



HIMALAYAN ECOLOGY

ISSN: 2277-9000 (Print)
ISSN: 2455-6823 (Online)

Vol. 12 (4), 2015

Bioprospecting Himalayan Microbial Diversity

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Cyanobacterial mats colonizing a hot spring in Himalaya Photo: Kailash N. Bhardwaj

The Indian Himalayan region (IHR) presents great variation, particularly in respect of topographic, geographic and the climatic conditions. This variation, in turn, supports a wide variety of habitats involving the colonization of microorganisms. One of the fascinating attributes of microorganisms is that some have evolved to thrive under "extreme" conditions that are too harsh for animals and plants. Such microbes contain enzymes that function in extreme environments and have several biotechnological applications. While the low temperature environments are excellent sites for colonization of the psychrophiles, hot springs, the manifestation of geothermal activity, provide niche habitat for a diversity of microorganisms, thermophiles in particular. The rhizospheres of diverse vegetation, growing under low temperature environments, are colonized by the microorganisms carrying traits

relevant to plant growth.

Bioprospecting of microbial resources revolves around their ability to colonize various environments, their taxonomic and genetic diversity, and the discovery of bioactive compounds. Further, it requires the facility for preservation/conservation of the microbial resources. This issue of 'ENVIS Newsletter on Himalayan Ecology' is inspired by the unique microbial diversity of IHR, focusing on its ecological as well as biotechnological prospects for human welfare. It begins with the Articles (1&2) on the diversity of the psychrophilic microorganisms from the northwestern Himalaya including the states Jammu & Kashmir (up to Ladakh) and Himachal Pradesh. These microorganisms are source of cold active enzymes of various applications in biotechnology, industry, agriculture and medicine.



ENVIS Newsletter

A Quarterly Publication Vol. 12(4), 2015

The "ENVIS Centre on Himalayan Ecology" is at G.B. Pant Institute of Himalayan Environment and Development (GBPIHED), Kosi-Katarmal, Almora, Uttarakhand, which is an autonomous Institute of Ministry of Environment, Forest & Climate Change (MoEF&CC), Government of India, New Delhi, India.

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Dear Readers,

Microorganism live in every part of biosphere, including soil, water, hot springs, air and even inside the rocks. They are crucial for organic matter decomposition and nutrient release, thus constitute the major pathway of nutrient cycling in all the ecosystems. Microorganisms are also used in biotechnology, genetic engineering and disease control. In fact, they were the first form of life on the planet earth some 3-4 billion years ago. The cold adapted microorganism in the Himalaya also hold potential for plant growth and can be utilized for increasing plant productivity.

In the Himalayan mountains biodiversity encompasses from the smallest microorganisms to giant mammals and trees. This issue of the ENVIS Newsletter endeavours to enlighten the readers about the enormous diversity of microorganisms in the IHR, and inspire meaningful discussions to know the world of these tiny organisms and their contribution to sustain life on this planet earth.

Editors

Shifting cultivation is a predominant form of agricultural practice in the hills of northeast India. It basically refers to 'slash and burn', a cyclic process that involves clearing land through the burning of natural vegetation. Fire, either as a natural or anthropogenic activity, is likely to influence the microbial dynamics that, in turn, affects fertility of soil. Article 3 summarizes the microbiological aspects of this age-old practice highlighting the role of traditional knowledge as an effective tool that provides a link between the cultural and the biological diversity.

The Himalayan rivers make crucial source of water under mountain ecosystem. The mountain streams generally contain few organisms at the source, but as they flow further the number and types of organisms increase. Coliforms, in general, and *Escherchia coli*, in particular, are important among bacterial indicators that are used in monitoring of water quality. In this background, the focus of Article 4 is on the microbial diversity in Himalayan rivers under the influence of excessive anthropogenic activities and loss of self-purification capability of water bodies. Taking example of the state of Uttarakhand, Article 5 presents the emerging importance of the diversity of photosynthetic microorganisms that colonize various habitats in Himalaya. It also highlights the probability to promote these organisms for their large-scale cultivation.

Enhanced global preference for the naturals (organics) and the need to cut down on the use of chemical fertilizers has catalyzed research on the development of plant growth promoting (PGP) microorganisms. Two major benefits of this technology are: (i) improved plant nutrition, and (ii) biocontrol of a range of pathogens. Article 6 elaborates this concept emphasizing the bioprospecting of PGP microbes with respect to the mountain

ecosystems of the Andean Highlands and the IHR, that are limited by a common set of environmental constraints. It also highlights the issues that remain to be addressed to allow a more widespread utilization of this technology. This prospect is complemented by a success story through Article 7 that demonstrates the use of native PGP bacteria in plant growth and control of fungal pathogens of saffron, a commercially important crop of Jammu & Kashmir.

Establishment of Microbial Culture Collections, with a view to conserve microbial diversity under *ex situ* conditions, is important. These collections provide ready availability of essential raw material for carrying out specific investigations and developing the novel products. Article 8 summarises the role of Microbial Culture Collections in bioprospecting and conservation of microbial resources worldwide. It further gives specific information on the accessioning of microorganisms under general, safe, and patent deposits at Microbial Culture Collection, National Centre for Cell Science, Pune, under International Depositary Authority. This Centre is also recognized as Designated National Repository for Microorganisms by Ministry of Environment, Forest & Climate Change, New Delhi, India, under Biological Diversity Act 2002.

The distinguished authors are thanked for sharing their knowledge and views on one of the integral components of the Himalayan biodiversity, i.e., microbial diversity, through their contributions.

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Microbial diversity of Indian Himalayan Region:

Jammu & Kashmir.....

Microbial diversity is the functional backbone of any ecosystem and essential for life since they perform numerous functions which are essential for the biosphere. The management and proper exploitation of microbial diversity including mainly bacteria, fungi and actinomycetes, which are mostly soil inhabiting, has an important role in sustainable development towards large-scale industrial and commercial applications. Researchers around the globe are putting a lot of thrust towards harnessing and understanding the pathways involved in production of novel secondary metabolites from them. Further, recent research studies are focused to understand their species richness, functional and phylogenetic diversity and response under changing abiotic and biotic factors as they are responsible for functioning of the ecosystem. Throughout the world the majority of these microorganisms especially in extreme habitats still remain hidden and need to be explored and utilized for better use of humankind in particular, and the environment in general. Therefore, microbial culture collections are encouraged worldwide to create novel and better techniques for bioprospecting of these novel microorganisms.

Himalayan mountain region in the subcontinent of India extends from west to east for about 2,500 km and is one of the richest bioresource of unique microflora particularly bacteria, fungi and actinomycetes which are of immense biotechnological potential. The region of northwestern Himalaya that passes through Jammu and Kashmir upto Ladakh is a composite of various climatic zones with varying high altitude peaks and different soil textures (Fig. 1). These characteristic features like different soaring heights with lush green meadows, valleys, alpine glaciers and series of different elevation zones harbour an amazing plethora of microorganisms. The deep gorges which are covered with frozen snow throughout the year and alpine glaciers are a rich reservoir of extremophilic microorganisms particularly psychrophilic/psychrotolerant bacteria and actinomycetes.



