Biol Fertil Soils (1991) 10:276–280

Biology and Fertility of Soils

Changes in bacterial populations along roots of wheat (*Triticum aestivum* L.) seedlings

E. Liljeroth[1]*, S.L.G.E. Burgers[2], and J.A. Van Veen[1]

[1] Institute for Soil Fertility Research, P.O. Box 48, 6700 AA Wageningen, The Netherlands
[2] Agricultural Mathematics Group, P.O. Box 100, 6700 AA Wageningen, The Netherlands

Received June 11, 1990

Summary. In this study the bacterial populations on root tips (1–2 days old) of wheat (*Triticum aestivum* L.) were compared with the populations on root segments about 1 week older (root base). The isolates were characterized with a set of physiological tests and the test results were used to group the bacteria by means of cluster analysis. Some clusters contained bacteria that occurred mainly on the root tips and were characterized by the ability to produce acid from different sugars and by the presence of the enzymes nitrate reductase, lipase, and oxidase; they were sensitive to high salt concentrations in the media. Another cluster included significantly more isolates from the root-base segments; these bacteria were characterized by a negative reaction to most of the physiological tests; the colonies formed by these bacteria had yellow pigmentation. Possible mechanisms for the changes in the bacterial populations are discussed.

Key words: Cluster analysis – Physiological tests – Rhizoplane bacteria – Root age – *Triticum aestivum* L. – Wheat

An exponential growth of bacteria can be expected to occur along a growing root from the root tip towards the root base in response to the release of organic substrates from the root (Newman and Watson 1977). In studying wheat roots, Van Vuurde and Schippers (1980) found an exponential increase in bacterial numbers on the rhizoplane, from 2-day-old root segments to 5-day-old root segments. Thereafter, the numbers levelled off, followed by a second increase around day 8. The bacteria on the rhizoplane generally increased in number with increasing age of the root segment; bacterial numbers were about 15 times higher on 8-day-old segments than on 1-day-old segments. Differences in the release of organic substrate between root segments of different age, presumably due to cortical cell lysis, were proposed to explain the particular dynamics of the bacterial populations (Van Vuurde and Schippers 1980). An exponential increase in the numbers of bacteria along roots of rape was reported by Olsson et al. (1987).

The populations of bacteria on the root tip may consist of strains met by the root during its growth through the soil, which then adhere to the root surface or mucigel. Any proliferation of cells on the root tip is limited because the tip moves relatively fast and is only 1–2 days old. After the initial colonization of the tip the same bacteria might be able to grow on the elongating roots, but other populations of slower growing bacteria that are not able to colonize the rapidly passing tip might be able to proliferate along the roots, also. Thus, it is likely that changes in the composition of bacterial populations occur along a growing root. However, very little evidence has been presented to support this assumption.

More information on which bacteria can best proliferate on the root surface is required so that beneficial strains of bacteria can be successfully applied to the rhizosphere (Scher et al. 1984). In the present study we compared the bacterial populations on root tip (1–2 days old) and root base (7–8 days old) segments of wheat seedlings from two different wheat lines. These lines, C-R_5B and C-R_5D, had been reported to differ in the type of microbial population growing in the rhizosphere (Neal et al. 1973). The lines had also been shown to differ in the rate of root cortical cell death (Deacon and Lewis 1982) and were therefore expected to stimulate bacterial growth in different ways at different stages of root development.

Materials and methods

Soil and plant growth

A loamy sand (Ede, Netherlands; organic C, 2%; total N, 0.13%; pH(KCl) 6.2) was used in the experiment. The soil has been described in more detail by Van Elsas et al. (1986). The soil moisture content was

* Present address: The Swedish Unversity of Agricultural Sciences, Department of Crop Genetics and Breeding, S-26800 Svalöv, Sweden

Offprint requests to: E. Liljeroth

kept at 12% (−63 kPa). After thorough mixing, the soil was placed in 500-mm long plastic tubes with a diameter of 60 mm, to a bulk density of 1.2 g cm^{-2} (wet weight).

