Diversity of plant growth and soil health supporting bacteria

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The global necessity to increase agricultural production from a steadily decreasing and degrading land resource base has placed considerable strain on the fragile agro-ecosystems. Current strategies to maintain and improve agricultural productivity via high-input practices places considerable emphasis on 'fail-safe' techniques for each component of the production sequence with little consideration to the integration of these components in a holistic, systems approach. While the use of mineral fertilizers is considered the quickest and surest way of boosting crop production, their cost and other constraints deter farmers from using them in recommended quantities. In recent years, concepts of integrated plant nutrient management (IPNM) have been developed, which emphasize maintaining and increasing soil fertility by optimizing all possible sources (organic and inorganic) of plant nutrients required for crop growth and quality. This is done in an integrated manner appropriate to each cropping system and farming situation. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil physical properties, both of which rely on soil biological processes and soil biodiversity. An understanding of microbial diversity perspectives in agricultural context, is important and useful to arrive at measures that can act as indicators of soil quality and plant productivity. In this context, the long-lasting challenges in soil microbiology are development of effective methods to know the types of microorganisms present in soils, and to determine functions which the microbes perform *in situ*. This review describes some recent developments, particularly in India, to understand the relationship of soils and plants with the diversity of associated bacteria, and traces contributions of Indian scientists in isolating and defining the roles of plant growth promoting bacteria to evolve strategies for their better exploitation.

Need and ways of analysing bacterial diversity in soil/rhizosphere

SOIL is a dynamic, living matrix that is an essential part of the terrestrial ecosystem. It is a critical resource not only for agricultural production and food security but also towards maintenance of most life processes. The functions of soil biota are central to decomposition processes and nutrient cycling. Soil is considered a storehouse of microbial activity, though the space occupied by living microorganisms is estimated to be less than 5% of the total space. Therefore, major microbial activity is confined to the 'hot-spot', i.e. aggregates with accumulated organic matter, rhizosphere (RS)[2,3]. Microbial ecologists have, in particular, studied microbial community composition since it exerts important control over soil processes[4,5]. Diversity and community structure in the rhizosphere is however influenced by both, plant and soil type[6]. Plant-species-specific selective enrichment of microflora in the rhizosphere milieu has been exploited in legumes from the point of view of N_2-fixation under nitrogen limiting conditions[7–10]. Likewise, non-leguminous crops select specific bacterial groups in the rhizosphere[11,12]. For example, colonization in maize rhizosphere by specific groups of bacteria was consistent and comparable when studied by two groups located at two distinct geographic locations, France and Canada[13,14].

Soil microorganisms play an important role in soil processes that determine plant productivity. For successful functioning of introduced microbial bioinoculants and their influence on soil health, exhaustive efforts have been made to explore soil microbial diversity of indigenous community, their distribution and behaviour in soil habitats[15]. The era of molecular microbial ecology has uncovered only a part of novel microbiota, most of which is based on rRNA and rDNA analysis[16]. The molecular methods used globally for diversity assessment of different cropping systems include, phospholipid fatty acid (PLFA) analysis[17,18], terminal-restriction fragment length polymorphism (T-RFLP)[19], single-strand conformation polymorphism (SSCP)[20–22], and denaturing/temperature gradient gel electrophoresis (DGGE/

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TGGE)[23–25]. The quantitative description of microbial communities in terms of gene expression of particular function is now possible through the development of DNA microarray technology and its applications in the study of microbial community structure of agro/natural ecosystem[26–30]. In conjunction with DNA microarray, direct RNA-based analysis of community dynamics to measure the functionality of environmental microbial populations without PCR amplification has been developed and it is equally applicable to direct detection and characterization of 16S rRNA of microbial species, and analysis of environmental samples[23,31–33]. To understand the dynamics of community life on a broader scale, metagenomics (study of collective genome of an ecosystem) provide insights of functional information through genomic sequences and expression of traits[16]. This component is discussed independently by Sharma and others in this special section.

Diversity of plant growth promoting bacteria

Plants play an important role in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, depending on the nature and concentrations of organic constituents of exudates, and the corresponding ability of the bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere[34]. There is a continuum of bacterial presence in soil → rhizosphere → rhizoplane → internal the plant tissues[35]. Bacteria living in the soil are called free-living as they do not depend on root exudates for their survival. Rhizospheric bacterial communities however have efficient systems for uptake and catabolism of organic compounds present in root exudates[36]. Several bacteria have the ability to attach to the root surfaces (rhizoplane) allowing these to derive maximum benefit from root exudates. Some of these are more specialized, as they possess the ability to penetrate inside the root tissues (endophytes) and have direct access to organic compounds present in the apoplast. By occupying this privileged endophytic location, bacteria do not have to face competition from their counterparts as encountered in the rhizosphere, or in soil.

Bacteria associated with plants can be harmful and beneficial. Plant growth promoting (PGP) bacteria may promote growth directly, e.g. by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron, or production of plant growth regulators (hormones)[37]. Some bacteria support plant growth indirectly, by improving growth-restricting conditions either via production of antagonistic substances or by inducing resistance against plant pathogens. Since associative interactions of plants and microorganisms must have come into existence as a result of co-evolution, the use of latter group as bioinoculants must be pre-adapted, so that it fits into a long-term sustainable agricultural system. A number of bacterial species associated with the plant rhizosphere belonging to genera *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are able to exert a beneficial effect on plant growth.