Fig. 1. High altitude peaks of Himalaya in Sonamarg

Psychrophilic microorganisms existing in permanently cold habitats and at high altitudes or polar zones are a great source of cold active enzymes. Among these cold active proteases, lipases, amylases and alkaline phosphatases etc. constitute an important group of enzymes with various applications in a wide range of industries, including food processing, dairy, agricultural and medical processes.

In the Kashmir region to the northeast lies Ladakh and to the east lies Zaskar the second coldest place of the world. A part of Himalayas in the west divides Kashmir and Himachal Pradesh from Ladakh. They all form part of Kashmir as a state, but for scientists, they are different geographical entities with hotspots of unique microbial biodiversity. The high saline soils and the dry high plains of the Ladakh region are a huge resource of halophilic microorganisms, which require

salt as an essential nutrient for their growth and radio resistant bacteria and fungi. These organisms have resistance to high levels of ionizing radiation, most commonly ultraviolet radiation.

The greater part of the Himalayas, however, lies below the snow line where mostly mesophilic bacteria, fungi, actinomycetes and potential endophytes associated with medicinal plants and trees occur. These low elevation areas and slopes generally have a fairly thick soil cover, supporting dense forests along with a variety of medicinal plants and grasses. *Atropa belladonna*, *Dioscorea deltoidea*, *Artemisia* spp., *Thymus linearis* etc. are some of the high value medicinal plants growing under the shade of these forests. Temperate mixed forests contain conifers like *Taxus* species including *Taxus wallichiana* and broad-leaved temperate trees. The forest soils at these elevations are dark brown in colour and silt loam in texture. They are ideally suited for the growth of rich medicinal plant like *Sassurea costus*, *Rheum emodi*, *Picrorhiza kurroa*, *Arnebia benthamii* etc. All the medicinal plants in different elevations of this zone are a tremendous source of bioactive endophytic bacteria and fungi.

Some of the major high altitude areas of Kashmir Himalaya like Thajiwas glacier, Kolahoi, Haramukh, Amarnath glacier and the Apharwat and its environs are a great source of psychrophilic microorganisms. Shrubs of *Junipers* and *Rhododendron* are widespread especially on the steep and rocky slopes and the dried areas are a source of novel endophytes (Fig. 2). Lithotrophic microorganisms mostly inhabit the imperfectly weathered rock fragments in this area.



Fig. 2. Collection for endophytes from Apharwat peak

Looking into the extent of microbial biodiversity of the entire Indian Himalayan region, particularly in Kashmir, the chance of finding novel enzyme producing microorganisms which may be exploited for their use in biotechnological applications is certain. Studying the diversity of Archaea, Bacteria and Eucarya inhabiting the various climatic zones of Indian Himalaya with its temperate, subtropical, tropical and cold environments will give an insight into the breadth and type of biological products and processes that might be exploited in future for whole of mankind.

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Microbial diversity of Indian Himalayan Region:

Himachal Pradesh.....

Himachal Pradesh (HP) has enormous climatic, geographic and socio-cultural diversity. This state is divided into four agroclimatic zones viz. Shivalik Hill, Mid Hill, High Hill and Cold Dry zones, which depict subtropical, sub humid, temperate wet and temperate dry climatic conditions, respectively. Geoclimatic variations have enabled the state to cultivate a range of conventional and non conventional grains, pulses, oil plants, vegetables, flowers and fruits. Its large area is under forest cover comprising hardwood and softwood forests. A number of rivers originate from the state and there are many artificial and natural lakes. This has made HP as one of the biodiversity rich regions of India.

The biodiversity has become an important subject in view of global warming, environmental pollution, food security, health issues and economic growth. Microbial diversity encompasses a spectrum of microscopic organisms including bacteria, actinomycetes, algae, fungi, protozoa and viruses. The microbial diversity of HP becomes more interesting in view of microbes associated with endemic plants, animals, foods and specialized ecological niches e.g. thermal springs, cold deserts, glacial lakes, salt mines, etc. (Fig. 1). The microbiologists of HP (HP University, CSK Himachal Pradesh Krishi Vishvavidyalaya, Dr Y.S. Parmar University of Horticulture and Forestry, ICAR-Central Potato Research Institute, CSIR-Institute of Bioresource Technology, CRI Kasauli, ICAR-Directorate of Mushroom Research and several private Universities) and outside have explored the microbial diversity; some of the organisms explored are listed below:

Mesophilic bacteria: Species belonging to *Acetobacter*, *Acidomonas*, *Acidophilum*, *Acinetobacter*, *Actinomyces*, *Aerococcus*, *Alcaligenes*, *Azotobacter*, *Bacillus*, *Campylobacter*, *Carnobacterium*, *Cellulomonas*, *Cornybacterium*, *Flavobacterium*, *Kurthia*, *Listeria*, *Mesophilobacter*, *Micrococcus*, *Renibacterium*, *Rhizobium*, *Streptococcus*, *Burkholderia*, *Enterobacter*, *Enterococcus*, *Kocuria* and *Lactobacillus* (Thakur et al., 2015), *Lactococcus lactis*, *Leuconostoc mesenteroides*, *Microbacterium saperdae*, *Pediococcus pentosaceus*, *Pseudomonas putida*, *P. synxantha*, *Serratia marcescens*, *Staphylococcus* sp., *Staphylococcus sciuri*, and *Weissella cibaria* (Fig. 2 a,b).

Thermophilic bacteria: *Bacillus licheniformis*, *B. megaterium*, *B. sporothermodurans*, *Brevibacillus thermoruber*, *Geobacillus pallidus*, *Geobacillus subterraneus*, *Hydrogenobacter* sp., *Paenibacillus* sp., *Pyrobaculum aerophilum*, *P. caldifontis*, *Thermus brockianus*, *T. thermophilus*.

Halophilic bacteria: *Halobacillus* sp., *Halomonas* sp., *Marinomonas* sp. and *Shewanella* sp.

Psychrophilic and psychrotrophic bacteria: *Janthinobacterium lividum*, *Serratia quinivorans* A5-2 and *S. quinivorans* B8.

Actinomycetes: *Frankia* sp., *Gordoniaterrae*, *Micromonospora endolithica*, *Nocardia globulera*, *Rhodococcus rhodochrous*, *Streptomyces griseus*, *Streptomyces* sp.

Thermophilic cyanobacteria: *Aphanocapsa*, *Calothrix brevissima*, *C. parietina*, *Chroococcus*, *Coelosphaerium*, *Entophysalis granulose*, *Leptochaete hansqirgi*, *Lynqbya*, *Mastigocladus*, *Microcytic stagnalis*, *Oscillatoria*, *Phormidium*, *Plectonema notatum*, *Scytonema leptobasis*, *Spirulina subsalasa* and *Synechococcus elongates*.

Yeast: *Candida bombicola*, *C. chiropterorum*, *C. glabrata*, *C. parapsilopsis*, *C. tropicalis*, *Debaromyces* sp., *Endomyces fibuligera*, *Geotrichum candidum*, *Kluyveromyces thermotolerans*, *Pichia anomala*, *P. burtonii*, *P. kudriavzevii*, *Saccharomyces cerevisiae*, *Saccharomycopsis fibuligera* (Fig. 2c), *Saccharomyces* spp. and *Zygosaccharomyces bisporus* (Thakur et al., 2015).

