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Survival of *Azorhizobium caulinodans* in the Soil and Rhizosphere of Wetland Rice under *Sesbania rostrata*-Rice Rotation

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The survival of indigenous and introduced strains of *Azorhizobium caulinodans* in flooded soil and in the rice rhizosphere, where in situ *Sesbania rostrata* was incorporated before the rice crop, is reported. The azorhizobia studied were both root and stem nodulating. In a pot experiment, two crop cycles each of inoculated and noninoculated *Sesbania*-rice were compared with two crop cycles of flooded fallow-rice. In a field experiment, the effect of repeated incorporation of in situ *S. rostrata* in the *Sesbania*-rice sequence was studied. Soils in which inoculated *S. rostrata* was incorporated contained about 3,000 times more azorhizobia than did soils in the flooded fallow treatment and about 50 times more azorhizobia than did soils in the noninoculated *Sesbania* treatment. Azorhizobial numbers in the inoculated *Sesbania* treatment declined toward rice harvest but remained much higher than in the flooded fallow-rice treatment. Repeated incorporation of *S. rostrata* increased the population density of indigenous soil azorhizobia, whereas the population of inoculated strain ORS571 (Str^r Spc^r) declined to an undetectable level; this finding suggested low competitiveness by the introduced strain. In the incorporated *Sesbania* treatment, the rice rhizosphere harbored significantly more *A. caulinodans* and supported higher nitrogenase activity per plant than did the rhizosphere of the flooded fallow-rice treatment. Sterile rice seedlings inoculated with *A. caulinodans* showed nitrogenase activity comparable to that of seedlings inoculated with *Azospirillum lipoferum* 34H, a rice root isolate. Rhizobia from *Sesbania aculeata*, *Sesbania sesban*, a *Trifolium* sp., and *Vigna unguiculata* did not support appreciable nitrogenase activity.

Green manure crops can supply a substantial portion of the N required by rice (13, 21). Rice farmers in the tropics can cultivate a green manure during the 40- to 50-day transition period between the dry and wet seasons. Lowland rice fields during the transition period may be flooded intermittently to continuously. *Sesbania rostrata*, a fast-growing and flood-tolerant legume, has recently been found to be an excellent candidate for green manuring in lowland rice systems (6, 18). This plant is also attractive since it supports symbiotic N_2 fixation and produces stem nodules as well as root nodules (6).

Agricultural soils planted to grain legumes often require inoculation with rhizobia (17). However, inoculation of root-nodulating crops is not always successful because the inoculated strain may not compete well with native soil microbes or the soil environment may be unfavorable for the introduced strain (1, 2, 14, 20). On the other hand, legumes that produce nodules on the aerial part of the plant may be relatively free from soil-related problems and consequently more responsive to inoculation under certain conditions (10, 13).

To develop an inoculation technology for *S. rostrata*, knowledge of the survival of azorhizobia in soils, particularly wetland rice soils, is essential. Although the population kinetics and survival of root-nodulating rhizobia in non-flooded soils as affected by the succeeding legume or nonlegume crop have been well studied (1, 2, 4, 9), little is known about the ecology of rhizobia for either root- or stem-nodulating legumes in flooded rice soils. Flooded soil is normally not a favorable environment for rhizobia (15, 16). However, Tuzimura and Watanabe (22) found that *Astragalus sinicus* rhizobia survived well under flooded conditions. The rhizobial population was established at 10^5/g (dry weight) of soil in a field continuously under *Astragalus sinicus*.

The rhizobium of *S. rostrata* has recently been classified as *Azorhizobium caulinodans* (8). Azorhizobia are particularly interesting because they can grow and fix N_2 without added combined N under free-living conditions (7).

This study reports on the survival of inoculated and indigenous *A. caulinodans* in flooded rice soils as affected by the incorporation of *S. rostrata* and the planting of a succeeding rice crop. Stimulation of growth and N_2 fixation of *A. caulinodans* by the rice rhizosphere was also examined.

MATERIALS AND METHODS

Pot experiment. (i) Soil and pot preparation. The experiment was conducted in pots each containing 2.5 kg of dry Maahas clay soil (Mollisol, pH 6.5; total N, 0.14%; organic matter, 2.9%; cation exchange capacity, 40 meq/100 g; free iron oxide, 2.9%) in a greenhouse. The wet soil was sieved through a 20-mesh screen, thoroughly mixed, and placed in porcelain pots (15-cm diameter), which were kept continuously flooded. Each pot received a basal dose of triple superphosphate (37.5 mg of P_2O_5), muriate of potash (25 mg of K_2O), and ammonium molybdate (0.625 mg) before each *Sesbania* planting.

(ii) Treatment. Two cycles alternating between *Sesbania* and rice were grown in continuously flooded conditions during the wet season (WS; May through October 1986) and the dry season (DS; November 1986 through April 1987). The following treatments and crop sequence were established: (1) flooded fallow followed by rice (F/R-F/R); (2) no inoculation of *Sesbania* followed by rice (S−/R-S−/R); and (3) soil, seed, and stem inoculations in the first crop and stem inoculation in the second crop of *Sesbania* followed by rice

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(S++/R-S+/R). The plots of all treatments were arranged in a completely randomized design. Results for the soil and agronomic aspects of the *Sesbania*-rice sequence of this experiment will be reported elsewhere (J. K. Ladha, S. Miyan, and M. Garcia, Biol. Fertil. Soils, in press).

(iii) Plant culture and green manure incorporation. Six scarified seeds of *S. rostrata* coated with live or heat-killed rhizobia were sown. The plants were thinned to three per pot 5 days after planting. *S. rostrata* was incorporated in situ 45 days after emergence for the WS rice (first crop) and 55 days after emergence for the DS rice (second crop). The shoots were cut into small pieces (3 to 6 cm) and mixed thoroughly along with roots into the soil. Amounts of *S. rostrata* (grams [dry weight] per pot) incorporated were as follow: 17.4 in WS and 15.6 in DS in the S−/R-S−/R treatment and 18.3 in WS and 25.2 in DS in the S++/R-S+/R treatment. Weeds growing in the fallow pots were similarly incorporated. Three 15-day-old seedlings of rice genotype IR29723-88-2-3-3 were transplanted in each pot 5 to 10 days after incorporation of green manure. Rice duration excluding 15 days in the seedbed averaged 135 days.

(iv) *Azorhizobium* culture and inoculation. *A. caulinodans* ORS571, which nodulates both the root and the stem of *S. rostrata*, was obtained from O.R.S.T.O.M., Dakar, Senegal (8). A spontaneous mutant resistant to 200 μg each of streptomycin sulfate and spectinomycin (Sigma Chemical Co., St. Louis, Mo.) per ml was obtained by standard microbiological procedures. Nodulation and N_2 fixation efficiencies of the antibiotic-resistant strain were similar to those of the wild-type strain. The mutant strain, referred to as ORS571 (Str^r Spc^r), was grown in TGYE broth (10 g of tryptone [Difco Laboratories, Detroit, Mich.], 5 g of glucose, 5 g of yeast extract, 0.9 g of $CaCl_2 \cdot 2H_2O$, 1 liter of distilled water) at 30 ± 2°C for 3 days on a rotary shaker. The cells were centrifuged, washed twice with sterile 0.01 M phosphate-buffered saline (0.85% NaCl, pH 7.2), suspended in sterile water to a cell density of 10^8/ml, and used for inoculation.

In the soil, seed, and stem inoculation (S++) treatment of the first crop, soil was inoculated with 10 ml of cell suspension per pot. Treatments S− received an equal amount of boiled cell suspension. The soil inoculum was thoroughly mixed in soil before seeds were sown. Stems were inoculated by spraying 2 ml of live or boiled cell suspension mixed with 10% gum arabic on the aerial portion of each plant when it attained a height of 30 to 40 cm (25 days after emergence in the WS crop and 35 days after emergence in the DS crop). Because *S. rostrata* is sensitive to photoperiodism, its growth was slower in DS than in WS.