Nitrogen-fixing bacteria

Biological nitrogen fixation is estimated to contribute 180×10^6 metric tons/year globally[38], of which eighty per cent comes from symbiotic associations and the rest from free-living or associative systems[39]. The ability to reduce and siphon out such appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil is confined to bacteria and Archaea[40]. These include, a) symbiotic nitrogen fixing (N_2-fixing) forms, viz. *Rhizobium*, the obligate symbionts in leguminous plants and *Frankia* in non-leguminous trees, and b) Non-symbiotic (free-living, associative or endophytic) N_2-fixing forms such as cyanobacteria, *Azospirillum*, *Azotobacter*, *Acetobacter diazotrophicus*, *Azoarcus*, etc.

Symbiotic nitrogen fixers. Two groups of nitrogen-fixing bacteria, i.e. rhizobia and *Frankia* have been studied extensively. *Frankia* forms root nodules on more than 280 species of woody plants from 8 different families[41], however its symbiotic relationship is not as well understood. Species of *Alnus* and *Casuarina* are globally known to form effective symbiosis with *Frankia*[42–45]. In India, a technique for isolation of *Frankia* by single spore culture technique was developed, and PCR-RFLP markers were identified for screening actinorhizal symbionts[46,47].

In the context of rhizobia, considerable change in taxonomic status has come about during the last years. Sahgal and Johri[48] outlined the current status of rhizobial taxonomy and enlisted 36 species distributed among seven genera (*Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Methylobacterium*, *Rhizobium* and *Sinorhizobium*) derived, based on the polyphasic taxonomic approach. Although most *Rhizobium* isolates can nodulate more than one host species and also several different bacterial species are often isolated from a single legume, it is only from a few legumes that the symbionts have, so far, been investigated thoroughly[49]. The family Fabaceae (formerly Leguminoseae) is important both ecologically and agriculturally, since it is a major source of biological nitrogen fixation[50]. Species of *Parasponia* and *Tremma* are the only non-legumes that form an effective symbiosis with *Rhizobium* or *Bradyrhizobium*[51]. There appears a common evolutionary origin, as on the basis of chloroplast genome sequence data they all form a single clade within the angiosperms[52]. A few aquatic legumes bear stem nodules in addition to the normal root nodules. This peculiarity is restricted to 15 of the 250 species of *Aeschynomene*, 1 out of 15 species of *Neptunia* (*N. oleracea*),

and 1 out of 70 species of *Sesbania* (*S. rostrata*)[53–55]. *Aeschynomene aspera* and *A. indica* form nodules in their native environment[53]. The stem nodulation is more prevalent in waterlogged conditions and is not affected by mineral nitrogen in soil or water. *Neptunia natans*, an aquatic legume indigenous to tropical and subtropical regions and in African (Senegal) soils is nodulated by *Allorhizobium*, which includes a single species *A. undicola*[56]. A critical examination of the Indian isolates of *N. natans* revealed that they were not related to *A. undicola* but belonged to genus *Devosia*[57,58]. Members of the genus *Ochrobactrium*, till recently, were considered as nosocomial opportunistic human pathogens. Verma *et al.*[59] reported their presence as non-nitrogen fixing endophytes in deep water rice. But, latest reports on characterization of isolates from root nodules of *Acacia mangium* collected from Thailand and Philippines revealed that members of the genus *Ochrobactrum* possessed complete symbiotic ability to form nitrogen-fixing nodules[60]. Waelkens *et al.*[61] demonstrated that *Azorhizobium caulinodans*, specific for stem nodulation in *Sesbania rostrata*[62] can also nodulate *Phaseolus vulgaris*.

Legumes of economic importance are grown in India under different agro-climatic conditions and presence of native rhizobia has therefore been anticipated. An extensive survey of nodulation status of legumes, viz. chickpea, pigeonpea, moongbean, soybean and groundnut with native rhizobia during 1967–72 (refs 63, 64) and in 1977–80 (ref. 65) under the All India Coordinated Pulse Improvement Programme has belied this assumption since except for groundnut, most legumes nodulated poorly at more than 50 per cent of the places surveyed. There was a deficiency of specific *Rhizobium* even in traditional legume-growing areas. Another survey determined the serological types of the native rhizobial population, frequency of effective types and the fate of the introduced antigenic type in competition with the native types in chickpea[63,66,67], moongbean[68], groundnut[69,70] and clover[71] and revealed that only 20–30% of indigenous rhizobia were effective. A detailed eco-serological survey of chickpea in 13 major soils of India revealed three broad serogroups, of which serogroup I was widely distributed. Serogroup II was limited to grey and brown soil types, and serogroup III, which recognizes among strains of American origin, did not occur in any Indian soil. Field trials conducted in India showed that nearly 50% of nitrogenous fertilizer can be saved through rhizobial inoculations with considerable increase in yield depending on the legume, soil and agro-climatic conditions[72,73].

In order to tap the vast diversity of rhizobia in the country, it is important to screen legumes that are wild or are found in rare habitats. Until recently, it was generally accepted that legumes were nodulated only by the members of α-proteobacteria. The first report on nodulation of legumes by members of β-proteobacteria were by Moulin *et al.*[74] on the isolation of the members of *Burkholderia* from the African legumes *Aspalanthus carnosa* and *Machaerium lunatum*, and by Chen *et al.*[75] on isolation of *Ralstonia taiwanensis* from *Mimosa pudica* and *M. diplotricha*. Almost in a parallel attempt, Tripathi[76] in India also observed *R. taiwanensis* in *Mimosa pudica*. On the basis of recent observations of widespread occurrence of β-proteobacteria nodulating legume plants, rhizobia are now divided as α-rhizobia and β-rhizobia[77,78]. The genus *Ralstonia*, which includes *R. taiwanensis*, has recently been given a new name, *Wautersia*[79]. Ogasawara *et al.*[80] reported new species, *Sinorhizobium abri* from *Abrus precatorius* and *S. indiaense* from *Sesbania rostrata* in the Himalayan region of India. Considerable genetic diversity amongst rhizobia of five medicinal plants of the sub-Himalayan region was reported by Pandey *et al.*[81]. Notable differences in the whole cell protein patterns of root nodule isolates of *Dalbergia sissoo*, collected from five states of India showed the extent of diversity of microsymbiont[82].