Major genera and species of fungi: *Alternaria*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Cunninghamella*, *Curvularia*,

Drechslera, *Epicoccum*, *Fusarium*, *Gliocladium*, *Monilia*, *Mucor circinelloides*, *M. hiemalis*, *Rhizopus chinensis*, *Rhizopus oryzae* and *R. stolonifer* (Sagar, 2005).

Mushrooms: *Agaricus compestris*, *Amanita* sp., *Boletus* sp., *Cantharellus cibarius*, *Gyromitra* sp., *Helvella compressa*, *Hericium erinaceus*, *Humaria hemisphaeria*, *Hygrophorus* sp., *Laccaria* sp., *Lactarius deliciosus*, *Lycoperdon* sp., *Macrolepiota procera*, *Morchella conica*, *M. deliciosa*, *M. esculenta*, *Pleurotus cornucopiae*, *Ramaria botrytoides* and *Rhizopogon* sp. (Lakhanpal, 1996).

The microbial diversity explored/reported hitherto is only a bit of the mole. There is huge scope to explore the unexplored habitats, specialized ecological niches, rhizospheres and phyllospheres of the plants, animals, traditional fermented foods and beverages of Himachal Pradesh, to isolate, characterize and report the microbial diversity. The places which are beyond the reach providing undisturbed natural habitat and sites need to be focused for finding novel microorganisms. There is a need to establish microbial culture collections and identification facilities in the state for preservation of the invisible microbes for agricultural, industrial, environmental and medical applications.



Fig. 1. Some ecological niches (a) Cold desert area in Spiti (b) Dry mountains near Mani Mahesh lake, Chamba (c) Parashar lake, Mandi (d) Thermal springs, Manikaran in Himachal Pradesh for exploration of microbial diversity

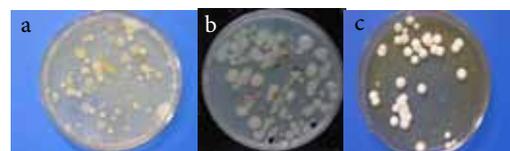


Fig. 2. Some microbes isolated from (a) soil (b) water and (c) traditional foods of Himachal Pradesh

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Microbial diversity of Indian Himalayan Region:

Jhum Agroecosystem of North East Himalaya.....

North East Himalaya (NEH) of Indian subcontinent is comprised of 65% mountainous area and 35% area under Brahmaputra and Barak valley. In IUCN's account, its biodiversity is covered within the two biodiversity hotspots namely, Indian region of Indo-Burma and Eastern Himalaya biodiversity hotspots. Six types of vegetation ranging from tropical moist deciduous to Alpine forests holds a large share of the plant diversity of the earth along with rich diversity of fauna. Compared to flora and faunal diversity, data and knowledge on microbial diversity and its role on functions of ecosystems in NEH is very limited. In about 5476 sq. km. (1.6 million ha) area of NEH, *jhum* farming is practiced which provide livelihood support to about 4.5 to 5.0 lakh households and it is the way of life for the different tribes in NEH.

Jhumming or Slash and burn agriculture (SAB) is a practice of farming in hills carried out by cutting all vegetation of an area, then burning during Jan-April and growing crop for successive two years. First year crop harvest in Oct-Nov is followed by a fallow period until next year Feb-March during which operation for 2nd year cropping begins. After 2nd year crop harvest, the *jhum* field is followed to allow natural revegetation for varying period of 3

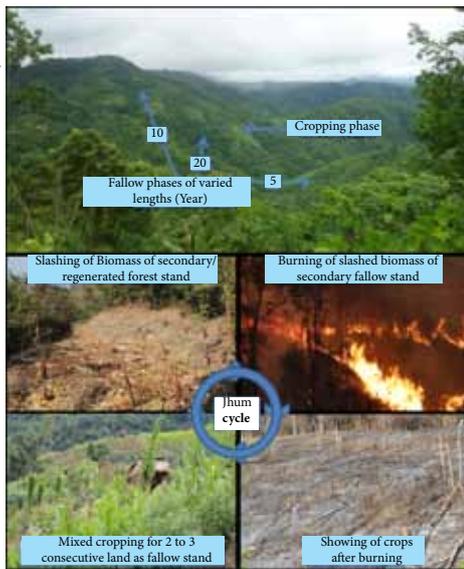


Fig.1. Various phases of *Jhum* cultivation

to 20 years (Fig. 1) However, there are several reports of adverse effects of *jhum* farming in the hills including degradation of natural resources, loss of biodiversity, both plants and beneficial microorganisms, due to rapid decline of *jhum* cycle from average 10-20 years to 5-6 years. Shorter fallow cycle leads to mountain ecosystem instability, soil erosion and siltation of rivers and frequent flood in valleys; but yet this agroecosystem maintains a rich agrobiodiversity base and is intricately linked with socio-cultural fabric of inhabitants of hills of NE India. Scientific research to improve the system has been limited. Although, it is known that cultivation of as many as 35 different crops are practiced in *jhum* farming, there are very little information on microbial diversity and dynamics in *jhum* crop fields of NEH.

Research on microbial diversity in fired plot soils generated initial data on bacteria and actinomycetes. A total of 35 actinomycetes in the *Streptomyces*, *Kitasatospora* and *Nocardia* and several other genera were isolated from such fired plots of Arunachal Pradesh and some of these isolates were capable of producing chitinases, glucanases, amylases, lipases and proteases enzymes. Similarly, several bacterial species in the genera *Bacillus* and *Pseudomonas* were isolated from the fired plots. Another study showed that firing caused only a transient rise of temperature up to 100°C within 0-5 cm with no significant effect on microbial diversity in the soil profile. These initial data pointed out the need for more systematic investigation on microbial diversity and its role on crops in agroecosystems.

A recent classical and molecular approach (PCR-DGGE) based analysis of soils of rice, maize, and arhar crop fields in the 2nd year of the cropping phase in the *jhum* fields of 5, 10 and 20 year fallow cycles at Changki village of Nagaland generated interesting data on diversity of types and population of Arbuscular Mycorrhizal Fungi (AMF). Roots

of these crops also showed differences in the extent of their colonization by AMF structures such as arbuscules, vesicles and hyphae. In general, AMF spore density (53-212 μm size range) and arbuscular colonization in roots decreased with the increase in the length of *jhum* cycles. Based on spore morphology, three dominant types of AMF spore were found

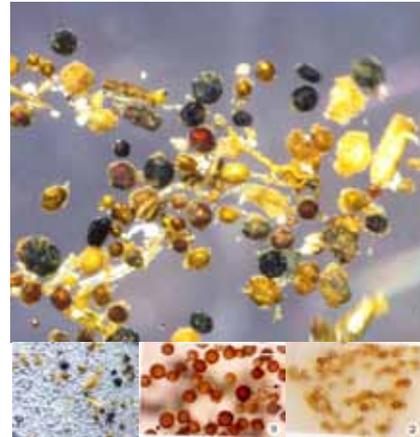


Fig. 2. AMF spore mixture and separation of 3 types of spores

in *jhum* field soils (Fig. 2). However, DGGE profile of AMF primer based PCR amplification of root DNA suggested greater diversity of AMF in different fields. Interesting data was also obtained on the diversity of soil and rhizosphere bacteria in the crop fields of the three different *jhum* cycles. It is known that distribution of microorganisms in the plough layer of a soil gets re-oriented when a crop root starts proliferating in it. However, influence of roots on soil at different distance is expected to be different and therefore, a strategic sampling procedure ensured separation of non-rhizosphere (NRS), loosely adhered rhizosphere (LARS) and strongly adhered rhizosphere (SARS) soils to see the diversity of bacteria. The number of bacteria in the interior of the same root from which LARS and SARS separated was also obtained. The general trend of bacterial population (cfu/g dry material) irrespective of crops of *jhum* cycle was: strongly adhered rhizosphere soil (SARS) > loosely adhered rhizosphere soil (LARS) > bulk soil (BS) > root interior. The population of phosphate solubilizing bacteria, fluorescent *Pseudomonas* and *Azospirillum* in SARS of different crop rhizosphere was found to decrease with age of *jhum* cycle. The rhizosphere soils of crop grown in the fields of longer fallow cycle also contained less AMF spores.

