SIXTY GLORIOUS YEARS OF CYANOBACTERIAL RESEARCH IN BARC

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Abstract

Tradition of cyanobacterial research in BARC has been quite old, spanning nearly six decades of excellent scientific contributions in terms of high-quality basic research as well as of several appropriate technologies. It is a pleasure to recall some of these, the discoverers and some of their significant publications of that time. Sixty years is a long-time to remember and in doing so, I may have missed some of the important contributions inadvertently. This article attempts to take stock of some of the notable research carried out in MBD, by about a dozen researchers including myself, which has led to recognition of BARC, as the top-most laboratory for modern state of art cyanobacterial research in India in the last 5 decades. It has also earned a comparable reputation globally.

1. The Beginning

In BARC, research on blue-green algae (as cyanobacteria used to be referred to before 1980s) started in the late 1960s when late Dr. Joseph Thomas (my mentor, research supervisor and Boss) joined the then Biology Division of the Bio-Medical Group. One of his previous expertise was in mass cultivation of green algae and he used it to set up fermenter-based mass cultivation systems for the green alga *Chlorella* and the blue-green alga *Anabaena*. His major achievement in this field was also to establish the first *continuous culture* system for *Anabaena* at BARC. Later he also developed methods for growing two blue green algal isolates from Trombay, *Anabaena* L-31 and *Nostoc*-4, on

huge plastic sheets overlayed with a thin layer of soil, that made their harvesting easier. This mass cultivation technique came in handy for later evaluation of nitrogen biofertilizer potential of these cyanobacterial strains using ^{15}N-labeled Urea and NH_4NO_3 in the 1980s, in collaboration with Rashtriya Chemicals and Fertilisers (RCF) and NOCIL. This aspect of Dr. Thomas' research earned him lot of attention and considerable fame nationally.

However, what really caught attention of youngsters like me joining BARC in the early 1970s were his outstanding contributions to the basic biology of heterocyst differentiation in *Anabaena*. Heterocysts in *Anabaena* filaments not only looked special – they were shown to be special using a unique equipment Absorption Microspectrophotometer, that existed in the division that time, and could record absorption spectra of individual cells *in situ*. These studies revealed that heterocysts lacked the Photosystem II (PSII) pigments and therefore would not photoevolve O_2 like the remaining photosynthetically active vegetative cells (Nature 228: 181-183, 1970). That they were indeed microaerophilic was shown soon thereafter, by Dr. Thomas with another senior colleague Dr. K.A.V. David (Nature New Biol. 238: 219-221, 1972) using tetrazolium blue and nuclear emulsion-based microscopy. Later, after the procurement of a fluorescence microscope in the group, it became possible to demonstrate that fluorescence from Phycocyanin (the most important component of PSII) was also absent in heterocysts. This established heterocysts as the sole sites of aerobic nitrogen fixation (oxygen being highly toxic for N_2 fixation) in cyanobacteria. There was one more paper in Nature (by late Prof. R.N. Singh of BHU, Varanasi), published 2 years before my birth, which reported reclamation of highly saline alkaline sodic soils using naturally occurring populations of cyanobacteria around Varanasi (Nature 165: 325-326, 1950). The work was highly impressive because of its sheer simplicity and yet high quality, and truly reflected a case of *appropriate technology* which was the buzzword at that time. Charmed by these 3 Nature papers on cyanobacteria, I decided to join Dr. Thomas' group in the Biophysics Section of the then Biology & Agriculture Division (B&AD). When I narrated the saline soil reclamation story and my aspiration to experiment with it further to Dr. Joseph Thomas, he immediately agreed to allow me to work on this aspect, in spare time.

2. Scientific Contributions of first 3 decades

When I joined the laboratory in 1974, my senior colleagues Dr. David was continuing to work on heterocyst differentiation, and Dr. Tonina Fernandes, was busy discovering factors that trigger sporulation in *Anabaena torulosa*, and obtaining UV-induced mutants of cyanobacteria. Another colleague Nagaraja, from the previous (15^{th}) batch of BARC Training School Biology & Radiobiology discipline, was devising methods to isolate heterocysts using density gradient centrifugation. His methodology, though never published, was used by us every year to prepare heterocysts (as pure photosystem-I samples) for Prof. Govindjee (of Photosynthesis fame) who used to visit Biophysics

Section in summer to do fluorescence and thermoluminescence-based investigations in the mid-1970s.

