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Characteristics of Woodland Rhizobial Populations from Surface- and Deep-Soil Environments of the Sonoran Desert

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A collection of 74 rhizobial isolates recovered from nodules of the desert woody legumes *Prosopis glandulosa*, *Psorothamnus spinosus*, and *Acacia constricta* were characterized by using 61 nutritional and biochemical tests. We compared isolates from *A. constricta* and *Prosopis glandulosa* and tested the hypothesis that the rhizobia from a deep-phreatic rooting zone of a *Prosopis* woodland in the Sonoran Desert of southern California were phenetically distinct from rhizobia from surface soils. Cluster analysis identified four major homogeneous groups. The first phenon contained slow-growing (SG) *Prosopis* rhizobia from surface and deep-phreatic-soil environments. These isolates grew poorly on most of the media used in the study, probably because of their requirement for a high medium pH. The second group of isolates primarily contained SG *Prosopis* rhizobia from the deep-phreatic rooting environment and included two fast-growing (FG) *Psorothamnus* rhizobia. These isolates were nutritionally versatile and grew over a broad pH range. The third major phenon was composed mainly of FG *Prosopis* rhizobia from surface and dry subsurface soils. While these isolates used a restricted range of carbohydrates (including sucrose) as sole carbon sources, they showed better growth on a range of organic acids as sole carbon sources and amino acids as sole carbon and nitrogen sources than did other isolates in the study. They grew better at 36°C than at 26°C. The FG *Acacia* rhizobia from surface-soil environments formed a final major phenon that was distinct from the *Prosopis* isolates. They produced very high absorbance readings on all of the carbohydrates tested except sucrose, grew poorly on many of the other substrates tested, and preferred a 36 to a 26°C incubation temperature. The surface populations of *Prosopis* rhizobia required a higher pH for growth and, under the conditions used in this study, were less tolerant of low solute potential and high growth temperature than were phreatic-soil isolates. SG *Prosopis* rhizobia from phreatic and surface soils were physiologically distinct, suggesting adaptation to their respective soil environments.

Deep-rooted woodland legumes such as *Prosopis glandulosa* (mesquite), *Acacia constricta*, and *Psorothamnus spinosus* (smoke tree) often dominate plant communities in Sonoran Desert ecosystems. Many woodland legumes form nitrogen-fixing symbioses with rhizobia (1) and are sources of fixed N to desert ecosystems that are characteristically N limited (27). Because of their ability to fix N_2 symbiotically, woodland legumes such as *Prosopis* and *Acacia* spp. are becoming important in agroforestry (6, 23). Optimized exploitation of these legumes depends on understanding the ecology and physiology of the rhizobia which nodulate them.

Recently, Jenkins et al. (14) developed a collection of indigenous fast-growing (FG) *Rhizobium* and slow-growing (SG) *Bradyrhizobium* isolates from soils associated with the distinct surface (depth, 0.6 m) and deep-phreatic (depth, 4 to 6 m) root systems of a *Prosopis glandulosa* woodland in the Sonoran Desert of southern California. The two absorbing root systems occur in contrasting soil environments. The water content and temperature of the surface soil fluctuate extremely (22), while the deep-phreatic-root environment is constantly temperate and moist from the capillary rise of water from the stable water table. Concentrations of soil nutrients such as N and P are much higher near the surface than they are in the deep-soil profile (30).

Historical evidence indicates that the establishment of this *Prosopis* woodland occurred between 200 and 500 years ago (33) and that the surface- and phreatic-root systems have been separated by 3 to 4 m of perennially dry soil for perhaps a few hundred years (W. M. Jarrell and R. A. Virginia, J. Arid Environ., in press). This dry soil layer would prevent surface rhizobia from being transported to the phreatic zone and interacting with the phreatic roots and associated rhizobial symbionts. Thus, it is likely that the phreatic and surface *Prosopis* rhizobia have been isolated from each other in recent times. The populations from the two depths differ; SG rhizobia predominate in the deep-root environment, while FG and SG rhizobia have been isolated in equal numbers from surface soil (14). In addition, colony morphologies of the surface and phreatic SG populations differ.

The objectives of this study were to examine the phenotypic diversity of woodland rhizobia from undisturbed natural ecosystems and to determine the physiological bases for the apparent distinction between populations of SG *Prosopis* rhizobia from the surface- and phreatic-soil environments. In this study, we measured responses to 61 nutritional and other physiological tests of rhizobia nodulating woodland legumes of the genera *Acacia*, *Psorothamnus*, and *Prosopis*. *Prosopis* rhizobia from surface- and phreatic-soil environments were compared.