These preliminary results of below ground microbial diversity in crop fields of the unique *jhum* agroecosystem suggest that increase in fallow period of a *jhum* cycle restores stability in terms of physico-chemical and biological properties. It appears that the fields used to grow crops after burning the long fallow period's vegetation is also congenial in terms of physico-chemical and biological properties and perhaps crops grown in it require less support from bacteria. Analysis of more samples in future will provide insight into ecological implication of the observed pattern of AMF and bacterial diversity in *jhum* agroecosystem. This preliminary data also indicated that the crops grown in the fields of the short duration fallow might respond more to inoculation with efficient strains of rhizobacteria. In fact, inoculation of a consortia of four rhizobacteria (posses efficient P-solubilization, N_2 fixation and IAA production ability) @4.0 kg inoculum/100 kg seeds increased grain yield of rice grown in a five year fallow plot after slash and burn by 25.0 and 21.6% over the uninoculated rice during 2013 and 2014, respectively. *Jhum* agro ecosystem has preserved crop diversity for the ethnic tribes of NEH from time immemorial. This research initiative to understand microbial diversity and its function in *jhum* agro-ecosystems is promising in terms of enhancing the crop productivity and ecosystem restoration. There is a need of intensified efforts in this direction.

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Microbial diversity of Indian Himalayan Region:

Aquatic Microbial Diversity - Himalayan Rivers.....

The Himalayan Rivers have a paramount location in Indian culture and tradition. Uttarakhand is a state in northern part of India and privileged with a range of splendid Himalayan peaks that attracts nature lovers worldwide as well as offers a prehistoric part of nature, art, and culture, and also known for its natural beauty. The river Ganga is the composite of two major tributaries including Bhagirathi and Alaknanda that amalgamate at Dev Prayag in Uttarakhand. The origin of Bhagirathi is Gangotri glacier that is thought to be the original source of River Ganga, located between 30°56'N and 79°04'– 79°15'E whereas Alaknanda originates from holy shrine of Badrinath (Sood et al., 2008).

However, water standard of Himalayan Rivers is continuously worsening due to anthropogenic activities, dumping of non-treated wastes, poorly constructed drainage and sewage systems etc. Recently, the influence of such activities has been so substantial that directly led to loss of self-purification capability of water bodies. Furthermore, since mass bathing in water systems is considered as a historic ritual in India during festivals, individuals of country take holy dip in river. Therefore, it is the high time to recognise and understand the need of conserving and sustaining aquatic water systems or an ecosystem like Ganga.

Microorganisms consist of approx 60% of the Earth's biomass and its diversity and density can be utilized for indicating the acceptable quality of water. Most freshwater systems in India comprise of rich biodiversity spots which needs to be explored, exploited and conserved. The climate, geo-chemical, geo-morphological and pollution conditions governs the physico-chemical attributes of fresh water bodies. The water quality characteristics influence the ability of species living in a given river habitat. It is essential for sustaining all forms of life in aquatic systems for general well-being. Using bacteria to indicate quality of water has two perspectives. First, the presence of such bacteria gives the evidence of water contaminated with faecal coliforms and second it highlights the health risk that faecal impurity poses.

Aquatic biodiversity plays a prime role within the aquatic ecosystems so as to maintain the stable nature and cope up with any changes in its environment. Garhwal and Kumaun are the two regions that comprise Uttarakhand. Since it's a hill state, the geographical parameters completely differ from other Indian plain regions. Ganges water sources at various locations in Uttarakhand are shown in Fig.1.

Aquatic environments act as a natural habitat of diverse range of microorganisms, with both beneficial and pathogenic characteristics. The beneficial properties of microbes include clearing of wastes from water, removal of heavy metal of pesticide contamination, making the water suitable for daily use etc. Pathogenic bacteria are considered as etiological agents of infectious diseases to human and marine animals. There are number of parameters namely, turbidity, TDS, alkalinity, calcium and magnesium hardness, iron concentration that identifies the level of contamination in aquatic systems. Out of these parameters, higher level of iron concentration than desirable and permissible limits reflects anthropogenic and geogenic activities in that system. Similarly, aquatic systems highly contaminated with total and faecal coliform is indicative of contamination from sewerages. The riverine area of Indian Himalayan region consists of number of microorganisms including *Enterobacter*, *Proteus* and *Staphylococcus* etc. *Staphylococci* are considered to be the most dominant pathogenic species in hospital-acquired infections, as hospital outbreaks because of methicillin-resistant *Staphylococcus aureus* i.e. MRSA have become a serious problem in nosocomial infections (Shah et al., 2007). It is possible that contaminated sea and river systems may serve as potential reserves of healthcare and community-associated MRSA, if these sources become contaminated with such strains (Tolba et al., 2008). Also there is high count of *E. coli* in Gangetic river system of Uttarakhand signalling the presence of pathogenic microbes of intestinal origin. Alakhnanda, Bhagirathi, Ganga, Yamuna and Mandakini rivers are found to contain *E. coli*, *Staphylococcus aureus*, *Bacillus* and *Pseudomonas* spp.

Freshwater bodies, especially microbial diversity, of Himalayan Rivers are found to be contaminated with various types of pollutants resulting from disposal of domestic and municipal wastes causing undesirable change in them. Hence, the main purpose of analysing physical, chemical and microbiological characteristics of such aquatic systems is to determine its pollution status. Therefore, protection of these systems can be regulated by monitoring microbial load on periodic basis. Also, it is necessary to understand the pathogenic bacterial genera in Himalayan river system and to develop measures that may act as indicators of water quality and pollution.

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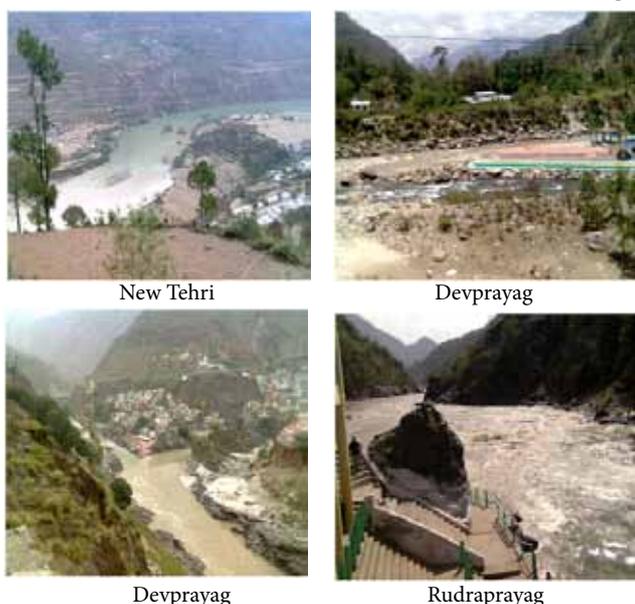
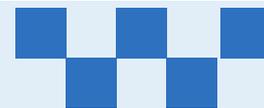


Fig. 1. Ganges water at various locations in Uttarakhand



Microbial diversity of Indian Himalayan Region:

Cyanobacteria & Algae- Bioprospecting Opportunities in Uttarakhand.....