Salinization/alkalization is known to limit nodulation and nitrogen fixation. Response of legumes to salinity varies greatly; some legumes, e.g. *Vicia faba*, *Phaseolus vulgaris* and *Glycine max* are more salt tolerant than others such as, e.g. *Pisum sativum*. Other legumes like *Prosopis*, *Acacia* and *Medicago sativa* are salt tolerant, but their rhizobia are more salt tolerant than the host plants[83]. Marked variations are also observed among salt tolerance of different species of rhizobia. While growth of a number of strains of *Bradyrhizobium japonicum* is inhibited at less than 100 mM NaCl, various strains of *Sinorhizobium meliloti* and *R. leguminosarum* grow at more than 300 mM NaCl. Rhizobia isolated from woody legumes like *Hedysarum*, *Acacia*, *Prosopis* and *Leucaena* can tolerate up to 500 to 800 mM of NaCl. Many species of rhizobia adapt to salinity stress by intracellular accumulation of compatible solutes. Exogenous supply of glycine betaine and choline enhance the growth of various rhizobia like *Rhizobium tropici*, *S. fredii*, *Rhizobium galegae*, *Mesorhizobium loti* and *M. haukkii* under salt stress. However, both the compounds are ineffective for relieving salt stress in *R. leguminosarum*, *R. etli* and *B. japonicum*[84]. *Sinorhizobium meliloti* has the remarkable ability to use glycine betaine as carbon and nitrogen source at low osmolarity but at high osmolarity the catabolism of glycine betaine is inhibited in order to accumulate it at desired level within the cells[85]. High salt tolerance aids in tolerance to high pH and temperature[86]. Several *Rhizobium* species have been reported from salt-stressed soils in India (Table 1) and around the world[83].

Non-symbiotic nitrogen fixers. Non-symbiotic nitrogen fixation is known to be of great agronomic significance. The main limitation to non-symbiotic nitrogen fixation is the availability of carbon and energy source for the energy intensive nitrogen fixation process. This limitation can be compensated by moving closer to or inside the plants,

Table 1. Tolerance of rhizobia to abiotic stresses

Stress	Host from which isolated
Saline-alkaline soil, pH 10.3	Indian clover (*Medicago parviflora*), Dhaincha (*Sesbania aculeata*), Berseem (*Trifolium alexandrium*), Guar (*Cyamopsis tetragonoloba*), Cowpea (*Vigna sinensis*) and lentil (*Lens esculenta*)[197]
Saline soil	Soybean[198]
Nodulation possible at 150 mM NaCl	*Acacia nilotica*[199]
Tolerant to 3% NaCl	Chickpea (*Cicer arietinum*)[200]
Survive 50°C and 5% NaCl	*Albizzia lebbek*[201]
Growth at pH 12.0 and 5% NaCl	*Sesbania formosa*, *Acacia farnesiana* and *Dalbergia sissoo*[201]
Alkaline soil	*Prosopis juliflora*[202]
Alkaline soil, 32% NaCl up to 8 h, 55°C upto 3 h, and 45°C +salt at pH 12	*P. juliflora*[203]
10% and 28% NaCl for 18 h at 30°C	*Sesbania*[204]

viz. in diazotrophs present in rhizosphere, rhizoplane or those growing endophytically. Some important non-symbiotic nitrogen-fixing bacteria include, *Achromobacter*, *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Azomonas*, *Bacillus*, *Beijerinckia*, *Clostridium*, *Corynebacterium*, *Derxia*, *Enterobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhodospirillum*, *Rhodopseudomonas* and *Xanthobacter*[87].

(i) Azotobacter. The family Azotobacteriaceae comprises of two genera[88] namely, *Azomonas* (non-cyst forming) with three species (*A. agilis*, *A. insignis* and *A. macrocytogenes*) and *Azotobacter* (cyst forming) comprising of 6 species[89], namely, *A. chroococcum*, *A. vinelandii*, *A. beijerinckii*, *A. nigricans*, *A. armeniacus* and *A. paspali*. *Azotobacter* is generally regarded as a free-living aerobic nitrogen-fixer. *Azotobacter paspali* which was first described by Dobereiner and Pedrosa[90], has been isolated from the rhizosphere of *Paspalum notatum*, a tetraploid subtropical grass, and is highly host specific. Various crops in India have been inoculated with diazotrophs particularly *Azotobacter* and *Azospirillum*[91,92]. Application of *Azotobacter* and *Azospirillum* has been reported to improve yields of both annual and perennial grasses[93]. Saikia and Bezbaruah[94] reported increased seed germination of *Cicer arietinum*, *Phaseolus mungo*, *Vigna catjung* and *Zea mays*. However, yield improvement is attributed more to the ability of *Azotobacter* to produce plant growth promoting substances such as phytohormone IAA and siderophore azotobactin, rather than to diazotrophic activity.