Seeds of two spring wheat varieties, *Triticum aestivum* L. C-R_5B and C-R_5D (Neal et al. 1973) were obtained from Dr. R.M.N. Kucey, Agriculture Canada Research Station, Lethbridge, Alberta. The seeds were surface-sterilized with sodium hypochlorite (Miller et al. 1989) and allowed to germinate on soft 1/10 strength tryptic soy agar (Oxoid). After 3 days sterile seedlings were selected, the criterion being that no viable microbial colonization of the agar was detected during germination, and planted in the tubes. The tubes were placed in a growth chamber with a day temperature of 20 °C and a night temperature of 15 °C. The day was 16 h long and the light intensity was about 500 μE m^{-2} s^{-1}. For each variety three replicate tubes were incubated in the growth chamber for 11 days.

Analysis

After 11 days of plant growth the soil core was removed from the tube and the roots were separated from the soil. The five longest roots from each plant were selected. The mean root lengths were 36.6 cm for C-R_5B and 33.5 cm for C-R_5D; they were not significantly different ($P > 0.05$). From all five seedling roots, 5 cm of the root tip was taken and bulked as the root-tip sample. These root samples were 1−2 days old since the linear growth rates of the seedling roots were 3−4 cm per day. The second sample was taken 5 cm below the seed, from all five seedling roots, and was bulked as the root-base sample; these root segments were estimated as 8−9 days old. The roots were gently washed (five times) with sterile demineralized water to remove adhering soil particles. Rovira et al. (1974) reported that by washing roots in this way only 3−4% of the bacteria in the rhizoplane were lost. The roots were then macerated and homogenized in a mortar containing 20 ml 0.1% sodium pyrophosphate. From this solution serial dilutions were made and plated on 1/10 strength tryptic soy agar. Five plates were used per sample per dilution. The number of colonies was determined after a 10-day incubation at 20 °C.

Some bacteria from these plates were isolated in order to characterize the populations. Agar plates with between 300 and 50 colonies were used and 30 colonies were randomly selected from one agar plate of each sample. In total, 360 bacteria were isolated and purified, using 1/10 strength tryptic soy agar. The colonies were stored at 20 °C, and transfers to new media were made every 3rd week. Each isolate was tested for a number of physiological characters, mostly determined in square petri dishes with 25 compartments. These tests are described below.

The metabolism of acetate, citrate, and succinate was determined. Simmons' citrate agar was used as described in Gerhardt (1981). For the acetate and succinate tests, Na-citrate was substituted for Na-acetate and Na-succinate, respectively. The results were recorded after 7 days of incubation at room temperature.

The acid production from xylose, mannose, galactose, cellobiose, sucrose, lactose, and mannitol was determined by substituting organic acids for the different sugars in the same media as above. Results were determined after 4 and 7 days of incubation at room temperature.

Tolerance to 3 and 10% NaCl was determined by adding NaCl to 1/10 strength tryptic soy agar. Bacterial growth was recorded after 2 weeks of incubation at root temperature.

The presence of phosphatase (as described in Gerhardt 1981), starch hydrolysase (Gehlen et al. 1985), urease (with Christensen urea agar as described in Gerhardt 1981); NO_3^--reductase (Harrigan and McCance 1966), lipase (1% tween 80, Sierra 1957), oxidase (method 1 in Gerhardt 1981) was determined after incubations at room temperature.

The ability of the bacteria to dissolve $CaHPO_4$ (Katznelson and Bose 1959) was tested. The isolates were also tested for colony color, slime production, radial growth rate on 1/10 strength tryptic soy agar, and colony morphology. All test results were scored as 0 or 1. In the color test, two groups, yellow and white/grey/cream were considered. The radial growth rate was determined as the colony diameter after 7 days at either 1−2 mm or >2 mm. Colony morphology was determined as regular or irregular. Some isolates were also characterized with the Gram stain technique and tested for the production of fluorescent pigment on King's B medium (King et al. 1954) and S1 medium (Gould et al. 1985).

The results of each test were analyzed separately, with the effect of the wheat line and the root position from which the bacteria were taken as the main variables. A generalized linear model was used (McCullagh and Nelder 1983), on the assumption that positive test results were binomially distributed.