Cyanobacteria and algae are photosynthetic microorganisms which by virtue of their remarkable features have emerged as promising bioresource for biotechnological applications (Box 1). However, as compared to bacteria and fungi, these photoautotrophic microorganisms have relatively been ignored since past many years in main stream of R&D. Consequently, fragmentary information persists on diversity and potential applications of these bioresources in Uttarakhand. In view of diverse habitats including some extremes like geothermal springs, which persist for these remarkable bioresources in Uttarakhand, they need worth attention of researchers and academicians.

Box 1: Inherent remarkable features of cyanobacteria and algae

- ⇒ Superior photo-conversion efficiency (PCE) [up to 3-9% as compared to ≤ 0.25 -3% achieved by traditional crops]
- ⇒ Utilize CO₂ in photosynthesis and help to achieve carbon neutral production process
- ⇒ Require inexpensive minimal nutrients for growth and less land area for cultivation
- ⇒ Residual biomass as a left-over after high-value products extraction that can be used as animal feed or organic fertilizer
- ⇒ Being prokaryotes, cyanobacteria can be easily manipulated for desired application

As far as the perspective of cyanobacterial diversity in Uttarakhand is concerned, a total of 53 species belonging to 26 genera have been reported from aquatic habitats. In addition, 31 thermophilic cyanobacterial species belonging to 14 genera have recently been reported from geothermal springs (Fig.1; Bhardwaj et al., 2011). A total of 346 species belonging to 122 genera of algae has also been reported from Uttarakhand. Prospecting of cyanobacteria and algae encompasses a great array of possibilities for further research and development among which algal biofuel production is the prominent one (Georgianna and Mayfield, 2012). Numerous bioactive compounds, such as carotenoid, phycobiliproteins, lutein, biohydrogen, omega-3 fatty acids, scytonemin, poly- β -hydroxybutyrates, anti-HIV protein, lipids, vitamins, antimicrobial, anticancer, etc. have been reported from cyanobacteria and algae (Chang et al., 2015). Bio-sequestration of atmospheric CO₂ and flue gas containing CO₂ and wastewater treatment are some other notable applications of these bioresources.

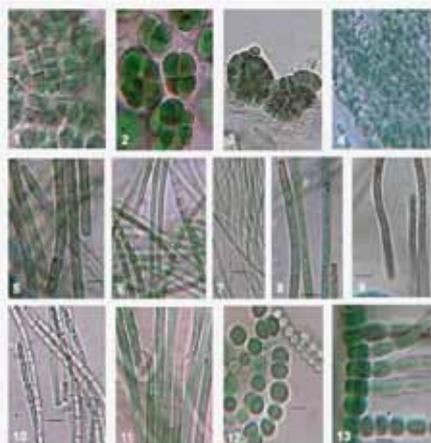


Fig. 1. Cultured thermophilic cyanobacteria - 1. *Chroococcus minimus*; 2. *Myxosarcina* sp.; 3. *Hydrococcus* sp.; 4. *Synechococcus elongates*; 5. *Phormidium corium*; 6. *P. bohneri*; 7. *P. frigidum*; 8. *P. abronema*; 9. *P. cebennense*; 10. *Pseudanabaena galeata*; 11. *Oscillatoria animalis*; 12. *Chlorogloeopsis* sp.; 13. *Mastigocladus laminosus* (*Fischerella*) (scale bars: 5 μ m)

Efforts have been made in large-scale cultivation of cyanobacteria and algae of potential biotechnological applications around the world applying open ponds (natural like lakes, lagoon, and ponds or engineered like paddle-wheel driven raceway and circular cemented ponds) and closed photobioreactors. Generally, open pond cultivation is cost-effective but associated with contamination issues, excessive space requirements and limited location possibilities due to prevailing climate. Closed systems are more capital intensive but provide precise control. Operating cost is relative to productivity. Photo-conversion efficiency (PCE) in photosynthesis has also been thrust area of research to enhance productivity. Minimizing or truncating the chlorophyll antenna size of the photosystems in genetically modified strains of photosynthetic organisms has been observed to improve PCE and productivity.

To assess the environmental, economical, and social sustainability of biotech industry based on these bioresources, there is a need for integrated resource assessments (IRA), techno-economic assessment (TEA) and life-cycle assessment (LCA). Improvements in critical cultivation parameters (PCE or content of bioactive compound) and market factors can bring favourable results for prospecting of these bioresources. Some of the companies have developed ventures for algae based commercial biotechnological applications: For ethanol and other biofuels (www.algenol.com/); antioxidant astaxanthin (www.algatech.com) oil (www.solazyme.com; www.syntheticgenomics.com/140710.html; www.kaibioenergy.com) in abroad, while for oil (www.altretgreenfuels.com; www.ebtiplc.com/default.htm; www.sea6energy.com) in India. Cultivation of nitrogen fixing algae and cyanobacteria for biofertilizer and *Spirulina* as protein for animal and fish feed in flooded paddy fields of Tarai region may be a potential option. It will not only make use of the surplus water but also greatly increase its oxygen content, thereby, controlling development of potential methane (GHG) emitting methanogens.

Bioprospecting of cyanobacteria and algae in Uttarakhand will have promising academic and commercial implications. Therefore, research and academic institutions in the state should adopt and strategize R&D on their diversity and bioprospecting. R&D will further open new vistas for their applications and promotion in biotech based industries. A symbiotic association of government and academia may bring remarkable success by translating research outcome to economy. In order to explore avenues in the field of bioprospecting of these bioresources, priority should be given to exhaustive assessment of their diversity in normal and extreme habitats, establishment of culture repository, assessment of potential biotechnological applications, exploration of commercial cultivation with their TEA and LCA.

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Plant growth-promoting microbes

Bioprospecting Opportunities in Cold-mountainous Ecosystems.....

Microbes thrive everywhere. We cannot see them but they are there: present in extremely high numbers in soils, rivers, lakes, oceans, even in our entire bodies. They are the “unseen majority” constantly remodeling the earth’s surface, driving the cycle of many nutrients, determining health (and disease) of other living beings, purifying waters and fertilizing soils.

Microbes were the first living beings to appear on earth, approximately 3.8 billion years ago. They were here long before the rise of humans, colonizing our same habitat. But some of them are also able to thrive under the most extreme conditions we can imagine: either in hot springs or in cold deserts of ice; in the presence of high concentrations of toxic metals or salts; several kilometers deep inside the earth or several kilometers high in the upper troposphere; in sediments located under the seafloor or in glaciers at the top of the mountains. That is why they are known as *extremophiles*.