(ii) Azospirillum. Members of the genus *Azospirillum* fix nitrogen under microaerophilic conditions, and are frequently associated with root and rhizosphere of a large number of agriculturally important crops and cereals. Due to their frequent occurrence in the rhizosphere these are known as associative diazotrophs. Sen[95] made one of the earliest suggestions that the nitrogen nutrition of cereal crops could be met by the activity of associated nitrogen-fixing bacteria such as *Azospirillum*. This organism came into focus with the work of Dobereiner and associates from Brazil[96–98], followed closely by reports from India[99–102]. After establishing in the rhizosphere, azospirilla usually, but not always, promote the growth of plants[103–105]. Despite their N_2-fixing capability (~1–10 kg N/ha), the increase in yield is mainly attributed to improved root development due to the production of growth promoting substances and consequently increased rates of water and mineral uptake[106–108]. Azospirilla proliferate in the rhizosphere of numerous plant species and the genus *Azospirillum* now contains seven species – *A. brasilense*[109], *A. lipoferum*[109], *A. amazonense*[110], *A. halopraeferens*[111], *A. irakense*[112], *A. dobereinerae* and *A. largimobile*[113].

An understanding of the mechanism of osmoadaptation in *Azospirillum* sp. can contribute towards long-term goal of improving plant–microbe interactions for salinity affected fields and crop productivity. The synthesis and activity of nitrogenases in *A. brasilense* is inhibited by salinity stress[114]. Tripathi *et al.*[115] reported accumulation of compatible solutes such as glutamate, proline, glycine betaine and trehalose in response to salinity/osmolarity in *Azospirillum* sp. Usually, proline plays a major role in osmoadaptation through increase in osmotic stress that shifts the dominant osmolyte from glutamate to proline in *A. brasilense*. Saleena *et al.*[116] have studied the diversity of indigenous *Azospirillum* sp. associated with rice cultivated along the coastline of Tamil Nadu. On the basis of mutational studies of *Azospirillum*, Kadouri *et al.*[117] suggested a role of PHB synthesis and accumulation in enduring various stresses, viz. UV irradiation, heat, osmotic pressure, osmotic shock and desiccation.

(iii) Acetobacter. *Acetobacter diazotropicus* (family Acetobacteriaceae), isolated from roots and stems of sugarcane, was first reported as an N_2-fixing bacterium from Brazil[118], and subsequently from Australia[119], India[120,121], Mexico[122], Uruguay[123], Canada and Cuba[124]. Isolation of this bacterium from most tissues of sugarcane, and its absence from the soils of sugarcane fields suggested these

to be systemic endophytes. The occurrence of this organism has been reported in sugar-rich plants like *Pennisetum purpureum* and sweet potato[125] and in insects like mealybugs[126] and leafhoppers[127]. The colonization of *A. diazotropicus* has also been reported in coffee plants grown through seeds and vegetative propagation[128]. This bacterium successfully colonizes sugarcane varieties in India where the chemical N fertilization is completely avoided for at least two successive years and replaced by organic manures[129]. *Acetobacter* has gained importance as an inoculant for sugarcane[130,131].

The family Acetobacteriaceae includes genera, *Acetobacter*, *Gluconobacter*, *Gluconoacetobacter* and *Acidomonas*[132]. Based on 16S rRNA sequence analysis, the name *Acetobacter diazotrophicus* has been changed to *Gluconoacetobacter diazotrophicus*[133]. In addition to *G. diazotrophicus*, two more diazotrophs, *G. johannae* and *G. azotocaptans* have been included in the list[134]. The genetic diversity of *G. diazotrophicus* isolated from various sources does not exhibit much variation[128,135]. However, Suman *et al.*[136] found that the diversity of the isolates of *G. diazotrophicus* by RAPD analysis was more conspicuous than that reported on the basis of morphological and biochemical characters. The SDS-PAGE and multilocus enzyme electrophoresis analysis also revealed certain differences among strains of *G. diazotrophicus* suggesting genotypic differences[137]. On the basis of DNA fingerprinting studies, existence of genetically distinct *G. diazotrophicus* strains in sugarcane cultivars has been reported from Louisiana[138]. Investigations of isolates of *G. diazotrophicus* from pineapple suggested that only certain genetically related groups of this bacterium or its ancestors have acquired the capability of colonizing plants by themselves or with the aid of the vectors such as insects or fungi[139]. *G. diazotrophicus* has been found to harbour plasmids[140] of 2–170 kb.

(iv) Azoarcus. *Azoarcus* gen. nov., an aerobic/microaerophilic nitrogen-fixing bacterium was isolated from surface-sterilized tissues of kaller grass (*Leptochloa fusca* (L.) Kunth)[141], and can infect roots of rice plants as well. Kallar grass is a salt-tolerant grass used as a pioneer plant in Pakistan on salt-affected low fertility soils. Repeated isolation of one group of diazotrophic rods[142] from kallar grass roots and the results of polyphasic taxonomy led to the identification of genus *Azoarcus*, with two species, *A. indigens* and *A. communis*, and three additional unnamed groups, which were distinct at species level. Nitrogen-fixation by *Azoarcus* is extremely efficient (specific nitrogenase activity, one order of magnitude higher than those found for bacteroids). Such hyper-induced cells contain tubular arrays of internal membrane stacks that can cover a large proportion of the intercellular volume. These structures are considered as vital for high efficiency N_2-fixation[141].

Phosphate solubilizing microorganisms

Phosphorus (P) is a major essential macronutrients for biological growth and development. P in soils is immobilized or becomes less soluble either by absorption, chemical precipitation, or both. A survey of Indian soils revealed that 98% of these need phosphorus fertilization either in the form of chemical or biological fertilizer. Although P content in an average soil is 0.05%, only 0.1% of the total P present is available to the plants because of its chemical fixation and low solubility. Application of chemical phosphatic fertilizers is practised though a majority of the soil P reaction products are only sparingly soluble. Under such conditions, microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants.

