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Microbial Diversity of Extreme Regions: An Unseen Heritage and Wealth

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Extreme environments represent a unique ecosystem which harbour novel microbial flora. India is one among 12 mega-biodiversity countries and 25 hotspots of the richest and highly endangered eco-regions of the world. Thermophiles from hot springs can be a source for enzymes that are active at high temperatures. They can also be used for decomposition process. Psychrophiles can be a source of anti freezing compounds. Halophiles and osmophiles can be a source of genes coding for osmolytes and can be used for the development of transgenic plants tolerant to salt and drought stress.

Different surveys were carried out to collect soil, plant and water samples for analysis of microbial diversity from the cold desert of Leh and Rohtang, from mangroves of Sunderbans, West Bengal and Bhitarkanika, Orissa; thermal springs of Rajgir, Manikaran, Bakreshwar, Balrampur and Vashisht, hypersaline soils of Rann of Kutch, Pullicat lake (Tamil Nadu), Sambhar salt lake and Chilka lake; acidic soils of Manipur, Kerala, Meghalaya and Mizoram for isolation of microbes growing under extreme conditions. All the collected isolates were characterised through biochemical analysis for PGP traits, and identified on the basis of 16s rRNA gene sequencing. Many novel genera and species not reported from country were identified. A database of predominant genera was developed and base line information generated for extreme environments in the country.

During these surveys a total of 1,540 bacteria, 157 archaea, 260 fungi and 200 actinomycetes were isolated and the occurrence of *Bacillus*, *Bacillus* derived genera and other predominant genera are depicted in Fig 1,2 (Yadav *et al*., 2015). The archaeal and bacterial isolates were further screened for molecular and functional diversity. A huge database comprising microbes tolerant to high salinity, low and high temperature, drought and low pH was generated.

Microorganisms isolated from extreme environments can be developed as inoculants for crops grown in these stressed environments. Eubacteria and archaea isolated from saline habitats were used as inoculants to alleviate salt stress and to influence the growth and yield of crop plants. Bacterial isolates obtained from Sambhar salt lake and capable of exhibiting plant growth promoting traits at high salt concentration improved the growth of wheat in saline soils. Inoculation induced significantly higher proline, total soluble sugar accumulation in plants and reduced Na:K ratio (Upadhaya *et al*., 2012). Likewise, a preliminary investigation on archaea isolated from hypersaline environment of Rann of Kutch, Gujarat, India suggests its role in supporting the growth of plants in saline soils. Among 157 archaeal isolates, twenty exhibited phosphate solubilization both in plates and broth. The ability of archaeal isolates to solubilise tricalcium phosphate ranged from 51.92 ± 0.8 to 1986.86 ± 1.8 µg mg^{-1} protein, the maximum being produced by *Halococcus hamelinensis* strain IARI-SNS2 (1986.9 mg l^{-1}) followed by *Natrinema* sp. strain IARI- WRAB2 (1341.5 mg l^{-1}) (Yadav *et al*., 2015). In addition archael isolates solubilizing potassium, capable of producing IAA, Zeatin, gibberellic acid and siderophore at high salt concentration were reported for the first time. Acid tolerant bacteria isolated from soils of Kerala were used as inoculant to improve the growth of horticultural crops like coconut, areca nut, cocoa and vanilla. Psychrophilic bacteria isolated form Leh and Rohtang Pass significantly

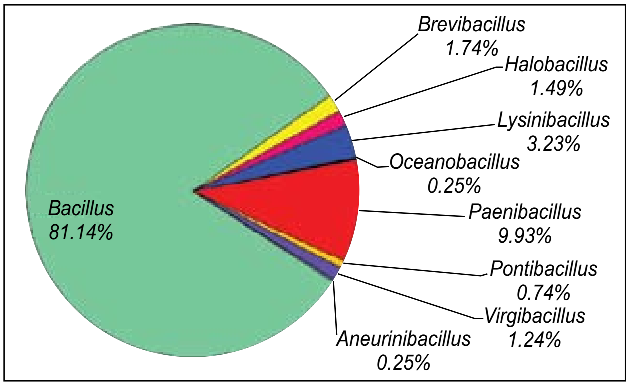

Fig. 1. Abundance of different *Bacillus* and *Bacillus* derived genera in the samples surveyed (Source: Yadav *et al.*, 2015)