Microbiologists love to search for these unexpectedly strange living beings. Every biotope on earth is being actively prospected in order to detect, isolate, culture, identify and preserve extremophiles. There is an enormous interest in unraveling the mechanisms and strategies they have evolved to cope with such extreme conditions. But the main motor behind this search is the desire to discover potentially useful microbes for the development of new (bio)technologies.



Andean Agriculture

Extremophiles and mountainous agriculture development

Mountainous agriculture, both in the Indian Himalayan Region (IHR) and in the Andean Highlands, is limited by a common set of environmental constraints, namely little access to irrigation, low fertility of soils, threat from specialist herbivores (pests and pathogens) and cool temperatures. In addition, small farm-holders have limited access to fertilizers, pesticides, improved crop varieties, mechanized tillage and irrigation. Therefore, although sustainable, mountainous agriculture remains a low input, low production and subsistence activity.

In 1992, the International Center for Integrated Mountain Development (ICIMOD) highlighted the necessity of considering mountain biodiversity as a repository of elements for future prospection and insisted on the urgent need of protecting this biodiversity to harness its potential for developing technological options for sustainable agriculture (Jodha et al., 1992). Following these recommendations, in the early 2000s Indian scientists started to bioprospect diverse Himalayan biotopes to explore their microbial wealth and diversity and began to isolate microorganisms with potential applications in mountainous agriculture (Trivedi et al., 2012). Indeed, microbes colonizing the high-mountains have adapted, superbly, through millions of years, to the prevailing, harsh climatic conditions. It is no surprise –therefore– that one of the most important and promising groups of mountainous microorganisms are cold-adapted or “psychrophiles”.

For several years, psychrophiles have been employed to develop cold-active detergents, cosmetic treatments, improved foods

and pharmaceuticals. But in the past 15 years, psychrophiles started to be employed to develop a radically different product: *microbial inoculants*. These are mixtures of living microorganisms able to promote plant growth and development by different mechanisms. For example, some of these microbes act as *biofertilizers*, providing plants with some important nutrients (like nitrogen or phosphorus); others protect plants from pathogens (*biocontrollers*); others produce some chemical compounds that stimulate plant growth in a direct way (*phytostimulators*). Very often, the same microbe exhibits –indeed– a combination of these abilities.

Bioprospecting high mountain ecosystems for plant-growth promoting micro organisms

Where can such kind of microorganisms be found? Intuitively, the most logical place to look for plant-growth promoting (PGP) microorganisms is the soil immediately surrounding the roots of plants, the so-called *rhizosphere*. If the crops have been cultivated for centuries, it is highly probable that strong mutualistic relationships –beneficial to both biological partners– may have evolved over time. Therefore, many studies have been conducted searching for potential PGP microorganisms in the rhizosphere of many important crops. For example, this is the case of potato or fava bean fields in the Andean Highlands (Fonte et al., 2012), and maize, lentil or amaranth in the IHR.

Many sites from different altitudes in the IHR, including forest soils and cold deserts, have also been investigated for PGP microorganisms. Bacteria, actinomycetes and fungi have been isolated and shown to exhibit different degrees of PGP activities. In several cases, the PGP effect has been confirmed in greenhouse and in field experiments. This search, both extensive and systematic, permitted –among others– the creation of a culture collection of high altitude bacteria where they are preserved adequately (Trivedi et al., 2012).

Up to date some success has been achieved using psychrophilic or psychrotolerant PGP microbes to promote growth of many crops. However, there are still a number of issues that remain to be addressed to allow a more widespread utilization of this promising biotechnology. For example, more information should be gained concerning the microbial wealth of mountainous ecosystems and their prospection to find potential microbial inoculants. On the other hand, new ways to formulate, store and apply these microbial inoculants need to be explored. Moreover, studies focusing on the plant-microbe interactions and on the effect of introduced microorganisms on resident microflora interactions will be of paramount importance. Finally, and perhaps one of the most important aspects to consider: a tremendous effort should be directed towards educating the potential users of this technology –the small farmers– to convince them of the many benefits inherent to it and to fight the popular misconception that consider microbes only as agents of disease.

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Plant growth-promoting microbes

Native Bacilli for Saffron in Jammu & Kashmir.....

The name saffron comes from an Arabo-persian word “za’faran”. Iran is the leading producer of saffron followed by Spain, India, Morocco and Italy. In India, saffron is produced only in Kashmir and Kishtwar regions of J&K. Saffron scientifically termed as *Crocus sativus* is a much valued spice endowed with some interesting medicinal properties. The flower in saffron consists of three stigmas and the stigma when dried becomes saffron, the spice. The stigma contains important constituents like crocetin, picrocrocin, safranal. Safranal is the main component for the particular aroma of saffron while the orange yellow colour is due to crocin and its flavouring property is contributed by picrocin. In ancient Ayurveda, saffron is reported to be used to cure various chronic diseases like arthritis, skin diseases, asthma, liver and spleen enlargement, kidney disorders etc. Thus it's aptly named as the **golden condiment** as well.

Being a sterile plant, saffron fails to produce viable seeds, therefore, it reproduces vegetatively by underground bulb-like starch storing organ known as corm. It has unique life cycle stages. One complete cycle consists of the dormant phase followed by the flowering and then the vegetative phase (Fig.1). The sowing of the seed / corm is done in the dormant stage (June–August) and at this stage the corm does not bear roots or leaves. The roots start emerging from the corms in the beginning of September and by November they get fully developed. The herb flowers from mid October to mid November (flowering stage) while the leaves start developing in the beginning of January with emergence of narrow grass-like green leaves from the head of the corm (Ambardar and Vakhlu, 2013).

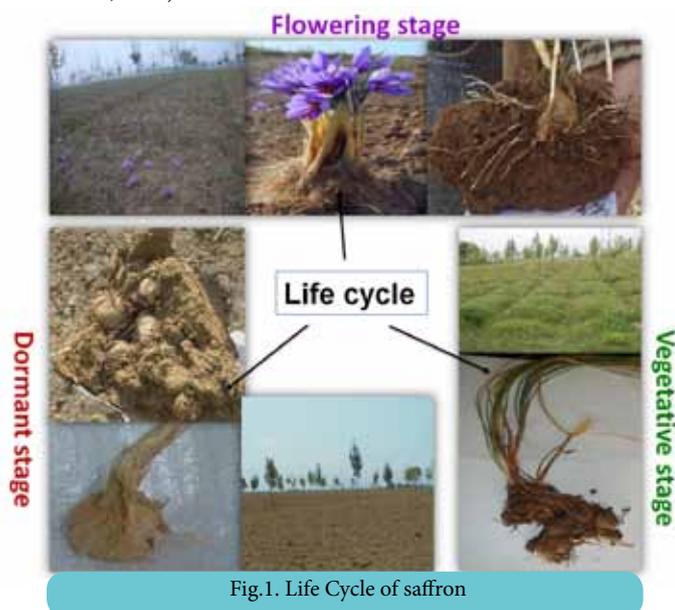


Fig.1. Life Cycle of saffron

Total world's production of saffron is estimated to be around 250 tons, 90% of which is being produced in Iran and rest 10% is contributed by others including India but its production has declined from 16 metric tons to 9.5 metric tons during the period 1996–97 to 2009–10. The probable reasons behind this decline can be a number of biotic as well as abiotic factors like environment, temperature, moisture content of the soil and microflora of the soil. One of the most prevalent factors responsible for this decline are the pathogens. A fungal pathogen, *Fusarium*, infects the corm of the plant and causes the most prevalent corm rot disease. With a disease incidence of 100% and severity ranging from 6 to 46%, corm rot diseases result in reduced plant growth and yield of saffron (Gupta and Vakhlu, 2015). As the infection initiates through corm, which is an organ for propagation in plant, the disease also affects quality and quantity of the material available for propagation. The microbes live in a symbiotic association with the belowground and above ground parts of the plant making certain nutrients freely