Field experiment. Rice plant and soil samples were obtained from a long-term biofertilizer field experiment at the International Rice Research Institute farm (Los Baños, Philippines) with Maahas clay soil (Mollisol, pH 6.6; total N, 0.18%; cation exchange capacity, 40 meq/100 g of soil). The experiment was started on March 1985, and since then two crops of rice per year (WS and DS) have been growing (26; J. K. Ladha, P. Padre, G. Punzalan, M. Garcia, and I. Watanabe, Plant Soil, in press). There were four treatments arranged in a completely randomized block design with four replications each: (i) control (fallow-rice with no inorganic or organic N fertilizer), (ii) urea N (fallow-rice with 50 kg of N as urea per ha, split application, 25 kg of N basal and 25 kg of N as topdressed at 50 days after transplanting), (iii) *Azolla*-rice (*Azolla microphylla* was grown for 30 days and incorporated three times before transplanting and once 30 days after transplanting), and (iv) *Sesbania*-rice (*S. rostrata* was grown for 45 days in WS and 55 days in DS and incorporated 1 day before rice transplanting). For this experiment, samples were obtained from treatments i, ii, and iv.

Soil and root sampling and enumeration of azorhizobia and total heterotrophs. Soil rhizosphere samples were obtained from the soil attached to the roots. Nonrhizospheric soil in the field was obtained from the centers of four rice hills with use of a core sampler (15 cm long, 1.7-cm inner diameter). Two cores were taken from each pot. The floodwater was discarded, soil was mixed, and plant debris was removed. A mixture of four cores from two pots (two cores per pot) represented one replicate. At least eight cores per plot were obtained from the field. The soils from either two or four plots, depending on whether counts were made in two or one replicate, respectively, were mixed. Similarly, freshly washed roots were taken in duplicate from composite samples of two plants per replicate. Serial 10-fold dilutions of macerated root or soil suspension were prepared as described earlier (28).

Total and inoculated (ORS571 [Str^r Spc^r]) *A. caulinodans* organisms were counted by the plant infection most-probable-number (MPN) and antibiotic plate count methods. TGYE agar medium (5 g of tryptone, 3 g of glucose, 1 g of yeast extract, 10 g of Noble agar [Difco], 1 liter of H_2O) with antibiotics (200 μg each of streptomycin and spectinomycin per ml) was used for counting *A. caulinodans* ORS571 (Str^r Spc^r); TGYE agar medium without antibiotics was used for counting total heterotrophs. MPN counting by the plant infection method was as follows. Scarified and surface-sterilized seeds of *S. rostrata* were pregerminated on sterilized water agar plates, and then seeds were transferred to agar tubes (200 mm long, 25-mm inner diameter) as described by Jensen (11). The tubes were incubated in a KG cabinet (phytotron with 84 W of light irradiation per m^2 [20 klx] and a 14-h photoperiod, 70 to 80% relative humidity, temperatures of 26°C [day] and 20°C [night]; Koito Co., Tokyo, Japan), and seedlings were allowed to grow for about 2 weeks before inoculation. Presence or absence of nodules was recorded about 3 weeks after inoculation with soil or root suspension in four replicate tubes per dilution.

In the pot experiment, soil counts were made once at the beginning (1 day after inoculation [initial]) and twice during the crop cycle (1 day after *Sesbania* incorporation and after harvest of the first and second crops of rice) in F/R-F/R, S−/R-S−/R, and S++/R-S+/R treatments. Counts in soil with no plants but with incorporated *Sesbania* were made at 1, 65, 110, and 140 days after *Sesbania* incorporation. Counts of bacteria associated with rice roots were taken at maximum tillering and heading.

In the field experiment, soil counts were made 30, 45, 65, and 95 days after rice transplanting during DS 1988; root counts were made at maximum tillering and heading of each of the third (WS 1986), fourth (DS 1987), fifth (WS 1987), and sixth (DS 1988) crops.

All counts in the pot and field experiments were made in duplicate. However, the nonrhizospheric and rhizospheric counts in the field were made in a composite sample in one replicate.