In addition, the data were subjected to multivariate classification analysis in order to identify different groups of bacteria according to the results of the different tests. The isolates were grouped by cluster analysis. An initial classification was obtained by Twinspan (Hill 1979), a Fortran program for arranging multivariate data in an ordered two-way table. This classification was used as a basis for the maximal predictive classification method of Gower (1974), which is a non-hierarchical method. The analysis was carried out with the Genstat 5 statistical program, release 1.3, Lawes Agricultural Trust (Rothamsted Experiment Station). For each cluster the statistical significance of the difference in the numbers of root-tip and root-base isolates was investigated with the Wilcoxon matched-pairs test.

In another analysis the test results for each isolate were compared with the test results of all the other isolates. Isolates that differed in less than five tests were considered to be similar. If a particular isolate had many characteristics in common with other isolates found in the same place (tip or base) it was classified as typical for that place. The criteria for a typical base or tip isolate were that more than 10 other similar isolates were found, with at least three times as many from the same place, base or tip, as the typical isolate, compared with those from the other location.

Results

The numbers of colony-forming units were about 15 times higher on the root-base segments than on the root tips (Table 1). No significant differences ($P > 0.05$) between the two wheat lines were found. In a pilot study (data not shown) the numbers of actinomycetes, determined with the chitin oatmeal method (Miller et al. 1989), were less than 0.5% of the bacterial numbers counted on 1/10 tryptic soy agar.

The percentages of positive results from the different tests, within each treatment, are given in Table 2. Some of the tests showed significant differences in the percentage of positive test results between bacteria isolated from the root tip and from the root base. Significantly more isolates from the tip were able to use citrate and produce acid from galactose than isolates from the root base. The same trends were observed for mannitol and xylose but the differences were not statistically significant. More isolates from the root base tolerated 10% NaCl in the growth medium and produced acid form cellobiose. Furthermore, relatively more of the base isolates had a yellow colony color while relatively more isolates from the root tip were white, grey or cream. Significant differences between the two varieties were only observed in the frequency of phosphatase-positive isolates, more being found on the strains isolated from C-R_5D.

Table 1. Bacterial numbers on the seminal roots of two wheat lines determined on the root tip (root segments 1−2 days old) and the root base (root segments 8−9 days old)

Wheat line	Root tip	Root base
C-R_5B	0.20 ± 0.01 (× 10^4)	2.16 ± 0.38 (× 10^4)
C-R_5D	0.17 ± 0.10 (× 10^4)	3.09 ± 1.09 (× 10^4)

Mean ± SD bacteria per cm root, three replicates

Table 2. Results of physiological and morphological tests on bacterial isolates from the root tip and root base of two wheat lines

Test	Tip		Base		Significant effect of	
	CR_5B	CR_5D	CR_5B	CR_5D	Place (tip/base)	Line
Citrate	42	40	6	21	*	
Acetate	6	3	6	7		
Succinate	43	46	22	38		
Xylose	38	43	20	33		
Mannose	27	27	18	20		
Galactose	44	43	29	36	*	
Cellobiose	1	3	14	7	*	
Sucrose	0	9	7	3		
Lactose	33	38	8	21		
Mannitol	39	41	16	23		
NaCl 3%	48	47	43	56		
NaCl 10%	3	2	10	14	*	
Phosphatase	30	38	26	49		*
Amylase	22	18	18	22		
Urease	28	10	14	22		
NO_3^--reductase	40	44	24	46		
Lipase	38	49	36	32		
Oxidase	47	38	43	32		
P-dissolution	0	0	1	3		
Slime	18	20	10	18		
White/grey/cream	87	86	70	73	***	
Yellow	26	22	37	32	*	
Regular	92	93	97	92		
1–2 mm	12	20	24	28	*	
>2 mm	30	11	20	27		

All tests were scored as 0 or 1, and the numbers given in the table are the percentages of isolates with positive test results. Means of three replicate plants, with 30 bacteria isolated per plant
* $P<0.05$; *** $P<0.001$, see Materials and methods

The bacteria were first clustered with the Twinspan method. This method indicated an optimal number of 11 clusters. These 11 clusters were used as start clusters for the maximal predictive classification method of Gower (1974). The results of the cluster analysis are shown in Table 3. The predicted test result pattern is given for each group. Group 3 contained significantly more isolates from the root tips ($P<0.05$) than from the base. This group was characterized by positive test results for organic acids and several sugars (mannitol, lactose, xylose, and galactose), and by the presence of the enzymes nitrate reductase, lipase, and oxidase. Only a few isolates were able to produce acid from disugars and most of the isolates did not tolerate salt (NaCl). A different group (no. 10) contained significantly more isolates from the root base. This group was characterized by negative test results for most of the physiological tests, and all colonies formed by these isolates had yellow pigmentation. With 80 isolates, group 6 contained the largest number of bacteria, and all physiological tests were predicted to be negative, although 24% of the isolates were able to grow on 3% NaCl and 21% showed nitrate reductase activity. In this group, root-base isolates did not differ from those of the root tip.