available to the plant and also protecting the plant against certain pathogens while, in turn, survive on the sugars and proteins released by the roots and on the starch in the corm. There are certain microbes that influence the growth and production of the plant and these are termed as PGPs i.e. plant growth promoters. These PGPs have been reported to enhance the growth by various direct and indirect mechanisms. Directly they increase the nutrient cycling such as biological nitrogen fixation, solubilization of phosphorous, production of siderophores important in iron chelation to the plant thus making the iron available to the plant, synthesis of phytohormones like auxins. Indirect mechanisms include the synthesis of certain biocontrol compounds that inhibit the phytopathogens thus protecting the plant. In our lab we have been working on isolating the microbial strains as potent PGPs from the saffron. Among the various microbes native bacilli have been our target to be used as plant growth promoter and a biocontrol agent.



Fig.2. PGP isolation and sequencing

A total of 1000 bacillus isolates were isolated randomly from the rhizosphere and cormosphere of saffron on different nutrient media using the heat shock treatments at a high temperature (800 °C). Out of which 21 were selected after screening for multiple PGP activities like siderophore production, phosphate solubilization, antifungal activity, Indole acetic acid production (Fig. 2). These bacilli were then identified by sequence homology of V1–V3 region of 16S rRNA gene. Out of the identified bacillus isolates, consortia with three isolates *B. aryabhattai*, *B. megaterium* and *B. thuringiensis* with complimentary PGP traits and antagonism towards pathogenic fungus *Fusarium oxysporum* causative agent for corm rot, were checked for their efficacy *in-vivo* by pot assays. The increase in average number and length of roots and shoots was statistically significant. The incidence of corm rot disease was less (20%) in test as compared to control (60%). These strains showed positive results in pot trials and are under observation in field trials. If field trials also show effective results then this formulation can be a subject for commercial use as a substitute for chemical fertilizers.

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Microbial Culture Collection:

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Microorganisms are important constituents of ecosystems of earth because of their ability to grow in extreme conditions as well as maintaining sustainable biosphere and productivity. As with other forms of diversity, events like global warming, life style changes and anthropogenic activities are resulting in the loss of microbial diversity. The Himalayas are the main victims of these activities that have been seen in last couple of years. There is a great variation in the Himalayas with respect to the environmental conditions which makes them a biological hot spot and there is a concern that the Himalayan hotspots (especially glaciers and hot springs) are getting degraded because of global warming, landslides, dam & road constructions and other environmental threats. Being a diverse habitat, Himalaya is the important reservoir of microbial diversity especially, extremophilic microorganisms whose biotechnological importance has been realized in the recent years. Therefore, the preservation of microbial diversity, from highly susceptible ecosystems such as Indian Himalaya, is necessary to prevent the loss due to climatic change and environmental perturbation.

Recent technologies in molecular biology have resulted in the continued discovery of novel strains and there is a need to preserve these so as to make them accessible for biotechnological exploitation as well as for research and teaching purpose. Good infrastructure and expertise in long term preservation, characterization and identification of diverse group of microorganisms is necessary for a good service culture collection which is difficult for a research group or laboratory to maintain. To conserve these industrially important microorganisms, culture collections play a vital role to prevent this loss of microbial diversity by long term preservation. Worldwide, there are around 714 culture collections in 72 countries registered with World Data Centre for Microorganisms (WDCM) database till 2014, which are supported by different government and private funding agencies. There are around 11 culture collections registered with WDCM from Africa, 174 from America, 244 from Asia, 42 from Oceania and 231 from Europe (www.wfcc.info). There are around 31 culture collections in India registered with WDCM among which very few are active for providing services to the scientific community. Microbial Culture Collection (MCC), Pune is one among these few active culture collections which was established in 2007 by Department of Biotechnology, Govt. of India, New Delhi.

MCC has been recognized as International Depository Authority (IDA) by the World Intellectual Property Organization, Geneva, Switzerland under Budapest Treaty on 9th April 2011. It is also recognized as Designated National Repository for Microorganisms on 8th July 2013 by Ministry of Environment, Forest and Climate Change (MoEF&CC), New Delhi, India under Biological Diversity Act 2002 (Sharma and Shouche, 2014). MCC has declared as largest culture collection by WFCC because of high number of strain holding (Table 1).

Table 1. Largest culture collections with holdings

Rank	Collection	Country	Holdings
1	MCC	India	164652
2	NBRC	Japan	127694
3	NRRL	USA	96200
4	ATCC	USA	75079
5	CCUG	Sweden	40500
6	DSMZ	Germany	33784
7	JCM	Japan	24784
8	KCTC	Korea	23175
9	CCTCC	China	22073

Source: www.wfcc.info

MCC accepts microorganisms for deposit under general, safe and patent deposits under IDA (Fig. 1). At present, MCC holds around 1.9 million cultures including deposits, cultures collected under the DBT's microbial prospecting program and another 30,000 donated by Piramal Life Sciences. More than 200 microbial strains have recently been identified and deposited at MCC in collaboration with G.B. Pant Institute of Himalayan Environment and Development, Almora, Uttarkhand (Courtesy: Dr Anita Pandey). These strains have originally been isolated from different ecological niches in Indian Himalayan Region including extreme environments such as hot springs, cold deserts and glaciers (Dhakar et al., 2014; Pandey et al., 2015).

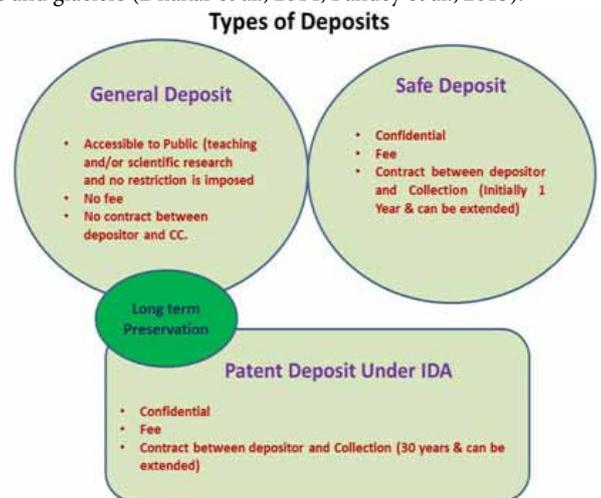


Fig.1. Type of deposits MCC is accepting

MCC preserves cultures in three different forms: -80°C, liquid nitrogen and in lyophilized form. MCC has expertise to handle all the major groups of bacteria including extremophiles, cyanobacteria, anoxygenic photoautotrophic bacteria and anaerobes. Currently, it accepts only BSL-2 category organisms. It also provides various services to Institutes/Universities and Industries like supply of cultures, identification services (16S rRNA gene sequencing, phylogenetic tree construction, MALDI-TOF, FAME, DNA-DNA hybridization, lyophilization, G+C mol% (Tm & HPLC) etc. MCC also conduct educational services (workshops in Colleges and Universities, hands on trainings etc). It, thus, serves to preserve microbial wealth of nation and also generates trained manpower in the area of microbial isolation, identification and preservation.

