# BIOPROSPECTING MICROBIAL DIVERSITY FROM NORTH EAST GENE POOL

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*Microbial diversity is an unseen treasure that deserves greater attention from the conservationists and policy makers. North East gene pool is endowed with huge potentialities for exploration and exploitation for the benefit of the society. Inspite of these vast potentialities, we have done a little for the betterment of the society. CSIR-NEIST, Jorhat is actively engaged in exploration of microbial diversity in the North-East Gene Pool and screening for specific functional diversity of microbes to be utilized for gainful purposes in the society. In this article, we have outlined the microbial diversity in this biodiversity hot-spot and their bio-prospecting in the field of bioremediation/ eco-restoration, biotransformation, hydrocarbon prospecting, plant growth promotion, bio-catalysis and pharmaceutical leads/hits.*

Microbes dominate world not only as plant and animal pathogens, but also as a source of food and other useful products and more importantly as the critical components of natural and agricultural ecosystems. They are numerous and ubiquitous and undertake many roles, both independently and in association with other organisms. Microbial diversity encompasses the spectrum of variability among all types of microorganisms, i.e. bacteria, fungi, viruses and many more in the natural world and also altered in genomic level by human intervention. Since microorganisms are very small, they are least known, and this gap of knowledge is particularly apparent for bacteria and other procaryotic organisms. Microorganisms are the pivotal to the development and sustenance of mankind. Presently, microbes have contributed much to the development of various industrial chemicals, materials and processes, agri-food commodities and human health products. Therefore, microbial diversity is fundamental to maintenance and conservation of global genetic resources. As extreme environments are explored, the richness of microbial diversity is increasingly evident. North Eastern Region of India is a huge potential area for bio-prospecting, since the region is best known for her rich biodiversity and its un-tapped bioresources has been identified as the Indo-Burma Mega Hot Spot by Conservation International[1].

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## *Microbes as Pharmaceutical Leads/Hits and Enzymes*

Bioresources of North-Eastern India and its potential in developing new products and process of pharmaceutical and industrial importance needs no explanation. Therefore, the area has already merited as a promising genetic hub due to its vast untapped resources prevalent in various geographical niches of North-Eastern India. Microbial diversity and its versatility in transforming drug intermediate into value added products of pharmaceutical importance is another priority R&D area of the institute. Apart from that, screening novel anti-infective and enzymes where novel drug molecules might be developed in addition to the existing drug regime to fight the dreaded

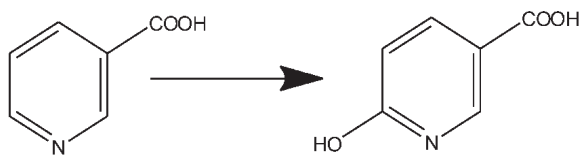
diseases, posing as a challenge and threat to the existence of human beings. CSIR-NEIST, Jorhat has successfully isolated novel antibiotic from microbial strain endowed with anti-TB activity[2-4]. A series of new derivatives were synthesized from the lead molecules through molecular modeling and assayed for targeted pharmacological parameters.

We have isolated several hundreds of rare actinobacteria from North East gene pool, and some of them are showing promising antimicrobial activity against various plant and human pathogens[5,6]. We have also isolated a good number of endophytic fungus having antifungal and antibacterial properties, e.g. endophytic *Fusarium* sp. DF2 isolated from *Taxus wallichiana* of Arunachal Pradesh, India[7]. The fungus was optimized for growth and maximum production of the antimicrobial agent.

### *Biotransformation*

Apart from these, CSIR-NEIST is also actively engaged in biotransformation of some industrially important compounds like conversion of some nitrile compounds into corresponding acids and amides also conversion of epoxides by biocatalysts. Nitriles are precursor for a number of industrially important compounds and in nature they occur as diverse structural variants, such as cyanolipids, cyanohydrins, cyanoglycosides, amino- and mandelonitriles and other nitrile compounds. Owing to their ubiquitous presence, the microbial utilization and bioconversion of these nitriles are of practical interest. Chemical hydrolysis of nitriles can be accomplished by numerous methods using strong acid or alkali. This chemical conversion suffers from various disadvantages such as low yields, undesirable side products, high energy use and the requirement of strongly acidic or basic conditions. The biotransformation of nitrile to carboxylic acid is achieved by both direct bioconversion by nitrilase as well as indirect bioconversion by nitrile hydratase through the formation of amide that in turn is degraded by amidase to the corresponding carboxylic acid and ammonia.

CSIR-NEIST has biologically converted nicotinic acid to 6-hydroxynicotinic acid. The process for production of 6-hydroxynicotinic acid developed at CSIR-NEIST, Jorhat is based on enzymatic hydroxylation of nicotinic acid using whole cell of indegenous *Pseudomonas* sp. in a single step reaction.




