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Sinorhizobium indiaense sp. nov. and *Sinorhizobium abri* sp. nov. Isolated from Tropical Legumes, *Sesbania rostrata* and *Abrus precatorius*, Respectively

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Abstract

Strains of root nodulating bacteria isolated from the leguminous plants *Sesbania rostrata* and *Abrus precatorius* growing in the sub Himalayan tract in the western Uttar Pradesh, a tropical region of India, were compared with the reference strains of *Sinorhizobium*, *Rhizobium*, *Azorhizobium* and *Agrobacterium*. The phylogenetic analysis based on 16S rRNA gene sequences showed that the isolates from *S. rostrata* and strains from *A. precatorius*, were the members of the genus *Sinorhizobium*. The 16S rRNA gene sequence similarity values of representative strain Ra-3 (from *S. rostrata*) and HA-1 (from *A. precatorius*) showed low values in species level, namely those of 97.1% to *Sinorhizobium arboris* and 96.1% to *S. fredii* and *S. xinjiangense*, respectively. Similarity values of both strains and other *Sinorhizobium* spp. were mostly lower than those of the above species. On the basis of the results, with the data of phenotypic characteristics, cellular fatty acid compositions (major, 18:1

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acid), ubiquinone system (major, Q-10) and DNA-DNA relatedness, we propose the name *Sinorhizobium indiaense* for strains from *S. rostrata* and *S. abri* for strains from *A. precatorius*.

Keywords: *Sinorhizobium indiaense*, *Sinorhizobium abri*, tropical legumes, phylogeny

1. Introduction

With the advancement of technology, methodology for taxonomic characterization has advanced. Nowadays most widely used methods for taxonomic characterization of root nodulating bacteria is the study of nucleic acids including both DNA and RNA. Due to the improved methods of characterization the classification of rhizobia has undergone several drastic changes and the phylogenetic analysis of the family Rhizobiaceae and related genera upgraded (Chen et al., 1988; Willems and Collins, 1993; Yanagi and Yamasato, 1993; De Lajudie et al., 1994; Holt et al., 1994; Rana and Krishnan, 1995; Martinez-Romero and Mellado, 1996) since the first partial 16S rRNA gene sequence for rhizobia was reported by Young et al. (1991).

The genus *Sinorhizobium* was first of all proposed for *Rhizobium fredii*, the fast growing rhizobia nodulating soybean (Chen et al., 1988), and later the partial 16S rRNA gene sequence of *Sinorhizobium fredii* was shown to be similar to that of *Rhizobium meliloti* (Jarvis et al., 1992). Therefore, both species were assigned to confirm the individualities as species of the genus *Sinorhizobium* (De Lajudie et al., 1994). Now, the genus *Sinorhizobium* includes eight species, *S. meliloti*, *S. fredii*, *S. terangae* (De Lajudie et al., 1994), *S. saheli* (De Lajudie et al., 1994), *S. xinjiangense* (Chen et al., 1988), *S. medicae* (Rome et al., 1996), and *S. arboris* and *S. kostiense* (Nick et al., 1999).

Most of the work on the taxonomy of rhizobia has been limited to agriculturally important legumes. Other tropical legumes with less economic value have been neglected. In the present study two strains of root nodulating bacteria isolated from *Sesbania rostrata* and *Abrus precatorius* were elaborately characterized so as to determine their taxonomic position. Both host plants are commonly found in the sub Himalayan region in India and especially *Abrus precatorius* known as ratti, has an immense value as the Ayur-Vedic medicine. *Sesbania rostrata* is used as green manure by the farmers. In order to determine the exact position of both strains, studies were performed including 16S rRNA gene sequencing and DNA-DNA hybridization, in relation to other species of *Sinorhizobium*, *Rhizobium leguminosarum*, *Azorhizobium caulinodans* and *Agrobacterium tumefaciens*. The results of all studies led to the proposal of two new species: *Sinorhizobium indiaensis* sp. nov. (from the

isolates from *S. rostrata*) and *Sinorhizobium abri* sp. nov. (from the isolates from *A. precatorius*).

