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Diversity of Root Nodule Bacteria: Strengthening R&D and Inoculant Supply

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There is a renewed global interest in biological nitrogen fixation (BNF) for the last several decades, particularly in assessing the diversity of root nodule bacterial populations in diverse geographies. This article focuses on the current status and argues for the establishment of centres of excellence on Nitrogen Fixation, Microbial Inoculants and Soil Ecology to provide sharper focus and impetus for the research and development effort needed for rhizobial strain improvement and for streamlining inoculants supply to help achieve improved soil health and N sustainability.

Introduction

The world nitrogen fertilizer demand continues to grow but the scenario has undergone a significant change with China and India accounting for nearly half the consumption. Overuse and imbalanced application, discontinuance of traditional practices like crop residue recycling, composts and green manuring, legume rotations etc., have allled to adverse effects on soil physical properties, reduced soil organic matter content and diminished soil biodiversity. Climate change will further exacerbate these problems. Rightly, therefore, the ecofriendly processes like biological nitrogen fixation (BNF) by legume-rhizobia symbiosis are regaining importance because BNF can supply a major portion of N in low external input agriculture.

Legume BNF

Global agricultural BNF is estimated at 50-70 Tg yr^{-1}; cultivated legumes supply 24 Tg of N annually with soybean alone accounting for over three-fourths of it. The BNF input added by various crops of India was estimated at 5.20-5.76 Tg yr^{-1} (Rao and Balachandar, 2017) representing 9.5 to 10.6 % of the global agricultural BNF, with legumes averaging about 2.24Tg yr^{-1}. About 50 million hectares of crop legumes, equivalent to about 25% of the area sown globally are inoculated with rhizobia each year with average yield benefits ranging from 15-20%. The primary goal of any legume rhizobial inoculant programme is to select those strains that compete well with native rhizobia for nodule occupancy, are stress-tolerant and most effective in improving BNF and yields. An overview of the status of BNF in Indian Agriculture is given in Rao (2014).

The bacteria which form nitrogen-fixing symbiosis with legumes are collectively called rhizobia. Until 1992, there were only four genera of root nodulating bacteria: *Rhizobium, Bradyrhizobium, Sinorhizobium* and *Azorhizobium*. Later, four more were added, *Mesorhizobium, Allorhizobium, Methylobacterium* and *Burkholderia.* The classification of rhizobia is in great flux for the last 3 decades. The nomenclature of some of the old species is under revision and many new genera and species are being continuously discovered. The majority of legume nodulating bacteria (LNB) described so far belong to α-*Proteobacteria* class. The isolation and characterization of new and often unusual LNB including *Agrobacterium* on diversified plant hosts have resulted in the naming of many new rhizobial species. On the basis of the 16S rRNA gene sequence, the currently described legume symbionts of α, β and γ-Proteobacteria belong to 238 species in 18 genera and two clades. The number of rhizobial species that can nodulate cultivated legumes has expanded, e.g., for common bean (14 species) soybean (11), cowpea (6), chickpea (5), peanut (5), lentils (4), faba bean (3) and pea (2 species) (Shamseldin *et al.,* 2017). The currently recognized important genera and species of rhizobia are shown in the box.

Diversity of Rhizobia

The outcome of the huge amount of work done on the diversity of *Rhizobium* in the last four decades is apparent in the constant flux in the taxonomy of root nodulating rhizobia owing to the rapid progress in molecular biology-based omics technologies. The recent use of whole-genome sequencing-based taxonomy will

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obviously again change the current taxonomy. The increase in legume explorations in different geographical regions is leading to isolation and characterization of more rhizobial species and the discovery of newer ones. The major focus in the last three decades has been on soybean rhizobia in China, Brazil and India.

Rhizobial Collections

Extensive, organised collections of root nodule bacteria were made all over India beginning from the late 1960's at IARI, New Delhi under a PL-480 project and under the ICAR's AICRP on Pulses Improvement Project from the 1970's. ICRISAT, Hyderabad from the mid-1970's created an extensive collection of rhizobia not only from India but of many elite global strains, these were later transferred to IARI under the DBT-*Rhizobium* germ plasm collections in early 1990's. Extensive surveys were made on nodulation of tree legumes at CSSRI, Karnal during mid-1990's in a DBT project and rhizobia deposited in the above collection. However, intensive efforts on biogeography of rhizobia were made only later, under the AINP on Soil Biodiversity-Biofertilizers, IISS, Bhopal first for soybean in Madhya Pradesh from 2002 onwards and later for all the major legumes grown in MP, Haryana, Rajasthan and Gujarat from 2009 onwards. The improved strains of soybean *Bradyrhizobiun japonicum* are in supply for inoculant manufacture in central India. In the DBT-funded India-UK Nitrogen Fixation Centre (IUNFC) from 2016, effective strains of *Brady rhizobium yuan mingense* and *Sinorhizobium* sp. of pigeonpea were isolated and field tested in central India at IISS, Bhopal and in North Indian Plains at NBAIM, Mau. The improved strains of rhizobia from the studies in the last 15 years in India are available in the depository of microbial germplasm at ICAR-NBAIM, Mau.