Nicotinic acid 6-hydroxynicotinic acid

Scheme 1 : Biotransformation of Nicotinic acid to 6-hydroxynicotinic acid.

6-hydroxy nicotinic acid is used as a starting material for synthesis of new generation insecticides "imidicloprid" and for synthesis of drug: pyridyl-pyridazinone compounds effective for the treatment of congestive heart failure**.** The technology is an import substitute, since we are to import 6-hydroxynicotinic acid from abroad. The technology was transferred to industry - M/S. Jubilant Organosis Ltd, Noida, India. Also CSIR-NEIST Jorhat has successfully demonstrated the biotransformation of potential anti-malarial compound artemisinin into C-9 acetoxy artemisinin using indigenous strain *Penicillium simplissimum*. The absolute stereochemistry of the newly generated chiral center has been ascertained using COSY and 1D NOESY tools. The derivative exhibited anti-cancer activity *in vitro*. This was the first report of direct acetoxylation of artemisinin using microbial cells .

### *Bioremediation of Degraded Land Ecosystem*

North-East India is a land with rich mineral resources. Among the different natural resources crude oil, natural gases and coal, are the main mineral resources of this region. Crude oil drilling, mining of coal, deforestation, etc. have degraded the land ecosystem of North Eastern Region. Degradation of land in North Eastern region also occurs due to deforestation, human habitation, industrialization, agricultural practices, coal mining, crude oil drilling etc. Amongst these causes coal mining and crude oil drilling are the major contributors. Mining transform fertile, cultivable land into wasteland as mining activity generate a vast quantity of solid waste which are deposited at the surface and occupy a huge areas of land. The sites become desert like in nature and disturb the original topography and ecology. These activities directly lead to unsightly landscape, loss of cultivated land, forest and pasture land amounting to overall loss of production. The indirect effects can be soil erosion, air and water pollution, toxicity, geo-environmental disasters, loss of biodiversity and ultimately loss of economic wealth. Mining waste usually includes waste rock, boulders, pebble, cobble and other sulphide, minerals which are of great environmental concern. Conventionally site restoration, incineration or land filling, are common practice in remediation of such sites which are expensive and not eco-friendly. Alternately, the adoption of bioremediation process is cost effective and eco-friendly. Bioremediation involves the action of microorganisms or biological agents along with introduction

Area before Treatments

Area before Treatment

After Treatment

**Figure 1.** Bioremediation in drilling sites in ONGC oil fields, Assam.

of plant species to revive the degraded land to its original state. CSIR-NEIST Jorhat has made tremendous contribution towards eco-restoration of degraded land by the application of various bio-formulations along with plantation of tree species. However, further research is still needed to develop bioremediation methods which can be effective over diverse climatic conditions of the region. In this article a brief description of various causes of land degradation in Assam and their bioremediation R&D initiatives are discussed.

Bioremediation of coal mine over burdened (OB) using microbes or plant (phytoremediation) is very viable technique and holds advantages over physical and chemical methods as being eco-friendly and highly specific. In phytoremediation, plant species like *Cassia streata, Sesbania rostrata, Gmelina arborea, Dalbergia sisso,* etc. were found to be resistant to coal mine OB and could be effectively used. Bioremediation has been proven to be an alternative technology capable of achieving permanent remediation at waste site as recognized by US EPA for implementation of the Superfund Amendments and Reauthorization Act (SARA) of 1986. Phytoremediation is another perspective of bioremediation in which specific efficient plant species can be utilized for remediation of the degraded land ecosystem. Utilizing plants to absorb, accumulate and detoxify contaminants in the growth substrate through physical, chemical or biological processes is a wide spread practice. It involves the use of plants to stabilize hydrocarbon polluted soils and to enhance hydrocarbon degradation by stimulating soil microbes / microbial consortia with hydrocarbon degradation capabilities in the rhizosphere. This technology has been applied to both organic and inorganic pollutants present in soil, water or the air. Phytoremediators have been reported in cleaning up heavy metals like aluminium (Al), cadmium (Cd), chromium ($Cr^{3+}$ and $Cr^{6+}$), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb) and zinc (Zn). It has also been tested for clean-up of explosives like 2, 4, 6-trinitrotoluene (TNT), trichloroethylene (TCE), other volatile organic chemicals and organic compounds such as petroleum compounds. CSIR- NEIST Jorhat, team is actively also extensively engaged in bioremediation of crude oil contaminated site in upper Assam oil fields of ONGC, Ltd., and has achieved spectacular success in CSIR-ONGC joint venture.