2. Materials and Methods

In total ten strains each were isolated from the root nodules of different plants of *S. rostrata* and *A. precatorius*, by the method of Vincent (1970). The plants were growing wildly in the sub-Himalayan tract about 200 km northeast of New Delhi. This area is a deciduous rain forest with a temperature range of 4°C to 45°C. The strains were maintained on YM agar medium containing, 3 g of yeast extract, 10 g of mannitol, 0.5 g of K_2HPO_4, 0.1 g of NaCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.025 g of Congo red and 15 g of agar in 1,000 ml water, pH 6.8 at 4°C. One representative strain of each type, Ra-3^T (from *S. rostrata*) and HA-1^T (from *A. precatorius*) were deposited in the Japan Collection of Microorganisms (JCM), Institute of Physical and Chemical Research, Wako, Japan, as JCM 10304^T and JCM 10305^T, respectively. Type or representative strains of *R. leguminosarum*, *Sinorhizobium* species and *A. tumefaciens* were included in the study for a comparison with the isolated strains.

Physiological and biochemical characteristics were determined by inoculating exponentially growing cultures using YM broth medium (YM agar medium without Congo red and agar), YM agar plates or other media as required and mentioned in earlier reports (De Lajudie et al., 1994). Utilization of sugar and organic acids was conducted on the medium as mentioned by White (1972), while sole nitrogen source utilization was determined on the basal salt medium, and mannitol was used as carbon source. Requirement for vitamins, tolerance to NaCl and the pH range of growth were examined as mentioned by Chen et al. (1988). Acid or alkali production was determined on YM agar medium supplemented with bromothymol blue indicator. Cell dimensions, morphology and type of flagellation of the isolates were determined by scanning electron microscopy according to Miller and Pepper (1988). Rest of the study was done on the two representative strains Ra-3^T and HA-1^T.

For determination of fatty acid contents, cells were collected by centrifugation in the exponential growth phase, washed with distilled water and freeze-dried. Approximately 50 mg of freeze-dried cells were methylated with 3 ml of 5% (w/v) HCl-methanol at 100°C for 3 h. The reaction mixture containing fatty acid methyl esters was washed with distilled water, and then dehydrated with anhydrous Na_2SO_4. The GC was performed in a Shimadzu gas chromatograph GC-8A (Shimadzu Co., Japan) equipped with an Advance-DS (Shinwa Kakou Co. Ltd., Japan) capillary column (0.25 mm × 25 mm) at 170°C. The detector used was flame ionized. GC peaks were processed by Shimadzu Chromatopac C-R3A.

Quinone composition

Ubiquinones were extracted with chloroform-methanol (3:1, v/v) solution from approximately 1 g of freeze dried cells. Crude ubiquinones were purified by TLC using silica gel B-O (Wako Pure Chemical Industries Ltd., Japan) plates (0.5 mm thick). The bands on the TLC plates developed with benzene were scrapped off under UV light. After extraction of ubiquinones from the scrapped silica powder with acetone, the molecular species of ubiquinones were determined by HPLC.

DNA base composition

The isolates were grown in YM broth medium at 27°C with shaking. The cells were suspended in 0.1 M saline EDTA (0.15 M NaCl. 0.1 M EDTA; pH 8.5), and then lysed at 60°C for 10 min with 0.5% sodium dodecyl sulfate as a final concentration. DNA was obtained from the lysed samples by treating with Tris-phenol (1:4,v/v; pH 9.0), and purified according to the methods of Saito and Miura (1963).

To measure guanine plus cytosine (G+C) content of DNA, a 0.1 ml portion of a 0.05 to 0.1 mg DNA solution in 0.1 × SSC (1 × SSC: 0.15 M NaCl and 15 mM trisodium citrate) was placed in a 1.5 ml tube and heated in boiling water for 5 min and rapidly cooled in an ice water bath. Then 0.01 ml of 0.1 mg ml^{-1} P1 nuclease (Seikagaku Co., Japan) in 40 mM CH_3COONa and 2 mM $ZnSO_4$ (pH 5.3) was added. The sample was incubated at 50°C for 1 h. The nucleotide composition was analyzed by HPLC equipped with a column Shim-Pac WAX-1, 4.0 mm × 50 mm (Shimadzu Co.) and detected at 260 nm. The mobile phase was 50 mM phosphate buffer (pH 2.83). The G+C contents were corrected by the value of the peak area of the sample nucleotide divided by the peak area of the standard nucleotides.

^3H-labeled preparation

A ^3H-labeled hybridization probe DNA was prepared with a DNA Labeling Kit (Nippon Gene Co. Ltd., Japan). The reagents, the DNA sample and 1.85 MBq of ^3H-deoxy (1',2',5'-^3H) cytidine 5'-triphosphate (ammonium salt) were mixed, incubated at 37°C for 2 h. The ^3H-labeled DNA obtained was dissolved in 0.1 × SSC solution. DNAs were hybridized using the membrane filter method described by Denhardt (1966) and Bonner et al. (1967). After unlabeled DNA was denatured by heating, the DNA concentration was adjusted to ca. 1.0×10^4 cpm ml^{-1} in 2 × SSC solution. One ml of this probe DNA solution and 4 µl of 25% (v/v) sodium dodecyl sulfate solution was added to filter-fixed, unlabeled DNA in a vial. The hybridization reaction was carried out at 65°C for 43 h.

After the reaction, the filter was washed with 2 × SSC solution and with 5 mM Tris buffer, and dried at room temperature. Its radioactivity was measured with a liquid scintillation counting system LSC-700 (Aloka Co., Japan).

Amplification of 16S ribosomal RNA gene

Amplification of 16S rRNA gene was performed on a quick Thermo Personal QTP-1 (Nippon Genetics Co. Ltd., Japan) in 100 µl reaction volume containing 100 ng of chromosomal DNA, 10 µl of 10 × Ex Taq buffer (Takara Shuzo Co Ltd., Japan), 200 mM of each dNTP, 1 mM of each primer and 2.5 U of Takara Ex Taq (Takara Shuzo Co. Ltd.). The primers were 5'-AGTTTGATCCTGGCTC-3'[*Escherichia coli* numbering system (Brosius et al., 1978): positions 10–25] and 5'-AAGGAGGTGATCCAGCC-3' (positions 1525–1541). Amplification of the 16S rRNA gene sequencing of the amplified DNA fragments were performed as described previously (Suzuki and Yamasato, 1994). The amplified DNA fragments were purified by gel electrophoresis on 1% Agarose S (Nippon Gene Co. Ltd., Tokyo, Japan), and recovered with glass powder using Prep-A-Gene DNA purification Systems (Bio-Rad Laboratories, California, USA). The sequences determined and the sequences of reference bacterial species were aligned by using the program Clustal W version 1.7 (Thompson et al., 1994). Phylogenetic analysis was performed by using the PHYLIP phylogeny inference package version 3.57c (Felsenstein, 1995). A distance matrix was calculated with DNADIST by using the Kimura 2-parameter, and a phylogenetic tree was reconstructed by using the NEIGHBOUR. The stability of the clusters was ascertained by performing a bootstrap analysis (1,000 replications) with DNABOOT, DNADIST, NEIGHBOUR and CONSENSE.

The 16S rRNA gene sequences of strains, Ra-3^T and HA-1^T has been assigned (DDBJ) accession number AB015420 and AB015421, respectively in the DDBJ, EMBL and GenBank nucleotide sequence databases.

3. Results

Cells of strains from *S. rostrata* were 0.7 to 0.9 µm wide and 1.3 to 2.0 µm long while cells of strains from *A. precatorius* were 0.5 to 0.8 µm wide and 1.5 to 2.5 µm long. Electron micrographs of negatively stained cells of all the isolates revealed that each possessed one or two subpolar flagella (Fig. 1). When grown at 27°C for 3 days, strain Ra-3^T formed 1 mm and 0.5 mm colonies on YM agar and NT agar media, respectively. However, strain HA-1^T formed much bigger colonies in comparison, 5 mm and 2 mm on YM agar and NT agar, respectively.