MATERIALS AND METHODS

Origin and maintenance of rhizobia. Rhizobia from the woody legumes *Prosopis glandulosa*, *Psorothamnus spinosus*, and *A. constricta* used for this study are listed in Table

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TABLE 1. *Rhizobium* (FG) and *Bradyrhizobium* (SG) isolates from nodules of *Prosopis glandulosa*, *Acacia constricta*, and *Psorothamnus spinosus*

Depth (m) of root environment	Host	Rhizobia	Source
0 to 0.3 (surface)	*Prosopis glandulosa*	FG isolates: 1B, 1F, 10A, 10C, 10D, 10I, 10K, 27A, 27C, 27E; SG isolates: 1A, 1C, 1D, 1J, 10B, 10E, 10F, 10G, 10H, 27B, 27D	Inoculated seedlings (14)
	Prosopis glandulosa	FG isolates: 3A, 4E, 6K	Field nodules (14)
	Psorothamnus spinosus	FG isolates: 14A, 14B	Field nodules (14)
0.2 to 0.3 (surface)	*Acacia constricta*	FG isolates: A1, A3, A4, A5, A12, A16, A18, A19, A21	Inoculated seedlings (31)
2.1 to 3.9 (intermediate)	*Prosopis glandulosa*	FG isolates: 17B, 17C, 22A, 22B, 22D, 22K; SG isolates: 17D, 17E, 22G, 22I, 22J	Inoculated seedlings (14)
3.9 to 4.5 (phreatic)	*Prosopis glandulosa*	FG isolates: 8D; SG isolates: 23E, 23F, 43A, 43B, 43D, 43E, 8A, 8B, 8C, 8E, 8F, 8I, 31A, 31B, 31D, 31F, 31G, 31H, 44A, 44D, 44F, 44G, 44H, 44J, 43C, 8G	Inoculated seedings (14)

1. The *Acacia* isolates were obtained from a rock and gravel hillside in the Sonoran Desert of Pinal County, Arizona (31). The *Prosopis* and *Psorothamnus* isolates originated from Harper's Well in the Sonoran Desert of southern California (14, 30). The study site at Harper's Well is in an extensive *Prosopis* woodland with distinct surface (depth, 0 to 0.6 m), intermediate (depth, 0.6 to 3.9 m), and phreatic (depth, 3.9 to 4.5 m) root zones. Fine roots capable of supporting nodules are found in the surface- and phreatic-soil zones, while only large conducting roots are present in the constantly dry intermediate zone. Reference strains were *Bradyrhizobium* sp. (*Lupinus*) ATCC 10319, *Bradyrhizobium* sp. (*Lotus*) ATCC 10325 and 10326, *Rhizobium leguminosarum* biovar *trifolii* ATCC 14480, and the *Prosopis*-nodulating strain WR1001 (12).

The isolates were maintained on mannitol salts medium containing the following, per liter: 10 g of mannitol, 1.0 g of sodium glutamate, 0.2 g of $MgSO_4 \cdot 7H_2O$, 0.5 g of K_2HPO_4, 0.1 g of NaCl, 0.08 g of $CaCl_2 \cdot 2H_2O$, 170 μg of Fe-EDTA, 14 μg of H_3BO_3, 10 μg of $MnSO_4 \cdot 4H_2O$, 2 μg of $ZnSO_4 \cdot 7H_2O$, 0.8 μg of $CuSO_4 \cdot 5H_2O$, 1 μg of $CoCl_2 \cdot 4H_2O$, 0.5 μg of $Na_2MoO_4 \cdot 2H_2O$, 10 mg of calcium pantothenate, 10 mg of thiamine hydrochloride, 0.3 mg of biotin, and 15 g of agar (13).

Nutritional and physiological characterization. For nutritional and physiological characterization of isolates, NaCl, $CaCl_2$, and all trace elements except Fe were omitted from mannitol salts medium. The effects of temperature (26 and 36°C), added solute potential (−0.5, −1.0, and −1.5 MPa), and pH (5, 6, 7, 8, and 9, obtained by adjusting it with H_3PO_4 or KOH) were tested. Solute potentials were obtained by adding 200, 390, and 580 mM sucrose to mannitol salts broth.

To measure growth on different compounds as the sole carbon source, glutamate was replaced with 0.32 g of NH_4Cl liter^{-1} and mannitol was replaced with the test substrate. Adonitol, arabinose, cellobiose, galactose, glucose, glycerol, lactose, mannitol, mannose, rhamnose, sorbitol, and sucrose (3, 20) were added at 5.0 g liter^{-1}. Sodium salts of acetic, citric, formic, lactic, malic, malonic, pyruvic, and succinic acids and glyoxylic, 2-ketoglutaric, and *cis*-oxalacetic acids were tested at 2 and 10 mM (28). Catechol and the sodium salts of anthranilic, benzoic, and salicylic acids were provided at 1.0 mM (21, 24).

The ability to use amino acids as the sole carbon and nitrogen source was determined by replacing mannitol and glutamate with alanine, cysteine, glutamic acid, histidine, leucine, lysine, ornithine, proline, serine, or tyrosine at 1.0 g liter^{-1} (20). A carbon- and nitrogen-free control was also tested.

Growth factor requirements were determined by replacing the growth factors in the medium with (per liter) 30 μg of *p*-aminobenzoic acid, 250 μg of calcium pantothenate, 60 μg of pyridoxine, 125 μg of thiamine, 5 μg of biotin, 100 μg of riboflavin, 3 μg of choline, and 10 mg of inositol (3). These factors were then eliminated one at a time. A control containing all of the growth factors and a growth factor-free control were also included.

The media were filter sterilized and dispensed into flat-bottom, 96-well tissue culture plates (0.25 ml per well). Cultures were grown to the mid-exponential phase at 26°C, washed, and diluted with 0.2 g of $MgSO_4 \cdot 7H_2O$ liter^{-1} and 0.5 g of K_2HPO_4 liter^{-1} (pH 7.0) to 3×10^5 cells per ml, as determined by a standard curve of absorbance against viable counts.

