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Diversity in a promiscuous group of rhizobia from three *Sesbania* spp. colonizing ecologically distinct habitats of the semi-arid Delhi region

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Abstract

Sesbania-rhizobia associations have immense significance in soil amelioration programs for diverse habitats. Diversity in symbiotic properties, LPS profiles, *Sym* plasmid and rhizobiophage sensitivity of 28 root- and stem-nodulating bacterial isolates of three *Sesbania* species (*S. sesban*, *S. aegyptica* and *S. rostrata*) inhabiting six ecologically distinct sites of semi-arid Delhi region was analyzed. The isolates were highly promiscuous among the symbiotic partners (*Sesbania* spp.). The root nodules formed by all the isolates were morphologically similar but they differed in their symbiotic efficiency and effectiveness. 16S rDNA sequence analyses revealed that root nodule isolates of sesbanias belong to diverse rhizobial taxa (*Sinorhizobium saheli*, *S. meliloti*, *Rhizobium huautlense*) whereas stem-nodule isolates were strictly *Azorhizobium caulinodans*. *Sinorhizobium* spp. seem to dominate as microsymbiont partner of *Sesbania* in the Delhi region. The genetic diversity revealed by cluster analyses based on NPC-PCR reflects sorting of isolates across the ecological gradient. Parallel diversity was also observed in the grouping based on LPS profiles and *sym* plasmid (NPC-PCR). Segregation of different rhizobial taxa into distinct types/clusters based on LPS and NPC-PCR analyses suggest its significance in the circumscription of the taxa. However, subtypes and subclusters showed their sorting across the ecological gradients. *Sesbania* rhizobia showed extremely high specificity to rhizobiophages. Enormous diversity in LPS profiles and high specificity of rhizobiophages might be the result of environmental selection pressures operating in ecologically distinct habitats. The ability of sesbanias to enter into effective symbioses with different rhizobial taxa and colonize diverse habitats with various biotic and abiotic stresses appears to contribute to its wide ecological amplitude.

Keywords: *Sesbania* spp.; Diversity in root- and stem-nodulating bacteria; Promiscuity; LPS; Phage sensitivity; NPC-PCR; Ecological niches

1. Introduction

Legume-nodulating bacteria belong to at least 11 genera and 47 species. Of these, 41 species belonging to five genera of the family *Rhizobiaceae* (*Rhizobium*, *Mesorhizobium*, *Azorhizobium*, *Sinorhizobium*, *Bradyrhizobium*) are commonly known as rhizobia [26,35,37]. The rhizobia form nitrogen-fixing nodules on roots and occasionally at stem-located root primordia of leguminous species. Successful symbiosis between the legume and rhizobial partner contributes to the ecological success of a leguminous species. Major steps in symbiosis include bacterium-plant recognition, nodule formation and nitrogen fixation [31]. These processes are partner-specific and generally, a particular rhi-

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zobial species can only nodulate a limited and defined range of legumes [7].

The root infection and development of the nodule begins with the recognition between plant root lectin and outer membrane lipopolysaccharides (LPS) of rhizobia [11]. The LPS also leads to successful suppression of the host defense reaction and helps rhizobia to colonize the legume root [8]. In fact, LPS mutants of *R. phaseoli* were found to be defective in infection thread development. Ability of rhizobia to evolve strategies to combat biotic stress, and particularly rhizobiophags (soil-borne *Rhizobium* phage), is important for their colonization of the legume rhizosphere [27]. Symbiotic genes are plasmid-borne in rhizobia, except for *Azo*(*Brady*)*rhizobium*, where they are located on the chromosome [17]. The intimate association of symbionts with many partners (promiscuity) and the development of effective symbioses (nodulation, nitrogen fixation and biomass enhancement) have been proposed to determine the nitrogen input into various ecological niches [21]. However, promiscuity between the two partners and diversification of rhizobia in functional adaptive traits across the ecological gradients is poorly understood.

Sesbanias are a unique group of legumes native to the tropics and subtropics. They inhabit a wide range of ecologically diversified habitats from lowland, wet tropical ecosystems to semi-arid ecosystems and from seacoasts to freshwater swamps. These habitats vary in soil type, nutrient status and abiotic and biotic stresses. Sesbanias are excellent legume green manure (LGM) plants cultivated in rice paddies and alley cropping. In fact, the value of N accumulated in a root nodulating LGM crop of *Sesbania* ranges from 146 to 267 kg N per hectare [25]. They also yield firewood and fodder, in addition to improving soil fertility in arid and semi-arid regions. [15]. *S. sesban*, *S. aegyptica* and *S. rostrata* are among the major agroforestry species in the Delhi region which are used to ameliorate salinity and habitat processing in rehabilitation programs [10]. Soil amelioration and habitat processing properties of sesbanias are associated with its nitrogen-fixing symbiotic partner, i.e., rhizobia.

Diverse rhizobial taxa are known to nodulate *Sesbania* spp. in different parts of the world; however, the diversity among rhizobial taxa nodulating sesbanias in the semi-arid Delhi region has not been investigated [2,3]. Since sesbanias occupy a wide range of ecological niches, the study on symbiotic properties and functional traits of associated rhizobia would help in understanding the role of microsymbiont attributed to their wide ecological amplitude. Assessment of diversity in these traits will also help in the development of strategies to effectively utilize *Sesbania*-rhizobia symbioses in various soil amelioration programs. Therefore, in the present study symbiotic properties, surface polysaccharides, plasmid genotype and rhizobiophage sensitivity of 28 isolates from three *Sesbania* sp. (*S. sesban*, *S. aegyptica* and *S. rostrata*) inhabiting six ecologically distinct sites were examined.

2. Materials and methods

2.1. Study area and its ecological characteristics

Six ecologically distinct sites were selected from the Delhi region for the present investigation (Fig. 1). The soil characteristics were investigated by determining the pH, NPK and organic matter [1]. Observations were also recorded on the landscape features, land use pattern, anthropogenic activities and the vegetation types.

2.2. Legume-nodulating bacterial strains and their culture conditions

Legume-nodulating bacterial isolates were taken from root and stem nodules of different species of *Sesbania* (*S. sesban, S. rostrata* and *S. aegyptica*) collected from the Delhi region (Table 1). ORS571 and WE7 [20] were obtained from the *Rhizobium* Germplasm Collection (RGC) maintained at the University of Delhi. The isolates have already been characterized using standard microbiological methods (Sharma, R.S., 1999, Ph.D. thesis). The cultures for all the isolates were raised in YM/YL broth at $28 \pm 1\,^{\circ}$C, 130 ± 5 rpm [32].

2.3. Plant nodulation assay

Seeds of *Sesbania* spp. were surface-sterilized (A. Mohmmed, Ph.D. thesis), germinated on 1.5% water agar plates and transferred to acid (0.1 M HCl) washed sterile quartz sand in pots (15 cm diameter) filled up to 10 cm depth. The pots were maintained for 14 weeks in a growth chamber with a temperature of 28/20 °C (day/night) and 14 h photoperiod. The plants were watered with sterile distilled water every 2 days, and sterile 1:4 diluted Jensen N-free nutrient media [32] once a week.

The host range of 28 isolates of rhizobia was tested by the root nodulation assay on *S. sesban, S. aegyptica and S. rostrata* in all possible combinations. The root and stem of plantlets were inoculated with 5×10^{10} bacterial cells on the 7th and 21st day after sowing. In the case of *S. rostrata*, the stem nodulation assay was considered positive if nodules were observed within 4–5 weeks. Surface-sterilized seeds without inoculation were sown as negative control. Plants were uprooted after 45 days and checked for nodulation. The location, number and shape of nodules were also recorded.

2.4. Symbiotic efficiency and effectiveness

Symbiotic efficiency and effectiveness of the isolates were assessed on the homologous hosts. The symbiotic efficiency was measured in terms of nitrogenase activity tested by the acetylene reduction assay [29]. The healthy intact nodules were weighed, surface-dried and kept in an airtight vial (15 ml). 10% air of the vial was replaced with pure acetylene and the vial was incubated for 1 h at 28 °C. The