All the strains produced oxidase, catalase, urease and tryptophan deaminase. Strains from *S. rostrata* did not produce lysine decarboxylase and

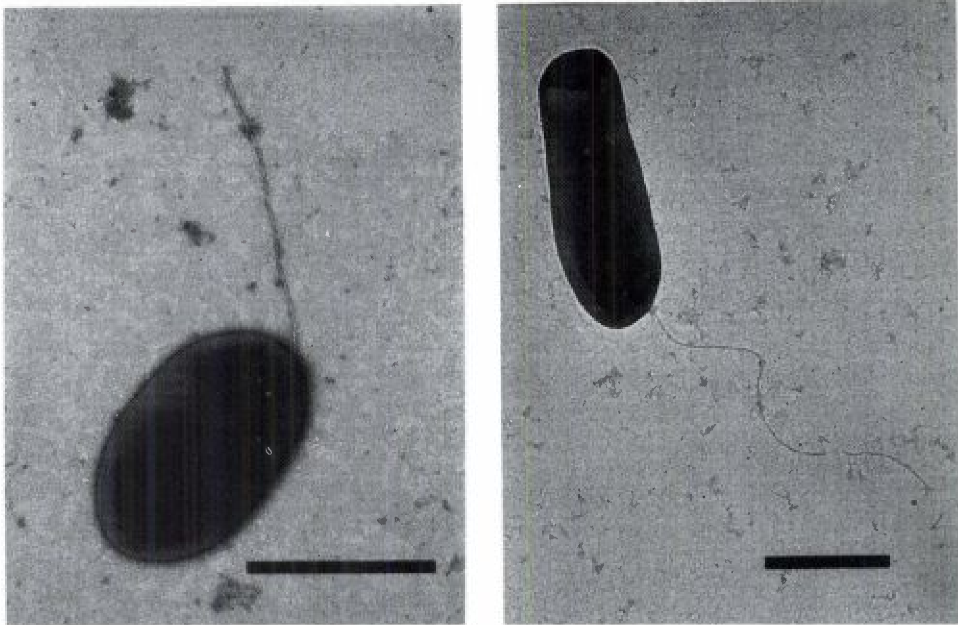

Figure 1. Electron micrographs of negatively stained cells of strains Ra-3T (left) and HA-1T (right). Bar = 0.5 μm.

arginine dihydrolase. Phenylalanine deaminase was not produced by strains from *A. precatorius*. None of the strains produced ornithine decarboxylase. All the strains hydrolyzed litmus milk but did not hydrolyze starch and Tween 80. Strains from *A. precatorius* produced acid in litmus milk. When grown with different concentrations of NaCl, strains from *S. rostrata* were more tolerant showing growth even at 3.5%, and strains from *A. precotorius* showed growth in presence of 2% NaCl. Optimum growth of all the strains occurred at pH between 6 to 8, however both strains were able to grow up to pH 9. All the strains grew at 37°C, and strain Ra-3T could grow weakly at 41°C while strain HA-1T did not grow at this temperature. All the strains utilized D-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-ribose, L-rhamnose, D-xylose, lactose, maltose, raffinose, inositol, 2-ketogluconate, DL-malate, DL-asparagine and L-histidine as sole carbon or nitrogen sources. Strains from *S. rostrata* could utilize benzoate and gluconate as well. Except L-valine, all other nitrogen sources such as L-arginine, DL-asparagine, L-glutamate, L-lysine, and L-histidine were utilized by all the strains. In case of strains from *A. precatorius* acid production was noticed on D-glucose, lactose and sucrose whereas strains from *S. rostrata* could produce acid only on sucrose.

