled ready to-eat meat products with extended shelf-life by using a combination of gamma radiation and other hurdles. The technology is ready for commercial exploitation.





A technology for RTE meat/poultry products for storage at ambient temperatures has been developed, using various hurdles such as reduced water activity, vacuum packaging and thermal or radiation processing.

These RTE meat products have a high sensory acceptability up to six months. The microbiological safety of these products has been validated by various challenge studies.

Kanatt, Chander and Sharma, **Meat Sci**. 69: 269-275, 2005. Kanatt, Chawla, Chander and Bongirwar, **J. Food Protect**. 65: 1628-1631, 2002.

Chawla and Chander, Food Control 15: 559-563, 2004.

#### Protein hydrolysates/proteases from chicken and fish waste

Poultry and fish processing industry waste are rich sources of protein and protein hydrolyzing enzymes known as proteases.

Chicken intestine is the major component of poultry waste comprising about 30 % of the total waste. The protein content varies between 10-30%. Similarly, the fish waste comprising 25-30 % of the total processed material of which around 8-10 % is protein.



CHICKEN INTESTINE FROM POULTRY WASTE

PROTEIN HYDROLYSATE FROM CHICKEN INTESTINE



Typically, protein hydrolysate contains 70-80 % peptides/amino acids, 0.7-0.8 % lipids, and 5-10 % moisture. Protein hydrolysate from both the sources (poultry and fish waste) exhibited very good biochemical, nutritional, organoleptic and functional properties, and could be used as food and feed supplement.

The CIPH and FWPH wastes are also rich sources of inherent proteases that could be utilized for converting these proteins to



a highly digestible and nutritious protein hydrolysate. Among the proteases aspartic proteases and cathepsin B, and cathepsin H were the major enzymes.

The poultry and fish waste could be hygienized by radiation and stored for some time before processing, without affecting the enzyme activity.

Jamdar S.N. and P. Harikumar, J. Food Sci Technol. 39: 72-73, 2002.

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Pardesi, Dandekar, Jamdar and Harikumar, Ind. J. Clin. Biochem. 19: 84-90, 2004.

Jamdar and Harikumar, Bioresource Technol. 96: 1276-1284, 2005.

#### 5.3 MICROBIAL BIOTECHNOLOGY

Microbes, both naturally occurring as well as genetically engineered strains, are being evaluated for their biotechnological potential either for bioremediation of nuclear waste or as nitrogen biofertilisers in paddy cultivation.

#### Bioremediation of nuclear waste through biosorption and bioaccumulation

The NA&BTD, BARC has been exploring the possibility of using biosorption and bioaccumulation processes for removal of cobalt, uranium, thorium, cesium, strontium and nitrate from the nuclear waste. A large number of biosorbents (bacteria, fungi, agrobiomass, plant seeds, plant and bacterial proteins) have been evaluated for their efficacy and suitable bioresins have been prepared and tested.

Methods have been standardized for pelletization of biomass in various forms, such as Powder, Biobeads, Biofilm and Biofloat.

Metal	Biomass	Loading capacity (mg/g)
Uranium	Pseudomonas sp.	540
	Rhizopus arrhizus	319
	Aspergillus fumigatus	423
	Mucilaginous seeds	161

Using 2.1 kg of bioresin at a flow rate of 5.5 m<sup>3</sup>/h, decontamination of  $^{60}\text{Co}$  from pool water was recently accomplished.

Sar, Kazi and D'Souza, Int. Biodeter. Biodeter. 54: 193-202, 2004. Melo and D'Souza, Bioresource Technol. 92: 151-155, 2004.