ARA associated with rice. Acetylene reduction activity (ARA) assays were done at maximum tillering and heading of the rice plants. Plants were cut 20 cm above the soil level, and the floodwater was removed with a hand suction pump. Surface soil and part of the soil adhering to the roots were removed carefully by hand until the cut plant-soil sample weighed about 500 g. The samples were placed in 15-

TABLE 1. Population of total and inoculated azorhizobia of *S. rostrata* in soil as affected by green manuring and rice cropping (WS 1986, DS 1987)

Treatment	Population (log_{10} cells/g [dry wt] of soil)									
	Initial		1 day after 1st *Sesbania* incorporation		1 day after 1st rice harvest		1 day after 2nd *Sesbania* incorporation		1 day after 2nd rice harvest	
	MPN	Plate count	MPN	Plate count	MPN	Plate count	MPN	Plate count	MPN	Plate count
Flooded fallow-rice (F/R-F/R)	3.4	<2.0	3.1	<2.0	3.5	<2.0	2.1	<2.0	1.3	<2.0
Sesbania (uninoculated)-rice (S−/R-S−/R)	3.4	<2.0	4.9	<2.0	4.8	<2.0	6.1	3.5	3.5	<2.0
Sesbania (inoculated)-rice (S++/R-S+/R)	7.1	7.7	6.6	7.1	5.5	3.2	6.4	4.9	3.6	<2.0
Least significant difference at the 5% level	1.3		1.2		0.8		1.0		0.5	

by-30-cm plastic bags, which were then doubly heat sealed. Each bag was evacuated once and filled through the gas port with 500 ml of a gas mixture containing 25% (vol/vol) acetylene, 74.99% air, and 0.1% propane. The bags were then incubated at 35°C in the dark. The zero-time and final samples were collected after 30 min and 6.5 h of exposure. Analyses of gases and amount of ethylene produced were done as described previously (3).

Rice-bacterium associations under gnotobiotic conditions. Dehulled seeds of variety IR42 were sterilized with 70% ethanol for 5 min and with undiluted commercial hypochlorite for 20 min and then with acidified 0.1% mercuric chloride for 2 to 3 min. Each treatment was followed by several washings with sterile distilled water. Seeds (15 to 20) were transferred to a sterile water agar (1.5%) plate and allowed to germinate in the dark at 32°C for 2 to 3 days. Three contaminant-free seedlings were transferred aseptically into each sterile cotton-plug glass tube (200 by 25 mm) containing 30 ml of the rice culture semisolid medium of Yoshida et al. (30) amended with 20 ppm of N (20 μg of N per liter) as NH_4NO_3. The plants were grown in a KG cabinet as described above. *Azospirillum lipoferum* 34H (12), *Azospirillum brasilense* ATCC 29145, and *A. caulinodans* ORS571 (8) were cultivated in TGYE medium; *Rhizobium* sp. strain TAL 382 (*Trifolium* sp.) and rhizobia strains isolated from *S. aculeata*, *S. sesban*, and *Vigna radiata* were cultivated in mannitol-yeast extract liquid medium for 48 h with shaking at ambient temperature. The cells were harvested by centrifugation and washed three times with 0.01 M phosphate-buffered saline (pH 7) solution. A 300-μl sample of bacterial suspension (10^9 cells per ml) as inoculant was placed in each tube 2 days after the transfer of seedlings.

ARA associated with rice seedlings was measured 8 days after inoculation in at least 10 replicates per treatment. Cotton plugs were replaced with rubber caps, and ARA assays were done in an atmosphere of air with 15% C_2H_2 for 24 h at 28°C under light in a KG cabinet. Ethylene produced was determined as described previously (3).

RESULTS

Populations of azorhizobia and total heterotrophs in soil and rice root and rice-associated ARA. (i) Pot experiment. The initial MPN of indigenous azorhizobia in soil was 2,500 cells per g (dry weight) of soil (Table 1). In flooded fallow-rice soil (F/R-F/R), this MPN level remained constant until the end of the first crop and then declined to 20 cells per g (dry weight) of soil during the second crop. The population in the S−/R-S−/R treatment increased about 30-fold after *Sesbania* incorporation and remained stable up to harvest of the first rice crop. In the second crop, the population increased sharply to 500 times the initial level after *Sesbania* incorporation and then declined to the initial level at the time of rice harvest. Both MPN and antibiotic plate counts in the S++/R-S+/R treatment increased 5,000-fold after inoculation. MPN counts remained at this high level until harvest of the second rice crop, after which the population dropped to the initial level. Counts in both *Sesbania*-rice treatments (S−/R-S−/R and S++/R-S+/R) were significantly higher than in the F/R-F/R treatment. Only after *Sesbania* incorporation in the first crop cycle, however, were the counts in the S++/R-S+/R treatment significantly higher than in the S−/R; S−/R treatment. No antibiotic-resistant colony was found at dilutions of 10^{-2} and higher in the F/R-F/R and S−/R-S−/R treatments until the end of the experiment. The counts of the Str^r Spc^r strain in the S++/R-S+/R treatment declined from the beginning to the end of the experiment. The population of total heterotrophs was 10 to 100 times higher in both *Sesbania*-rice treatments than in the F/R treatment (data not shown).

