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MESQUITE RHIZOBIA ISOLATED FROM THE SONORAN DESERT: COMPETITIVENESS AND SURVIVAL IN SOIL

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Summary—The competitiveness of a mesquite *Rhizobium* (AZ-M1) and its ability to survive in desert soils was compared to a selected commercial strain (31A5). In a greenhouse study, the native isolate out-competed strain 31A5 in nodule occupancy, when applied as a mixed inoculant to seed germinated and grown in sand culture, and irrigated with N-free nutrients. A high incidence of nodule double occupancy was found when double strain inoculants were used. The survival rate of the two strains was tested in three desert soils in a controlled laboratory study. The desert strain AZ-M1 grew and survived in all the soils for 1 month. The commercial strain 31A5, did not grow, and the population decreased in 14 days from 10^8 cells g^{-1} dry soil to below 10^4 cells g^{-1}. Both strains survived to a lesser extent in a saline-sodic soil. A significant morphological change from a rod to a coccus was observed 2 days after strain 31A5 had been introduced into the desert soils.

INTRODUCTION

Mesquite (*Prosopis*) is a tree legume prominent in the southwestern part of the United States, and other semi-arid regions of the world. The nitrogen fixation ability of this legume has been confirmed in controlled conditions (Bailey, 1976; Eskew and Ting, 1978; Felker and Clark, 1980; Rundel *et al.*, 1982; Shoushtari and Pepper, 1985).

Nitrogen fixation effectiveness is an important criterion used in the selection of strains of rhizobia for inoculants. Studies on the competitiveness of strains and their persistence in soil have introduced additional criteria for the selection of good inoculants (Amarger, 1981; May and Bohlool, 1983). The competitiveness of a strain is a measure of its ability for nodule occupancy, in the presence of other strains of the same species. Highly competitive strains will be found in most nodules on a legume, whereas uncompetitive strains will become eliminated during the nodulation process, regardless of how efficient they are at fixing N_2.

The successful introduction of any new rhizobial strain into soil depends on environmental factors, as well as interactions between indigenous microorganisms present in the soil and the introduced strain. Survival and multiplication in the soil and rhizosphere of a legume plant are necessary for a successful symbiosis to be initiated (Smith and Miller, 1974; Pugashetti and Wagner, 1979, 1980). Currently, little is known of the natural populations of mesquite rhizobia in Sonoran desert soils. We have evaluated the competitiveness and survival of a native strain AZ-M1 isolated from the Sonoran Desert against a best selected commercial strain, 31A5.

MATERIALS AND METHODS

Competitiveness of strains

A greenhouse experiment was conducted to evaluate the competitiveness of the 2 selected strains of mesquite rhizobia: the native strain AZ-M1 and a commercial strain 31A5 (courtesy Nitragin Company, Milwaukee) were shown to be highly efficient at fixing N_2 (Shoushtari and Pepper, 1985). These individual strains and mixtures of the strains were used as inoculants for mesquite seed. A completely randomized design was used with each inoculation treatment replicated 4 times. The total volume of inoculant was 1 ml. Inocula were made by growing rhizobia to log phase in yeast extract mannitol broth (YEM broth) and the inoculation treatments used are shown in Table 2.

Eleven liter plastic buckets filled with crystal silica sand (particle size 0.5–1.0 mm), were each planted with 15 scarified mesquite seeds (Shoushtari and Pepper, 1985), and seedlings were later thinned to 10 per bucket. Plants were watered twice weekly with 250 ml N-free macronutrients (Sieger, 1969) and micronutrients (Vincent, 1970). Plants were harvested after 7 weeks and evaluated for N_2 fixation. Data collected included shoot weight, root weight, nodule weight, total shoot N and the C_2H_2 reduction assay (Hardy *et al.*, 1968).