Phosphate solubilizing microorganisms (PSM) include largely bacteria and fungi, which can grow in media containing tricalcium, iron and aluminium phosphate, hydroxyapatite, bonemeal, rock phosphate and similar insoluble phosphate compounds as the sole phosphate source. Such microbes not only assimilate P but a large portion of soluble phosphate is released in quantities in excess of their own requirement[143]. The most efficient PSM belong to genera *Bacillus* and *Pseudomonas* amongst bacteria and *Aspergillus* and *Penicillium* amongst fungi. The reported bacilli include, *B. brevis*, *B. cereus*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. mesentricus*, *B. mycoides*, *B. polymyxa*, *B. pumilis*, *B. pulvifaciens* and *B. subtilis* from the rhizosphere of legumes, cereals (rice and maize), arecanut palm, oat, jute and chilli[144–155]. *Pseudomonas striata*, *P. cissicola*, *P. fluorescens*, *P. pinophillum*, *P. putida*, *P. syringae*, *P. aeruginosa*, *P. putrefaciens* and *P. stutzeri* have been isolated from rhizosphere of *Brassica*, chickpea, maize, soybean and other crops, desert soils and Antarctica lake[154,156–160]. In addition, *Escherichia freundii*, *E. intermedia*, *Serratia phosphaticum* and species of *Achromobacter*, *Brevibacterium*, *Corynebacterium*, *Erwinia*, *Micrococus*, *Sarcina* and *Xanthomonas* are active in solubilizing insoluble phosphates. Cyanobacteria, viz. *Anabaena* sp., *Calothrix brauni*, *Nostoc* sp., *Scytonema* sp. and *Tolypothrix ceylonica* can also solubilze phosphate[160].

Among phosphate solubilizing fungi, *Aspergillus niger*, *A. flavus*, *A. nidulans*, *A. awamori*, *A. carbonum*, *A. fumigatus*, *A. terreus* and *A. wentii* have been reported from the rhizosphere of maize, soybean, chilli, tista soils, acidic lateritic soils and compost[161–163]. *Paeciliomyces fusisporus*, *Penicillium digitatum*, *P. simplicissimum*, *P. aurantiogriseum*, *Sclerotium rolfisii* and species of *Cephalosporium*, *Alternaria*, *Cylindrocladium*, *Fusarium* and *Rhizoctonia* are other solubilizers of insoluble phosphate. Amongst yeasts, *Torula thermophila*, *Saccharomyces cerevisiae* and *Rhodotorula minuta* can solubilize inorganic phosphate[164]. PSM inoculants include species

of *Aspergillus*, *Bacillus*, *Escherichia*, *Arthrobacter* and *Pseudomonas*[165–166] which can add 30–35 kg P_2O_5 ha^{-1} (ref. 176).

Goldstein *et al.*[168] demonstrated that an efficient mineral phosphate solubilizing phenotype in Gram-negative bacteria resulted from extracellular oxidation of glucose to gluconic acid via the quinoprotein glucose dehydrogenase. A unique bacterial population isolated from the roots of *Helianthus annus jaegeri* growing at the edge of an alkaline dry lake in the Mojave Desert in Israel showed no mineral phosphate solubilizing activity and no gluconic acid production. Addition of a concentrated solution containing material washed from the roots to these bacteria in culture however resulted in production of high levels of gluconic acid. This suggested that signalling between bacteria and plant root regulated expression of direct oxidation pathway in this bacterium. The resultant acidification of the rhizosphere played a key role in nutrient availability and/or other ecophysiological parameters essential for the survival of this desert plant. The establishment and performance of PSM is however affected severely under stressed conditions such as high salt, pH and temperature prevalent in degraded ecosystems represented by alkaline soils with tendency to fix phosphorus[169]. In a screening of 4800 bacterial isolates from the root-free soil, rhizosphere and rhizoplane of *P. juliflora* growing in alkaline soils, 857 morphotypes solubilized phosphate in agar. The incidence of PSB was highest in the rhizoplane, followed by rhizosphere and root-free soil. Phosphate solubilizing ability of strain NBRI4 was higher than control in the presence of salts (NaCl, $CaCl_2$ and KCl) at 30°C and it further increased at 37°C (ref. 176). Strain NBRI2601 (ref. 171) isolated from the rhizosphere of chickpea and alkaline soils could solubilize phosphorus in presence of 10% salt, pH 12, at 45°C suggesting that extensive diversity searches in appropriate habitats may lead to recovery of effective bacteria.

The mechanism of osmotic stress adaptation in *P. aeruginosa* PAO1 was investigated by D'Souza-Ault *et al.*[172]. By using natural abundance ^{13}C nuclear magnetic resonance spectroscopy, osmotically stressed cultures were found to accumulate glutamate, trehalose, and *N*-acetylglutaminylglutamine amide, an unusual dipeptide previously reported only in osmotically stressed *Rhizobium meliloti* and *P. fluorescens*. The intracellular levels of these osmolytes were dependent on the chemical composition and the osmolality of the growth medium. It was also demonstrated that glycine betaine, a powerful osmotic stress protectant, participated in osmoregulation in this organism.

Other plant growth promoting rhizobacteria

Other microorganisms that are known to be beneficial to plants are the plant growth promoting rhizobacteria (PGPR). In addition to supplying combined nitrogen by biological nitrogen fixation, certain bacteria affect the development and function of roots by improving mineral (NO_3, PO_3^{-3} and K^+) and water uptake. Considerable research is underway globally to exploit the potential of one such group of bacteria that belong to fluorescent pseudomonad (FLPs). FLPs help in maintenance of soil health, protect crop from pathogens and are metabolically and functionally most[173,174]. *P. corrugata*, a form that grows at 4°C under laboratory conditions[175], produces antifungals such as diacetylphloroglucinol and/or phenazine compounds that aid in phosphate solubilization. According to Gaur *et al.*[176], 50–60% of fluorescent pseudomonads recovered from the rhizosphere and endorhizosphere of wheat grown in Indo-Gangetic plains were antagonistic towards *Helminthosporium sativum*. Field trials of a pseudomonad strain (GRP3) lead to yield increase[177] from 5.6 to 18%.

