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HOST-PLANT SPECIFICITY IN THE INFECTION OF CEREALS WITH *AZOSPIRILLUM* SPP

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Summary—The specificity of the infection of maize, wheat and rice roots by N_2-fixing *Azospirillum* spp was studied in four greenhouse experiments using pots with unsterilized soil and in two field experiments. In all experiments *A. lipoferum* was most frequently isolated from externally sterilized roots of maize, and *A. brasilense* nir$^-$ (nitrite reductase negative) from wheat and rice. In pot experiments, *A. brasilense* nir$^+$ was isolated with moderate frequency from within maize roots but rarely from within wheat or rice roots. Inoculation of the pots with a mixture of representative strains of the three *Azospirillum* groups had no effect on the proportion of strains recovered from each plant species. In the field experiments, inoculation with spontaneous streptomycin-resistant mutants of two of the representative strains confirmed the apparent specificity of *A. lipoferum* for maize roots and of *A. brasilense* for wheat but the results were partially obscured by the unexpectedly high proportion of streptomycin-resistant strains isolated from within the roots of uninoculated plants.

INTRODUCTION

Host-plant specificity in bacterial plant diseases and in the legume symbiosis is the result of close interactions between plant and bacteria. Specificity in the association of *Azotobacter paspali* with one ecotype of *Paspalum notatum* was shown many years ago (Döbereiner, 1966). This bacterium has not been found anywhere else except in association with this grass and wherever the grass grows the organism was found (Döbereiner, 1970). Host requirements of *A. paspali* are, therefore, more specific than those of most *Rhizobium* strains. Nitrogen fixation (C_2H_2) (Döbereiner *et al.*, 1972) and $^{15}N_2$ incorporation (De-Polli *et al.*, 1977) have been shown but the aspect of specificity in this plant has not been explored further. Specific stimulation of a N_2-fixing *Bacillus* sp. in the rhizosphere of certain wheat lines was reported by Neal and Larson (1976). In the many reports on the isolation of other N_2-fixing bacteria from various grasses and cereals (Balandreau, 1975; Barber and Evans, 1976; Nelson *et al.*, 1976; Barber *et al.*, 1978) no specificity and in most cases not even plant-bacterial interactions have been shown. For the association of *Azospirillum* spp with *Digitaria* sp. and maize, highly significant correlations of root-piece nitrogenase activity with *Azospirillum* enrichment-culture activity were shown (Döbereiner and Day, 1976; Bülow and Dobereiner, 1975) which strongly suggests this organism is the major one responsible for N_2-fixation in these grasses. Furthermore, *Azospirillum* spp generally occur in the rhizosphere soil and roots of maize, sorghum, wheat, rice and many other forage grasses grown in tropical and subtropical regions (Döbereiner *et al.*, 1976; Nayak and Rajaramamohan Rao, 1977). These bacteria also occur in cereal roots in temperate soils (Reynders and Vlassak, 1976; Pedersen *et al.*, 1978).

Observations of infection of *Digitaria* (Döbereiner and Day, 1976) and maize roots by *Azospirillum* sp. (Bülow and Döbereiner, 1975; Okon *et al.*, 1977) indicate the need for a better understanding of host bacteria interactions. Confirmation of the infections of inner cortex and stele tissues (Patriquin and Döbereiner, 1978) which may extend into the stem of various cereals (Magalhaes *et al.*, 1979; Kavimandan *et al.*, 1978) motivated a systematic search for host-plant specificities.

We present experimental evidence of host-plant specificity in the association of maize, wheat and rice with *Azospirillum* spp and confirm the infection of roots with this N_2-fixing bacterium under field conditions.

MATERIALS AND METHODS

Four pot experiments were carried out in the greenhouse during January–April 1978, one with maize, one with wheat, one with flooded and one with non-flooded rice. Each experiment contained 6 treatments which were with and without inoculation and three harvests (heading, grain filling and ripening) and four replicates. The 24 pots of each experiment were distributed in randomized complete blocks. The same red-yellow podzolic soil was mixed and distributed uniformly into all the pots of the four experiments. Fertilization consisted of 30 μg N g^{-1} as NH_4NO_3, 40 μg P g^{-1} as triple super-phosphate and trace elements (1 ml kg^{-1} soil of the following solution: 150 g $MgSO_4 \cdot 7H_2O$; 15.8 g $CuSO_4 \cdot 5H_2O$; 8.9 g $ZnSO_4 \cdot 7H_2O$; 0.5 g H_3BO_3; 0.5 g $Na_2MoO_4 \cdot 2H_2O$; 20 g $FeSO_4 \cdot 7H_2O$; 20 g citric acid; 1000 ml H_2O).

Two field experiments were planted in June 1978 one with maize and one with wheat to confirm the results with the pot experiments and to study establishment of marked strains in the field. The experiments consisted of 12 treatments and four replicates and the plots were distributed in randomized complete blocks. Treatments were three inoculants: con-

trol, *A. lipoferum* isolated from maize, *A. brasilense* nir$^-$ (nitrite reductase negative) isolated from wheat and four harvests (heading, full flowering, grain filling and ripening). Basic fertilization consisted of 30 kg N ha^{-1} as $(NH_4)_2SO_4$; 30 kg P ha^{-1} as superphosphate; 100 kg K ha^{-1} as KCl; 40 kg F.T.E. ha^{-1} as fritted trace elements, formula Br 12; 500 kg ha^{-1} of ground limestone. Plots were 6 × 6 m for maize and 1.2 × 2 m for wheat. There were 6 rows per plot, three of which were uninoculated guard rows. The plants were harvested from the central row of each plot.

Culture media

The media used in this study were: N-free semisolid malate medium (NFb): 5 g malic acid; 0.5 g K_2HPO_4; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.1 g NaCl; 20 mg $CaCl_2$; 2 ml trace element solution; 2 ml alcoholic solution of Bromothymol Blue (5%); 4 ml FeEDTA; 1 ml vitamin solution; 4 g KOH; 1.75 g agar; 1000 ml H_2O; NaOH to adjust pH to 6.8. The trace element solution was: 200 mg $Na_2MoO_4 \cdot 2H_2O$; 235 mg $MnSO_4 \cdot H_2O$; 280 mg H_3BO_3; 8 mg $CuSO_4 \cdot 5H_2O$; 24 mg $ZnSO_4 \cdot 7H_2O$; 200 ml H_2O. The vitamin solution was: 10 mg biotin; 20 mg pyridoxin; 100 ml H_2O.

Potato Infusion agar for non-selective growth and purity checks (BMS): potatoes 200 g; malic acid 2.5 g; KOH 2.0 g; sucrose 2.5 g; vitamin solution (as above) 1 ml. Washed potatoes were boiled for 30 min and the solution was then filtered through cotton. Malic acid (2.5 g) was dissolved in 50 ml H_2O, adding two drops of Bromothymol Blue (0.5% soln in ethanol), and adjusted with KOH until green (pH 7.0). This solution, sucrose, agar and vitamins were added to the potato filtrate and made to 1000 ml.

Cultures and inoculants

Cultures are identified as *Azospirillum lipoferum*, and *A. brasilense* (Tarrand *et al.*, 1978) rather than as *Spirillum lipoferum*. In the greenhouse experiments, all pots of the inoculated treatments, independent of host plant, were inoculated with the same mixture of three separately-grown strains (2 ml pot^{-1}; 2 × 10^9 cells ml^{-1}). The growth medium was aerated liquid NFb supplemented with 10 mM NH_4Cl and the strains were the following: *A. lipoferum* strain Sp Br 17 isolated from non-sterilized maize roots at the Centro de Pesquisa do Cerrado, EMBRAPA, Brasilia; *A. brasilense* nir$^-$ (nitrite reductase negative) strain Sp Br 14 isolated from non-sterilized wheat roots also collected at CPAC, Brasilia and *A. brasilense* nir$^+$ strain Sp Ph 1 isolated from non-sterilized rice roots collected at IRRI, Los Banos, Philippines. The three strains were selected because they had been obtained from fields where there was for at least 3 yr continuous cultivation of maize, wheat or rice respectively. The soil, before inoculation contained 3 × 10^7 cells of *Azospirillum* g^{-1} soil (MPN).

In the two field experiments, three inoculation treatments were used: control (no inoculation), inoculation with a streptomycin-resistant maize strain and inoculation with a streptomycin-resistant wheat strain. The maize strain (Sp 108 st^r) was one of the *A. lipoferum* strains isolated from maize roots (following 60 min immersion in 1% Chloramine T) from control pots of the previous experiment and the wheat strain (Sp 107 st^r) was one of the *A. brasilense* nir$^-$ strains isolated from wheat roots (15 min immersion in 1% Chloramine T) of the corresponding control pots. Spontaneous streptomycin-resistant mutants of these two strains and several others were obtained by plating them on potato agar containing 20 μg streptomycin ml^{-1}. The frequency of spontaneous mutants in these cultures was about 10^{-6}. The mutants showed the same growth and nitrogenase activity in semisolid NFb medium as the parent strains. The relatively low concentration of streptomycin (20 μg ml^{-1}) was selected after preliminary tests showed that 5 μg ml^{-1} was sufficiently strong to inhibit the growth of *Azospirillum* spp. Also the inhibition zone of 6 parent strains with 10 μg streptomycin Sensi-Discs was 11–15 mm while all mutants or strains isolated on medium containing 20 μg ml^{-1} streptomycin (14 were tested) showed no inhibition zone at all. Inoculation immediately after sowing, consisted of watering with 150 or 1125 ml m^{-2} for maize (5 seeds) or wheat (50–70 seeds) respectively, using liquid cultures grown as for the pot experiments and containing 1.4 × 10^{10} cells ml^{-1}. The number of *Azospirillum* spp in the soil before planting was 3.5 × 10^7 g^{-1} soil, and within this population the frequency of streptomycin-resistant mutants was about 10^{-6}.

Isolation and identification of strains

A uniform procedure to isolate and identify strains was used for pot and field experiments. Freshly-harvested roots were washed in sterile water and as appropriate sterilized intact (with the cut ends above the surface of the sterilant), for varying periods by immersion, in 1% Chloramine T. The roots were then washed in sterile water, and in 25 mM phosphate buffer, followed by 3 more washings. The entire washing procedure always took as long as the period of exposure to Chloramine T. The roots were then cut into 5–8 mm pieces which were macerated with forceps and introduced into semisolid NFb medium (4 ml in 6 ml serum bottles). N_2ase activity was checked, after incubation for 40 h at 32°C, by closing the bottles (without disturbing the pellicle) with rubber seals and injecting 15% C_2H_2. Two such cultures were prepared for each pot and sterilization treatment. Most cultures reduced C_2H_2 and N_2ase-negative cultures were discarded. Further enrichment was obtained in new 24 h cultures in NFb medium, which were then streaked out on NFb agar plates containing 20 mg yeast extract l^{-1}. After 1 week, typical small, white dense, single colonies were picked and transferred into semi-solid FNb medium. Pellicle formation in this medium indicated the success of isolation. For final purification these cultures were streaked out on potato agar (BMS) and the typical pink, often wrinkled colonies transferred for storage and identification tests. For this, one loop of semisolid NFb culture was inoculated into one vial with NFb, one with NFb in which malate and indicator were replaced by 0.5% glucose (added after sterilization) and one with NFb supplemented with 5 mM NH_4NO_3, and incubated at 32°C. Table 1 shows the characteristics used for identification. Attempts were made to obtain two strains from each sterilization treatment from each pot or plot in the field but this was not always possible, therefore, in the

Table 1. Characteristics used for identification of *Azospirillum* spp and subspecies from pot and field experiments

	A. lipoferum	*A. brasilense* nir$^+$	*A. brasilense* nir$^-$
Growth in semisolid NFb	good	good	good
Growth in semisolid NFb without malate or indicator with 0.5% glucose*	good	poor	poor
Growth in semisolid NFb with 5 mM NH_4NO_3†	good, usually gas after mixing	good, heavy gas minutes after mixing	good, no gas
Cell form in alkaline medium‡	large polymorph	very motile normal form	very motile normal form
Semisolid NFb with 20 μg ml^{-1} streptomycin	normal growth when streptomycin resistant only		

* The glucose test is best observed 2 or 3 days after inoculation. Then all *A. lipoferum* strains show a heavy pellicle on top of the medium and *A. brasilense* shows no pellicle. After 24 hr, observations can be misleading because *A. brasilense* strains may produce a fine pellicle below the surface which later disintegrates.

† The test for dissimilation of NO_3^- must be performed when the pellicle is at the surface and the lower medium is still green. Then the bottles are carefully mixed and incubated again. *A. brasilense* nir$^+$ strains produce large amounts of gas within a few minutes after mixing, while nir$^-$ strains produce no gas. *A. lipoferum* strains are variable and can produce as much gas as *A. brasilense* nir$^+$ but usually only produce gas after several hours; some strains produce no gas from NO_3^-.

‡ Most conveniently cells from the NH_4NO_3 vials (1 day after the bubble test) are used to make wet mounts and phase-contrast microscope observations.

tables the number of isolates from which the percentage was calculated is stated. To identify the inoculated streptomycin-resistant strains from the field experiments, one additional test vial was used with NFb (with NH_4NO_3) containing 20 μg ml^{-1} streptomycin in addition to the vials stated above. The same purified single colony isolates were used for identification of streptomycin-resistance as for identification of species and sub-species and growth was evaluated in parallel tests. Therefore a selection for resistant mutants in the streptomycin vials is unlikely.

RESULTS

The series of pot experiments sought information about the infection of the three major cereals, maize, wheat and rice with *Azospirillum* spp. For this the different cereals were planted in the same soil and inoculated with the same mixture of three strains. These strains had been selected in order to include one which might preferentially infect one of the cereals. Tables 2 and 3 show that there was little discernible effect of inoculation in the pot experiments, since uninoculated soil contained all three types of *Azospirillum* spp. However, there is strong evidence of specificity between host plants and *Azospirillum* type. In the pots planted with maize *A. lipoferum* was the predominant form in rhizosphere soil, and in unsterilized and sterilized roots. In maize roots (sterilized by immersion in 1% Chloramine T for 1 h), some strains were *A. brasilense* nir$^+$ but none nir$^-$. With wheat and rice, specificity seemed to be still more marked. All three types of *Azospirillum* were present in rhizosphere soil but most isolates from within surface-sterilized roots were identified as *A. brasilense* nir$^-$ and none was *A. lipoferum*. The possibility of introducing a bias either by differences in tolerance to Chloramine T or by selective multiplication of a specific group must be discarded. All strains are equally killed instantaneously upon exposure to a 1% Chloramine T solution and survival in roots after increasing times of exposure is due to progressive penetration of disinfectant into root tissue (Patriquin and Döbereiner, 1978). Even if diffusion gradients of the Chloramine T developed within the roots and the three groups varied in their tolerance to Chloramine T, this could not explain the higher recovery of *A. lipoferum* from maize and *A. brasilense* nir$^-$ from wheat and rice. Also, as shown in Tables 2, 3, 4 and 5 the specificity was also apparent in non-sterilized roots as well as in the rhizosphere soil of maize. Selective multiplication of one group during the isolation procedures is also very unlikely because exactly the same methods were used for all plants and, therefore, there is no reason to assume that in maize we selected for one group and in wheat and rice for the other.