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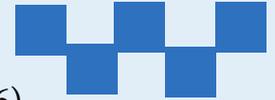
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Innovations/Discoveries in Science (January - March, 2016)

India

- An anti-diabetes herbal drug, BGR-34, jointly developed by two Council of Scientific and Industrial Research (CSIR) laboratories National Botanical Research Institute (NBRI) and Central Institute for Medicinal and Aromatic Plants (CIMAP). The drug has been derived from the medicinal plant extracts as described in the ancient Ayurveda text. The drug is available in the form of a 500mg pill which needs to be consumed twice a day by a patient suffering from Type 2 diabetes. BGR-34 was recently approved by the Indian ministry that looks after traditional Indian medicines, AYUSH.

- A new species of bird has been described from northeastern India and adjacent parts of China by a team of scientists from Sweden, India, China, the US, and Russia. The bird has been named Himalayan Forest Thrush (*Zoothera salimalii*).



Zoothera salimalii

- Prof. Sathyabhama Das Biju and his team of researchers has discovered a new genus of tree hole breeding frogs *Frankixalus jerdonii* in forests of north-east India and China.



Frankixalus jerdonii

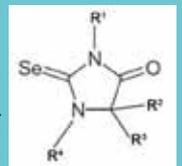
- A new species of narrow-mouthed frog has been discovered in the laterite rock formations of India's coastal plains by a team of researchers from Gubbi labs, Ashoka trust for Research in Ecology and the Environment (ATREE) India and the National University of Singapore (NUS). The frog, which is the size of a thumbnail, was named *Microhyla laterite* after its natural habitat.



Microhyla laterite

Worldwide

- A group of scientists from Moscow universities led by Yan Ivanenkov, the head of Laboratory of Medical Chemistry and Bioinformatics in Moscow Institute of Physics and Technology (MIPT), has succeeded in synthesizing a set of novel selenohydantoins with anticancer and antioxidant activity. In the study, the scientists have synthesized novel selenium-containing hydantoin derivatives.



Selenohydantoins

- Biologists have described a new species of extinct plant *Strychnos electri*, based on two fossil flowers that were trapped in chunks of amber for at least 15 million years. This plant belongs to the genus whose tropical shrubs, trees and vines are famous for producing the deadly toxin strychnine.



Strychnos genus



Boiling river in the Peruvian Amazon

- A mythical boiling river that stretches for four miles has been discovered in the Peruvian Amazon at Mayantuyacu by geoscientist Andrés Ruzo.



Fluorescent polyps

- An international team of biologists, which included researchers from the Lomonosov Moscow State University and the Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, found and explored new species of fluorescent polyps living in colonies on the shells of gastropods (*Nassarius margaritifera*). For the first time they showed that the localization of glow in certain parts of the body can help to distinguish different species of organisms that have identical structure. The collections of the MSU have been enlarged by these new fauna species and their DNA.

- A cathode material for li-ion batteries with a very high charge rate down to 90 seconds has been created by the researchers team led by Prof. Evgeny Antipov of MSU research scientists together with their Russian and Belgian colleagues, retaining more than 75% of an initial capacity. The discovery may stipulate the development of batteries where expensive lithium could be replaced with cheaper potassium

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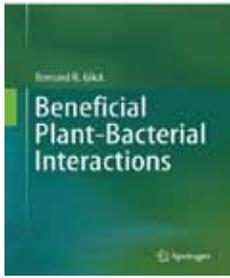
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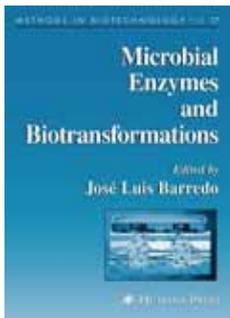
Books on Microbial Diversity



Beneficial Plant-Bacterial Interaction

This monograph provides an overview of beneficial plant-bacterial interactions in a straightforward and easy-to-understand format, and includes a wealth of unique illustrations elaborating every major point. Study questions that emphasize the key points are provided at the end of each chapter

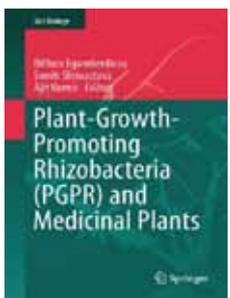
Authored by: Bernard R. Glick
Published by: Springer | Year: 2015
ISBN- 978-3-319-13921-0



Microbiological Enzymes and Biotransformations

The application of recombinant DNA technology to industrial fermentation and the production of enzymes over the last 20 years has produced a host of useful chemical and biochemical substances

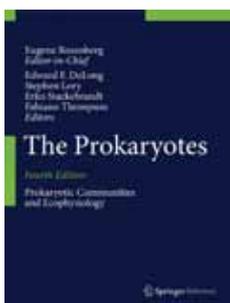
Edited by: J.L. Barredo
Published by: Humana Press | Year: 2005
ISBN- 978-1-59259-846-5



Plant growth promoting Rhizobacteria (PGPR) and Medicinal Plants

This book describes the various applications of microorganisms in improving plant growth, health and the efficiency of phytochemical production

Edited by: D. Egamberdieva, S. Shrivastava and A. Varma
Published by: Springer | Year: 2015
ISBN- 978-3-319-13401-7



The Prokaryotes

The Prokaryotes is a comprehensive, multi-authored, peer reviewed reference work on Bacteria and Achaea. This fourth edition of The Prokaryotes is organized to cover all taxonomic diversity, using the family level to delineate chapters

Editor-in-chief: E. Rosenberg
Edited by: E.F. DeLong, S. Lory, E. Stackebrandt and F. Thompson
Published by: Springer | Year: 2013
ISBN- 978-3-642-30140-7

Forthcoming Events

International

1-2 APRIL 2016

“International Conference on Nanotechnology and Environmental Issues (ICNEI’16)”
Venue: Prague, Czech Republic

7-9 APRIL 2016

“ICNSAM 2016 International Conference on Natural Science and Applied Mathematics - Scopus”
Venue: Dubai, United Arab Emirates

8-9 APRIL 2016

“2016 2nd International Conference on Biotechnology and Agriculture Engineering (ICBAE 2016)”
Venue: Tokyo, Japan

21-22 APRIL 2016

“2016 International Climate Conference on Climate Change: Impacts and Responses – A Common Ground Conference”
Venue: Hanoi, Vietnam

National

1-2 APRIL 2016

“International Conference on “Prospects and Competitive Challenges in Global Hospitality and Tourism Industry” ”
Venue: Phagwara, Punjab, India

2-3 APRIL 2016

“Third International Conference on Information Technology, Control, Chaos, Modeling and Applications (ITCCMA-2016)”
Venue: Chennai, Tamil Nadu, India

10 APRIL 2016

“International Conference On Emerging Technologies in Civil Engineering, Architecture and Environmental Engineering for Global Sustainability (CEAEGS- 2016)”
Venue: New Delhi, Delhi, India

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