CSIR-NEIST team has reported elevated level of heavy metals in crude oil contaminated soil in some areas of Sibsagar district, Assam. Aresenic, cadmium, chromium, mercury and lead were found at concentration of 2.43 ppm,

4.75, 7.72, 10.63 and 7.98 respectively which were much higher than the uncontaminated control. Application of bioformulation developed by NEIST Jorhat showed a significant decrease in the heavy metal concentration of those areas. It was further reported decreased number of beneficial microbes in soil (e.g. phosphorous solubilizers, sulphur oxidizers, cellulose degraders) in crude oil contaminated soil which showed an increase during and after remediation. A highly alkaline condition of the soil (pH 10.0-10.5) was reported in their study which might be the reason being low in the presence of beneficial microbes. NEIST-Jorhat has successfully reclaimed 6 sites in ONGC oil fields in Upper Assam using technology developed by this institute for eco-restoration of crude oil contaminated sites.

### *Geomicrobiological Technique for Hydrocarbon Prospecting*

Globally, hydrocarbon (HC) explorers are always in search for new oil field/reservoirs so as to maintain a steady flow of hydrocarbons:oil or gas, to sustain the civilization. Initially surface seepage of oil or gas was taken as indicator to drill well. As time goes on, visible seeps became less conspicuous, costlier geophysical and geological methods for locating HC prospect came in. But the cost of drilling a well became prohibitive day by day and even after drilling a well, the ratio of dry well to productive well remains 50:50. As such, search continued to find newer methods for locating HC prospect and that to at a lesser cost.

Over the years the NEIST, Jorhat has developed a high sensitive micromanometric technique for the monitoring of micro flora which thrive on lighter hydrocarbon gases as their sole source of carbon, under the active guidance of Dr.H D Singh then HOD of Biochemistry Division. Validity of this technique was established after extensive field studies on known petroliferous areas of upper Assam with the active help from M/s Oil India Ltd. Duliajan and M/s Assam Oil Company (presently Indian Oil Corporation, Assam Oil Division).and known non-petroliferous areas of upper and lower Assam. Such field studies shows nearly 78 -100% correct correlation for oil producing areas and 80- 100% correct correlation in non-petroliferous areas.

As the geomicrobiological methodology has been well established, the oil industries like Oil India Limited (OIL) and Oil and Natural Gas Corporation Limited (ONGCL) came forward for application in their virgin fields. The CSIR-NEIST team has successfully completed five such projects in various Oil India Ltd. and ONGC oil fields.

### *Plant Growth Promoting Rhizobacteria and Termite Control*

Plant growth promoting rhizobacteria (PGPR) have been explored successfully through a series of experiments starting from isolation, screening and optimization. The R&D team has developed bio-formulation/ products for promoting agricultural crops, such as tea and vegetable crops[8]. The CSIR-NEIST team also developed formulation for controlling Termites which causes serious damages to the tea and related agriculture crops. The product developed from PGPR provides biocontrol activities in addition to the mentioned above.

### *Microbial Repository of CSIR-NEIST*

The institute is maintaining a stock cultures of more than 3000 microbial strains isolated from the various ecological niches of North East gene pool. These stock cultures are being used in various on-going R & D projects of the institutes for diverse functionalities. Also the center is developing a Microbial database of indigenous strains for various utilities.

### *DBT- Sponsored Bioinformatics Hub*

Bioinformatics hub set up at the institute under the financial assistance of DBT has been catering to the needs of the students community of this region, since the year 2009. Several batches of P. G students and graduate students from neighbouring institutes, such as, Dibrugarh University and Nagaland University have already completed dissertation and summer training programme in the hub. The center organizes seminar and workshops for the benefit of R&D researchers especially in protein and drug designing area. Also student community from various local colleges have availed the facility to enhance their knowledge base.

### *Microbial database and Repository:*

CSIR-NEIST is maintaining a stock cultures of more than 3000 indigenous microbial strains isolated from North East Gene pool and developing a Microbial database.

### *Microbial analysis of water samples: a service to the society:*

Biotechnology Division of the institute is providing a much needed service to the society by regularly analyzing the microbial loads of drinking water samples following ISI and WHO's standard. We provide these services to the industries, NGO, public health organizations and individuals as regular consultants.

## *Industrial Linkage*

The foremost Industry engaged in coal mining in Assam as well as in many other parts of India is the Coal India Limited. It is a government of India enterprise and is an active partner in the environmental preservation campaign of the Ministry of Environment and Forestry. Projects have been undertaken at our Institute to revive the mine degraded lands. During such projects emphasis were given on the development of technologies which can be implemented for remediation of degraded land ecosystem in and around mine sites. These technologies can be commercialized to public entrepreneurs. Similarly, R & D team of CSIR-NEIST, Jorhat has achieved spectacular success in bioremediation of crude oil contaminated drill sites in Assam, especially in ONGC oil field and the company is an active partner in these research. They provide the sites for bioremediation study as well as sponsor the entire research programmme. The oil industry development board (OIDB) also sponsored research programs for bioremediation related scheme. Over the years, CSIR-NEIST team of Biotechnology Division has successfully completed 5 nos. of projects in specific sites in ONGC oil fields of upper Assam.

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