During my deputation on an IAEA fellowship, I chose to work with Prof. W.D.P Stewart on CYANOBACTERIA, in the University of Dundee in Scotland, U.K. I must admit it was here that I really received my core training in cyanobacterial physiology and biochemistry. I worked there for 15 months on a new pathway of electron donation to nitrogenase in the heterocysts of *Anabaena*. This publication (Proc. Roy. Soc, London B 200: 1-25, 1978) remains one of my most cited paper which also earned my mentor the Fellowship of Royal Society in 1977. We published 2 more papers on the regulation of this pathway and purification of major components of the pathway.

Before I returned to India, my Ph. D. guide, Dr. Thomas, went on Sabbatical Leave to Prof. Peter Wolk's laboratory in Michigan State University, USA, to do the most significant work of his career – discovery of the pathway of ammonia assimilation in *Anabaena* using the cyclotron-generated but very short-lived radioisotope of nitrogen (^{13}N) at MSU-DOE-Plant Research Laboratory at Michigan USA. This work identified the glutamine synthatase-glutamate oxoglutarate aminotransferase (GS-GOGAT) as the principal pathway of assimilation of nitrogen fixed in heterocysts (J. Biol. Chem. 251: 5027-5034, 1976) and led to several important research questions, such as how heterocyst pattern is regulated, or why they don't differentiate in nitrogen-supplemented media, and others.

Around that time, David and I initiated an interesting piece of work. We noticed that reduction of acetylene to ethylene, which was the routine assay for nitrogenase activity, was exponentially enhanced several-fold if the assay was carried out for more time than 30 min, resulting in erroneous over-estimates of nitrogen-fixed in field experiments, where prolonged incubation with acetylene was a norm. Using enzyme kinetics approach, we showed that this was due to conformational changes occurring in nitrogenase complex upon prolonged incubation with acetylene. This was first ever demonstration of conformational changes in nitrogenase in any organism (Biochem. Biophys. Res. Commun. 82: 39-45, 1978; Biochem. Biophys. Res. Commun. 83: 1157-1163, 1978) and immediately caught attention of N_2 fixation community. For us this was even more special since such conformational changes *in vivo* were demonstrated using cyanobacteria as the model system.

We followed this work with another interesting paper on acetylene reduction assay. In this nitrogenase activity assay, wherein a great access of acetylene is used as substrate, while the reduction product ethylene was detected within 30 seconds by gas chromatography, one had to wait for 5 min for acetylene to exit the column before the next injection could be made. This meant a tediously long day for researchers who had 100s of such samples to be assayed each day, such as in agricultural research in the field. With the help of Dr. Ashok Bannerjee (Bio-organic Division), we shortened the analysis time to less than a minute by completely precipitating acetylene as silver acetylide with the help of ammoniacal silver nitrate solution (Appl. Env. Microbiol, 39: 1078-1080, 1980) before analysis. This became instantly popular among field workers on nitrogen

fixation. An additional note put in the paper stated that such assay vials should be washed as soon as possible else it turned the glass vials black, and in case the precipitate dried up there was small risk of a mini-explosion upon friction. The warning note was appreciated even more than the paper itself, and made the life of researchers significantly easier. The aforesaid three papers gave us a lot of popularity in years to come.