Rangarajan *et al.*[178] analysed populations of *Pseudomonas* for their biochemical characters and genetic diversity using molecular tools including RAPD and PCR-RFLP and found that increased salinity caused selection of *P. pseudoalcaligenes* and *P. alcaligenes*, irrespective of the host rhizosphere. *Xanthomonas oryzae* pv. *oryzae* and *Rhizoctonia solani* – the bacterial leaf blight (BB) and sheath blight (ShB) pathogens of rice (*Oryza sativa*) were suppressed by indigenous *Pseudomonas* strains isolated from rhizosphere of rice cultivated in the coastal agri-ecosystem under both natural and saline soil conditions[179]. Schnider-Keel *et al.*[180] found that AlgU was a crucial determinant in the adaptation of *P. fluorescens* to dry conditions and hyperosmolarity, the two major stress factors that limit bacterial survival in the environment.

Recently, concern was shown on the use of FLPs in crop plants as the antifungal substances released by the bacterium, particularly 2,4-diacetylphloroglucinol (DAPG) could affect the arbuscular mycorrhizal fungi[181]. Gaur *et al.*[174] confirmed that DAPG producing pseudomonads recovered from wheat rhizosphere did not adversely affect AM colonization. However, given the toxicity of DAPG, such an inhibition may probably be dependent on the amounts released by the bacterium.

Bacterial diversity in rice–wheat cropping systems

Rice and wheat, the two most staple food crops of India, are cultivated as wheat–rice cropping sequence worldwide by farmers. However, intensive use of chemical fertilizer has resulted in increased soil salinity leading to deterioration of soil health. Moreover, the cultivation practices of two food grains are completely different; as rice requires waterlogging, which creates microaerophilic to anaerobic environment, that may change the rhizosphere microbial community. When wheat is sown in the same field, the microbial community structure has to change to aerobic resulting in alteration in soil biological equilibrium. Wheat–rice ecosys-

tem is therefore of central interest to explore for sustainable agriculture.

In view of its global significance in agriculture production and human health, wheat agroecosystem has been studied extensively from the point of view of bacterial diversity during the last 15–20 years across various regions of the world – Algeria[182], Canada[183], India[184] (Mittal and Johri, unpublished), France[185] and The Netherlands[186,187]. In a study involving rhizosphere of *Triticum monococcum* (an ancient wheat cultivar), *T. aestivum* cv Red File (a historical cultivar), and *T. aestivum* cv CDC Teal (a modern cultivar)[183], a continuum in microbial diversity from the ancient races to modern cultivar, was observed. The endophytic community of the more modern cultivar was more diverse than the ancient race. Pseudomonad population was more numerous and diverse in root interior than rhizosphere, however there was greater abundance of *P. fluorescens* in the latter niche; bacilli were predominant in rhizosphere. Genera *Aureobacter* and *Salmonella* were recovered only within the roots of ancient wheat cultivar.

In an interesting study of long-term organic cultivation of summer and winter wheat in the Netherlands, culture-dependent methods showed presence of diverse Gram-positive bacteria; there were few proteobacteria and green sulphur bacteria however pseudomonads were the second most dominant member[24]. Data from culture-independent methods showed that a large proportion of the sequences belonged to the division Acidobacterium, followed by proteobacteria; no sequences belonged to Gram-positive forms. While genera *Arthrobacter, Corynebacterium* and *Micrococcus* were recovered all through the crop season, *Bacillus* was recovered only in July, a period with most reduced diversity spectrum. Selective enrichment of FLPs in wheat rhizosphere has long been known from take-all diseased plots[188] and a general life pattern for such pseudomonads has been described recently[189]. Diversity of *Paenibacillus polymyxa* was studied in Durum wheat in fields with cropping history of 5 yr (H5, Z26), 70 yr (D70) and 2000 yr (K2000, T2000)[182]. In general, phenotypic bacterial diversity declined with extended period of wheat cultivation. In contrast, occurrence of N_2-fixing forms was more frequent in plants with short cultivation history (H5, Z26, D70) showing similar genetic structure however those recovered from T2000 and K2000 were genetically distinct from such bacterial populations. Long term cropping history therefore appeared to influence the genetic make up of *P. polymyxa* populations. The influence of soil type, climate, wheat cultivar and crop management practices would have however played a role in the long history of strain evolution.

In the Indian context, the rhizosphere community structure of wheat crop and influence of genotype on community structure has been studied quite extensively for the Indo-Gangetic region[183,190–192]. It was observed that wheat genotype did not appreciably influence the total bacterial and pseudomonad populations. Population structure was only marginally different in rhizosphere (RS) and rhizoplane (RP) fractions, which could be explained on the basis of wheat genotype-dependent influence[192]. Analysis of culturable genetic diversity by ARDRA and REP-PCR showed that for any one variety, distribution of various bacterial morphotypes was fairly even (Figure 1) RP fraction was generally more diverse than RS fraction. Diversity indices showed var. UP2338 to be most rich (E) and var. HD2687 to be most diverse (H'). Numerical analysis of phenotypic characters (morphological, biochemical, physiological and functional) revealed that most of the isolates exhibiting greater similarity with *Pseudomonas* reference strains belonged to var. UP2338; this was later on confirmed by 16S rDNA sequencing[192]. Sequencing data also revealed that among *g*-proteobacteria, *Pseudomonas* was most dominant, however, *Pseudoxanthomonas* and *Stenotrophomonas* were other common inhabitants of wheat rhizosphere. Plant species-specific distribution of bacterial groups in different wheat varieties was explained by exclusive presence of *Enterobacter amnigenus* and *Vogesella indigofera* in var. PBW343, *Hydrogenophaga* in var. K8027, *Aeromonas, Arthrobacter* and *Thermonas* in var. HD2687, and *Rhizobium* and *Brevundimonas* in var. UP2338 (GenBank accession numbers AY677123 – AY677127, AY682627–AY682677).