The field experiments were planned to confirm with two of the cereals the host-plant specificity of the three groups of strains under field conditions, and furthermore to make an attempt to establish selected strains within the roots of field-grown plants. The results in Table 4 confirm the observations made in the pot experiments except that *A. brasilense* nir$^+$ seemed to be less-frequent in the soil and therefore did not represent a large proportion of the isolates from soil or roots of either plant species. As in the pot experiments, soil from the maize rhizosphere contained a higher proportion of *A. lipoferum* than that from the wheat rhizosphere and the infection of inner

Table 2. Distribution of *Azospirillum* spp groups among isolates from inoculated pots in glasshouse experiments*

Treatment	Sterilization in Chloramine-T (min)	No. of Isolates	% of isolates identified as *A. lipoferum*	*A. brasilense* nir$^+$	*A. brasilense* nir$^-$
Maize					
Soil	0	23	79	21	0
Roots	0	21	75	14	11
Roots	0.5	21	92	8	0
Roots	60	22	54	46	0
Wheat					
Soil	0	24	14	29	57
Roots	0	22	37	4	59
Roots	0.25	21	0	10	83
Roots	15	20	0	8	92
Flooded rice					
Soil	0	23	46	29	25
Roots	0	24	12	42	42
Roots	0.25	19	0	25	75
Roots	15	20	0	0	100
Non-flooded rice					
Soil	0	24	33	17	50
Roots	0	23	12	46	42
Roots	0.25	18	4	42	54
Roots	15	22	4	25	71

* For each cereal, 12 pots with red yellow-podzolic soil were used, all inoculated with the same mixture of three *Azospirillum* strains.

root tissues was limited almost exclusively to *A. lipoferum* in maize and to *A. brasilense* nir$^-$ strains in wheat.

The interpretation of the effects of inoculation with streptomycin-resistant (sr^r) strains in the field was obscured by the high porportion of streptomycin-resistant cultures isolated from surface-sterilized roots from uninoculated treatments (Table 5). Nevertheless, there were clear increases in the proportions of inoculant types recovered from rhizosphere soil and from unsterilized roots. There were also discernible increases in the proportions of isolates of *A. lipoferum* str^r recovered from surface-sterilized roots of maize plants inoculated with this strain and of *A. brasilense* nir$^-$ str^r from surface-sterilized roots of wheat inoculated with that strain. Furthermore maize plants inoculated with *A. brasilense* nir$^-$ str^r (the wheat strain) showed increasing proportions of *A. lipoferum* str^r in

Table 3. Distribution of *Azospirillum* spp groups among isolates from uninoculated pots in greenhouse experiments*

Treatment	Sterilisation in Chloramine-T (min)	No. of isolates	% of isolates identified as *A. lipoferum*	*A. brasilense* nir$^+$	*A. brasilense* nir$^-$
Maize					
Soil	0	19	47	19	34
Roots	0	21	70	30	0
Roots	0.5	22	83	8	8
Roots	60	20	58	42	0
Wheat					
Soil	0	21	33	39	28
Roots	0	18	21	33	46
Roots	0.25	23	15	31	54
Roots	15	22	0	0	100
Flooded rice					
Soil	0	24	33	37	29
Roots	0	21	46	25	29
Roots	0.25	20	33	25	42
Roots	15	23	0	4	96
Non-flooded rice					
Soil	0	21	63	8	29
Roots	0	24	33	0	67
Roots	0.25	18	62	0	38
Roots	15	22	7	13	80

* For each cereal, 12 pots with red-yellow podzolic soil were used uninoculated.