Meanwhile another enthusiastic young researcher Rakesh Tuli had joined our laboratory (from 19th batch of BARC TRaining School) and started working on regulation of glutamine synthetase (GS) – Dr. Thomas's favourite subject at that time. He painstakingly purified glutamine synthetase from *Anabaena* using conventional ion exchange, molecular sieve and affinity chromatography – today's rapid technique of expression of recombinant His-tagged proteins and their purification using NiNTA affinity chromatography was not available then. He biochemically characterized GS and studied its regulation in detail, including adenylylation/deadenylilaton based inactivation/activation of the enzyme. Later he was deputed to the laboratory of Prof. Robert Haselkorn at the University of Chicago where he cloned the *glnA* gene – the first recombinant DNA work in our group. His elegant work was published in couple of excellent publications. Dr. H. S. Misra who joined Dr. Tuli's laboratory a little later did some work on the non-heterocystous filamentous cyanobacterium *Plectonema boryanum* and its diurnal regulation of nitrogen fixation and assimilation. Later this groups interest in cyanobacteria somewhat dwindled and their attention shifted to plant molecular biology.

My old interest on cyanobacteria-based remediation of saline soils was revived in the late 1970s and early 1980s. We found a novel and unique requirement of trace quantities (micromolar levels) of sodium for normal growth and metabolism of two filamentous, heterocystous (*Anabaena torulosa and A.* sp. strain L-31) and one filamentous non-heterocystous (*Plectonema boryanum*) cyanobacteria (Curr. Microbiol. 3: 291-293, 1980; J. Gen. Microbiol. 130: 1161-68, 1984). Upon severe starvation of Na^+ growth came to a complete halt due to inhibition of the vital processes of both photosynthesis and nitrogen fixation in cyanobacterial strains. Effects of Na^+ deficiency were bacteriostatic in nature – photosynthesis (measured by photochemical reactions as well as carbon fixation), nitrogen fixation (estimated as C_2H_2 reduction or total Kjeldahl nitrogen) and growth resumed within minutes of re-addition of Na^+ ions. The effect was irrespective of combined nitrogen sources and was observed even in nitrate or ammonium-supplemented media, wherein nitrate assimilation and ammonium ion recirculation were significantly inhibited. Sodium deficiency also lowered aerobic respiration and reduced intracellular phosphate pools and ATP levels (J. Biosci. 6: 771-794, 1984). Such dependence on an otherwise unwanted cation (Na^+) was not known before for any mesophile.

In order to understand the molecular basis of such Na^+ dependence, two *out of box* experiments were carried out. Purification of nitrogenase was attempted from 1500L of *Anabaena* culture grown under Na^+-starved as well as Na^+-supplemented conditions. This experiment was performed over a 3 month visit to the AFRC Unit of nitrogen fixation at Brighton, U.K. aided by the award of a short-term fellowship from the Nuffield

Foundation U.K. The enzyme purified from Na^+-supplemented cultures showed nitrogenase activity while that purified from the Na^+-starved cultures did not. When subjected to ESR spectroscopy (to detect the 3.7*g* unique signal from the MoFe protein or dinitrogenase), the signal was detected in both preparations. This was a strong indication that nitrogenase was synthesized in Na^+-starved condition, but was inactive.

To address this issue, another elegant experiment was carried out by way of detecting presence of Fe (an active component of nitrogenase active site, the FeMoco) in nitrogenase proteins synthesized by Na^+-supplemented and Na^+-starved cultures. Cells were radio-labelled with ^{59}Fe, proteins were extracted and electrophoretically resolved anaerobically by SDS-PAGE. Then 1 mm gel slices were cut and radioactivity was monitored in each slice. Activity bands of the expected molecular mass which were absent in NH_4^+-supplemented media were identified as dinitrogenase and dinitrogenase reductase bands. Both the Na^+-starved cultures as well as the Na^+-supplemented cells exhibited the bands corresponding to both the nitrogenase proteins. This clearly established that Na^+ deprivation did not affect nitrogenase synthesis but adversely affected nitrogenase activity. Recovery of activity within minutes of Na^+ re-addition amply supported this result. A limitation of available ATP, caused by reduced phosphate uptake, thus appeared to result in loss of nitrogenase activity during Na^+ starvation.