With the other classification method (see Materials and methods) 51 typical tip and 17 typical base isolates were identified (Table 4). It was clearly easier to find typical tip isolates than typical base isolates and, the result was well correlated with the other cluster method. Of the 17 typical base isolates, 13 belonged to group 10 in the cluster analysis and the other 4 to groups 8 or 9. In the cluster analysis most of the typical tip isolates were found in groups 3 and 4 (23 and 18 of the 51 isolates, respectively), with 4 in group 2, and 3 in group 7.

About 20 isolates from the root tip (groups 3 and 4) and 20 isolates from the root base (groups 9 and 10) were randomly selected for an analysis of the Gram reaction, and to determine cell morphology and the production of fluorescent pigment. The Gram reaction was not correlated with any of the groups determined by the cluster analysis. Of the base isolates, 33% were Gram-negative, and

Table 3. Class predictors from cluster analysis of 360 rhizoplane bacterial isolates, clustering in 11 groups

	Cluster group (no. of isolates)										
	1 (14)	2 (28)	3 (33)	4 (27)	5 (19)	6 (80)	7 (25)	8 (37)	9 (25)	10 (41)	11 (31)
Citrate	0	1	1	1	1	0	0	0	0	0	0
Acetate	0	0	0	0	0	0	0	0	0	0	0
Succinate	0	1	1	1	1	0	0	0	1	0	0
Xylose	1	1	1	1	0	0	0	0	0	0	0
Mannose	1	1	0	1	1	0	0	0	0	0	0
Galactose	1	1	1	1	1	0	0	0	0	0	0
Cellobiose	1	0	0	0	0	0	0	0	0	0	0
Sucrose	1	0	0	0	0	0	0	0	0	0	0
Lactose	1	1	1	1	0	0	0	0	0	0	0
Mannitol	1	1	1	1	0	0	0	0	0	0	0
NaCl 3%	0	1	0	1	1	0	1	1	1	0	0
NaCl 10%	0	0	0	0	0	0	0	0	0	0	0
Phosphatase	0	1	0	1	1	0	0	1	0	0	0
Amylase	0	0	0	1	1	0	0	0	0	0	0
Urease	0	0	0	0	0	0	0	0	0	0	1
NO_3^--reductase	0	1	1	1	0	0	0	1	0	0	0
Lipase	0	1	1	1	0	0	1	0	1	0	0
Oxidase	1	0	1	0	0	0	0	0	0	1	1
P dissolution	0	0	0	0	0	0	0	0	0	0	0
Slime	0	1	0	0	0	0	0	0	0	0	0
White/grey/cream	1	1	1	1	1	1	1	1	1	0	1
Yellow	0	0	0	0	0	0	0	0	0	1	0
Regular	1	1	1	1	1	1	1	1	1	1	1
1–2 mm	0	0	0	1	0	0	0	0	1	0	0
>2 mm	0	1	0	0	1	0	0	0	1	0	0
Site of isolates											
Root base,	No. of isolates										
$C\text{-}R_5B$	7	2	2	2	2	16	7	10	13	18	11
$C\text{-}R_5D$	2	9	1	8	8	19	0	15	7	13	8
Root tip,											
$C\text{-}R_5B$	1	10	14	6	6	19	9	7	2	6	10
$C\text{-}R_5D$	4	7	16	11	3	26	9	5	3	4	2

Cluster groups 3 and 10 showed a significant difference ($P<0.025$) between isolates at the root tip or base by the Wilcoxon matched pairs test; this test could not be used for comparison of the wheat lines since only three replicates were taken

Table 4. Percentages of positive test results for bacterial isolates selected as typical root-base or typical root-tip isolates by the second method of classification (see Materials and methods)