The MPN of azorhizobia and total heterotroph counts in soil containing *S. rostrata* but without rice plants are shown in Fig. 1. The population of azorhizobia declined from about 10^7 cells per g (dry weight) of soil at 1 day after *Sesbania* incorporation to about 10^3 cells per g (dry weight) of soil at 140 days after *Sesbania* incorporation. The number of total heterotrophs remained almost unchanged up to 110 days after *Sesbania* incorporation and then declined about 10-fold at 140 days.

Table 2 shows the average bacterial counts of rice roots of two crops of rice (WS and DS). Since the counts did not significantly differ, averages were taken. Rice roots in both *Sesbania*-rice treatments (S−/R-S−/R and S++/R-S+/R) harbored a significantly higher MPN of azorhizobia than did those in the F/R-F/R treatment at both maximum tillering and heading stages of rice growth. The MPN of the antibiotic-resistant strain was higher in the S++/R-S+/R treatment. As in the soil, the heterotrophic counts in roots were 10 to 100 times higher in both *Sesbania*-rice treatments than in the flooded fallow-rice treatment (data not shown).

Rice rhizospheric ARA was measured at maximum tillering and heading in WS and DS (Table 3). Plants in the S−/R-S−/R and S++/R-S+/R treatments had significantly higher ARA than did those in the F/R-F/R treatment at both stages and seasons. In WS, however, ARA in the S++/

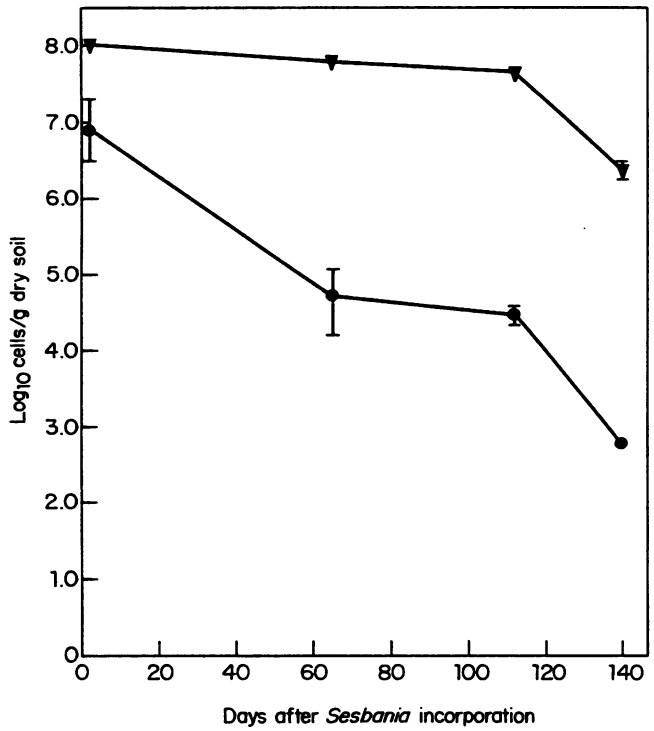

FIG. 1. MPN of azorhizobia (●) and total heterotrophs (▼) in soil with incorporated *S. rostrata* but not planted to rice (pot experiment, DS 1987). Bars indicate standard deviations.

R-S+/R treatment was significantly higher than in the S−/R-S−/R treatment.

(ii) Field experiment. The MPN of azorhizobia in association with roots of four consecutive rice crops as affected by flooded fallow or incorporation of in situ *Sesbania* sp. as a crop preceding the rice is shown in Table 4. The population ranged from about 1×10^3 to 2×10^4 cells per g (dry weight) of root in the flooded fallow-rice treatment and from 2×10^5 to 3×10^6 cells per g (dry weight) of root in the *Sesbania*-rice treatment. Numbers in the *Sesbania*-rice treatment were higher at heading than at maximum tillering.