Rhizobial Taxonomy

Rhizobium strains
Class α-Proteobacteria
I Order Rhizobiales
I Family Rhizobiaceae
Genus *Rhizobium* (98); Genus *Ensifer* (formerly *Sinorhizobium*) (21); Genus *Allorhizobium* (1); Genus *Shinella* (1); Genus *Pararhizobium* (5).
II Family Phyllobacteriaceae
Genus *Mesorhizobium* (40); Genus *Phyllobacterium* (8); Genus *Aminobacter* (1)
III Family Bradyrhizobiaceae
Genus *Bradyrhizobium* (37); Genus *Blastobacter* (2); Genus *Photorhizobium* (1)
IV Family Hyphomicrobiaceae
Genus *Devosia* (1); Genus *Azorhizobium* (3)
V Family Methylobacteriaceae

Notwithstanding the above, it can be asserted that rhizobial research in India has been comparatively neglected by microbiologists, attributable mainly to excessive attention towards plant growth-promoting rhizobacteria (PGPR). The rigorous efforts required in extraction of rhizobia from root nodules, differentiation from non-rhizobia, batteries of nodulation tests, MPN assays for soil rhizobial populations, ARA assays of nitrogenase activity in root nodules, continuous green-house testing of the strains every year, measurements of competition with native rhizobial strains for nodule occupancy by inoculant strains in fields using marked strains or their intrinsic antibiotic resistance, field measurements of N fixation either by N difference with non-legumes, isotope dilution methods or long-term N balance over several years summarize some of the reasons for the diversion towards more convenient research avenues.

Perspective

The neglect of rhizobial research is dangerous and needs urgent redressal. The only solution is to establish a centre on the pattern of countries like Australia devoted exclusively to research on rhizobia, maintain the culture collections, undertake continuous testing and centralise mother culture supply each crop season to inoculant manufacturers as per the need of various geographies.

Such a centre also needs to conserve rhizobia from wild legume relatives of cultivated legumes, tree legumes etc. It needs to be adequately staffed with scientists and technicians and equipped with advanced instruments for BNF measurements by isotope abundance.

There is an urgent need to improve rhizobial inoculant quality standards in India, both in terms of rhizobial counts and fewer contaminants. Also, develop technologies for manufacturing high-count inoculants and novel delivery systems.

During the PM's Vaibhav Summit in the vertical on "Microbial Resources for Sustainable Agriculture" held on Oct 10, 2020, I concluded my presentation *(Soil Ecological Stewardship-BNF, Microbial Inoculants, Soil Biological Indicators*) with a need for creating a long-term institutional framework, firstly, strengthen the existing networks of AINP Soil Biodiversity-Biofertilizers; AMAAS project and Crop and Commodity AICRPs; and secondly establish 3 advanced centres of excellence at NBAIM, Mau, IARI, New Delhi and IISS, Bhopal with collaborations with ICAR institutes and SAU's.

- India Nitrogen Fixation Research Centre (INFRC)
- Indian Microbial Inoculants Technology Centre (IMTC)
- India Soil Ecology Research Centre (ISERC)

As a follow-up action of the summit, the establishment of the above centres along with assured core manpower and budget and linking up with entrepreneurial start-ups for inoculant manufacture would help achieve the goal of timely supply of quality inoculants to farmers through assured supply chains and translate into improved BNF, soil biodiversity and soil health, and ultimately to lesser use of externally applied fertilizers, greater input use efficiency and improved factor productivity.

References

Rao DLN (2014) Recent advances in biological nitrogen fixation in agricultural systems. *Proc. Ind. Nat. Sci. Acad.* **80**: 359-378.

Rao DLN and D Balachandar (2017) Nitrogen inputs from Biological Nitrogen Fixation in Indian Agriculture. In: YP Abrol *et al.* (eds) The Indian Nitrogen Assessment. Sources of reactive nitrogen, environmental and climate effects, management options and policies. Elsevier, pp. 117-132.

Shamseldin A, A Abdelkhalek, MJ Sadowsky (2017) Recent changes to the classification of symbiotic, nitrogen-fixing, legume-associating bacteria: a review. *Symbiosis* **71**:91-109.