Table 1. Differential characteristics of strains from root nodules of *S. rostrata* and *A. precatorius*

Characteristic	Strains from *S. rostrata*										Strains from *A. precatorius*									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Gram reaction	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Colony diameter (mm)																				
27°C, 3 days:																				
on YM agar	1	1.1	1.1	1.2	1	0.9	0.8	1	1.1	1	5.2	5.1	5.1	4.7	5	5	4.9	5.2	4.9	5
on NT agar	0.5	0.5	0.4	0.5	0.6	0.5.	0.6	0.5	0.5	0.4	2.1	2.1	2.0	2.0	1.8	2.1	2.0	2.0	1.9	2.0
Growth at pH 5.1	w	w	w	w	w	w	w	w	w	w	–	–	–	–	–	–	–	–	–	–
Growth at 41°C	w	w	w	w	+	w	w	w	w	w	–	–	–	–	–	–	–	–	–	–
Growth at 3% NaCl	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Acid product. in litmus milk	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+
Acid from glucose	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+
Acid from lactose	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+
Oxidase production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Assimilation of gluconate	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Assimilation of benzoate	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Requirement for:																				
Biotin	+	+	+	+	+	+	+	+	+	+	–	+	–	–	–	–	–	–	–	–
Pantothenate	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Phenylalanine deaminase	+	+	+	+	–	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
Lysine decarboxylase	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	–	+	+
Arginine dihydrolase	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+
OF test	–	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+	+
G+C (mol%)	63.3	63.4	63.3	63.5	63.2	63.2	63.3	63.2	63.3	63.3	61.0	61.1	61.3	60.9	61.0	61.1	61.1	61.2	61.0	61.1

+, positive; w, weak; –, negative.

Table 2. Cellular fatty acid compositions of representative strains

	Saturated							Unsaturated				Cyclo-propane	3-hydroxy			
	12:0	14:0	16:0	17:0	18:0	20:0	22:0	12:1	16:1	17:1	18:1	19:0	14:0	16:0	18:0	18:1
Ra-3T	–	–	8.9	–	9.1	–	–	–	–	–	69.7	5.8	3.7	–	2.8	–
HA-1T	–	–	5	–	2.5	–	–	–	t	–	72.5	14.3	3.6	t	2.1	t
12609T	–	–	4.6	–	19.9	–	–	–	–	–	43	23	6.5	1.4	3.6	–

t, trace amount (less than 1%). Number preceding colon indicates number of carbon atoms; after colon designates degree of unsaturation. The cells were grown in YM broth medium.

Biotin and pantothenate were required as growth factors for *S. rostrata* strains (Table 1).

The major fatty acid of both the representative strains was 18:1 acid (69.7–72.5%), and the others were cyclo 19:0, 16:0, 18:0, 3-OH 14:0, and 3-OH 18:0 acids presented in small amount (2.1–9.1%). In minor acids, strain Ra-3^T showed comparatively higher content of 18:0 (9.1%) than strain HA-1^T, on the other hand starin HA-1^T showed higher content of cyclo 19:0 (14.3%) than strain Ra-3^T (Table 2).

Quinone composition

In both strains, Q-10 was the major quinone while Q-8 and Q-9 were present in small amounts in strain Ra-3^T whereas Q-8 was absent in strain HA-1^T (Table 3).

Table 3. Quinone compositions of strains Ra-3^T and HA-1^T

Type	Ra-3^T (%)	HA-1^T (%)
Q-8	1.8	–
Q-9	1.0	1.3
Q-10	97.2	98.7

Table 4. Homology values of the DNA preparations

Source of unlabeled DNA	Homology (%) with labeled DNA from	
	Ra-3^T	HA-1^T
Ra-3^T	100	48
HA-1^T	44	100
S. fredii IAM 13625^T	23	33
S. meliloti IAM 12611^T	26	33
S. terangae LMG 7834^T	31	42
S. saheli LMG 8309^T	24	39
R. leguminosarum IAM 12609^T	15	20

DNA base compositions

G+C contents of the DNA were 63.3 and 61.1 mol% for strains Ra-3^T and HA-1^T, respectively.

DNA-DNA hybridization

Both isolates were reassociated to the other type strains of *Sinorhizobium* species with low homology values (Table 4). The DNA homology values between strains Ra-3^T and HA-1^T were 44 to 48%.