Table 4. Distribution of *Azospirillum* spp groups among isolates from uninoculated maize or wheat grown in the field*

Treatment	Sterilized in Chloramine T (min)	No. of isolates	% of isolates identified as *A. lipoferum*	*A. brasilense* nir$^+$	*A. brasilense* nir$^-$
Maize					
Soil	0	32	84	12	3
Roots	0	32	59	9	31
Roots	0.5	30	78	19	3
Roots	60	29	96	0	4
Wheat					
Soil	0	32	57	0	43
Roots	0	31	21	19	60
Roots	0.25	32	37	6	57
Roots	15	31	0	12	88

* Results are from the uninoculated control plots (4 replicates) of the two field experiments. Plants were harvested 45, 60, 75, 95 days after planting and the root systems of two plants per plot were pooled for isolation and identification.

surface-sterilized roots although the inoculated strain predominated in the rhizosphere soil. A comparable situation was also observed when wheat was inoculated with *A. lipoferum* str^r (the maize strain). All these observations support the host-plant specificity aspect but in order to confirm establishment of inoculated strains within roots double-marked strains or resistance to higher streptomycin concentrations will have to be used.

The frequency of streptomycin-resistant strains of *Azospirillum* spp in the soil, before the experiment was planted, was 10^{-6}. From Table 5 it can be seen that although few resistant strains were isolated from rhizosphere soil, the mean frequency was $4.4 \pm 4\%$ which indicates a marked preferential increase of naturally-occurring streptomycin-resistant strains in the rhizosphere. The proportion increased considerably within the roots.

Table 5. Establishment of inoculated *Azospirillum* spp in soil and roots of maize and wheat grown in the field and occurrence of spontaneous streptomycin-resistant forms*

	Maize				Wheat			
	Soil	Roots	Roots	Roots	Soil	Roots	Roots	Roots
Sterilization in Chloramine-T (min)	0	0	0.5	60	0	0	0.25	15
Uninoculated control								
No. of isolates	32	32	30	29	32	31	32	31
			(% Streptomycin resistant isolates)					
A. lipoferum	6	3	12	81	9	3	3	9
A. brasilense nir$^-$	0	0	0	3	6	0	12	81
A. brasilense nir$^+$	3	0	0	0	3	0	0	0
Total	9	3	12	84	18	3	15	90
Inoculated with *A. lipoferum* str$^+$†								
No. of isolates	32	30	28	30	32	30	29	32
			(% Streptomycin resistant isolates)					
A. lipoferum	91	59	78	90	70	57	3	9
A. brasilense nir$^-$	0	0	3	4	12	39	97	88
A. brasilense nir$^+$	0	3	0	0	0	0	0	0
Total	91	62	81	95	82	96	100	97
Inoculated with *A. brasilense* nir$^-$ str$^+$†								
No. of isolates	31	28	32	28	32	29	31	31
			(% Streptomycin resistant isolates)					
A. lipoferum	8	3	35	84	12	16	3	0
A. brasilense nir$^-$	50	22	8	6	86	78	97	94
A. brasilense nir$^+$	4	0	0	0	0	3	0	0
Total	62	25	43	90	98	97	100	94

* The experiment included 3 inoculant treatments, 4 harvests and 4 replicate plots.

† Soil inoculated at sowing with 10^{11} cells per seed of *A. lipoferum* or *A. brasilense* nir$^-$ isolated from surface-sterilized roots of maize or wheat respectively and marked with streptomycin resistance.