The effects of fallow or incorporation of in situ *Sesbania* sp. or *Azolla* sp. preceding the rice crop on the population of total azorhizobia and percentage of azorhizobia in nonrhizospheric soil, rhizospheric soil, and the roots of rice were also studied. Counts of nonrhizospheric soil were made at 30, 45, 65, and 95 days after transplanting (Fig. 2), and counts of rhizospheric soil and rice roots were made at 45 (maximum tillering) and 65 (heading) days after transplanting (Table 5). Generally, (i) the counts of azorhizobia were several hundredfold higher in the *Sesbania*-rice than in the flooded fallow-rice or *Azolla*-rice treatment and (ii) the population declined from maximum tillering to heading in nonrhizospheric soil but remained almost constant in rhizospheric soil and rice root. At maximum tillering, the relative abundance of azorhizobia (percentage of total heterotrophs) in rhizospheric and nonrhizospheric soil and root in the *Sesbania*-rice treatment ranged from 2.5 to 3.7%; the value was less than 0.01 to 0.001% in flooded fallow-rice and *Azolla*-rice treatments. The percentage of rhizobia in the *Sesbania*-rice treatment at heading declined to less than 0.6 but was about 10 times higher in rhizospheric soil and roots than in nonrhizospheric soil.

The population of azorhizobia in nonrhizospheric soil declined from about 10^7 cells per g (dry weight) of soil after *Sesbania* incorporation to about 10^4/g (dry weight) of soil at rice harvest but still was much higher than in the flooded fallow-rice and *Azolla*-rice treatments (Fig. 2).

ARA of rice seedlings inoculated with free-living and symbiotic bacteria under gnotobiotic conditions. Seedlings of strain IR42 showed substantial ARA when inoculated with *Azospirillum lipoferum* 34H isolated from wetland rice root or with *A. caulinodans* ORS571 isolated from stem nodules of *S. rostrata* (Table 6). *A. caulinodans* produced activity similar to that of *Azospirillum lipoferum*. Seedlings with *Azospirillum brasilense* ATCC 29145 from *Digitaria* showed significantly higher ARA than did the uninoculated seed-

TABLE 2. Population of total and inoculated azorhizobia of *S. rostrata* associated with rice root at maximum tillering and heading of rice (averages of WS and DS crops)

Treatment	Population (log_{10} cells/g [dry wt] of rice root)			
	Maximum tillering		Heading	
	MPN	Antibiotic plate count	MPN	Antibiotic plate count
Flooded fallow-rice (F/R-F/R)	2.8	<2	3.7	<2
Sesbania (uninoculated)-rice (S−/R-S−/R)	4.9	<2	6.2	<2
Sesbania (inoculated)-rice (S++/R-S+/R)	5.9	6.0	6.5	5.3
Least significant difference at the 5% level	2.2		1.5	

TABLE 3. Rice plant-associated ARA as affected by incorporation of *S. rostrata* (WS 1986, DS 1987)

Treatment	ARA (nmol of C_2H_4/plant per h)			
	1st crop (WS)		2nd crop (DS)	
	Maximum tillering	Heading	Maximum tillering	Heading
Flooded fallow-rice (F/R-F/R)	37	35	65	123
Sesbania (uninoculated)-rice (S−/R-S−/R)	95	138	202	252
Sesbania (inoculated)-rice (S++/R-S+/R)	117	208	267	303
Least significant difference at the 5% level	15	31	96	100

TABLE 4. MPN of *A. caulinodans* associated with roots of rice from four consecutive crops as affected by fallow and *Sesbania* incorporation before rice

Growth state and treatment	MPN (log_{10} cells/g [dry wt] of root)			
	WS1986	DS 1987	WS 1987	DS 1988
Maximum tillering				
Flooded fallow-rice	3.0	4.4	4.2	4.3
Sesbania-rice	5.7[a]	6.5[a]	5.5[a]	5.9[a]
Heading				
Flooded fallow-rice	3.9	4.4	3.5	4.4
Sesbania-rice	6.7[a]	7.0[a]	6.1[a]	6.4[a]

[a] Significantly different from control value at 5% level.