Phylogenetic analysis

Phylogenetic tree was drawn on the basis of 16S rRNA gene sequence analysis (Fig. 2) and revealed that strains Ra-3^T and HA-1^T belonged to the genus *Sinorhizobium*, forming separate lines of descent. Strain Ra-3^T showed 16S rRNA gene sequence similarities to the extent of 97.1% with *S. arboris* and 96.9% with *S. meliloti*, while strain HA-1^T showed 96.1% with *S. fredii* and *S. xinjiangense*. The 16S rRNA gene sequence similarity values among strains Ra-3^T, HA-1^T and the strains of other *Sinorhizobium* spp. were 95.0 % to 96.1%. Also, those among stains RA-3^T , HA-1^T and the strains of closely related genus *Mesorhizobium* were 92.4 to 93.4%. The similarity between strains Ra-3^T and HA-1^T were 96.8%.

4. Discussion

Sinorhizobium was originally proposed by Chen et al. (1988) with two species namely *S. fredii* and *S. xinjiangense* for fast growing soybean root nodulating bacteria due to their very low similarities in numerical analysis to either *Rhizobium* or *Bradyrhizobium*. Lately, De Lajudie et al. (1994) proposed that *R. meliloti* was reclassified in the genus *Sinorhizobium* as *S. meliloti* in addition to two new species, *S. saheli* and *S. terangae*. The latter organisms were obtained from nodules of *Sesbania* spp. and *Acacia* spp. in Senegal. Dreyfus et al. (1988) characterized *Azorhizobium caulinodans*, a stem nodulating nitrogen fixing bacterium isolated from *Sesbania rostrata*. Rana and Krishnan (1995) reported a new root-nodulating symbiont of *Sesbania*, *Rhizobium* sp. SIN-1 based on phylogenetic characters and fatty acid profiles. Thirty-one species, in five genera, are currently known. The number is still lower in comparison to the number of legume species mentioned in the literature. Young and Haukka (1996) stated the reasons of having a similar number of rhizobia to the number of legumes. During the present investigations, it was

