

# DNA Homologies among Members of the Genus *Azorhizobium* and Other Stem- and Root-Nodulating Bacteria Isolated from the Tropical Legume *Sesbania rostrata*

G. RINAUDO,<sup>1,2\*</sup> S. ORENGA,<sup>3</sup> M. P. FERNANDEZ,<sup>3</sup> H. MEUGNIER,<sup>4</sup> AND R. BARDIN<sup>3</sup>

*Laboratoire d'Ecologie Microbienne, Université Claude Bernard Lyon 1, F-69622 Villeurbanne Cedex,<sup>1</sup> Office de Recherche Scientifique et Technique Outre Mer<sup>2</sup> and Unité de Recherche Associée au Centre National de la Recherche*

TABLE 1. Origin of strains tested

Strain(s) <sup>a</sup>	Location	Isolated from:
Strains from North Senegal <i>A. caulinodans</i> ORS 571 <sup>T</sup> (C. J. M. G. 6165)		Stem nodules

the pH was 4.25, the flow rate was 1.0 ml/min, and absorption was measured at 260 nm.

**DNA-DNA hybridization and thermal stability of duplexes.** DNA-DNA hybridization tests were carried out at 70°C (the optimal temperature for DNA reassociation) with tritium-

TABLE 2. Levels of DNA hybridization among strains

Source of unlabeled DNA	G+C content (mol %)	% Reassociation at 70°C with labeled DNA from:				
		Strain ORS 571 <sup>T</sup>	Strain SD02	Strain SG28	Strain RT12	Strain RT14
Typical <i>Azorhizobium</i> strains						
Genomic species 1						
ORS 571 <sup>T</sup>	67	100 <sup>a</sup>	47	49	2	5
SV01	66	91				
SV06	66	98				
SV07	67	103		53		
SV08	66	96				
SV20	66	96				
SV25	68	84	53	55		
SV26		96			3	
SV31	68	87				
SV33		87 (1.3) <sup>b</sup>				
SV34		95	58			
BD01	66	98				
BD05	67	92				
RT01	66	88				
RT06	67	94				
RT10		104	51	46		
RT15	66	84				
RT18	67	92	51			
FY01	66	93				
FY10	68	81				
FY18	67	96				
FY29	67	89	48	50		
PR01	66	97	55	44		
SG01	66	97				
SG07	67	82				
SG10	67	94				
SG24	67	91				
SG26	66	85				
SG27	67	101	49	54		
SG31		106	53			
DP04	67	92				
DP13	67	92				
DP18	66	98	57	53		
DP19	67	105				
DP20		85	57			
DP22	67	98	55			
DP23	67	81				
KL03	66	94				
KL06	67	82 (1.5)	51 (17.8)			
KL11	68	99	53		2	7
KL14	66	90				4
KL16	66	101				
KL17	67	92	59	58		
SD01	67	104				
SD03	67	83 (1.7)	55 (15.2)		2	
SK02	66	99				
IRG10	67	92				
IRG40		89	50			
IRG44	68	102				
IRG23	67	100				
Genomic species 2						
SD02	67	53 (8.8)	100	87		
SD04	67	44 (7.9)	86		5	
SG28	66	47 (7.2)	92	100		
SG05	67	44 (9.1)	79 (2.2)	90	5	
SG06	66	46 (9.7)	94		7	
SG09	66	51 (6.7)	76 (1.6)	92		
SG19	66	51 (6.6)	83 (3.0)			
SG22	67	48 (7.0)	89	86		
SG25	67	50 (6.4)	90			
Other strains						
Genomic species 3						
RT12	63	0	2	1	100	
Genomic species 4						
RT14	60	4	0	1	3	100
						7

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TABLE 2—Continued

Source of unlabeled DNA	G+C content (mol %)	% Reassociation at 70°C with labeled DNA from:					
		Strain ORS 571 <sup>T</sup>	Strain SD02	Strain SG28	Strain RT12	Strain RT14	Strain TAL 674
Genomic species 5							
TAL 674	59	4	3	2	11		100
Unclassified							
RT09	61	4	0	1	5	10	7
RT11	61	2	-1	2	5	10	10
DP21	61	0	6	1	6	8	10
KL13	61	-2	4	6	-2	9	15

<sup>a</sup> Level of relatedness at 70°C.

<sup>b</sup> The numbers in parentheses are  $\Delta T_m$  values (in degrees Celsius).

than 86% related to strain SG28 (Table 2). These strains were only 44 to 53% related to strain ORS 571<sup>T</sup> ( $\Delta T_m$ , 6.4 to 9.7°C).

The remaining seven strains (strains RT12, RT14, TAL 674, RT09, RT11, DP21, and KL13) had lower G+C contents (59 to 63 mol%) and exhibited very low levels of DNA binding with strain ORS 571<sup>T</sup> (less than 4%) or strains SD02

expressed nitrogenase activity both in vitro and in symbiosis with the host plant.

According to Grimont (11), strains that exhibit less than 60% reassociation and more than 7°C divergence do not belong to the same genomic species. Thus, the results obtained with the nine strains belonging to genomic species 2 showed that these organisms are sufficiently different from

TABLE 3. Levels of DNA hybridization between strain ORS 571<sup>T</sup> and typical *Azorhizobium* strains belonging to genomic species 1

Source of unlabeled DNA	% Reassociation at 70°C with labeled DNA from strain ORS 571 <sup>T</sup>	Source of unlabeled DNA	% Reassociation at 70°C with labeled DNA from strain ORS 571 <sup>T</sup>
SV02	97	SG03	96



- in rhizobia in absence of plant host. *Nature (London)* **256**:407-408.
20. Ladha, J. K., I. Watanabe, and S. Saono. 1988. Nitrogen fixation by leguminous green manure and practices for its enhancement in tropical lowland rice, p. 165-183. *In Sustainable agriculture: green manure in rice farming*. International Rice Research Institute, Los Banos, The Philippines.
  21. McComb, J. A., J. Elliot, and M. J. Dilworth. 1975. Acetylene reduction by *Rhizobium* in pure culture. *Nature (London)* **256**:409-410.
  22. Pagan, J. D., J. J. Child, W. R. Scowcroft, and A. H. Gibson. 1975. Nitrogen fixation by *Rhizobium* cultured on a define medium. *Nature (London)* **256**:406-407.
  23. Peyret, M., J. Freney, H. Meugnier, and J. Fleurette. 1989. by nick translation with DNA polymerase I. *Int. J. Syst. Bacteriol.* **113**:237-251.
  25. Rinaudo, G., D. Alazard, and A. Moudiongui. 1988. Stem-nodulating legumes as green manure for rice in West Africa, p. 97-109. *In Sustainable agriculture: green manure in rice farming*. International Rice Research Institute, Los Banos, The Philippines.
  26. Saint Macary, H., E. A. Marqueses, R. O. Torres, and R. A. Morris. 1985. Effect of flooding on growth and nitrogen fixation of two *Sesbania* species. *Philipp. J. Crop Sci.* **10**:17-20.
  27. Scholla, M. H., and G. H. Elkan. 1984. *Rhizobium fredii* sp. nov., a fast-growing species that effectively nodulates soybeans. *Int. J. Syst. Bacteriol.* **34**:484-486.
  28. Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont.