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Content

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From the Editor's Desk

Dear Readers,

Over last two decades, while biofertiliser's production, number of production units and usage is continuously increasing, research out put is decreasing. There is hardly any break through in product formulations, shelf life improvement and super strain development. Private industry is coming up with its own formulations without any scientific validation and database. The trend need to be checked and the technologies must be authenticated before releasing in the field.

Bringing biofertilisers under fertilizers control order, has initiated the regulatory process and let us hope, it will usher the biofertilisers into new and effective quality scenario. In the last two decades molecular advancements has revolutionized many disciplines. Bacterial taxonomy has also gone under sea change. Every month new species are being described and old ones are re-classified. Rhizobium – root nodule bacteria has also gone many changes. The term rhizobia now refers to all the genera and species which form nitrogen fixing nodules in legume roots.

The rhizobia as a group now possess 63 validly published species belonging to 11 genera. The present issue gives an up-to-date status of rhizobial taxonomy with complete list of published species and their hosts. Other fixed columns are there in usual format with latest developments, research, happenings and book reviews. I hope the readers will find this issue informative.

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Changing Face of Rhizobial Taxonomy

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Introduction

Symbiosis between leguminous plants and soil bacteria commonly referred to as rhizobia are of considerable environmental and agricultural importance since they are responsible for most of the atmospheric nitrogen fixed on land. Rhizobia are able to elicit on most of the 18,000 species of the Leguminosae family, the formation of specialized organs, called nodules, in which they reduce atmospheric nitrogen to ammonia to the benefit of the plant. The term 'rhizobia', in the strictest sense, refered to members of the genus *Rhizobium*. Over the years, however, the term has come to be used for all the bacteria that are capable of nodulation and nitrogen fixation in association with legumes and that belong to a genus that was at one time part of the genus *Rhizobium* or closely related to it.

The family Rhizobiaceae in the 1984 edition of Bergey's Manual of Systematic Bacteriology, was composed of the rhizobia (at that time just including *Rhizobium* and *Bradyrhizobium*), *Agrobacterium* and *Phyllobacterium* (Jordan, 1984).

History of Rhizobial Taxonomy

By the end of the 19th century, it was realized that atmospheric nitrogen was being assimilated through the root-nodules of legume plants. In 1888, Beijerinck reported isolation of the root nodule bacteria. He named these bacteria *Bacillus radicicola* (Beijerinck, 1888). Later, Frank changed the name to *Rhizobium* with originally just one species, *R. leguminosarum* (Frank, 1889). Extensive testing of nodulation of diverse legume hosts by different bacteria in the beginning of the 20th century, led to the establishment of cross-inoculation groups, with rhizobia from one plant in a cross-inoculation group supposed to infect all other plants in the group (Fred *et al*., 1932). This concept was used in rhizobial taxonomy for some time, but later it was discarded mainly due to problem of rhizobial promiscuity among plant groups. Then the bacteriologists, in the early sixties, started using a large number of diversified morphological, nutritional, metabolic, serological and simple DNA characteristics in numerical studies demonstrating relatedness of *Rhizobium* and *Agrobacterium* and clear distinction between fast and slow growing rhizobia (Graham, 1964). Subsequently, the slow growing rhizobium was placed in a separate genus '*Bradyrhizobium'* (Jordan, 1982) .

Impact of molecular techniques on rhizobial taxonomy

Since 1980s, the shape of the whole bacterial taxonomy has undergone

tremendous changes and made a quantum jump in terms of progress mainly because of introduction of more genetic characteristics. Before that, the prokaryotic taxonomy was dependant on phenotypic and biochemical methodologies for many years and constrained to provide a meaningful framework for the classifications of bacteria (Cumming *et al* 2001). Recent technological advancements (DNA–DNA and DNA–rRNA hybridizations, rRNA catalogues, rDNA sequencing) give appropriate techniques to determine the relationships between different bacterial lineages. Particularly, the use of molecular chronometers like 16srRNA and their sequencing has provided a mechanism by which the evolutionary relationship among bacteria can be established. Most recent taxonomic studies have made use of a polyphasic approach with genetic, phenotypic, chemotaxonomic, phylogenetic data combined to establish a comprehensive picture of the relationships of the bacteria, and to propose a suitable classification. Therefore, more diversity among rhizobia has been discovered and their relationship with other groups of bacteria has become evident. This led to a gradual increase in the number of genera. At the same time, there is a significant increase in the number of validly published species with 63 species of rhizobia now recognized. The other reason is, earlier the main emphasis were concentrated to legumes that were food and pasture species. But now many different species of legume are being studied. Even now, only about 20% of the total of about 18,000 species and 57% of about 650 genera of legume plants have been studied for nodulation (Sprent, 1995). This leaves a large number of legume species to be studied and potentially many more species and genera of rhizobia to be described.

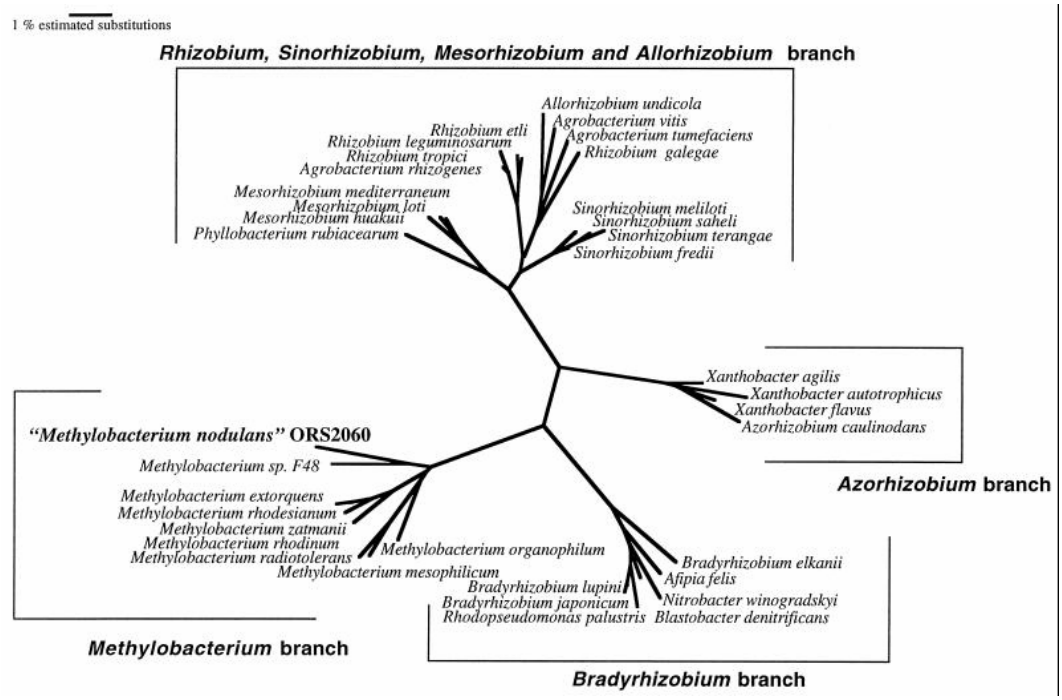

Fig.1 Unrooted phylogenetic tree of rhizobial branches on the alpha-subclass of proteobacteria .(Source : Sy *et al* 2001)