For reclamation of saline soil, the relevant cyanobacteria need to accumulate Na^+ and yet be salt tolerant. The relationship between Na^+ uptake and accumulation and salt tolerance, if any, was therefore investigated in detail. Na^+ transport was measured, using ^{22}Na or ^{24}Na, in two differentially salt tolerant *Anabaena* strains along with membrane electrogenesis (Eur. J. Biochem. 154: 395-401, 1986). Voluminous work on both the influx as well as the efflux of Na^+ revealed that curtailed Na^+ influx and high efflux resulting in low intracellular concentration of Na^+ was responsible for higher salt tolerance. Conditions which reduced the Na^+ influx, such as high external K^+, alkaline pH and presence of combined nitrogen enhanced the inherent salt tolerance of *Anabaena* strains further (Appl. Env. Microbiol. 53: 1934-1939, 1987; Plant Physiol. 89: 204-210, 1989). These studies also provided clues for enhancing nitrogen fixation in saline environment, without genetic manipulation.

However, the above-mentioned work pointed out that the basis of cyanobacterial salt tolerance was Na^+ exclusion, and not Na^+ accumulation, and this raised serious questions about the ability of these microbes to remove Na^+ from saline soils. Detailed analysis of cellular distribution of Na^+ showed that while *Anabaena* strains did remove Na^+, much of it remained adsorbed in the extracellular mucilaginous sheath and did not enter the interior of the cells. Subsequent to the death and decay of *Anabaena* cells, such as during mulching in rice fields, the sequestered sodium was released back in to the soil. Thus, late Prof. R. N. Singh's reclamation success was somewhat short-lived, i. e. during the cropping season Na^+ would remain cell-bound and rendered unavailable thereby supporting better crop growth. But the method cannot offer a permanent solution to the problem of soil salinity. Indeed, this conclusion was supported by the experiments of late Dr. G, S. Venkataraman and his colleagues at IARI, New Delhi, who practiced such

bioremediation of saline soils, but had to do fresh inoculation of cyanobacteria in every cropping season. Dr. Venkataraman was my Ph. D. examiner and as my results were contradictory to his group's practices, we had a lot of argument in my Ph. D. viva wherein he graciously agreed with my findings and granted me a Ph. D. in 1985. Much later, we published these results in a prestigious soil journal (Plant and Soil 189: 205-211, 1997), which finally brought the curtain down on this exciting story.

3. Cyanobacterial research in the last 3 decades

Around late 1980s, our group seemed to somewhat disintegrate with Dr. Thomas quitting BARC (to join as a Vice President in SPIC), Dr. Tuli departing to greener pastures in NBRI, Lucknow and Dr. David choosing to work on an entirely different but interesting aspect of PS-II as a second target of UV in the unicellular cyanobacterium *Anacystis nidulans*. This gave us a chance to reorganize our activities around my choice subject - the molecular basis of stress responses in *Anabaena* strains - which I and my colleagues continued working with till my superannuation in 2014. We were initially joined by (a) Dr. Arvind Bhagwat who had been doing some fascinating work on Rhizobium-legume molecular interactions leading to nodulation and symbiotic nitrogen fixation with Dr. Thomas, and (2) Dr. Tonina Fernandes, who had been busy carrying out field work with Dr. Thomas around that time. A couple of students also joined me for their Ph.D. work on stress response of *Anabaena*. Together we initiated a strong program of stress proteomics in *Anabaena*.

To visualize stress-induced proteins immediately following short or long exposure to stress we devised the [^{35}S] methionine pulse radiolabeling technique for proteins and resolving them by 5-15% gradient SDS-PAGE. This gave the best resolution of *Anabaena* proteins. The first excellent publication using this methodology was on detection of salinity stress-induced proteins in two differentially salt tolerant *Anabaena* strains (J. Bacteriol. 171: 909-915, 1989) The importance of this paper lied in the fact that this was the first demonstration of *stress-induced gene expression in cyanobacteria through transcriptional ac*tivation which showed how salt-sensitive and salt tolerant strains responded to salinity within minutes of exposure to as low as 1-2 mM NaCl. The paper was not only appreciated for its scientific content but also for the quality of protein resolution in *Anabaena*. It was followed up with another paper using 2-dimensional resolution of radio-labelled proteins in *Anabaena* for the first time to show that different stresses (salinity, osmotic and heat stresses) evoked induction of some stress-specific proteins as well as some proteins that were commonly induced by all stresses (J. Bacteriol. 171: 5187-5189,1989) These two papers, published back-to-back, provided important leads for later work on (a) cloning of salinity stress-induced genes, and (b) testing the possibility of cross protection against multiple stresses by pre-exposure to one of them.

In 1989-90 while on my post-doctoral assignment to Prof. Robert Haselkorn's laboratory, we used our finding of transcriptional activation of salinity stress-induced genes to design a strategy to fish out genes differentially expressed during exposure to stress, by a

subtractive RNA hybridization procedure (Plant Molec. Biol. 15: 723-733, 1990). This enabled us to clone multiple salinity stress-induced genes (in a single experiment) from a cosmid library of *Anabaena* that was constructed for the purpose. The technique became a trend setter. The second lead from the aforesaid J. Bacteriol papers was also used to design experiments where pre-exposure to a sub-lethal level of one stress (for example, heat shock) not only enhanced tolerance to lethal dose of the same stress (heat shock) but also to a totally unrelated stress (such as salinity or purely osmotic stress triggered by sucrose). These experiments established the concept of cross protection by stresses, which is common in nature where exposure to multiple stresses occurs successively.

The publication of the above-mentioned studies and methodology, led to a spree of analysis of proteins induced by a variety of stresses across many different organisms, such as salt tolerant fungi and rhizobia, pesticide degrading bacteria, salt tolerant rice varieties and even mammalian cell proteins induced by radiation stress, by many workers (both from other divisions of BARC and from quite a few non-DAE institutes as well). Impressed by the power of this technique, Department of Science & Technology persuaded us to take up a project on Stress Proteomics of *Anabaena* strains and supported us with a major grant. We expanded studies on identification of ionic and osmotic stress-induced proteins (Appl. Env. Microbiol. 59: 899-904, 1993; J. Bacteriol. 176: 5868-5870, 1994), potassium starvation-induced novel proteins in *Anabaena* (J. Biosci. 29: 153-161, 2004), heat-shock proteins (Arch. Microbiol. 179: 423-429, 1993), and oxidative stress proteomics (Proteomics 14: 1895-1904, 2014; J. Proteomics 127: 152-160, 2015). These studies led to important projects pursued by new training school graduates, KSKRAs and students who joined us for their Ph.D. subsequently.

Dr. Alahari discovered a novel and obligatory requirement for potassium in two *Anabaena* strains and in non-heterocystous *Plectonema boryanum* (Ind. J. Biochem. Biophys., 31: 267-279, 1994). Potassium is normally needed for maintenance of intracellular pH and turgor pressure in bacteria, in addition to role in some steps of protein synthesis. In *Anabaena*, pleiotropic effects of potassium deficiency on growth, photosynthesis, nitrogen fixation and nitrate assimilation were revealed (Microbiol. 154: 1557-1563, 1998). The effects were quickly reversed upon re-addition of K^+, almost like the effects of Na^+ deficiency reported earlier. In particular, potassium starvation resulted in synthesis of nearly a dozen new proteins, which were not known until then and were termed the potassium deficiency proteins, or PDPs. Using immunodetection approach, some of them were also identified as Kdp proteins, which constitute a potassium-dependent ATPase (Kdp-ATPase) in bacteria, and their regulation was elaborated in *Anabaena* for the first time (J. Bacteriol. 183: 5778-5781, 2001).

This work was subsequently followed up to find very exciting things about the Kdp-ATPase in *Anabaena*. While details of this work are elaborated in a latter article, the key elements of the work deserve a mention here itself. Unlike *E. coli*, *Anabaena* was found to contain two *kdp* operons (Appl. Env. Microbiol. 71: 5297-5303, 2005). Both had the structural genes (*kdpABC*). One of them had a truncated *kdpD* – the transmembrane sensor, but lacked *kdpE* – the cytosolic regulator of *kdp* expression, and did not express

kdp. The other one lacked both *kdpD* and *kdpE*, but possessed interesting genes that looked like another two-component regulatory system (comprising of a sensory kinase, SK; and response regulator, RR) in the neighborhood, and expressed *kdp*. The very fascinating work involving protein engineering of truncated *kdpD* of *Anabaena* with the C-terminal half of the *kdpD* of *E. coli* to obtain a chimeric *Anacoli* KdpD protein (J. Bacteriol. 187: 4921-4927, 2005), and to investigate who and how regulated the functional *Anabaena kdp* operon (Arch. Biochem. Biophys. 474: 65-71, 2008) has been nicely reviewed recently (J. Biosci. 32: 559-568, 2007).