To determine the competitiveness of each strain, the serological method of the fluorescent antibody technique (FA) was employed (Schmidt *et al.*, 1968) to determine proportional nodulation due to each strain. Twenty nodules were randomly selected from each replicate for FA identification against the 2 antisera. Roots and attached nodules were washed in distilled water containing 0.1% Tween 80 on the day of harvest, and then dried in an oven at 60°C for 24 h. Each individual nodule was crushed in 1 ml of sterile water and a loopful of the homogenate was smeared on a cleaned microscope slide. One drop rhodamine gelatin isothiocyanate (RHITC) was applied onto each slide and dried in an oven at 60°C, followed by addition of 1 drop of specific FA. Slides were then held in a humid chamber (a Petri dish with a moist filter paper) in the dark for 30 min at room tem-

Table 1. Some chemical and physical properties of the soils used in the survival study

Soil	Gothard[1]	Stewart[2]	Laveen[3]
Sand %	19.2	63.1	38.8
Clay %	34.0	26.1	23.0
Silt %	46.8	10.8	38.2
Texture	Silty clay loam	Sandy clay loam	Loam
pH[4]	8.2	9.5	7.7
ECe dSm^{-1}	7.2	11.2	1.9
Na[5] m-equiv l^{-1}	28.9	87.3	8.2
ESP %	7.5	26.6	4.3
% organic C	1.0	0.3	0.6

[1]Fine loamy, mixed thermic Typic Natragids: saline.
[2]Loamy mixed, thermic shallow Typic Durorthids saline-alkali.
[3]Coarse-loamy mixed, hyperthermic Typic Calciorthids: non-saline, non-alkaline.
[4]Saturated paste.
[5]Measured in saturated extract.

perature. Physiological concentration of saline water was later used to rinse the excess FA off the slide. Slides were then soaked in saline water in the dark for 15 min, and finally rinsed in distilled water and air dried. Later, they were examined under a Zeiss ultra-violet microscope for a positive or negative response against the appropriate antiserum.

Survival of mesquite rhizobia

Survival of the two strains of mesquite rhizobia was compared in three different Sonoran Desert soils. These surface soils (0–15 cm) represent some of the common soils of the Sonoran Desert (Table 1) and include a wide range of physical and chemical properties. All soils were light textured with high pH, typical of Sonoran Desert soils. Electrical conductivity (EC_e) varies from 1.9 dSm^{-1} (non-saline) to 11.2 dSm^{-1} (saline). Exchangeable sodium % varies from 4.3 to 26.6, with the latter soil being classified as sodic. Organic C values were low, except for the 1% value of the Gothard, which would be fairly high for a desert soil. Fresh soil samples collected from the field were stored in plastic bags at 4°C. Later, the samples were air dried at room temperature and sieved (< 2 mm). Ten g air-dried soil samples were transferred into 150 ml milk dilution bottles, followed by addition of 1 ml of log phase culture of either strain, and enough sterile water to bring each soil sample to near field capacity (33 kPa tension). Soils were thoroughly mixed, and the sample bottles were completely randomized and held in the dark at 25°C. Treatments included three soils maintained for 0, 2, 7, 14 or 28 days with either strain, and replicated 4 times. Before this experiment all 3 soils were tested for indigenous cross-reacting bacteria. None were found for either strain in any soil.

To recover the bacteria from the soil at each sampling, the density centrifugation method of Wollum and Miller (1980) modified by M. S. Miller (unpublished Ph.D Dissertation, University of Arizona, 1985) was employed. The recovered bacteria were enumerated by means of the FA technique (Schmidt *et al.*, 1968) using the Zeiss ultra-violet microscope based on an average of 20 fields slide^{-1} after applying the appropriate antiserum.

RESULTS

Competitiveness of strains

The native strain AZ-M1 out-competed the commercial strain 31A5 in nodule occupancy (Table 2). Even when 10^7 cells of AZ-M1 had been combined with 10^5 cells of 31A5, the nodules contained 47% strain AZ-M1 compared to 25% 31A5 with 26.3% of the nodules being double infected. The proportional recovery of AZ-M1 in the nodules increased as the number of cells of AZ-M1 increased in the mixed inoculum. Most uninoculated plants were without nodules but occasional large nodules were found on a few plants. Overall contamination was minimal. Nodules from the uninoculated treatment were 95% contaminated with AZ-M1 and 5% unknown where none of the strains were detected. This contamination was probably caused by airborne transfer of AZ-M1 in the greenhouse. Contamination is also the likely cause of AZ-M1 in nodules, when 31A5 was the sole inoculant. The fact that airborne contaminant cells of AZ-M1 could cause nodulation when 31A5 was the sole inoculant is a further indication of the competitiveness of AZ-M1. Where both strains were applied as a mixed inoculant, they were found as double occupants of nodules. The highest proportion

Table 2. Percentage of nodules formed on mesquite by inoculum strains

Inoculum (cells plant^{-1})		Strains recovered in nodules (% of total)			
AZ-M1	31A5	AZ-M1	31A5	AZ-M1 + 31A5	Unknown
0	0	95.0[1]	0.0	0.0	5.0
10^4	0	98.8	0.0	0.0	1.3
0	10^5	1.7[1]	80.0	13.3[1]	5.0
10^5	10^4	86.3	2.5	11.3	0.0
10^5	10^5	67.5	8.5	24.0	0.0
10^4	10^5	47.0	25.0	26.3	1.8

[1]Contamination by the native strain AZ-M1.

Table 3. Effectiveness as affected by mixtures of the 2 inoculants

Inoculum (cells plant^{-1}) AZ-M1	31A5	Acetylene reduction (nmol plant^{-1} h^{-1})	Nodule weight (mg plant^{-1})	Shoot weight (mg plant^{-1})	Root weight (mg plant^{-1})	total plant N (mg plant^{-1})
0	0	1.6a	0.49a	117.86a	119.98ab	1.12a
10^8	0	52.4a	5.86b	193.11bc	110.41ab	3.11ab
0	10^8	8.1a	2.20ab	128.95a	130.51ab	2.09a
10^8	10^8	27.6a	3.27ab	142.20ab	146.90bc	2.46a
10^8	10^8	15.5a	3.13ab	170.33abc	97.44a	2.50a
10^4	10^8	151.4b	13.56c	218.64c	184.10c	4.91b

Values in columns followed by the same letter do not differ significantly ($P = 0.05$)

of double occupancy of nodules was observed when both strains were applied as inoculants with at least 10^8 cells of 31A5. Double occupancy decreased as the ratio of AZ-M1 to 31A5 increased.

Effectiveness (N_2 fixation) of mesquite rhizobia as influenced by mixtures of the two strains in the inoculum is presented in Table 3. C_2H_2 reduction was not significantly different ($P = 0.05$) between the treatments except for the inoculum which contained 10^4 cells of AZ-M1 and 10^8 cells of 31A5. Nodules formed in this treatment showed significantly higher C_2H_2 reduction values and were 47% AZ-M1, 25% 31A5, with 26.3% showing double occupancy (Table 2). With the presence of both strains in many of the nodules of the plants, little difference in C_2H_2 reduction was expected between the treatments. No significant differences in nodule mass were observed between any treatments, except when 10^4 cells of AZ-M1 and 10^8 cells of 31A5 were applied as inoculant. Again note that when AZ-M1 dominated nodule occupancy, nodule mass tended to increase relative to when 31A5 was dominant. Shoot weight followed similar trends, but root weight data showed no discernible trends.

Survival of mesquite rhizobia

The survival rate of mesquite rhizobia in the three Arizona soils was directly related to the strain introduced into the soil. Figure 1 shows the survival rate of both strains in the soils.

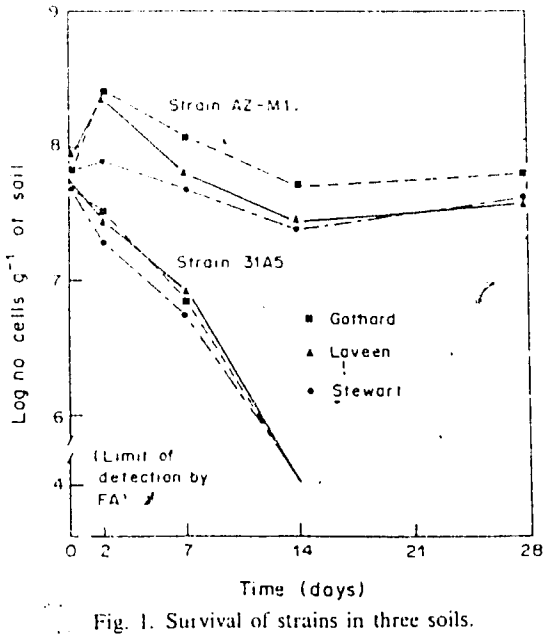

Fig. 1. Survival of strains in three soils.

The commercial strain survived poorly in all soils. The number of cells recovered from the Stewart soil was significantly lower than from the other soils suggesting the influence of soil factors such as salinity and sodicity, pH and organic C (Table 1). Two days after strain 31A5 was introduced into all desert soils, it changed from a rod to a coccus. The desert strain AZ-M1 survived well in all soils, but survival was also significantly lower in the Stewart soil. The number of cells of the native strain increased in the Gothard and Loveen soils after 2 days, and then gradually decreased thereafter. Overall, AZ-M1 showed significantly better survival in the Gothard soil. The population of the desert strain was in general, fairly stable in the soils after 2 weeks (Fig. 1).