Present Status of Rhizobial Taxonomy

The rhizobial species described so far are very diverse and do not form an evolutionary homogenous clade. They belong to three distinct branches within the alpha-2 subclass of Proteobacteria and are phylogenetically intertwined with non-symbiotic bacteria (Young and Haukka,1996). A first large branch groups the genera *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Allorhizobium* with *Agrobacterium*, a pathogenic bacterium of plants. A second branch contains the genus *Bradyrhizobium* together with photosynthetic free-living *Rhodopseudomonas*, whereas the third branch includes the genus *Azorhizobium* as well as the chemoautotroph *Xanthobacter.* Each rhizobial species has a defined host range, varying from very narrow, as in the case of *Azorhizobium caulinodans*, to very broad, as in the case of *Sinorhizobium* sp. strain NGR234. Symbionts of legumes exhibiting ecological and agronomic potential should be characterized prior to their use in sustainable agriculture and environment management.

The *Rhizobium - Allorhizobium-Agrobacterium* -16S rDNA sequence data reveal that *Agrobacterium*, *Allorhizobium* and *Rhizobium* are rather more closely related in the alpha-Proteobacteria and are quite separate from *Bradyrhizobium* and *Azorhizobium*. *Sinorhizobium* and *Mesorhizobium* also form separate clusters. The proposal (Young *et al*., 2001) to abandon the genera *Agrobacterium* and *Allorhizobium* and incorporate them in *Rhizobium*, has not been widely accepted (*Farrand et al*., 2003).

Rhizobium

The genus *Rhizobium* (Frank 1889) was the first named (from latin meaning root living), and for many years this was a 'catch all' genus for all rhizobia. Some species were later moved in to new genera based on phylogenetic analysis. It currently consists of 17 rhizobial species (Table 1). From the 16S rDNA phylogeny (Figure 1), it is clear that *Rhizobium* and *Agrobacterium* are highly related and their species are interwoven. In particular, biovar 2 groups with the majority of *Rhizobium* species. Biovar 1 consists of several smaller groups representing different genospecies. *A. rubi* and *A. larrymoorei* are closely related to these biovar-1 genospecies. *A. vitis* is close to *Allorhizobium*. *Rhizobium giardinii* is the most peripheral of the whole group. Young *et al*. (2001) proposed to transfer all these taxa to *Rhizobium*. They proposed to unite *A. radiobacter* and *A. tumefaciens* in *R. radiobacter*, which thus represents biovar-, while *A. rhizogenes* becomes *R. rhizogenes* and represents biovar-2. *A. rubi* and *A. vitis* are transferred to *Rhizobium* as distinct species and also *A. larrymoorei* is transferred as *R. larrymoorei* (Young, 2004). This proposal solves the species matching the biovars and the placement of *A. rhizogenes* in *Rhizobium* or *R. vitis,* is clearly justified, but it is not widely accepted (Farrand *et al.,* 2003). However, as these species (formerly under *Agrobacterium*) do not enter into symbiotic N fixing nodulation with legumes, are not included in the list of rhizobial species under *Rhizobium.*

Table 1. List of approved *Rhizobium* species

Species	Host	Year	Reference
R. cellulosilyticum	isolated from sawdust of *Populas alba*	2007	Garcma-Fraile *et al.* (2007)
R. daejeonense	*Medicago sativa*	2006	Zhexue *et.al 2005*
R. etli	*Phaseolus vulgaris*	1993	Segovia *et al.* (1993)
R. galegae	*Galega*	1989	Lindstrom (1989)
R. gallicum	*Phaseolus vulgaris*	1997	Amarger *et al.* (1997)
R. giardinii	*Phaseolus vulgaris*	1997	Amarger *et al.* (1997)
R. hainanense	*Desmodium, Stylosanthes, Centrosoma, Tephrosia, Acacia, Zornia, Macroptilium*	1997	Chen *et al.* (1997)
R. huautlense	*Sesbania herbacea*	1989	Wang *et al.* (1998)
R. indigoferae	*Indigofera*	2002	Wei *et al.* (2002)
R. leguminosarum (Type)	*Pisum, Lathyrus, Vicia, Lens, Phaseolus, Trifolium*	1879	Frank (1879)
R. loessense[1]	*Astragalus*	2003	Wei *et al.* (2003)
R. lusitanum[2]	*Phaseolus vulgaris*	2006	Valvarde *et al.* 2006
R. mongolense	*Medicago ruthenica*	1998	van Berkum *et al.* (1998)
R. sullae[3]	*Hedysarum hedysary*	2002	Squartini *et al.* (2002)
R. tropici	*Phaseolus vulgaris, Leucaena*	1991	Martinez-Romero *et al.* (1991)
R. undicola[4]	*Neptunia natans*	2001	Young (2001)
R. yanglingense	*Coronilla*, *Gueldenstaedtia*, *Amphicarpaea*	2001	Tan *et al.* (2001)

1.formerly "*R.. huanglingense* 2. formerly *Herbaspirillum lusitanum* ; 3 formerly *Rhizobium hedysari* 4. formerly *Allorhizobium undicola*

***Ensifer* (formerly *Sinorhizobium*)**

A separate genus for the fast-growing soybean rhizobia, renaming *R. fredii* as *Sinorhizobium fredii*, and a second species, *S. xinjiangense* was proposed by Chen *et al.* (1988). This new genus was controversial at first since genetic evidence to justify its creation and to separate it from *R. fredii* was not presented at the time (Jarvis *et al.1992).*

Later, phylogenetic data were presented to support a third genus of rhizobia, not restricted to the fast-growing soybean rhizobia (de Lajudie *et al.,* 1994) and the genus definition was emended. *R. meliloti* was transferred to *Sinorhizobium* as *S. meliloti* and two additional species, *S. saheli* and *S. terangae*, were proposed for isolates from *Acacia* and *Sesbania* from Senegal. It has recently become evident from 16S rDNA comparisons that *Ensifer adhaerens* is also phylogenetically a member of the *Sinorhizobium* lineage (Balkwill, 2005), therefore, *Sinorhizobium* and the genus *Ensifer* (Casida, 1982) belong to a single taxon. Since, *Ensifer* is the earlier heterotypic synonym (it was named first), thus, takes priority (Young, 2003). This means that all *Sinorhizobium* spp. must be renamed as *Ensifer* spp. according to the Bacteriological code. The taxonomy of this genus was verified in 2007 by Martens *et. al.* (2007). The genus currently consists of 15 species.