Another important aspect that was followed by younger colleagues was characterization of heat-shock response (HSR) in *Anabaena*. While the HSR had been characterized in the unicellular cyanobacterium *Synechocystis* earlier, not much was known about it in the filamentous, heterocystous, nitrogen-fixing *Anabaena* strains, especially its regulation. They were instrumental in cloning the two major heat shock chaperone genes/operon, *groESL* and *cpn60*, (Biochim. Biophys. Acta 1519: 143-148, 2001; Microbiol. 154: 317-325, 2008**)** and elucidating their contribution to the thermotolerance of *Anabaena* (Arch. Microbiol. 179: 423-429, 2003). For the cpn60, this work also demonstrated that the differential expression of this gene by nitrogen status and heat proportionately influenced thermotolerance of *Anabaena*. Presence of the negative regulatory element CIRCE upstream of both the chaperone genes and its regulation by the HrcA was demonstrated. A *hrcA* null mutant constitutively synthesized high levels of both Cpn60 and GroES and GroEL and notably enhanced thermotolerance. The most creditworthy finding was the regulation of *groESL* operon in *Anabaena* both by heat and light (Ach. Microbiol. 192: 729-738, 2010**)**. Further, identification and cloning of a stress induced heme-erythrin DNAse and its regulation in *Anabaena* (Arch. Biochem. Biophys. 505: 171-177, 2011) was demonstrated. Subsequently with more younger colleagues joining the attention of this group shifted to DNA repair genes and enzymes of *Anabaena*. Details of their work and significant findings on HSR and DNA repair in *Anabaena* are elaborated in an article that follows.

In 2004, the Department of Biotechnology wrote to Director, BARC requesting that our laboratory undertake work on genetic transformation of *Anabaena* by developing new tools and techniques for the purpose of enhancing nitrogen fixation potential of cyanobacteria. While they funded us well, they set up for us quite a challenging task. With the induction of a very able Research Associate (Dr. Anjali Parasnis) and a new dedicated JRF (Akhilesh Chaurasia) we set out to (1) develop an integrative expression vector for *Anabaena* (2) design a protocol for its transformation, and (3) construct some recombinant strains with higher nitrogen fixation capabilities. The strategy used to design the vector was as follows : (a) identification of an innocuous integration site in the genome wherein there were no genes whose function could be impaired upon integration (termed the F region) (b) the 900bp 'F' sequence was divided roughly in to two halves (F1 and F2) which flanked the cloning site for desired gene(s) (c) placement of an eco-friendly, light-inducible *Anabaena* promoter preceding the cloning site (d) incorporation of a suitable antibiotic resistance (neomycin resistance, Nm^r) gene with its own promoter

following the cloning site, for selection in *Anabaena*, and (e) inclusion of the entire cassette (F1-promoter-cloning site-Nm^r gene-F2) in a suicide vector [pBluescript IISK (+)] to ensure its non-replication and integration in *Anabaena*. Several trials and errors yielded a suitable electroporation buffer good for *Anabaena* electro-transformation with high efficiency. The vector was electro-transformed in to *Anabaena* and evaluated for its integration in the desired site, proper functioning of the light inducible promoter and overexpression of the candidate gene (*hetR* – which is the master regulator of heterocyst differentiation in *Anabaena*). Indeed, the transformed cells showed double the number of heterocysts and 3 to 4-fold higher rates of nitrogenase activity for a prolonged time compared to wild type *Anabaena* cells. That is how, the first integrative expression vector for *Anabaena* was created successfully (J. Microbiol. Methods 73: 133-141, 2008).