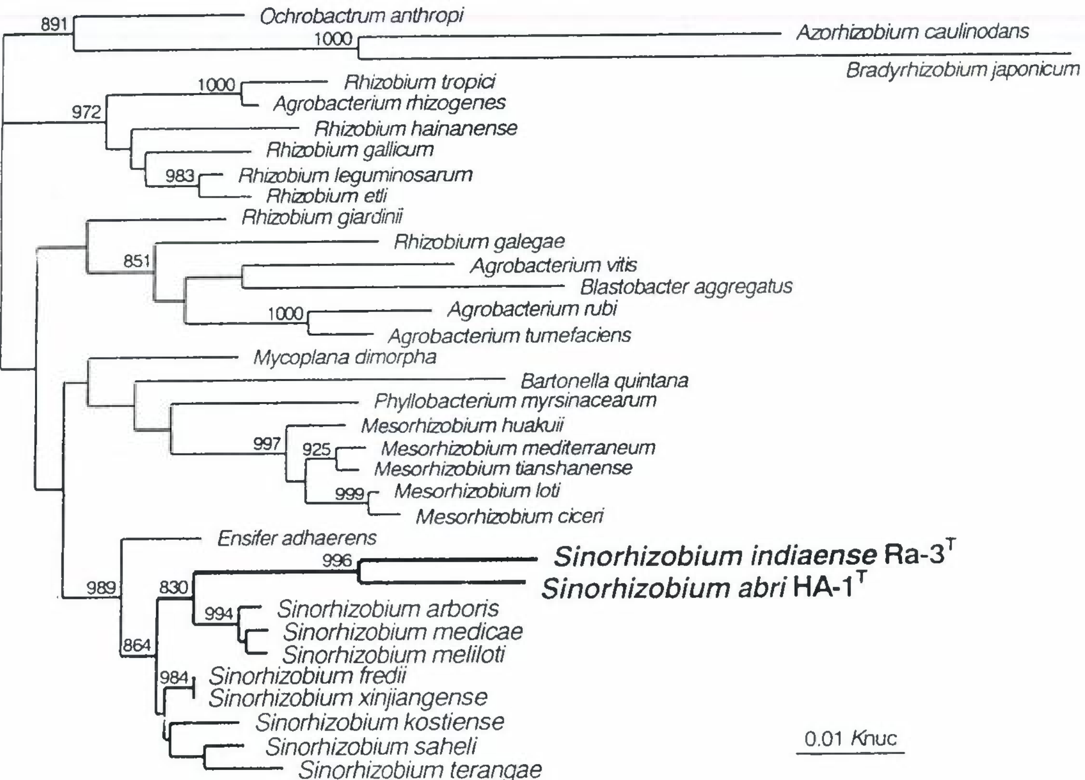

Figure 2. Unrooted phylogenetic tree derived from the analysis of the 16S rRNA sequences of *Sinorhizobium indiaensis* Ra-3T, *Sinorhizobium abri* HA-1T and other members of the α subclass of the Proteobacteria. The numbers at the nodes indicate the levels of bootstrap based on 1,000 resamplings. Only values above 800 significance are indicated.

Table 5. Differential characteristics of representative strains Ra-3^T, HA-1^T and *Sinorhizobium* spp.

Characteristic	Ra-3^T	HA-1^T	1	2	3	4	5	6	7
Flagella arrangement									
Polar or subpolar	+	+	+	+	+	+	−	+	+
Peritrichous	−	−	+	+	−	−	+	−	−
Growth in 2% NaCl	+	+	d			d	+	+	−
Growth at 41°C	+	−		d	+	+	+	+	−
Carbon sources:									
Acetate	−	−	+	d	+	+	d		
DL-asparagine	+	+	d	−	+	+	−		
Dulcitol	−	−	−	−	−	−	+		
Gluconate	+	−	−		d	d	−		
Nitrogen sources:									
L-lysine	+	+	d	−	+	+	−		
L-valine	−	−	d	−	+	+	−		
Growth at pH 5.5	+	+	d	−	+	+	−		−
Growth at pH 8.5	+	+	d	−	+	+	+	+	−
Nitrate reduction	+	+	d	−	+				
Denitrification	+	+	−		−	−			

+, 95% or more strains are positive; d, between 5 and 94% of the strains are positive; −, negative. 1, *S. fredii*; 2, *S. xinjiangense*; 3, *S. saheli*; 4, *S. terangae*; 5, *S. meliloti*; 6, *S. arboris*; 7, *S. kostiense*.

found that the *Sinorhizobium* species with strains Ra-3^T and HA-1^T formed one branch on the phylogenic tree (Fig. 2).

The taxonomic relation of the strains based on morphological, physiological, biochemical and genetic studies including DNA-DNA hybridization and 16S rRNA gene sequencing, clearly indicated that the strains showed marked similarities with the strains of *Sinorhizobium* spp. A total of 32 carbon sources were tested and acetate was not assimilated by all of the strains. Although, most of the *Sinorhizobium* spp. reported earlier assimilated acetate. On the other hand, all of the strains assimilate DL-asparagine similar to *S. fredii*, *S. saheli* and *S. terangae* but dissimilar to *S. xinjiangense* and *S. meliloti*. It was observed that both strains assimilate L-lysine also similar to *S. fredii*, *S. saheli* and *S. terangae* (De Lajudie et al., 1994) but dissimilar to *S. xinjiangense* and *S. meliloti*. Further the strains could grow at pH 8.5 as also observed in