Mesorhizobium

The genus *Mesorhizobium*, characterized by a growth rate, intermediate between the fast- and slow growing rhizobia, was initially proposed for five rhizobial species (*R. loti, R.*

huakuii, R. ciceri, R. mediterraneum and R. tianshanense) that are phylogenetically related and distinct from the large phylogenetic grouping of *Rhizobium*, *Agrobacterium* and *Sinorhizobium* (Jarvis *et al.,* 1997). On the basis of 16S rDNA sequence data, *Mesorhizobium* is phylogenetically separated from the fast-growing rhizobia by the genera *Bartonella, Defluvibacter, Aquamicrobium Phyllobacterium, Aminobacter* and *Pseudoaminobacter* and perform photosynthesis (Alazard, 1985; Evans *et al.,* 1990; Molouba *et al.,* 1999). Some photosynthetic bradyrhizobia have also been reported as endophytes of African wild rice (Chaintreuil *et al.,* 2000). Several species were moved from *Rhizobium* to this genus. It currently consists of 11 species.

Table 2 *Ensifer* (fomerly *Sinorhizobium*)

Species	Host	Year	Reference
E. abri	*Acacia, Prosopis chilensis*	2003	Ogasawara *et. al.* (2003), Nick *et al.* (1999)
E. americanum	*Acacia spp*. (Mexico)	2003	Toledo *et al.* (2003)
E. arboris	*Acacia, Prosopis*	1999	Nick *et al.* (1999)
E. fredii (formerly *R. fredii*, Type)	*Glycine, Vigna, Cajanus*	1984	Scholla and Elkan (1984)
E. indiaense	*Sesbania rostrata*	2003	(Ogasawara, *et. al.* 2003),
E. kostiense	*Acacia, Prosopis*	1999	Nick *et al.* (1999)
E. kummerowiae	*Kummerowia stipulacea*	2002	Wei *et al.* (2002)
E. medicae	*Medicago*	*1996*	Rome *et al.* (1996)
E. meliloti formerly *Rhizobium meliloti*	*Melilotus, Medicago, Trigonella*	1926	Dangeard (1926)
E. mexicanum	*Acacia angustissima*	2007	Lloret *et. al.* (2007)
*'Sinorhizobium morelense'**	*Leucaena leucocephala*	2002	Wang *et al.* (2002)
*E. adhaerens****	*Medicago sativa L. leucocephala*, *Pithecellobium dulce*	2002	Willems *et al.* (2002)
E. saheli (also known as *S. sahelense)*	*Sesbania, Acacia*	2002	de Lajudie *et al.* (1994)
*E. terangae***	*Sesbania*	1994	de Lajudie *et al.* (1994)
E. xinjiangense	*Glycine*	1988	Chen *et al.* (1988)

*This species is distinct from *Ensifer adhaerens* but cannot yet be named *Ensifer)*

** This species is incorrectly known as *Sinorhizobium teranga*

*** This name is a earlier heterotypic synonym *of Sinorhizobium morelense* (see Young, 2003))

Bradyrhizobium

Genus *Bradyrhizobium* with a single species, *B. japonicum*, was proposed for symbionts of soybean (Jordan 1982). Later, Hollis and coworkers (Hollis *et al* 1981) separated *B. japonicum* into three DNA homology groups with species *B. elkanii* for one group and *B. liaoningense* for another group comprising extra slow growing *Glycine* isolates, retaining the name *B. japonicum* for slow-growing isolates of *G. max*.

A major factor complicating the evaluation of the taxonomic status and interrelationships of bradyrhizobia is the high similarity of 16S rDNA gene sequences. Many strains have 16S rDNA

sequence divergences of 0.1–2.0%. Only sequences for *B. elkanii* and related strains differ by up to 4% from those of other *bradyrhizobia* (Willems *et al*., 2001a). On the basis of 16S rDNA similarities and total DNA homology values, *B. elkanii* is considered distinct from *B. liaoningense* and could represent a separate genus; *B. liaoningense* is phylogenetically closer to *B. japonicum* which is closer to genera *Afipia*, *Agromonas*, *Blastobacter*, *Nitrobacter* and *Rhodopseudomonas*. The genus *Bradyrhizobium* currently consists of 7species

Table 3. *Mesorhizobium* species

Species	Host	Year	Reference
M. amorphae	*Amorpha fruticosa*	1999	Wang *et al.* (1999)
M. chacoense	*Prosopis alba*	2001	Vela´ zquez *et al.* (2001)
M. ciceri (formerly *Rhizobium ciceri)*	*Cicer arietinum*,	1994	Nour *et al*. (1994)
M. huakuii	*Astragalus*	1991	Chen *et al.* (1991)
M. loti (formerly) *Rhizobium huakuii*	(formerly *Rhizobium loti*, Type species *Loti*) *Lotus, Lupinus, Anthyllis, Leucaena*	1982	Jarvis *et al.* (1982)
M. mediterraneum (formerly *R. mediterraneum)*	*Cicer arietinum*	1995	Nour *et al.* (1995)
M. plurifarium	*Acacia, Prosopis, Chamaecrista, Leucaena*	1998	de Lajudie *et al.* (1998b)
M. septentrionale	*Astragalus adsurgens*	2004	Gao *et al*. 2004
M. temperatum	*Astragalus adsurgens*	2004	Gao *et al* 2004
M. tianshanense (formerly R. tianshanense	*Glycyrrhiza, Sophora, Caragana, Halimodendron, Swainsonia, Glycine*	1995	Chen *et al.* (1995)
M. thiogangeticum	Rhizosphere of *Clitoria ternatea*	2006	Ghosh and Roy (2006)

Table 4. *Bradyrhizobium* species

Species	Host	Year	Reference
B. elkanii	*Glycine max*	1992	Kuykendall *et al*. (1992)
B. japonicum[1]	*Glycine max*	1982	(Jordan, 1982).
B. liaoningense	*Glycine max*	1995	Xu *et al.* (1995)
B. yuanmingense	*Lespedeza*	2002	Yao *et al.* (2002),
B. canariense	from genistoid legumes from the Canary Islands	2005	(Vinuesa *et al*. (2005).
B. denitrificans.	*Aeschynomene indica*	2002	van Berkum & Eardly, (2002).
B. betae	from the roots of *Beta vulgaris* afflicted with tumor-like deformations	2004	Rivas *et al.* (2004)

[1](formerly *R. japonicum*, Type species

Azorhizobium

The ***Azorhizobium*** genus was described by Dreyfus *et al*. in 1988. It currently consists of 2 species (Table 5).

New entrants in rhizobial group

Recently, a number of isolates, capable of nitrogen fixation but phylogenetically located outside the traditional groups of rhizobia in the alpha-Proteobacteria, but do carry nod genes similar to those of rhizobia, have been reported from legume nodules. These genes encode for Nod factors, signal molecules in the bacterium–legume communication to enforce nodulation. These new legume symbionts include *Methylobacterium*, *Devosia*, *Ochrobactrum* and *Phyllobacterium* in the alpha-Proteobacteria and *Burkholderia*, and *Cupriavidus* in the beta-Proteobacteria. Most of the new nodulating bacteria belong to genera that have at least some plant-associated species and that are therefore, likely to have the molecular strategies to overcome plant defenses. Recent reports suggest that it is highly probable that more such bacteria, capable of effective nodulation will come up outside traditional rhizobia (Barret and Parker, 2006; Rasolomampianina *et al.,* 2005; Zakhia *et al*., 2006).

Table 5. List of *Azorhizobium* species

Species	Host	Year	Reference
Azorhizobium caulinodans	*Sesbania rostrata*	1988	Dreyfus *et al.* (1988)
Azorhizobium doebereinerae[1]	*Sesbania virgata*	2006	Moreira *et al,* 2006

[1]formerly *Azorhizobium johannae*

Methylobacterium

The ***Methylobacterium*** genus currently contains only one rhizobial species.

Species	**Host**	**Year**	**Reference**
Methylobacterium nodulans	*Crotolaria*	2004	Sy *et al.* 2000

Burkholderia

The ***Burkholderia*** genus currently contains five named rhizobial members and others as *Burkholderia* sp.

Species	**Host**	**Year**	**Reference**
Burkholderia caribensis	*Mimosa*	2005	Chen *et al* 2005
Burkholderia cepacia now *B. dolosa*	*Alysicarpus*	2004	Vermis *et al.*2004
Burkholderia mimosarum	*Mimosa*	2006	Chen *et al.*2006
Burkholderia phymatum	*Machaerium*	2001	Mouline *et al.,* 2001
Burkholderia tuberum	*Aspalathus*	2001	Mouline *et al.* 2001

Cupriavidus

Cupriavidus formerly *Wautersia,* formerly *Ralstonia,* has recently undergone several taxonomic revisions. This genus currently contains a single rhizobial species.

Species	Host	Year	Reference
Cupriavidus taiwanensis	*Mimosa*	2001	Barrett and Parker 2006

Devosia

The ***Devosia*** genus currently contains only a single rhizobial species.

Species	**Host**	**Year**	**Reference**
Devosia neptuniae	*Neptunia natans*	2003	Rivas *et al.* 2003

Ochrobactrum

The ***Ochrobactrum*** genus currently contains two rhizobial species.

Species	Host	Year	Reference
Ochrobactrum cytisi	*Cystisus scorparius*	2007	Zurdo-Piqeiro *et al.* 2007
Ochrobactrum lupini	*Lupinus*	2005	Traujillo *et al.* 2005

Phyllobacterium

The *Phyllobacterium* genus currently contains a single rhizobial species.

Species	Host	Year	Reference
Phyllobacterium trifolii	*Trifolium, Lupinus*	2005	Valverde *et al.* 2005

Remarks

Taxonomy is a complicated and ever-changing discipline of science. The recent accelaration in the pace of these changes due to technological advancements, specially in molecular science, have made it more difficult to keep track of the taxonomic standing of any particular group of bacteria, like rhizobia. More so, because of the importance of rhizobia for their symbiotic association to legumes contributing to their productivity, many scientific workers are engaged to explore the field of legume research. The species listed above are so far validly published names of rhizobia consisting 63 species spread over 11 genera. Rhizobia are nitrogen-fixing bacteria that form nodules on the roots of leguminous plants. It may also be noted that, the list described so far, is not 'official' and this list will undergo as many changes in near future as we saw in the last decade.

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Research Notes and New Reports

Nitrogen-fixing and cellulose-producing *Gluconacetobacter kombuchae* sp. nov., isolated from Kombucha tea – A few members of the family *Acetobacteraceae* are cellulose-producers, while only six members fix nitrogen. Bacterial strain RG3^T, isolated from Kombucha tea, displays both of these characteristics. A high bootstrap value in the 16S rRNA gene sequence-based phylogenetic analysis supported the position of this strain within the genus *Gluconacetobacter*, with *Gluconacetobacter hansenii* LMG 1527^T as its nearest neighbour (99.1 % sequence similarity). It could utilize ethanol, fructose, arabinose, glycerol, sorbitol and mannitol, but not galactose or xylose, as sole sources of carbon. Single amino acids such as L-alanine, L-cysteine and L-threonine served as carbon and nitrogen sources for growth of strain RG3^T. Strain RG3^T produced cellulose in both nitrogen-free broth and enriched medium. The ubiquinone present was Q-10 and the DNA base composition was 55.8 mol% G+C. It exhibited low values of 5.2–27.77 % DNA–DNA relatedness to the type strains of related gluconacetobacters, which placed it within a separate taxon, for which the name *Gluconacetobacter kombuchae* sp. nov. is proposed, with the type strain RG3^T (=LMG 23726^T=MTCC 6913^T).