Availability of such a vector was quite a boon for strain improvement for field applications. Non-transferability of the transgene by lateral or horizontal gene transfer was an essential pre-requisite for this. Genome integration of the transgene ensured that. Several laboratories in India and a couple of them abroad requested for the vector and used it for their specific purpose. Our laboratory also constructed several agriculturally important improved strains of *Anabaena* over the next 5-6 years. Such strains were endowed with desired *cis* or *trans*-genes and constitutively over-expressed *groESL* for enhanced heat and salt tolerance (Appl. Env. Microbiol. 75: 6008-6012, 2009) or *hetR* for enhanced heterocyst frequency and nitrogen fixation (Appl. Env. Microbiol. 77: 395-399, 2011) or *linA* for pesticide lindane (γ-HCH) biodegradtion capability (Bioresource Technol. 149: 439-445, 2013) to *Anabaena*. A biosensor for detection of pesticide lindane was also designed (Anal. Chem. 84: 6672-6678, 2012). These developments more than fulfilled the DBTs mandate. The technology also paved the way for identification of oxidative stress tolerance genes and their integrative over-expression for increased stress tolerance in years to come.

The next target was tolerance to oxidative stress, which was central to all stresses since reactive oxygen species were invariably produced under all stress conditions. To start with, attention was paid to cloning and overexpression of superoxide dismutase (*sod*) genes and catalase (*kat*) genes. Two newly joined KSKRAs undertook the responsibility and did some commendable work. One of them chose to clone and overexpress the manganese and iron dependent MnSOD and FeSOD of *Anabaena* while the other targeted the peroxiredoxin (*prx*) genes of *Anabaena*. It was shown that the overexpression of MnSOD enhanced oxidative stress tolerance of *Anabaena* in nitrogen-fixing conditions, while that of FeSOD was beneficial under nitrogen-supplemented conditions (Plant Mol. Biol. 77: 407-417, 2011). Thus, the contribution of the two SODs to oxidative stress tolerance was nitrogen status dependent. An elegant paper also demonstrated N-terminal processing of membrane-targeted MnSOD and formation of multiple active molecular forms of this enzyme in different cell compartments from a single protein precursor (FEBS J. 280: 4827-4838, 2013). The membrane-targeting of MnSOD was found to be absolutely essential and offered a distinct physiological

advantage to *Anabaena* exposed to oxidative stress (Plant Mol. Biol. 88: 503-514, 2015). Two equally elegant papers revealed that a novel glutaredoxin domain containing peroxiredoxin (Biochem. J. 442: 671-680, 2012) and an unusual Mn-catalase (Environ. Microbiol. 14: 2891-2900, 2012) confer significant protection from oxidative stress to nitrogen-fixing *Anabaena* cultures. These and subsequent outstanding contributions have been elaborated in the following article.

Around the year 2002, and then onwards, we developed a new interest in *Deinococcus radiodurans*, the most radioresistant bacterium in this world. Its genome had just been sequenced in 1999 and opened up immense possibilities of doing basic research on its radiation and oxidative stress tolerance and DNA repair, and biotechnological research on its possible applications in nuclear industry, particularly in bioremediation of nuclear waste. We did both quite successfully for a decade publishing over a dozen excellent papers on the molecular basis of its radioresistance as well as on engineering this microbe for bioremediation of uranium from acidic/alkaline radioactive waste. But pertinent to the present discussion were the striking similarities between this heterotroph and *Anabaena* in terms of their radiation, desiccation and oxidative stress tolerance right up to molecular level of organization of certain operons (kdp) and their regulation. Possibilities of using photautotrophs like *Anabaena* for biotechnological exploitation were any day far more attractive than heterotrophic *Deinococcus*. Therefore, the excitement generated in *Deinococcus* trickled down to cyanobacteria as well and their radiation/desiccation tolerance and proteomics and utilization for uranium recovery were explored in some detail.