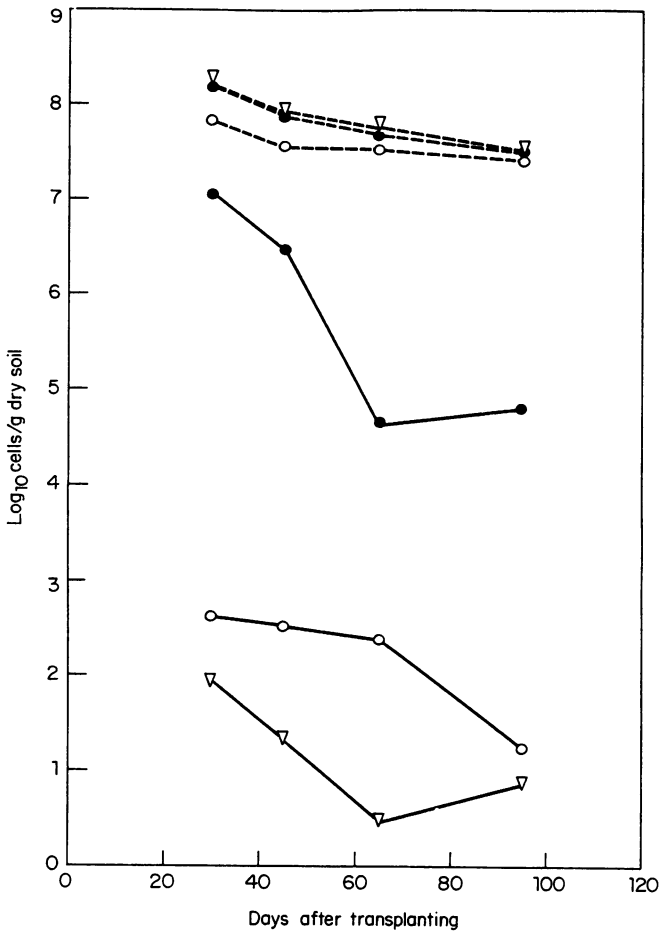

FIG. 2. MPN of azorhizobia (flooded fallow-rice [○——○], *Azolla*-rice [▽——▽], and *Sesbania*-rice [●——●]) and total heterotrophs (flooded fallow-rice [○- - -○], *Azolla*-rice [▽- - -▽], and *Sesbania*-rice [●- - -●]) in nonrhizospheric soil. MPN of azorhizobia at 45 and 65 days after transplanting are from Table 5. (Field experiment, DS 1988.)

lings, but the value was much lower than with *Azospirillum lipoferum* 34H and *A. caulinodans*. Rhizobium isolates from *S. aculeata*, *S. sesban*, *Trifolium* sp., or *Vigna unguiculata* failed to show appreciable ARA with rice seedlings.

DISCUSSION

The incorporation of *S. rostrata* increased several hundred- to several thousand-fold the soil population of azorhizobia because of the release of azorhizobia from degrading stem and root nodules. The magnitude of the increase may have depended on the level of nodulation. Incorporation of inoculated *S. rostrata* released about 50 times more rhizobia than did incorporation of noninoculated *S. rostrata* nodulated by indigenous azorhizobia and released about 3,000 times more than did the flooded fallow treatment. The difference between noninoculated and inoculated treatments was reduced at the end of the second rice crop (Table 1). Repeated incorporation seemed to increase the population of azorhizobia further (Fig. 2).

In general, the soil population of azorhizobia declined over time after the incorporation of *S. rostrata* regardless of whether rice plants were present. Similar declining trends were observed in soil in which rice was followed by either flooded fallow or *Azolla* incorporation. However, the population at rice harvest was still much higher in the *Sesbania*-rice treatment than in the flooded fallow-rice and *Azolla*-rice treatments (Fig. 2). Maintenance of a higher population of azorhizobia in the *Sesbania*-rice treatment suggests saprophytic growth, for which there is evidence in other rhizobia (5, 19, 23, 27).