MAIL: BURESS	Kinetics - 1 filter Biomass - 400 g; F.R 0.36m³/day	Volume perfused (m <sup>3</sup> )	Percent activity reduction in pool water (30 m <sup>3</sup> )
	Volume perfused (m <sup>3</sup> )	0	0
	Kinetics - 3 filter	8.0	14.0
	Biomass - 1.7 kg; F.R 0.6m²/day	21.0	18.0
tern the		44.0	23.0
	82 40- 82 0	55.0	25.0
	0 1 2 3 4 5 6 Volume perfused (m <sup>3</sup> )	90.0	26.0

#### Microbial granules for bioremediation of organic or inorganic waste

Self-immobilization of complex consortia microbial (especially aerobic heterotrophic bacteria) into granules is quite advantageous in several ways. Since close packing of a variety of cells at high densities occurs in such immobilised cell systems, high volumetric reaction rates and efficient substrate conversion are possible. When employed in bioreactors, microbial granules ensure efficient biomass retention, minimizing cell loss through washout. This also simplifies downstream processing of the effluent.

A program on bioremediation of organic and inorganic wastes based on aerobic microbial granules (granules cultivated under aerobic conditions) has been initiated at Water and Steam Chemistry Division, Kalpakkam.



Sequencing batch reactors (SBR) used to cultivate aerobic microbial granules



Aerobic microbial granules were successfully cultivated in laboratory scale sequencing batch reactor. Seed material for inoculation was obtained from activated sludge from a wastewater treatment plant.

The developed granules were successfully used for biodegradation of recalcitrant organic compounds such as nitrilo-triacetic acid (NTA) and tributyl phosphate (TBP), using laboratory scale column reactors.



The granules also exhibited significant biosorption ability and could remove uranium from solutions.



The ongoing/future work under the programme includes scaling up, molecular analysis of microbial diversity in the granules, improvement of biogranulation process and possible bioaugmentation of the granules using genetically modified organisms.

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#### Genetic engineering of microbes for metal bioprecipitation

A programme to isolate or construct bacterial strains with enhanced bioremediation potential, using recombinant DNA technology was initiated in the Molecular Biology Division three years ago. It has focused on genetic engineering of bacterial strains for metal bioprecipitation, and isolation of bacteria degrading tributylphosphate (TBP). A *Sphingomonas paucimobils* strain capable of degrading TBP has been isolated recently from the RSMS site, BARC and is being characterized.

Periplasmic phosphatases generate inorganic phosphate (Pi) (precipitant ligand) which, in turn, interacts with the metal to form insoluble metal-phosphate (MP) on the cell surface. These enzymes can be used to recover heavy metals, including uranium, from nuclear waste.

Crystal structure of a non-specific acid phosphatase (PhoN) from *Salmonella typhi* was determined earlier. The corresponding gene (*phoN*) has now been cloned into the most radioresistant bacterium, *Deinococcus radiodurans*.



Zymogram assays for expression and actiovity of acid phosphatase PhoN

+ DIX MG



In-gel zymogram assays of *Deinococcus* extracts showed less phosphatase activity in cells carrying the empty vector pRAD1, high activity associated with a 27kDa protein in the clone CL#27 and intermediate activity in another clone (DN). Compared to *E. coli* this strain shows 25% PhoN activity and is being tested for its uranium bioprecipitation potential.

Makade, Vinaykumar, Rao, Yadav and Mahajan, Acta Crystallographica 59: 515-518, 2005.

#### Transgenic cyanobacterial nitrogen biofertilisers for stressful environments

Under a DBT-sponsored project, a novel plasmid vector (pFPN) was constructed to transfer, integrate and express desired genes into *Anabaena*. Successful transformation by electroporation, integration into genome and expression from a selected cyanobacterial promoter has already been tested for the (a) *gfp* (green fluorescent protein) and (b) *hetR* (regulator of heterocyst differentiation) genes. Arrows indicate heterocysts whose frequency increased in the *hetR* transformants while the *gfp* transformants could be visualized by their green fluorescence as against the red fluorescence of *Anabaena* PCC7120 cells.



These are the first transgenic cyanobacteria constructed in the country and have paved the way for fundamental research as well as biotechnological application of cyanobacteria as nitrogen biofertilisers in stressful environments.

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