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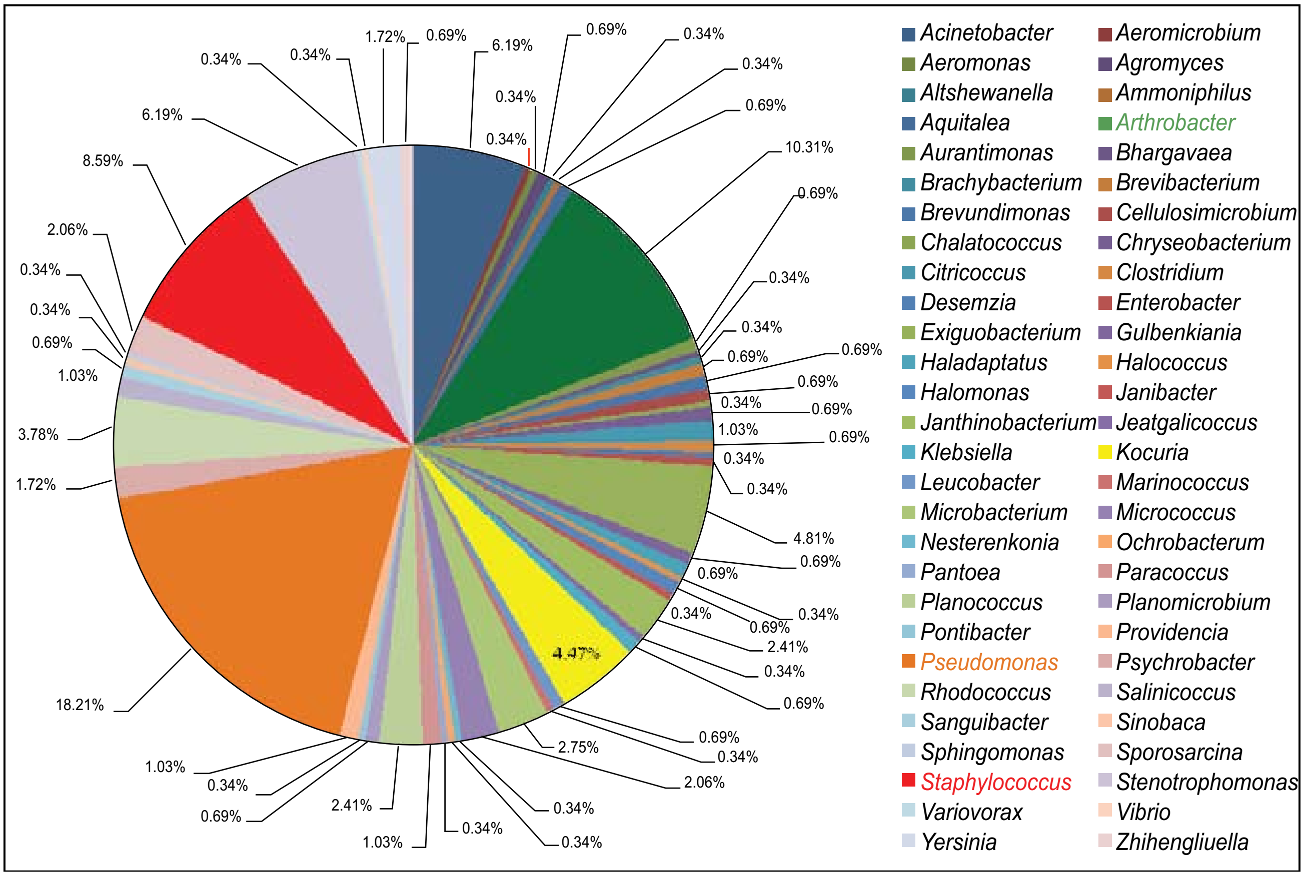

Fig. 2. Abundance of other predominant bacterial genera in the samples surveyed (Source: Yadav *et al*., 2015)

influence the growth of wheat on inoculation (Verma *et al*., 2015).

Low temperature tolerant microbial strains were screened for production of lignocellulolytic enzymes active at low temperature and a consortia consisting of two bacteria (*Bacillus atropheus* and *Bacillus* spp) and two fungi (*Eupenicillium crustaceum and Penicillium citrinum*) was developed for the preparation of compost at low temperature (4 to 10^{o}C) (Shukla *et al*., 2016). Pshycrophilic bacteria are a source of cold active enzymes. Psychrotrophic microbes isolated from samples collected from high mountain pass (Khardungla and Rohtang), rivers (Indus, Zanskar, I-Z confluence and Beas) and sub-glacial Lakes (Pangong, Dashair and Chandratal) of Indian Himalayas were found to produce amylase, β-glucosidase, pectinase, protease, cellulase, xylanase, β-galactosidase, laccase, chitinase and lipase enzymes active at 4^{o}C (Yadav *et al*., 2016). These cold active enzymes have importance for both industrial and agricultural application. Psychrophilic bacteria capable of producing anti freeze proteins could be used to counter ice nucleation protein produced by bacteria like *Pseudomonas syringae* and *Erwinia herbicola* and thereby protect the plants from frost damage. Within the last two decades, antifreeze proteins have been found in variety of bacteria from cold habitats (Wilson and Walker 2010; Kawahara *et al.,* 2007).

The microbes obtained from extreme environments are a good source of enzymes to be used in agriculture and industry. Many of the isolates were found to produce, cellulases, amylases and proteases (Sahay *et al*., 2012). Candidate genes for abiotic stress tolerance were amplified from many extremophiles and can be used for the development of transgenic plants tolerant to salt, drought or temperature stress (Kumar *et al*., 2013). The gene *dna*K from *Bacillus pumilus* strain B3; superoxide dismutase (*sod*D) gene from *Halobacillus* sp. and cyanobacteria *Lyngbya* sp.; pyrroline-5-carboxylate reductase gene from *Bacillus pumilus*; genes for glutamate dehydrogenase, ectoine biosynthesis and sorbitol dehydrogenase from *Oceanobacillus iheyensis*; mannitol-1-phosphate-5-dehydrogenase gene from

Bacillus licheniformis; genes for trehalose-6-phosphate synthase (TPS), 2-Cys peroxiredoxin (Prx2), hydroxy methyl butenyl diphosphate reductase (HDR), chloroplast lycopene beta-cyclase (LCYB), duplicated carbonic anhydrase (DCA1) from *Dunaliella viridis* were successfully amplified, cloned, sequenced and expressed in heterologus strain.

The database of microbes from extreme environments is a resource that can be tapped as and when required for screening of different molecules, enzymes, genes and alleles.

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