(Source - Debasree Dutta and Ratan Gachhui Int J Syst Evol Microbiol 2007, 57 : 353-357)

Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture - In the present study, the capacity of phosphate solubilizing bacterial strain, *Bacillus* (FS3) and fungal isolates, *Aspergillus* FS9 and FS11 have been tested in National Botanical Research Institutes Phosphate nutrient medium (NBRIP) broths containing two different phosphate sources, $Ca_3(PO_4)_2$ and rock phosphate (18% of total P_2O_5 and CaO 42.2%, of which 27% of P was soluble in 2% citric acid) at two different concentrations (10 and 20 g 250 ml^{-1}). NBRIP broth without microbial inoculation was used as the control. The phosphorus solubilizing bacteria and fungi inoculation decreased solution pH and increased electrical conductivity, and Ca and P concentrations in solution culture. The largest pH decrease was found with FS9 fungi inoculation at 20 g 250 ml^{-1} with three calcium phosphate (TCP) applications when compared to control. Similarly, the highest EC values, Ca and P concentrations were found in NBRIP broth with FS9 inoculation at the concentration of 20 g 250 ml^{-1} TCP when compared to control and other treatments. In addition, it was found that the highest Ca uptake was formed in solution culture inoculated with FS3 bacteria at 10 g 250 ml^{-1} rock phosphate (RP) application. The result suggested that phosphate solubilizing bacteria FS3 and fungal strain FS9 have great potential for bio-fertilizer development in agriculture.

(Source - Metin Turan, Nizamettin Ataoğlu and Fikrettin Sahin Journal of Sustainable Agriculture 2006, Vol 28 : 99 – 108)

Co-immobilization of *Azospirillum lipoferum* and *Bacillus megaterium* for successful phosphorus and nitrogen nutrition of wheat plants – The efficacy of strains of *Pseudononas fluorescens, Bacillus megaterium* and *Azospirilum* spp. in *in-vitro* solubilisation of Ca_3Po_4 was studied. *Pseudomonas fluorescens* and *Bacillus megaterium* strains were the most powerful phosphate solubilizers on Pikovskaya (PVK) plates and liquid medium. *Azospirillum lipoferum* strains showed weak zones of solubilization on the PVK plates. Phosphate solubilization by the tested organisms was accompanied with pH reduction of the culture medium. Maximum pH reduction was 2.8, 1.2 and 0.5 units for *Pseudomonas fluorescens, Bacillus megaterium* and *Azospirillum lipoferum* strain 137, respectively. Alginate and agar immobilization of the tested bacteria or coimmobilization of *A. lipoferum* 137 and *B. megaterium* significantly enhanced phosphorus solubilization for four consecutive 4-day cycles. In a pot experiment, phosphorus mobilization in wheat (*Triticum aestivum* L. cv. Beni Swif 1) inoculated with *B. megaterium* or *A. lipoferum* 137 as single or mixed inocula (as free or alginate immobilized beads) was studied in presence of Ca_3Po_4. Wheat inoculated with mixed inocula exhibited high shoot dry weight, total nitrogen (N) yield and the shoot phosphorus content increased by 37 and 53% compared to the plants inoculated with *A. lipoferum* and uninoculated ones, used as control, respectively. Maximum nitrogenase activity (measured by acetylene reduction assay) was observed in mixed inoculum treatment, and was increased by 500 and 32% compared to uninoculated and *A. lipoferum* inoculated plants. Results demonstrate the beneficial influence of coinoculation of *A. lipoferum* and *B. megaterium* for providing balanced N and P nutrition of wheat plants.
(Source – *El-Komy* 2005 Food Technol. Botechnol. 43(1) 19-27).

Co-inoculation effects of phosphate solubilizing microorganisms and *Glomus fasciculatum* on Green Gram-*Bradyrhizobium* symbiosis - Experiments were conducted to evaluate the effects of nitrogen fixing (*Bradyrhizobium* sp. (*Vigna*)), phosphate solubilizing bacterium (*Bacillus subtilis*), phosphate solubilizing fungus (*Aspergillus awamori*) and AM fungus (*Glomus fasciculatum*) on the growth, chlorophyll content, seed yield, nodulation, grain protein, and N and P uptake of green gram plants grown in phosphorus-deficient soils. The triple inoculation of AM fungus, *Bradyrhizobium* sp. (*Vigna*) and *B. subtilis* significantly increased dry matter yield, chlorophyll content in foliage and N and P uptake of green gram plants. Seed yield was enhanced by 24% following triple inoculation of *Bradyrhizobium + G. fasciculatum + B. subtilis*, relative to the control. Nodule occupancy, determined by indirect enzyme linked immunosorbent assay (ELISA), ranged between 77% (*Bradyrhizobium + A. awamori*) and 96% (*Bradyrhizobium + G. fasciculatum + B. subtilis*) at flowering (45 DAS), decreasing at the pod-fill (60 DAS) stage with each treatment. Replica immunoblot assay (RIBA) revealed a greater variation in the rhizobial

populations within nodules and the correlation between nodule occupancy and immunoblot counts was highly significant at 45 (r = 0.95) and at 60 DAS (r = 0.96). There was a negative effect on some of the measured parameters when *A. awamori* was used alone or added to the combination treatments. The present findings showed that rhizospheric microorganisms can interact positively in promoting plant growth, as well as N and P uptake of green gram plants, leading to improved yield.
(Source - Zaidi and Khan, 2006, Turk J Agric For 30 223-230)

An efficient microbiological growth medium for screening phosphate solubilizing microorganisms – A novel defined microbiological growth medium "National Botanical Research Institute's Phosphate growth medium (NBRIP)", which is more efficient than Pikovskaya medium (PVK), was developed for screening phosphate solubilizing microorganisms. In plate assay the efficiency of NBRIP was comparable to PVK; however, in broth assay NBRIP consistently demonstrated about 3-fold higher efficiency compared to PVK. The results indicated that the criterion for isolation of phosphate solubilizers based on the formation of visible halo/zone on agar plates is not a reliable technique, as many isolates wich did not show any clear zone on agar plates solubilized insoluble inorganic phosphates in liquid medium. It may be concluded that soil microbes shold be screened in NBRIP broth assay for the identification of most efficient phosphate solubilizers.

(Source – FEMS Microbiol Lett. 1999; 170(1):265-70, Patented composition, available online at http://www.freepatentsonline.com/6638730.html)

Effect of the herbicides oxadiazon and oxyfluorfen on phosphates solubilizing microorganisms and their persistence in rice fields – A field experiment has been conducted with two herbicides viz. oxadiazon [5-terbutyl-3-(2,4-dichloro-5-isopropoxyphenyl)-1,3,4-oxadiazol-2-one] and oxyfluorfen [2-chloro-1-(3-ethoxy-4-nitrophenyl)-4-(trifluoromethyl) benzene] at rates of 0.4 and 0.12 kg/a.i.ha^{-1}, respectively to investigate their effect on the growth and activities of phosphate solubilizing microorganisms in relation to availability of phosphorus and persistence of the herbicides in the rhizosphere soil of wetland rice (*Oryza sativa* L. variety IR-36). Application of herbicides stimulated the population and activities of phosphate solubilizing microorganisms and also the availability of phosphorus in the rhizosphere soil. Oxyfluorfen provided greater microbial stimulation than oxadiazon. Dissipation of oxyfluorfen and oxadiazon followed first order reaction kinetics with half-life ($T_{1/2}$) of 8.8 and 12 days respectively. Sixty days after application 0.5% and 3% of the applied oxadiazon and oxyfluorfen residues persisted, respectively, in the rhizosphere soil of rice.
Source – Das *et.al* 2003, Chemosphere, 53(3):217-221).