The effect of plant type on community composition was investigated by the study of rhizosphere microbial population of wheat (*Triticum aestivum)* and mandua (*Eleusine coracana*) grown at Chaukhutia, Almora. Phylogenetic analysis using 16S rDNA restriction profiles from the cereals distinctly placed in two separate clusters (Figure 2), although *Pseudomonas* and *Bacillus* (GenBank accession numbers AY389815, AY390771, AY392012, AY442189, AY498709, AY498710, AY498711) were the predominant rhizosphere inhabitants of both the crops (Mittal *et al*., unpublished).

Raised bed management practice of wheat cultivation is a new development to achieve sustainable agriculture and to maintain soil health. When bacterial diversity of two management practices, conventional (pf) and raised bed (rb) systems, were compared for wheat variety UP2338, higher diversity of *Pseudomonas* was observed in plain field based on ARDRA, sequencing and SSCP data whereas total diversity (SSCP) and functional diversity were greater in raised bed as revealed by Shannon's diversity index (H′). Most *Pseudomonas* isolates belonged to *P. fluorescens* bv I, II, III and IV, and *P. putida* bv B (Mittal and Johri, unpublished). The diversity data is to be corroborated with soil nutrient and soil health parameters to relate population structure to management practices. However, to come to terms with the deterioration of soil quality in this and other agroecosystems, it is now necessary to apply functional assays using microarrays since soil is indeed complex and interactions very diverse.

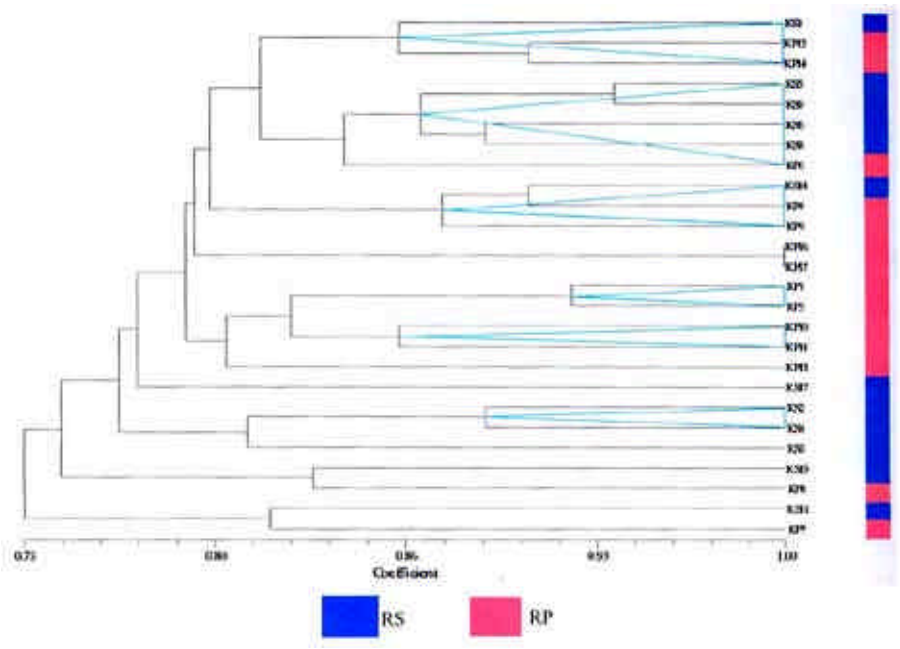

Figure 1. UPGMA dendrogram showing phylogenetic relationship among isolates recovered from rhizosphere (RS) and rhizoplae (RP) of wheat var. K8027.

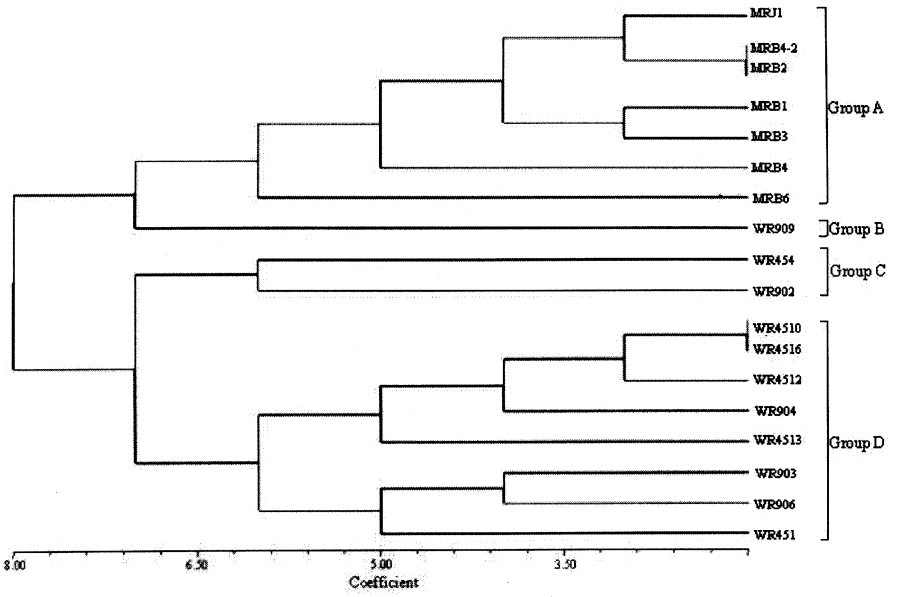

Figure 2. NJ tree of ARDRA profile generated after restriction digestion of 16S rDNA of bacterial isolates recovered from mandua rhizosphere (MR) and wheat rhizosphere (WR).