Thus, the last, (but not the least) of the stresses to be examined was ionising radiations from ^{60}Co. Earlier UV tolerance and mutagenesis was investigated in cyanobacteria by Dr. Tonina Fernandes, but nothing was really known about response to γ-rays in the hetercystous nitrogen-fixing cyanobacteria. It was found that *Anabaena* cultures exhibited very unusual resistance to ionizing radiation. No adverse effects were observed on the diazotrophic growth and nitrogen fixation in *Anabaena* exposed up to 5kGy dose of γ-rays. Higher doses affected growth and N_2 fixation, which however recovered after a short lag (J. Biosci. 35: 427-434, 2010). Such high radioresistance, as well as desiccation resistance, emanated from its phenomenal DNA repair capabilities (Photosynth. Res. 118: 71-81, 2013) which were quite comparable to those of *Deinococus radiodurans*. Equally interestingly, proteomic response of *Anabaena* to desiccation and radiation showed considerable overlap (The Protein J. 37: 608–621, 2018) as has been reported for *Deinococcus*. Addition of low concentration of ethanol during irradiation drastically reduced DNA damage *in vitro* and also protected live cultures of *Anabaena* from radiation damage (J. Biosci. 43: 15-23, 2018).

With the joining of another KSKRA, a new relevant venture was undertaken to explore the possibility of uranium removal from sea-water by selected resident marine cyanobacteria. For this, a *Synechococcus elongatus* strain BDU75042 (a unicellular cyanobacterium) was procured from the National Facility of Marine Cyanobacteria (NFMS) at Tiruchirapally. The microbe was found to remove 72% or 53.5 mg U g^{-1} dry

weight from test solutions containing 100μM U at the sea-water pH of 7.2. All the bound U was detected in the exopolysaccharide sheath using EDXRF. FT-IR spectroscopy showed that amide groups and deprotonated carbonyl groups were involved in U sequestration (Biores. Technol. 100: 2176-2181, 2009). Another brackish-water strain, *Anabaena torulosa*, similarly sequestered 48% or 56 mg $g^{-}1$ U in 30 min and 65% or 77.35 mg $g^{-}1$ U in 24h from same concentration of U (Biores. Technol. 116: 290-294, 2012**)**. Interestingly, in *A. torulosa*, the sequestered U was found to be bound to novel surface-associated polyphosphate bodies (Metallomics 5: 1595-1598, 2013). Of course, the capability of *S. elongatus* BDU 75042 to remove U from actual sea-water, where the U concentration is only 13 nM, was low and it could remove only 3 mg U g^{-1} dry weight. Impressively, from RO water (which contains 21 μM U) obtained from Desalination Division of BARC, the cyanobacterum could remove 13.3 mg g^{-1} U in 24h. Obviously, therefore, the inherent U removal capabilities were higher than sea-water concentrations and could be enhanced further by suitable manipulations, in future.

4. Future Perspective

Cyanobacterial research has had a glorious past in our country, and 4-5 national laboratories (including ours) have been globally well acclaimed in the past for the research carried out on blue-green algae. The advent of recombinant DNA technology in MBD, BARC gave us an edge over other laboratories in the country, and today MBD is recognized as a premier institution of cyanobacterial research in India. We should continue to work on these organisms particularly since (1) the interest of other institutes and universities in this area is on a decline in India, and (2) cyanobacteria are fascinating organisms which have served as model systems for studying all kinds of biological phenomena - from nitrogen fixation and photosynthesis to stress responses and circadian rhythms to biotechnologies for production of vitamins, pigments and many other natural products. Important leads have emerged from past work on cyanobacteria in MBD (for example, radio-resistance and oxidative stress tolerance) and these must be explored further, in view of their importance to the department. Outstanding quality basic research has been the strength of MBD and we should build on it further. Also, applications are a need of the hour and those of great relevance to DAE programs (such as uranium sequestration from sea-water) must be pursued vigorously. Now that the expertise is available in rDNA technologies right up to the CRISPR technology in the division, genome editing should be the way forward. I am hopeful that MBD will continue to pursue excellent programs on cyanobacteria and continue our glorious tradition in this field for few more decades.