Ten randomly selected isolates were tested in duplicate. The isolates and three uninoculated blanks were randomly assigned to plate wells. All wells (except for uninoculated blanks) received 5 μl (1,500 cells) of inoculum from a multipoint inoculator (Clonemaster). Plates were sealed, and because of the wide range in doubling times between FG and SG isolates, they were incubated for 22 days at 26°C to ensure maximum yield. The A_{660} was then determined with a spectrophotometer (Dynatech Laboratories, Inc., Alexandria, Va.) that was blanked against air.

Data analysis. Growth yield was corrected for the absorbance of plates and medium and expressed as $1{,}000 \times A_{660}$. Media on which no isolates grew (catechol and the substrate-free controls) were not used in the analyses. Cluster analysis was carried out by using Clustan 2 software (35) on a Cyber 176 computer. Absorbance data for each organism were standardized to a mean of zero and a standard deviation of 1.0. Standardized data were used to calculate the squared Euclidean distance between each pair of strains. The strains were then clustered by using the Ward method (32), which finds minimum variance spherical clusters. A distance coefficient (two times the Euclidian distance) was used to compare distances between and within clusters. Maximum likelihood factor analysis with orthogonal rotation was used to interpret the tests which differentiated the major phena. Sorted rotated factor loadings greater than 0.5 were used in interpreting the results. BMDP 4M software (4) on a Cyber 170 computer was used for factor analysis.

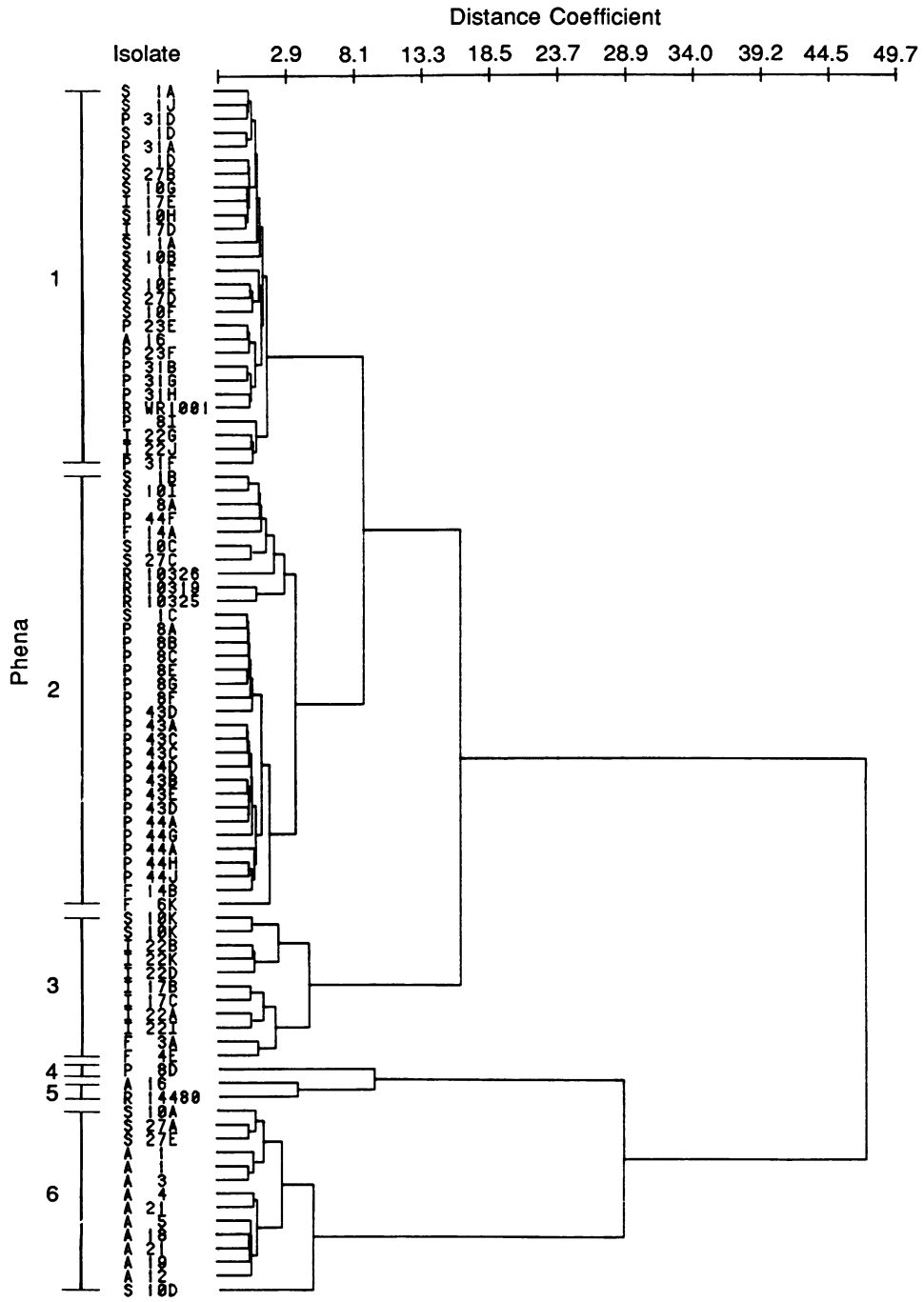

FIG. 1. Dendrogram of the woodland rhizobia examined based on Euclidean distance and grouped by the Ward method (32). Letters before the isolate identification number indicate the source of the isolates: S, *Prosopis* surface-root zone; I, *Prosopis* intermediate-depth root zone; P, *Prosopis* phreatic-root zone; F, field nodules; A, *Acacia* rhizobia; R, reference strains. The distance measure is two times the Euclidean distance.

In order to determine differences between SG *Prosopis* isolates from surface and phreatic soils, values for isolates tested in duplicate were averaged to prevent weighting. The growth yield data were incremented by a constant so that all values were positive, and the logarithm was taken. A *t* test was carried out on the transformed data by using Statview 512+ software (BrainPower, Calabasas, Calif.) on a Macintosh SE computer (Apple Computers).

RESULTS AND DISCUSSION

The rhizobial collection in this study segregated into six distinct groups, as shown in the dendrogram (Fig. 1). Within phena, distance coefficients ranged from 1.6 (phenon 1) to 4.9 (phenon 6), while between-phena distances were greater than 8.9. With the exception of the two cultures of A16, the other nine duplicate cultures tested fell into identical phena,

indicating the overall consistency and reproducibility of the tests.

Phenon 4 contained only isolate 8D, the sole FG *Prosopis* isolate obtained from the phreatic-soil environment. Phenon 5 contained one of the cultures of A16 and the FG reference *Rhizobium leguminosarum* biovar *trifolii* strain. The characteristics of these small groups were not examined in detail.

None of the major phena utilized anthranilate or salicylate as the sole carbon source or histidine or serine as the sole carbon and nitrogen source. The growth responses of the four major phena (Table 2) were summarized in three main factors (Fig. 2). Factor 1 represented growth on nonrestrictive carbohydrate-based media (all sugars except sucrose, pH 6 to 8, temperature, growth factor, and osmotolerance tests); on 10 mM lactate, succinate, and malonate as sole carbon sources; and on alanine and proline as sole carbon and nitrogen sources. Phenon 1 grew poorly on these media compared with the moderate growth responses of phena 2 and 3 and the very high yields of phenon 6. Factor 2 represented the use of sucrose, several organic acids (2 mM acetate, citrate, formate, glyoxalate, 2-ketoglutarate, malate, malonate, and pyruvate and 10 mM formate), and benzoate as sole carbon sources and the use of ornithine, cysteine, and tyrosine as sole carbon and nitrogen sources. Factor 3 represented growth on additional organic acids (10 mM glyoxalate, 10 mM 2-ketoglutarate, 2 mM lactate), on benzoate (the only substrate contributing strongly to more than one of the three factors), and on glutamate and lysine as sole carbon and nitrogen sources. Higher yields on these media allowed factors 2 and 3 to separate isolates in phenon 3 from the other isolates. Factor 3 also helped separate isolates in phenon 2 from the poorly growing isolates in phenon 1.

Phenon 1 contained SG *Prosopis* isolates from all three soil depths and the *Prosopis* reference strain WR1001 (Table 3). As discussed above, these isolates grew poorly on many of the media which favored growth of isolates in the other phena. The low yield of isolates in this group may be explained by a preference for alkaline pH. While they showed virtually no growth in medium with an initial pH of 6, their yield was comparable to that of the other groups at high pH. All of the SG rhizobia tested by Moffett and Colwell (20) grew at pH 4.0. The apparent osmosensitivity and low growth yields of the isolates in phenon 1 require retesting at a higher pH.

Phenon 2 was heterogeneous in origin, containing the two FG *Psorothamnus* isolates; 5 FG and 21 SG *Prosopis* isolates; and the SG reference strains ATCC 10139, ATCC 10325, and ATCC 10326. Most of the FG isolates in this phenon formed a subgroup with the reference strains. Although the *Bradyrhizobium* reference strains were phenotypically similar to the *Psorothamnus* and *Prosopis* isolates in this phenon, Lieberman et al. (18) have reported data indicating that *Prosopis* is not a compatible host for these *Bradyrhizobium* species. The two *Psorothamnus* rhizobia, on the other hand, effectively nodulated *Prosopis glandulosa* (15).

Isolates in phenon 2 grew well on mannitol-based, glutamate-containing medium, poorly on other sugars tested with NH_4Cl as the N source, and moderately well on a few organic acids. With the inclusion of 0.1 g of yeast extract $liter^{-1}$ in the medium, Stowers and Elkan (29) found that SG cowpea rhizobia grow well on all these sugars except for sucrose and lactose. In contrast to the other predominantly SG group, phenon 1, initial pH values from 6 to 9 allowed growth of isolates in phenon 2.

FG *Prosopis* rhizobia predominated in phenon 3, which contained all of the FG isolates from intermediate soil depths and two FG surface-soil isolates. This group was differentiated from the others by growth on a variety of organic and amino acids. The rhizobia in phenon 3 did not utilize lactose, arabinose, or glucose. This was the only phenon to grow, albeit poorly, on an aromatic compound (benzoate) as the sole carbon source. Like the isolates in phenon 1, these

TABLE 2. Growth yields of major phena on test media

Test	Growth yield (1,000 × A_{660}) (mean ± SD) of the following phena (no. of isolates):			
	1 (28)	2 (32)	3 (11)	6 (14)
Adonitol	5 ± 27	38 ± 64	50 ± 62	319 ± 112
Arabinose	15 ± 52	31 ± 48	17 ± 36	235 ± 69
Cellobiose	5 ± 22	27 ± 49	94 ± 109	250 ± 107
Galactose	12 ± 36	38 ± 58	73 ± 128	357 ± 75
Glucose	31 ± 83	47 ± 92	4 ± 8	380 ± 130
Glycerol	11 ± 35	55 ± 84	85 ± 101	372 ± 105
Lactose	13 ± 36	38 ± 67	13 ± 25	218 ± 52
Mannose	9 ± 60	44 ± 106	42 ± 87	284 ± 84
Rhamnose	11 ± 30	65 ± 105	99 ± 110	321 ± 125
Sorbitol	37 ± 104	76 ± 114	130 ± 120	419 ± 125
Sucrose	3 ± 13	3 ± 19	59 ± 29	1 ± 7
Acetate, 2 mM	7 ± 13	20 ± 11	66 ± 26	14 ± 5
Acetate, 10 mM	32 ± 28	58 ± 13	81 ± 35	62 ± 18
Citrate, 2 mM	3 ± 10	15 ± 27	57 ± 38	4 ± 19
Citrate, 10 mM	0 ± 5	28 ± 57	18 ± 30	15 ± 66
Formate, 2 mM	2 ± 4	18 ± 24	69 ± 32	14 ± 29
Formate, 10 mM	3 ± 6	19 ± 18	59 ± 36	12 ± 22
Glyoxalate, 2 mM	1 ± 4	8 ± 23	59 ± 55	9 ± 12
Glyoxalate, 10 mM	8 ± 8	43 ± 14	66 ± 23	25 ± 22
Ketoglutarate, 2 mM	3 ± 9	16 ± 17	60 ± 21	7 ± 16
Ketoglutarate, 10 mM	44 ± 40	104 ± 39	157 ± 31	45 ± 55
Lactate, 2 mM	50 ± 31	74 ± 30	96 ± 27	74 ± 35
Lactate, 10 mM	51 ± 47	105 ± 33	106 ± 33	252 ± 61
Malate, 2 mM	17 ± 11	26 ± 19	43 ± 13	22 ± 12
Malate, 10 mM	56 ± 26	57 ± 21	66 ± 14	68 ± 25
Malonate, 2 mM	3 ± 8	15 ± 12	60 ± 19	21 ± 13
Malonate, 10 mM	16 ± 29	30 ± 29	90 ± 37	87 ± 17
Oxalacetate, 2 mM	−1 ± 8	8 ± 13	26 ± 22	14 ± 7
Oxalacetate, 10 mM	64 ± 24	70 ± 20	84 ± 32	64 ± 14
Pyruvate, 2 mM	6 ± 7	18 ± 9	26 ± 12	15 ± 10
Pyruvate, 10 mM	65 ± 27	92 ± 19	90 ± 34	71 ± 31
Succinate, 2 mM	54 ± 16	50 ± 19	48 ± 8	48 ± 22
Succinate, 10 mM	47 ± 73	97 ± 61	147 ± 53	262 ± 39
Benzoate	1 ± 4	0 ± 11	40 ± 24	3 ± 21
Alanine	20 ± 43	37 ± 44	130 ± 22	197 ± 58
Cysteine	3 ± 7	5 ± 7	23 ± 13	7 ± 6
Glutamate	60 ± 58	104 ± 29	147 ± 65	105 ± 35
Leucine	7 ± 28	44 ± 34	135 ± 55	34 ± 34
Lysine	9 ± 38	16 ± 35	32 ± 40	23 ± 51
Ornithine	0 ± 5	0 ± 5	20 ± 28	−1 ± 4
Proline	10 ± 39	37 ± 58	38 ± 60	188 ± 57
Tyrosine	4 ± 10	5 ± 7	55 ± 36	9 ± 6
Growth factor-free	71 ± 53	298 ± 117	354 ± 153	570 ± 101
Choline-free	105 ± 98	355 ± 145	316 ± 188	614 ± 101
Pyridoxine-free	198 ± 171	326 ± 138	389 ± 201	548 ± 130
Thiamine-free	100 ± 68	286 ± 88	313 ± 130	568 ± 118
All growth factors	122 ± 62	273 ± 79	296 ± 116	526 ± 136
Sucrose, −0.5 MPa	38 ± 59	176 ± 58	178 ± 93	385 ± 129
Sucrose, −1.0 MPa	40 ± 71	209 ± 79	238 ± 146	365 ± 121
Sucrose, −1.5 MPa	15 ± 49	176 ± 72	175 ± 55	386 ± 135
pH 5	6 ± 31	36 ± 114	2 ± 5	9 ± 46
pH 6	11 ± 42	181 ± 84	199 ± 185	512 ± 186
pH 7	165 ± 78	249 ± 86	259 ± 118	458 ± 151
pH 8	275 ± 94	299 ± 140	347 ± 101	495 ± 143
pH 9	296 ± 99	320 ± 107	449 ± 134	439 ± 167
26°C	118 ± 102	290 ± 81	296 ± 110	566 ± 93
36°C	102 ± 172	222 ± 130	366 ± 141	744 ± 146

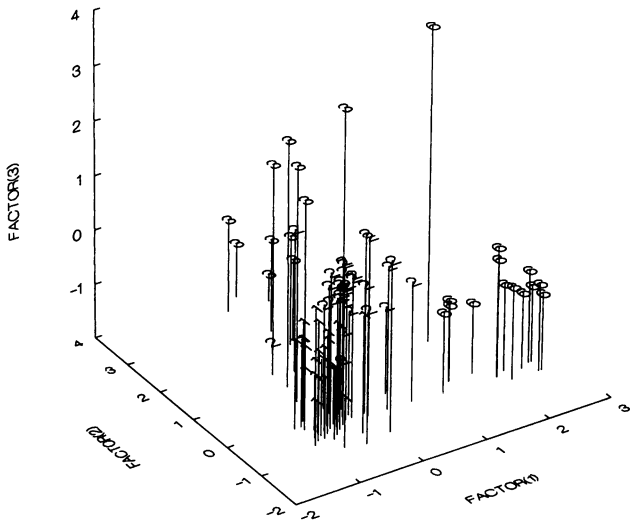

FIG. 2. Factor analysis of four main phena of desert woodland rhizobia. Tests contributing to the three major factors are described in the text.

isolates responded strongly to an increased initial pH of the medium; however, they did grow at pH 6. Rhizobia in phenon 3 grew better at 36°C than at 26°C ($P < 0.05$).

FG *Acacia* rhizobia predominated in the remaining major group, phenon 6, which also included four FG *Prosopis* rhizobia from surface-soil environments. The extremely high absorbance readings obtained for this group on some media may have resulted from extracellular polysaccharide production because of the high carbohydrate concentration and long incubation time. High growth yields were obtained for phenon 6 isolates on all but one of the carbohydrates tested. Unlike most FG rhizobia, which use sucrose as the sole carbon source (7–9, 20), isolates in phenon 6 failed to grow on this substrate. The response of phenon 6 isolates to organic acids was generally weaker than that of the other predominantly FG group, phenon 3. As in phenon 3, growth was better at 36°C than at 26°C ($P < 0.05$). Whereas FG isolates from *Acacia cyanophylla* and *Acacia melanoxylon* effectively nodulated *Prosopis chilensis* (11), the isolates from *A. constricta* failed to nodulate *Prosopis glandulosa* (31). This observation is, however, consistent with the high degree of host specificity reported in other *Acacia* species (5).

TABLE 3. Distribution of FG and SG rhizobia within phena by host and soil environment of origin

Host	Soil zone and rate of growth	No. of isolates in the following phena:					
		1	2	3	4	5	6
Prosopis glandulosa	Surface						
	FG	0	5	2	0	0	4
	SG	10	1	0	0	0	0
	Intermediate						
	FG	0	0	6	0	0	0
	SG	4	0	1	0	0	0
	Pheatic						
	FG	0	0	0	1	0	0
	SG	9	20	0	0	0	0
Psorothamnus spinosus	Surface						
	FG	0	2	0	0	0	0
	SG	0	0	0	0	0	0
Acacia constricta	Surface						
	FG	1	0	0	0	1	8
	SG	0	0	0	0	0	0

Phena 1 and 2 were predominantly SG rhizobia, while strains in phena 3 and 6 were almost all FG rhizobia. Whereas FG rhizobia generally have been reported to utilize a larger number of substrates than do SG rhizobia (2, 9, 26), the number of compounds used as the sole C source did not differ between the SG and FG woodland rhizobia examined in this study. However, the FG phena did grow on more amino acids as the sole C and N sources than did the predominantly SG phena. In contrast to the results of two previous studies (10, 19), our data indicate the FG rhizobia may be more tolerant of high temperatures than the SG rhizobia are. FG rhizobia in phenon 3 and the FG *Acacia* rhizobia in phenon 6, which were predominantly from surface-soil environments, grew better at 36°C than at 26°C. The only FG *Prosopis* isolate from the phreatic-soil environment failed to grow well at 36°C. The high growth optima of the FG rhizobia may indicate adaptation to the high temperatures often reached in the surface soils (34) from which most of the FG rhizobia in this study originated (Table 3).

The major phena shared several common characteristics. Growth yields on favored organic acids were comparable to yields with carbohydrates as the sole carbon sources, especially among the *Prosopis* rhizobia. This is in contrast to results obtained by Skotnicki and Rolfe (28), who generally obtained much better growth in carbohydrate-containing media than on media in which organic acids were the sole carbon sources. The *Prosopis* rhizobia showed a better response to organic acids than did cowpea rhizobia (29). In contrast to rhizobia from legumes in the Canadian high arctic region (25), most of the woodland rhizobia examined in this study grew with oxalacetate as the sole carbon source. Growth of the tree rhizobia on pyruvate is consistent with previous reports on a variety of rhizobia (8, 25, 28).

The general inability of the rhizobia tested in this study to grow on aromatic compounds was unusual. Muthukumar et al. (21) reported that all but one of the FG rhizobia they tested grew on salicylate. In another study, only one FG rhizobial strain, but all of the SG rhizobia tested, utilized anthranilate (24).

None of the major phena was dependent on growth factors. Because of contamination of some of the growth factor media, results were obtained for the elimination of only choline, pyridoxine, and thiamine; however, all phena grew well in vitamin-free mannitol salts medium compared with growth in the fully supplemented medium or media from which a single growth factor was eliminated. Growth factor responses and requirements vary within rhizobial species (3, 9, 29), and the possibility that some isolates within phena responded to growth factors cannot be ruled out.

The isolates generally tolerated −1.5 MPa of sucrose in the medium. The FG rhizobia from dry surface soil environments were no more tolerant of low solute water potentials than were SG rhizobia from the moist deep-phreatic-soil environment. The major phena grew well at initial medium pH values of 8 and 9, reflecting their adaptation to the neutral to alkaline soils characteristic of the Sonoran Desert.

Based on the distribution of colony types, surface and deep SG rhizobial populations from the Harper's Well mesquite woodland are distinct (14). Within both SG and FG

TABLE 4. Comparison of SG mesquite rhizobia from surface and phreatic soils

Test	Growth yield (1,000 × A_{660}) on:		P
	Surface soil	Phreatic soil	
Nutritional			
Formate, 2 mM	3	11	0.0080
Succinate, 10 mM	34	78	0.0060
All growth factors	132	247	0.0030
Pyridoxine-free	169	329	0.0004
Thiamine-free	98	246	0.0012
Growth factor-free	96	229	0.0050
Physiological tolerances			
Sucrose, −0.5 MPa	20	140	0.0001
Sucrose, −1.0 MPa	22	174	0.0001
Sucrose, −1.5 MPa	22	114	0.0001
pH 5	0	8	NS[a]
pH 6	13	112	0.0003
pH 7	143	235	0.0041
pH 8	290	276	NS
pH 9	321	322	NS
26°C	121	259	0.0007
36°C	38	207	0.0002

[a] NS, Not significant.

Prosopis rhizobia, isolates from the surface and intermediate (nonphreatic) soil environments tended to group separately. Although cluster analysis did not completely resolve *Prosopis* rhizobia from surface and phreatic environments, SG isolates from the two soil depths differed phenotypically (Table 4). The surface isolates were sensitive to acid pH, with decreased growth at pH 7 and below. Surprisingly, they also appeared to be less osmotolerant than the phreatic isolates were and grew poorly at 36°C, but these physiological differences may reflect the fact that tests were carried out at a pH level which stressed the isolates.

Recent studies have indicated that large populations of free-living rhizobia, nodulation, and N_2 fixation associated with *Prosopis* occur at depths (1 to 10 m) that are seldom studied (16, 30) and that the distributions of FG and SG symbionts vary with ecosystem type and soil depth (17). The tree rhizobia examined in this study from phreatic and nonphreatic (surface and intermediate depth) soil environments and from the *Prosopis* and *Acacia* hosts segregated into distinct groups. Although isolates within phena appeared to be phenotypically homogeneous, analysis of DNA restriction fragment length polymorphisms of selected *Prosopis* rhizobia indicates genetic diversity within phena (P. M. Thomas, K. F. Golly, R. A. Virginia, and J. W. Zyskind, unpublished data).

The spatial and temporal separation of the surface- and deep-soil rooting systems of mesquite has resulted in the development of distinct rhizobial populations. Thus, the phreatophytic mesquite system provides a model for studying the ecological differences between SG and FG rhizobia (14), as well as the divergence of natural rhizobial populations exposed to different soil environments while under the influence of a single host plant. The major phena formed by the rhizobia examined in this study were quite distinct, representing both *Rhizobium* and *Bradyrhizobium* species. Additional studies are needed to determine the taxonomic positions of these organisms.

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LITERATURE CITED

1. **Allen, O. N., and E. K. Allen.** 1981. The leguminosae. University of Wisconsin Press, Madison.
2. **Bromfield, E. S. P., and J. V. D. K. Kumar Rao.** 1983. Studies on fast and slow growing *Rhizobium* spp. nodulating *Cajunus cajan* and *Cicer arietinum*. Ann. Appl. Biol. **102**:485–493.
3. **Chakrabarti, S., M. S. Lee, and A. H. Gibson.** 1981. Diversity in the nutritional requirements of strains of various *Rhizobium* species. Soil Biol. Biochem. **13**:149–154.
4. **Dixon, W. J. (ed.).** 1988. BMDP statistical software manual. University of California Press, Berkeley.
5. **Dreyfus, B. L., and Y. R. Dommergues.** 1981. Nodulation of *Acacia* species by fast- and slow-growing tropical strains of *Rhizobium*. Appl. Environ. Microbiol. **41**:97–99.
6. **Felker, P.** 1979. Mesquite—an all purpose leguminous arid land tree, p. 89–132. *In* G. A. Ritchie (ed.), New agricultural crops. American Association for the Advancement of Science Symposium, vol. 38. Westview Press, Golden, Colo.
7. **Glenn, A. R., and M. J. Dilworth.** 1981. The uptake and hydrolysis of disaccharides by fast- and slow-growing species of *Rhizobium*. Arch. Microbiol. **129**:233–239.
8. **Graham, P. H.** 1964. Studies in the utilization of carbohydrates and Krebs cycle intermediates by rhizobia, using an agar plate method. Antonie van Leeuwenhoek **30**:68–72.
9. **Graham, P. H., and C. A. Parker.** 1964. Diagnostic features in the characterization of the root-nodule bacteria of legumes. Plant Soil **20**:383–396.
10. **Hartel, P. G., and M. Alexander.** 1984. Temperature and desiccation tolerance of cow pea rhizobia. Can. J. Microbiol. **30**: 820–823.
11. **Herrera, M. A., E. J. Bedmar, and J. Olivares.** 1985. Host specificity of *Rhizobium* strains isolated from nitrogen-fixing trees and nitrogenase activities of strain GRH2 in symbiosis with *Prosopis chilensis*. Plant Sci. **42**:177–182.
12. **Hua, S. T., V. Y. Tsai, G. M. Lichens, and A. T. Noma.** 1982. Accumulation of amino acids in *Rhizobium* sp. strain WR1001 in response to sodium chloride salinity. Appl. Environ. Microbiol. **44**:135–140.
13. **Jenkins, M. B., and P. J. Bottomley.** 1985. Composition and field distribution of *Rhizobium meliloti* in root nodules of field-grown alfalfa. Soil Biol. Biochem. **17**:173–179.
14. **Jenkins, M. B., R. A. Virginia, and W. M. Jarrell.** 1987. Rhizobial ecology of the woody legume mesquite (*Prosopis glandulosa*) in the Sonoran Desert. Appl. Environ. Microbiol. **53**:36–40.
15. **Jenkins, M. B., R. A. Virginia, and W. M. Jarrell.** 1988. Rhizobial ecology of the woody legume *Psorothamnus spinosus* in a Sonoran Desert arroyo. Plant Soil **105**:113–120.
16. **Jenkins, M. B., R. A. Virginia, and W. M. Jarrell.** 1988. Depth distribution and seasonal populations of mesquite-nodulating rhizobia in warm desert ecosystems. Soil Sci. Soc. Am. J. **52**:1644–1650.
17. **Jenkins, M. B., R. A. Virginia, and W. M. Jarrell.** 1989. Ecology of fast-growing and slow-growing mesquite-nodulating rhizobia in Chihuahuan and Sonoran Desert ecosystems. Soil Sci. Soc. Am. J. **53**:543–549.
18. **Lieberman, M. T., L. M. Mallory, S. Simkins, and M. Alexander.** 1985. Numerical taxonomic analysis of cross-inoculation patterns of legumes and *Rhizobium*. Plant Soil **84**:225–244.
19. **Marshall, K. C.** 1964. Survival of root-nodule bacteria in dry soils exposed to high temperatures. Aust. J. Agric. Res. **15**: 273–284.
20. **Moffett, M. L., and R. R. Colwell.** 1968. Adansonian analysis of the *Rhizobiaceae*. J. Gen. Microbiol. **51**:245–266.
21. **Muthukumar, G., A. Arunakumari, and A. Mahadevan.** 1982.

Degradation of aromatic compounds by *Rhizobium* sp. Plant Soil **69**:163–169.
22. **Nilsen, E. T., M. R. Sharifi, P. W. Rundel, W. M. Jarrell, and R. A. Virginia.** 1983. Diurnal and seasonal water relations of the desert phreatophyte *Prosopis glandulosa* (honey mesquite) in the Sonoran Desert of California. Ecology **64**:1381–1393.
23. **Oldeman, R. A. A.** 1983. The design of ecologically sound agroforestry, p. 173–206. *In* P. A. Huxley (ed.), Plant research and agroforestry. International Council for Research in Agroforestry, Nairobi, Kenya.
24. **Parke, D., and L. N. Ornston.** 1984. Nutritional diversity of *Rhizobiaceae* revealed by auxanography. J. Gen. Microbiol. **130**:1743–1750.
25. **Prevost, D., L. M. Bordeleau, S. Caudry-Reznick, H. M. Schulman, and H. Antoun.** 1987. Characteristics of rhizobia isolated from three legumes indigenous to the Canadian high arctic: *Astragalus alpinus*, *Oxytropis maydelliana*, and *Oxytropis arctobia*. Plant Soil. **98**:313–324.
26. **Sadowsky, M. J., H. H. Keyser, and B. B. Bohlool.** 1983. Biochemical characterization of fast- and slow-growing rhizobia that nodulate soybeans. Int. J. Syst. Bacteriol. **33**:716–722.
27. **Shearer, G., D. H. Kohl, R. A. Virginia, B. A. Bryan, J. L. Skeeters, E. T. Nilsen, M. R. Sharifi, and P. W. Rundel.** 1983. Estimations of N_2-fixation from variation in the natural abundance of ^{15}N in Sonoran Desert ecosystems. Oecologia **56**: 365–373.
28. **Skotnicki, M. L., and B. Rolfe.** 1977. Differential stimulation and inhibition of growth of *Rhizobium trifolii* strain TI and other *Rhizobium* species by various carbon sources. Microbios **20**: 15–28.
29. **Stowers, M. D., and G. H. Elkan.** 1984. Growth and nutritional characteristics of cowpea rhizobia. Plant Soil **80**:191–200.
30. **Virginia, R. A., M. B. Jenkins, and W. M. Jarrell.** 1986. Depth of root symbiont occurrence in soil. Biol. Fertil. Soils **2**: 127–130.
31. **Waldon, H. B.** 1987. Sonoran Desert rhizobia found to nodulate *Acacia constricta*. Desert Plants **8**:106–110.
32. **Ward, J. H.** 1983. Hierarchical grouping to optimise an objective function. J. Am. Statistical Assoc. **58**:236–244.
33. **Weide, D. L.** 1976. Regional environmental history of the Yuha Desert, p. 9–20. *In* P. J. Wilke (ed.), Background to the prehistory of the Yuha Desert region. Ballena Press, Ramona, Calif.
34. **Wilkins, J.** 1967. The effects of high temperature on certain root-nodule bacteria. Aust. J. Agric. Res. **18**:299–304.
35. **Wishart, D.** 1978. Clustan user manual. Inter-University Research Council Series Report no. 47. Inter-University Research Council, Edinburgh.