DISCUSSION

The desert strain AZ-M1 was more competitive than strain 31A5 when applied as a mixed inoculant. Competitiveness of rhizobia independent of effectiveness has been reported by May and Bohlool (1983), Amarger (1981) and Josephson and Pepper (1984). However the isolate AZ-M1 was competitive and effective. Competitiveness was independent of cell numbers as shown by the high proportional recovery of AZ-M1 from nodules (Table 2) even when strain AZ-M1 was greatly outnumbered in the inoculant. Robert and Schmidt (1983) and Josephson and Pepper (1984) reported the same finding when evaluating the competitiveness of *R. leguminosarum*, biovar *phaseoli*. Robert and Schmidt (1983) also reported no correlation between the competitiveness of a strain and the numbers of that strain in the rhizosphere. A high incidence of double-strain occupancy was found when both strains were applied together as the inoculant. May and Bohlool (1983) found a close relationship between the competitiveness of a strain and its involvement in the formation of doubly infected nodules. Josephson and Pepper (1984) reported that a highly competitive strain of *R. leguminosarum*, biovar *phaseoli* tended to form doubly infected nodules. In our study AZ-M1 was highly competitive, so that when both strains were present in the inoculant, but with AZ-M1 outnumbered, AZ-M1 still managed to infect the plant root, and be present in most nodules. The reverse was not true when 31A5 was outnumbered. Note also that contamination only of AZ-M1 was found. Plants inoculated with 10^4 cells of AZ-M1 and 10^8 cells of 31A5 gave greater biomass, with most nodules being occupied by the native isolate, and a large number of double infections. These data are from plants har-

vested after 49 days compared to those harvested after 60 days in a previous study (Shoushtari and Pepper, 1985). However, biomass data and N_2 fixation data from both studies correlate well. Burton and Allen (1949) and Dorosinskii and Makarova (1977) have also shown that plants inoculated with a mixture of effective strains, gave better yield and a higher N content than those with single strain inocula. Competitiveness of a strain may also depend on the host plant. Strain host interactions were noticed by May and Bohlool (1983) with *R. leguminosarum* and Russell and Jones (1975) with *R. trifolii*. *Rhizobium* strain traits appear to be predominantly determinant for infectivity, for defined efficiency of symbiotic N fixation, and possibly for the competitiveness of the strain.

Strain AZ-M1 survived better in all soils than 31A5. Since strain AZ-M1 is native to the soils of the southwest, and was isolated from a high pH soil, then this organism may have originated from a saline or sodic soil, and it appears to have some degree of tolerance to salinity and alkalinity, as well as to the lack of nutritional materials prevalent in desert soils. The differences in the survival rate of the desert isolate in the three Arizona soils are probably due to several factors. The soils varied greatly in their electrical conductivity (EC), exchangeable sodium percentage (ESP), pH and organic C. The fact that both rhizobia did poorly in the Stewart soil, may very well be due to the unfavorable conditions of this soil. This soil contains the least organic C, the highest EC, the most sodium and the highest pH. Pena-Cabriales and Alexander (1983) found that addition of organic matter to soil promoted the growth of rhizobia. The Gothard soil with 1% organic C may have furnished the native isolate with more nutrients than the other soils. The commercial strain 31A5 was not able to withstand the conditions of the desert soils. Rhizobia have no specific nutritional requirements (Vincent, 1977) and should be able to survive and to compete with other inhabitants of the soil for nutrients unless the strain itself lacks the natural tolerance to overcome the unfavorable conditions in soil. A significant morphological change was observed 2 days after the introduction of the commercial strain 31A5 into all soils. This was a morphological change in cell shape from rod to a coccus and has been recorded by Bohlool and Schmidt (1970) and Crozat *et al.* (1982) who worked with *R. japonicum*. The change to spherical shape, and later the dramatic decline of this strain 31A5 in the soils suggest that this change may be a defensive response to stress.

Strain AZ-M1 is an infective, effective, highly competitive strain, capable of surviving in desert soils. The presence of an indigenous strain of mesquite rhizobia in the Sonoran Desert may be important ecologically as a source of N.

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