New research may reduce global need for nitrogen fertilizers - Research published in June 2006 issue of the

journal "Nature" reveals how scientists at the John Innes Centre (JIC), Norwich and Washington State University, USA have managed to trigger nodulation in legumes, a key element of the nitrogen fixing process, without the bacteria, otherwise necessary. The research, funded by the Biotechnology and Biological Sciences Research Council (BBSRC), the Royal Society and the US National Science Foundation, have used a key gene that legumes require to establish the interaction with the nitrogen-fixing bacteria to trigger the growth of root nodules, even in the absence of the bacteria. Nodules are normally formed when the plant perceives the presence of the bacteria. The fact that we can induce the formation of nodules in the plant in the absence of the bacteria is an important first step in transferring this process to non-legumes. If this could be achieved we could dramatically reduce the need for inorganic nitrogen fertilizers, in turn reducing environmental pollution and energy use. However, we still have a lot of work before we can generate nodulation in non-legumes."
(Source - http://www.bbsrc.ac.uk/media/pressreleases/06_06_28_nitrogen_fertilizers.htm)

***Swaminathania salitolerans* gen. nov., sp. nov., a salt-tolerant, nitrogen-fixing and phosphate-solubilizing bacterium from wild rice (*Porteresia coarctata* Tateoka)** - A novel species, *Swaminathania salitolerans* gen. nov., sp. nov., was 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. Strains were Gram-negative, rod-shaped and motile with peritrichous flagella. The strains grew well in the presence of 0·35 % acetic acid, 3 % NaCl and 1 % KNO_3, and produced acid from L-arabinose, D-glucose, glycerol, ethanol, D-mannose, D-galactose and sorbitol. It oxidized ethanol and grew well on mannitol and glutamate agar. The fatty acids 18 : 1 ω7*c*/ω9*t*/ω12*t* and 19 : 0cyclo ω8*c* constituted 30·41 and 11·80 % of total fatty acids, respectively, whereas 13 : 1 AT 12–13 was found at 0·53 %. DNA G+C content was 57·6–59·9 mol% and the major quinone was Q-10. 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 *Acetobacteraceae*. Isolates were able to fix nitrogen and solubilized phosphate in the presence of NaCl. Based on overall analysis of the tests and comparison with the characteristics of members of the *Acetobacteraceae*, a novel genus and species is proposed for these isolates, *Swaminathania salitolerans* gen. nov., sp. nov. The type strain is PA51^T (=LMG 21291^T=MTCC 3852^T).
(Full paper available at http://ijsb.sgmjournals.org/cgi/content/full/54/4/1185)

***Azospirillum melinis* sp. nov., a group of diazotrophs isolated from tropical molasses grass** - Fifteen bacterial strains isolated from molasses grass (*Melinis minutiflora* Beauv.) were identified as nitrogen-fixers by using the acetylene-reduction assay and PCR amplification of *nifH* gene fragments.

These strains were classified as a unique group by insertion sequence-PCR fingerprinting, SDS-PAGE protein patterns, DNA–DNA hybridization, 16S rRNA gene sequencing and morphological characterization. Phylogenetic analysis of the 16S rRNA gene indicated that these diazotrophic strains belonged to the genus *Azospirillum* and were closely related to *Azospirillum lipoferum* (with 97.5 % similarity). In all the analyses, including in addition phenotypic characterization using Biolog Micro Plates and comparison of cellular fatty acids, this novel group was found to be different from the most closely related species, *Azospirillum lipoferum*. Based on these data, a novel species, *Azospirillum melinis* sp. nov., is proposed for these endophytic diazotrophs of *M. minutiflora*, with TMCY 0552^T (=CCBAU 5106001^T=LMG 23364^T=CGMCC 1.5340^T) as the type strain.
(Source – Peng *et al*, 2006, Int J Syst Evol Microbiol **56**, 1263-1271)

Azospirillum oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the roots of the rice plant *Oryza sativa - The taxonomic position of the free-living diazotrophic strain COC8^T isolated from rice was investigated based on phylogenetic analyses. 16S rRNA gene sequence analyses indicated that strain COC8^T was closely related to the genus *Azospirillum* (96% similarity). Chemotaxonomic characteristics (G+C content of the DNA 66·8 mol%, Q-10 quinone system, 18 : 1ω7*c* as the major fatty acid and 14 : 0 3-OH and 16 : 0 3-OH as the major hydroxy fatty acids) were also similar to those of the genus *Azospirillum*. Based on its physiological characteristics, strain COC8^T can clearly be differentiated from recognized species of *Azospirillum*. The name *Azospirillum oryzae* sp. nov. is proposed to accommodate this bacterial strain; the type strain is COC8^T (=IAM 15130^T=CCTCC AB204051^T).
(Source - Cheng-Hui Xie and Akira Yokota**,** Int J Syst Evol Microbiol 2005, **55** 1435-1438)

Nitrogen fixation in wheat provided by *Klebsiella pneumoniae* 342 - *Klebsiella pneumoniae* 342 (Kp342) has been reported to fix atmospheric nitrogen in association with wheat (*Triticum aestivum* L), the world's most important crop. Kp342 relieved nitrogen (N) deficiency symptoms and increased total N and N concentration in the plant. Nitrogen fixation was confirmed by ^{15}N isotope dilution in the plant tissue and in a plant product, chlorophyll. All of these observations were in contrast to uninoculated plants, plants inoculated with a nitrogen-fixing mutant of Kp342, and plants inoculated with dead Kp342 cells. Nitrogenase reductase was produced by Kp342 in the intercellular space of the root cortex. Wild-type Kp342 and the *nifH* mutant colonized the interior of wheat roots in equal numbers on a fresh weight basis. The nitrogen fixation phenotype described here was specific to cv. Trenton. Inoculation of cvs. Russ or Stoa with Kp342 resulted in no relief of nitrogen deficiency symptoms.
(Source- Leonardo et al 2004 The American Phytopathological Society 17(10) : 1078–1085).