DISCUSSION

The occurrence of host-plant specificity in *Azospirillum*-plant associations strongly supports close interactions between the host plant and these bacteria. It supports the observations of infection of living inner-root tissues (Patriquin and Döbereiner, 1978; Magalhaes *et al.*, 1979) which could be expected to be selective for specific strains. The reported differences in the distribution of the various strains in soil and surface-sterilized roots would be difficult to explain as a casual, superficial association. Whether the infections of maize with *A. lipoferum* and that of wheat and rice with *A. brasilense* nir$^-$ indicate basic differences between C_4 and C_3 plants cannot be said, but possibly the form of carbon substrate which is furnished by the plant plays a role. Ruscoe *et al.* (1978) showed that *Spirillum lipoferum* strains Sp 81 and Sp 82 (*A. brasilense* nir$^-$ strains) would grow and fix N_2 with trans-aconitate as carbon source, whilst strain Sp 7 (*A. brasilense* nir$^+$) would not. They observed that the organisms were located on the root surface or outer tissues. *A. lipoferum* was not tested. The question is whether the major contribution in the various cereals and grasses comes from N_2-fixing bacteria localized in the rhizosphere, on the root surface or outer root zones or from the organisms in the inner root tissues. Numbers within the inner root tissue are two orders of magnitude smaller during vegetative growth stages but become much closer to those on non-sterilized roots during the reproductive growth phase (Magalhaes *et al.*, 1979; Scott *et al.*, 1978), the period in the life cycle of various cereals that highest nitrogenase activities occur (Bülow and Döbereiner, 1975). Also many more infections of inner root tissues were observed during this period in maize (Magalhaes *et al.*, 1979). These findings support the possibility of a major contribution of the N_2-fixing bacteria localised within inner root tissues.

The possibility of establishing selected N_2-fixing bacteria in the rhizosphere and roots of normally field-grown plants makes possible a large field of research with all kinds of strains and mutants and investigations of their respective roles in the plant root. It also provides more justification for inoculation trials where strains superior to those occurring in the field could be selected under field or greenhouse conditions with normally-growing plants. All these aspects, to date could only be studied with monoxenic growth-chamber plants.

The reclassification of the *Spirillum lipoferum* group into a new genus *Azospirillum* with two species, *A. lipoferum* and *A. brasilense* (Tarrand *et al.*, 1978) based on DNA homology does not confirm earlier observations that two subgroups exist within *A. brasilense* (Neyra *et al.*, 1977). The difference in the dissimilatory nitrite reductase could be interpreted as a point mutation similar to these obtained by selection with chlorate (Magalhaes *et al.*, 1978) and therefore may not be detected by comparing DNA-homologies. The marked difference in host-plant specificity between *A. brasilense* nir$^+$ and nir$^-$ strains reopens the question of three groups because it indicates additional differences, possibly related to surface characteristics. Preliminary cross-reaction tests with fluorescent antibody confirm such differences (De-Polli, Bohlool and Döbereiner, unpublished results).

The observation that streptomycin-resistant strains seem to be selected in the rhizosphere and play a major part in root infection is most interesting. Resistance to this antibiotic was chosen simply as a marker and it cannot be said whether strains resistant to other antibiotics would be selected in the same way. The high proportion of streptomycin-resistant strains in the controls could not have been caused by cross-contamination between plots. The layout of the experiments with three uninoculated rows in between plots makes this unlikely. But even if it did not prevent cross-contamination, the frequencies of about 80% of resistant strains in surface-sterilized roots (Table 5) in the controls and even higher frequencies of homologous resistant strains in plots inoculated with heterologous strains would be impossible to explain by cross-contamination unless there were selection for such strains. Counts of total numbers of bacteria in potato agar with and without 20 $\mu g\ ml^{-1}$ streptomycin showed increases of the proportion of resistant forms from less than 0.1% in the soil to about 1% in the maize rhizosphere and to more than 50% in surface-sterilized roots (Döbereiner and Baldani, 1979). Brown (1961) also reported that populations of bacteria in the rhizosphere of some legumes contained unexpectedly large proportions of streptomycin-resistant types. Selective stimulation of actinomycetes in the rhizosphere and root surface has been shown (Krasil'nikov, 1958; Katznelson, 1965). Maize, rice and wheat are recommended as rotation crops for tomatoes and potatoes because they reduce the incidence of bacterial diseases (Robbs, 1960). The assimilation and accumulation of antibiotics, among them streptomycin, in plants has been reported (Krasil'nikov, 1958; Pramer, 1955). It seems, therefore, possible that cereal roots may contain concentrations of antibiotics which require a certain level of resistance among bacteria infecting them to permit multiplication within root tissues.

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