Whether the increase in azorhizobial population in soil results mainly from increased organic matter content due to the incorporation of *S. rostrata* or from the release of a large number of azorhizobia from degrading nodules is not known. Organic matter amendments often increase the number of rhizobia (14, 20), possibly as a result of the addition of some organic or inorganic nutrients or modification of soil pH. A control treatment with *Azolla* incorporation was included in this study to compare its effects with those of *Sesbania* incorporation. That *Azolla* incorporation did not stimulate

TABLE 5. MPN of *A. caulinodans* and total heterotroph plate counts in nonrhizospheric and rhizospheric soils and rice root as affected by different pre-rice treatments (DS 1988)

Treatment	Maximum tillering		Heading	
	MPN (log_{10} cells/g [dry wt])	% Azorhizobia of total heterotrophs	MPN (log_{10} cells/g [dry wt])	% Azorhizobia of total heterotrophs
Nonrhizospheric soil				
Flooded fallow-rice	2.52	<0.001	2.39	<0.001
Azolla-rice	1.33	<0.001	0.47	0.001
Sesbania-rice	6.47	3.7	4.6	<0.09
Rhizospheric soil				
Flooded fallow-rice	2.6	<0.001	3.22	<0.001
Azolla-rice	1.25	<0.001	1.76	<0.001
Sesbania-rice	6.31	3.4	5.6	0.7
Root				
Flooded fallow-rice	4.3	<0.01	4.39	<0.002
Azolla-rice	3.08	<0.001	3.59	<0.001
Sesbania-rice	6.98	2.5	6.3	0.6

TABLE 6. ARA of IR42 seedlings inoculated with free-living and symbiotic bacteria under gnotobiotic conditions

Sample no.	Treatment	C_2H_4 (nmol/tube per 24 h)	Source (host)
1	Uninoculated	3	
2	*Azospirillum lipoferum* 34H	365	Wetland rice root
3	*Azospirillum brasilense* (Sp. 7)	51	*Digitaria*
4	*Azorhizobium caulinodans* ORS571	368	*Sesbania rostrata*
5	*Rhizobium-Sesbania aculeata*	3	*Sesbania aculeata*
6	*Rhizobium-Sesbania sesban*	3	*Sesbania sesban*
7	*Rhizobium* sp. strain TAL 382-*Trifolium* sp.	3	*Trifolium* sp.
8	*Rhizobium*-mung bean	20	*Vigna unguiculata*
SEM		76	

the population of azorhizobia probably rules out the possibility that pH or organic matter alone caused the increase in the rhizobial population. The possibilities that *S. rostrata* incorporation provides soil with an inoculum of azorhizobia and also provides a specific factor(s) for growth and survival of *Azorhizobium* spp. are interesting and need further investigation.

The sharp decline in population of the inoculated strain ORS571 (Str^r Spc^r) to an undetectable level at the end of two crop cycles (Table 1) suggests that this strain may be less competitive than the indigenous strain under continuously flooded conditions even though it is more efficient in nodulation and N_2 fixation (Ladha et al., in press). The potential of rhizobia to survive flooding was ascribed to the ability to produce a dissimilatory nitrate reductase (9). Lack of the dissimilatory nitrate reductase in *A. caulinodans* ORS571 (8; Ladha et al., unpublished data) may partly explain the poor survival of this organism under flooded conditions. However, an increase in the population of azorhizobia in the rice rhizosphere or histosphere can be explained by the ability of rice plants to supply oxygen to root-associated microorganisms through the well-developed aerenchyma, thus overcoming the anaerobic condition caused by flooding (29).

Rhizobia can multiply in the rhizosphere of both host and nonhost plants (4, 24, 25). The degree of stimulation depends on plant variety, soil type, and *Rhizobium* species or strain (14). However, the degree of specificity of the stimulation is not clear (14). The ex planta ability of *A. caulinodans* to grow and fix N_2 with no supplemental N may be an additional advantage. The *S. rostrata-A. caulinodans* symbiosis as green manure for flooded rice is advantageous because it adds to the soil not only organic C and N but also a large population of azorhizobia, which multiply in the rice rhizosphere. The exact significance of and role played by *A. caulinodans* in the rice rhizosphere are not known. An active diazotrophic association between rice and *A. caulinodans* may be found. This possibility seems to be supported by the stimulation of nitrogenase activity of the rhizosphere of rice grown in *Sesbania*-incorporated soil and of sterile rice seedlings inoculated with *A. caulinodans*. Rhizobia from other hosts, such as *S. aculeata*, *S. sesban*, *Trifolium* sp., and *V. unguiculata*, failed to exhibit significant nitrogenase activity when inoculated in rice.

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