Seminar, Conferences and Workshops

15th International Congress on Nitrogen Fixation - The 15th International Congress on Nitrogen Fixation was organized during 21-26 January 2007 in Cape Town, South Africa – The important topics of discussion during the conference were: Genomics of Nitrogen Fixers and Associated Plants, Legumes in Agriculture and Forestry, Fundamentals of Nitrogen Fixation, Chemistry and Biochemistry of Nitrogenases, Rhizosphere Associations and Nitrogen-Fixing Endophytes, Genetics and Regulation of Nitrogen Fixation, Sustainable Agriculture and Limitations to BNF, Frontiers of BNF Research, Phylogeny of Nitrogen Fixers and Evolution of Symbioses, Nodule Organogenesis and Function (Legumes and Actinorhizal plants), Phytosynthetic Nitrogen Fixers, BNF and food security and BNF and Poverty Reduction. Detailed proceedings are awaited. Further details can be obtained from The Secretariat, 15th ICNF 2007, PO Box 2760, Clareinch 7740, South Africa **Telephone**+27 21 683 5522 **Fax:** +27 21 674 3269 **Email:** aecon.e@mweb.co.za

National Seminer on Microbes on Pharmaceutical Food and agriculture - was organized during 20 - 21st December 2006 at Department of Microbiology, Vidyasagar University, Kolkata. The Theme of the conference was on the recent developments in the field of Agriculture, Food and Pharmaceuticals by the application of microbial technology. Following areas were covered through interactive discussions among scientists, researchers, students and entrepreneurs.

- Biofertilizer
- Biopesticide
- Microbial food processing & preservation
- Food colourants and contaminants
- Production and advance application of microbial enzyme
- Microbial polymers
- Microbes mediated Pharmaceutical products
- Probiotics

International Workshop on EM (Effective Microorganisms) Technology is scheduled for 23-26 July 2007 throughat APNAN, Bangkok, Thailand. The workshop offers the following benefits:

1. Interaction with the Japanese Masters to learn more about this amazing technology.
2. Interaction with more than 40 people from different Nationalities to share their experience.
3. Seeing is believing, which also leads to greater confidence building. The entire 70 acre land and forest is using EM in every aspect of their lives. The results are truly amazing.
4. There are numerous field visits where you can see how Thai farmers and people manage their farms and sanitation. It is truly amazing.

For further details contact Mr. Sanjay Aggarwal, Mapel Orgatech (India) Ltd. Gandhi Road, Dehradun – 248 001. TELEFAX (0135) 2657119 AND 2654447

Book Review

The Prokaryotes - A Handbook on the Biology of Bacteria: Vols. 1-7 (Set), Editor-in-chief: Dworkin, Editors Martin Falkow, S.; Rosenberg, E.; Schleifer, K.-H. and Stackebrandt, E., Version: p+eRef (book + online access), 3rd ed., 2007, ISBN: 978-0-387-33488-2, Price $6,150.00 (full set) - The first edition of **The Prokaryotes**, published in 1981, was a bold step to become the first, most comprehensive and authoritative encyclopedic handbook on prokaryotes. The information was further upgraded with the second edition in 1992, when the chapters were organized on the basis of the molecular phylogeny as a rational, evolutionary basis for the taxonomy of the prokaryotes. By then, the two volumes of the first edition had expanded to four. With the decision to publish the handbook electronically, the third edition is the largest of all. The advantages are obvious and persuasive: essentially unlimited space, no restrictions on the use of color, and the inclusion of film and animated illustrations. Nevertheless, the affection for a printed handbook was highly under estimated and during the first 5 years of the continuously evolving online version, a growing demand for a new print edition was voiced by the scientific and corporate community. Springer is now publishing a third edition in printed form. In total, 7 volumes will make up this new fully revised and updated version. Compared to the second edition, this edition will contain 85% new contents, printed in color throughout. It will be ideally suited for research centers in academia and in the corporate world that need reliable and up-to-date information on the biology of the prokaryotic organisms.

Bergey's Manual of Systematic Bacteriology Editor-in-chief: Garrity, George M. Boone, David R.; Castenholz, Richard W. (Eds.) Published by Williams & Wilkins, 2nd ed., ISBN: 978-0-387-98771-2 - Bergey's Manual of Systematic Bacteriology, one of the most comprehensive and authoritative works in the field of bacterial taxonomy, has been extensively revised in the form of a five volume Second Edition. Since the first edition was published in 1984, the field has undergone explosive growth, with over 2200 new species and 390 new genera having been described. Numerous taxonomic rearrangements and changes in nomenclature have resulted from more than 850 published new combinations. These developments, which are attributable to rapid advances in molecular sequencing of highly conserved regions of the prokaryotic genome, most notably genes coding for the RNA of the small ribosomal subunit, have lead to a natural classification that reflects the evolutionary history of Bacteria and Archaea, and to the development of new, universally applicable methods of identifying these organisms. This new edition has been completely reorganized along phylogenetic lines to reflect the current state of prokaryotic taxonomy but still maintains the familiar layout of the First Edition. In addition to the detailed treatments, provided for all of the validly named and well-known species of

prokaryotes, new ecological information and more extensive introductory chapters have been added. Use of the manual is aided by a system of cross referencing between the phylogenetic groups and the phenotypic groups used in the First Edition. In keeping with the tradition of the First Edition, volumes will be available individually, and eventually as a complete set.

Volume One : The Archaea and the Deeply Branching and Phototrophic Bacteria. Editors David R. Boone and Richard W. Castenholz 2001, XXI, 721 p., 251 illus., Hardcover –The volume provides descriptions of 413 species in 165 genera that are assigned to the phyla Crenarchaeota, Euryarchaeota, Aquificae, Thermatogae, Thermodesulfobacteria, "Deinococcus -Thermus," Chrysiogenetes, Chloroflexi, Thermomicrobia, Nitrospira, Deferribacteres, Cyanobacteria, and Chlorobi. In addition, the volume contains an introductory chapter to nonoxygenic, phototropic species of Bacteria belonging to the Proteobacteria and Firmicutes, which will be repeated in more detail in subsequent volumes.

Volume Two: The Proteobacteria, (2004) Editors Don J. Brenner, Noel R. Krieg and James T. Staley The Volume 2, Proteobacteria culminates a four year effort by Bergey's Manual Trust and more than 150 internationally recognized authorities to provide a comprehensive view of the Proteobacteria, the largest prokaryotic phylum. Out of 6250 named species of Bacteria, the Proteobacteria represent the single largest phylum. Encompassing 72 families and including descriptions of 538 genera and over 2000 named species, the volume is further subdivided into three sub-volumes: The Gammaproteobacteria (Part A), The Alphaproteobacteria (Part B) and the Beta-, Delta-, and Epsilonproteobacteria. Also included are new introductory chapters specific to the phylum. The Proteobacteria also represent the most diverse group of bacteria, metabolically and ecologically. Moreover, the Proteobacteria contain many of the clinically relevant species of Bacteria and are of significance in human, animal and plant health. As a result, this volume caters to the broadest audience and the set is an essential reference for the microbiologist.