case of *S. fredii, S. saheli, S. terangae* and *S. meliloti,* but *S. xinjiangense* and *S. kostiense* did not grow at this pH. The study also revealed that strains showed evident differences within themselves and with other species reported earlier (Table 5). For the fatty acid compositions, similar to other *Sinorhizobium* species, both strains Ra-3^T and HA-1^T contained 16:0, 18:0, 18:1 and cyclo 19:0 fatty acids with the exception of 17:0 which is produced by few other species of *Sinorhizobium* (Jarvis and Tighe, 1994). The results obtained for both the strains agree well with the results described for *Sinorhizobium* species by previous workers (Chen et al., 1988; De Lajudie et al., 1994; Rome et al., 1996). However, both the strains can be differentiated from the other members of the genus *Sinorhizobium* by phenotypic characteristics and DNA homologies (to five type strains, Table 4). On the phylogenic tree, *Sinorhizobium arboris* recently proposed by Nick et al. (1999) related closely to strains Ra-3^T and HA-1^T, but the 16S rRNA gene sequence similarity values were comparatively low (97.1% to Ra-3^T, 95.3% to HA-1^T). For *S. arboris,* there are no data of DNA homologies to both strains, however the 16S rRNA gene sequence similarities were low, so it is considered that the DNA homologies among them are low. Hence, strains Ra-3^T and HA-1^T are found to constitute two new species of the genus *Sinorhizobium* due to their high level of divergence between both and the other *Sinorhizobium* species. Therefore, on the basis of above facts, it is concluded that both strains are being reported for the first time from India, and we propose the names *Sinorhizobium indiaense* sp. nov. for strains from *Sesbania rostrata* and *Sinorhizobium abri* from *Abrus precatorius.*

Description of Sinorhizobium indiaense sp. nov.
(in.di.a.en'se. L. neut. adj. indiaense, referring to India, the source of type strain)

Cells are aerobic, gram-negative and non-spore-forming rods (0.7–0.9 × 1.3–2.0 μm) that are motile with one or two subpolar flagella. Colonies on YM agar medium are circular, mucoid and 0.5 mm to 1 mm in diameter after 3 days incubation at 27°C. The strain grows well at 37°C and weakly at 41°C. The strain is tolerant to NaCl up to a concentration of 3.5% and able to hydrolyze litmus milk. Catalase, oxidase, urease, phenylalanine deaminase and tryptophan deaminase are produced. Lysine decarboxylase, arginine dihydrolase and ornithine decarboxylase are not produced. Nitrate reduction and denitrification are positive under microaerobic conditions. Wide ranges of carbon and nitrogen sources are assimilated including D-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-ribose, L-rhamnose, D-xylose, lactose, maltose, raffinose, inositol, benzoate, gluconate, 2-ketogluconate, DL-malate, DL-asparagine and L-histidine. The major

cellular fatty acid is 18:1. Quinone system is Q-10. The G+C content of DNA is 63.3 mol%. The strain is able to renodulate the host.

The type strain is strain Ra-3^T (= JCM 10304^T), which was isolated from root nodules of *Sesbania rostrata* grown in Indian soil.

Description of Sinorhizobium abri sp. nov.
(ab'ri. M. L. adj. abri, referring to Abrus, the source plant of the type strain)

Cells are aerobic, gram-negative and non-spore forming rods (0.5–0.8 × 1.5–2.5 μm) that are motile with one or two subpolar flagella. Colonies on YM agar medium are circular, mucoid, elevated and 2 mm to 5 mm in diameter after 3 days of incubation at 27°C. The strain is able to grow well up to 37°C and able to hydrolyze litmus milk with acid production but is unable to hydrolyze starch. Catalase, oxidase, urease, tryptophan deaminase, lysine decarboxylase, and arginine dihydrolase are produced. Phenylalanine deaminase and ornithine decarboxylase are not produced. Nitrate is reduced to nitrite in aerobic, microaerobic and anaerobic conditions besides exhibiting denitrification. D-Arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, D-ribose, L-rhamnose, D-xylose, lactose, maltose, raffinose, inositol, 2-ketogluconate, DL-malate, DL-asparagine and L-histidine are utilized. The major cellular fatty acid is 18:1. Quinone system is Q-10.

The G+C content of DNA is 61.1 mol%. The strain is able to renodulate the host. The type strain is strain HA-1^T (= JCM 10305^T) which was isolated from root nodules of *Abrus precatorius* growing in Indian soil.

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