Diversity of growth promoting bacteria associated with rice under deepwater and salinity

The race for producing more rice by adopting intensive agronomic practices and applying more nitrogenous fertilizers is thought to have had adverse effects on the diversity of nitrogen-fixing bacteria in the paddy fields. This could have enriched chemical nitrogen scavenging bacteria. It is therefore expected that modern varieties of rice may have higher nitrogen use efficiency in terms of their yield response to chemical fertilizer application but may have lost their associative nitrogen fixation ability. This trait of associative nitrogen fixing ability is expected to be present in wild and traditional rice varieties, which do not respond well to chemical fertilizer application but retain the associative nitrogen fixation ability. In India,

two typical paddy cultivation systems that are affected by submergence and salinity have been systematically investigated using state of the art molecular methods.

In North Eastern India, some varieties of paddy are traditionally grown in ponds and low-lying fields termed, deep water rice, such varieties grow in over a meter deep water for more than a month. Five varieties of deep-water rice grown in a large lake were investigated for endophytic bacterial diversity employing PCR-RFLP of 16S rDNA, BOX-PCR and 16S rDNA nucleotide sequencing. The endophytic bacterial community consisted of enterobacteria, *Pantoea*, *Citrobacter* and *Klebsiella*. In addition, other bacteria such as, *Ochrobactrum*, *Stenotrophomonas*, *Pseudomonas* and *Microbacterium* were also isolated from surface sterilized seeds and stems of five different rice varieties, grown in the same lake. Consistency of their association was confirmed by reisolation from the seeds harvested in three consecutive years, at two different locations. Endophytic occurrence of the members of Enterobacteriaceae was more consistent than others; interestingly, they were conspicuous by their absence in the soils/sediments of the lake in which these rice varieties grew. This strongly indicated that members of Enterobacteriaceae are transmitted from one generation of rice to the next, not by the contact of seeds with soil, but directly via seeds in a manner similar to seed-borne pathogens. Most of these endophytic bacteria produced IAA, and pectinase and cellulase that would help to invade plant tissues. Some were able to solubilize insoluble phosphate but only *Pantoea*, *Citrobacter* and *Klebsiella* possessed the ability to fix atmospheric nitrogen[59]. As Enterobacteriaceae are known to fix nitrogen anaerobically, it was logical that the submerged portions of rice under the deepwater facing nearly anaerobic condition may be the right locations for endophytic nitrogen fixation. One of the diazotrophic endophytes, i.e. *Pantoea* was genetically tagged with both *gus*- and *gfp*-reporters, and shown to vigorously colonize the inter-cellular spaces in the roots of the rice seedlings[193].

In South India, paddy is cultivated along the coastline of Tamil Nadu where salinity gradient dominate. Effect of salinity on the diversity of two important plant associated bacteria, i.e. *Azospirillum* and *Pseudomonas*, was investigated at several paddy fields with varying levels of salinity. An increase in salinity led to decrease in bacterial diversity. PCR-RFLP of 16S rDNA from 256 *Pseudomonas* strains isolated from five paddy cultivation sites revealed the occurrence of 18 different genotypes. Fluorescent pseudomonads dominated at non-saline sites whereas salt-tolerant species, in particular *Pseudomonas alcaligenes* and *P. pseudoalcaligenes* dominated the saline sites. Diversity of pseudomonads at saline sites was higher when organic farming was practised, showing positive effects of organic farming on the diversity of pseudomonads under saline conditions[194]. Taxonomic analysis of 402 strains isolated by enrichment in NFB medium from 12 paddy cultivation sites with varying salinity and soil texture, revealed that 302 of them belonged to *Azospirillum*. They were represented by 19 fingerprints (genotypes) based on PCR-RFLP of 16S rDNA. Of the 19 genotypes, 15 were specific to non-saline soils whereas only two genotypes were specific to saline soils[116]. Enrichments for *Azospirillum* on NFB media have to be taken with caution, as none of the bacteria isolated from rhizosphere of the rice grown in salinity-affected fields and enriched in NFB medium, however, turned out to be *Azospirillum*[195]. Identification based on nucleotide sequence of 16S rDNA revealed that the bacterial community in the rice rhizosphere from salt-affected rice consisted of *Alacaligenes xylosoxidans*, *Ochrobactrum anthropi*, *Serratia marcescens* and *Pseudomonas aeruginosa*.

A search for bacteria isolated from the rhizosphere, roots and stems of salt-tolerant, mangrove-associated wild rice (*Porteresia coarctata* Tateoka) using nitrogen-free, semi-solid LGI medium at pH 5.5 revealed close association of a novel genus and species, *Swaminathania salitolerans*[196]. This novel bacterium was able to fix nitrogen and solubilize phosphate in the presence of NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that these strains were related to the genera *Acidomonas*, *Asaia*, *Acetobacter*, *Gluconacetobacter*, *Gluconobacter* and *Kozakia* in the *Acetobacteriaceae*.

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