PGPR: Biocontrol and Biofertilization, Edited by Siddiqui, Zaki A. Springer 2006, Hardcover 2006, XIII, 318 p., ISBN 1402040024, Price £65.00 - PGPR (Plant growth promoting rhizobacteria) have gained world wide importance and acceptance for agricultural benefits. These microorganisms are the potential tools for sustainable agriculture and the trend for the future. Scientific researches involve multidisciplinary approaches to understand adaptation of PGPR to the rhizosphere, mechanisms of root colonization, effects on plant physiology and growth, biofertilization, induced systemic resistance, biocontrol of plant pathogens, production of determinants etc. Biodiversity of PGPR and mechanisms of action for the different groups: diazotrophs, bacilli, pseudomonads, and rhizobia are shown. Effects of physical, chemical and biological factors on root colonization

and the proteomics perspective on biocontrol and plant defence mechanism is discussed. Visualization of interactions of pathogens and biocontrol agents on plant roots using autofluorescent protein markers has provided more understanding of biocontrol process. Commercial formulations and field applications of PGPR are detailed.

Molecular Basis of Symbiosis, edited by Jorg Overmann, Springer 2006 Hardcover XIV, 310 p. 60 illus., 5 in colour, ISBN 3-5402821-06, Price Bp.100.00 – Symbiotic associations involving prokaryotes occur ubiquitously and are ecologically highly significant. In symbiotic associations, co-evolution of the partner organisms has led to specific mechanisms of signal exchange and reciprocal regulation, and resulted in novel physiological capabilities of the association as compared to those of the individual partners. Symbiosis research has recently entered an exciting era because molecular biology techniques are available for studying partner organisms in association and in a culture-independent manner. It is the goal of this book to contribute towards a broader perspective and an understanding of the function of symbiotic systems. 14 different model systems have been chosen, comprising well known symbioses as well as novel experimental systems which have only recently become amenable to experimental manipulation.

Microbial Root Endophytes. Edited by Schulz, Barbara, JE; Boyle, Christine JC; Seiber, Thomas N. Springer 2006. hardcover XX, 267 p., 29 illus. 4 in colour, ISBN 3-5403352-50, Price Bp.115.00 – Plant roots may not only be colonized by mycorrhizal fungi, but also by a myriad of bacterial and fungal root endophytes that are usually not considered by the investigators of classic sybioses. This is the first book dedicated to the interactions of non-mycorrhizal microbial endophytes with plant roots. The phenotypes of these interactions can be extremely plastic, depending on environmental factors, nutritional status, genetic disposition and developmental stages of the two partners. The book deals with diversity, life history strategies, interactions, applications in agriculture and forestry, methods for isolation, cultivation, and both conventional and molecular methods for identification and detection of these endophytes. The comprehensive reviews demonstrate the high diversity of interactions and will provoke further studies to better understand the mechanisms which determine whether a plant-microbial interaction remains asymptomatic, leads to disease or to a mutualistic interaction. The book is very useful to students and scientistis in botany, ecology, agriculture, forestry, microbiology and soil biology.

First International Meeting on Microbial Phosphate Solubilization, Series: Developments in Plant and Soil Sciences , Vol. 102 Edited by Velazquez-Perez, E.; Rodriguez-Barrueco, C., 2007, ISBN: 978-1-4020-4019-1 - The last decade has seen a significantly increased knowledge about phosphate solubilizing microorganisms. Sixty specialists from 13 countries met in Salamanca to discuss the problems of high P-unavailability as a soil nutrient for crops, and the hazards of an increasing phosphate input to aquatic habitats from

industrial and mining activities, sewage disposal, detergents, and other sources. Updated solutions to enhance P-uptake by plants, bioremediation potential in the rehabilitation of ecosystems, taxonomic characterization interactions with mycorrizae, the physiological and molecular basis of PSM, and possibilities of genetic modifications of rhizospheric microorganisms were among the contributions presented. Challenges in commercializing a phosphate solubilizing microorganism were also outlined by a relevant biotech company. Editors have tried to attract the attention of agronomists, environmentalists, technocrats and administrators holding responsibilities in the field of soil conservation and sustainable agricultural production.

Associative and Endophytic Nitrogen-fixing Bacteria and Cyanobacterial Associations (Nitrogen Fixation: Origins, Applications, and Research Progress) by Claudine Elmerich (Editor), **William E. Newton** (Editor) by **Claudine Elmerich** (Editor), **William E. Newton** (Editor) ISBN-13: 978-1402035418 Springer; (November 2006), Price $169.00 - This book is self-contained fifth volume of a comprehensive seven-volume series covering both fundamental and applied aspects of nitrogen-fixation research since the 19th century. It addresses the issues arising from bacterial colonization of either the plant-root surface or other tissues as well as their modes of doing so. These associations are less formalized than the rhizobia-legume symbiosis but, as more and more of them are discovered, their myriad of effects on their plant hosts is becoming understood. An understanding at the molecular level of the mechanisms by which these bacteria benefit crop productivity is an important issue in agriculture. This book describes the milestones in the discovery of the associative and endophytic nitrogen-fixing bacteria (*Azoarcus, Azospirillum, Gluconacetobacter, Herbaspirillum*, and others) found intimately involved with cereal crops, forage grasses, and sugar cane. There are also chapters describing the bacterial functions required for a bacterium to be competent and competitive in the rhizosphere; these include chemotactic response, adhesion and motility, enzymes and secondary-metabolite production, and synthesis of phytohormones, which play an important role in the association with the host plants. In addition, the plant's response to inoculation is reviewed. The book also provides an up-to-date analysis of the different associations of cyanobacteria with fungi, diatoms, bryophytes, cycads, *Azolla*, and *Gunnera*, including the complex regulatory network that controls the differentiation of vegetative cells into nitrogen fixing heterocysts. No other available work provides the up-to-date and in-depth coverage of this volume, which is intended to serve as an indispensable reference work for academic, government, and industrial scientists working in the areas of plant microbiology, ecology, and genetics, including those studying plant growth and biocontrol; to assist students to enter this challenging area of research; and to provide science administrators with ready access to vital relevant information.