Test	Root-base isolates (n = 17)	Root-tip isolates (n = 51)
Citrate	6	84
Acetate	6	4
Succinate	12	92
Xylose	0	90
Mannose	0	35
Galactose	6	90
Cellobiose	6	0
Sucrose	6	0
Lactose	0	88
Mannitol	0	90
NaCl 3%	71	55
NaCl 10%	18	0
Phosphatase	35	53
Amylase	12	37
Urease	24	10
NO_3^--reductase	18	82
Lipase	41	88
Oxidase	59	31
P dissolution	0	0
Slime	0	16
White/grey/cream	12	76
Yellow	82	39
Regular	100	96
1–2 mm	24	22
>2 mm	12	22

the others were Gram-positive or Gram-variable. Of those on the root tip, 48% were Gram-negative. No relationship between cell morphology (rods, cocci, coryneform, irregular) and the cluster groups was found. None of the isolates was fluorescent, on either King's medium B or S1 medium.

Discussion

The results of this study indicate that changes occur in the bacterial populations located along seedling wheat roots. There was a major difference between the populations on the root tips and those on the older root segments in that positive test results for monosugars (mannitol, lactose, mannose, xylose and galactose) were found significantly more often in populations from the root tips. The elongating root tip is considered an important site for the exudation of soluble C substances (McDougall and Rovira 1970; Rovira 1973); a significant part of the exudates may consist of simple sugars. Moreover, since these substances have not yet been consumed by microorganisms, the concentrations of soluble C are still relatively high, as predicted by Newman and Watson (1977). Peak concentrations of soluble C have also been found just behind the root tips of rape seedlings (Olsson 1987). Under these conditions the growth of bacteria that can use simple sugars and organic acids efficiently might be stimulated, which may explain the higher frequencies of those bacteria found on root tips in the present study.

An important aspect is whether the bacteria present on the root tips are simply transferred from the soil or whether bacterial cell proliferation over 1–2 days is sufficient to explain the observed colonization of new root tips. The growth rate in the rhizosphere varies between bacterial species. Bowen and Rovira (1973) reported that *Pseudomonas* spp. have a generation time of 5 h, while *Bacillus* spp. require 35 h. These authors (Bowen and Rovira 1973) planted sterile *Pinus* sp. seedlings in non-sterile soil and during the first few days the natural soil population showed a generation time of about 10 h. Thus, without a long lag-time for the soil inoculum, certain bacteria can proliferate several times within a 1- to 2-day period. However, it is also possible that these experimental results reflect metabolic stimulation, not cell proliferation, since metabolic stimulation will allow bacteria to grow on an artificial agar medium after inoculation.

In contrast to observations by Neal et al. (1973), no significant differences were found between the two wheat lines with respect to the isolate clusters, even though significantly more bacteria with phosphatase activity were isolated overall from line C-R_5D. This indicates that there were greater differences between different sites on the root system than between the two wheat lines used here.

It has been argued that Gram-negative bacteria form the largest part of the rhizosphere bacterial population (Debette and Blondeau 1980; Kleeberger et al. 1983; Scher et al. 1984; Lambert et al. 1987). In the present experiment the Gram stain was only applied to a few representative isolates from groups typical of either the root tip or the root base, and fairly equal numbers of Gram-positive and Gram-negative isolates were found. However, the isolation of the bacteria on nutrient-rich media may have favored Gram-negative bacteria as shown by Miller et al. (1989, 1990). When the bacteria were isolated on nutrient-poor (diluted) media, coryneform bacteria (Gram-positive and Gram-variable) formed a large percentage of the total bacterial flora and fluorescent pseudomonads were found in very low percentages, less than 1% of the total viable population. Turner et al. (1985) also used nutrient-poor media to isolate bacteria and found that *Pseudomonas* spp. were rare, while pleomorphic bacteria were more abundant than rods, in the rhizosphere of *Lolium rigidum*. In those investigations samples for bacterial analysis were taken from a mixed root sample, presumably containing both young and old root segments.

It is interesting that typical tip bacteria tend to show a preference for organic acids, such as citrate, and for simple sugars. It is known that associated rhizobacteria such as *Azospirillum* spp. prefer low molecular weight organic compounds, e.g., organic acids, as a substrate (Tarand et al. 1978). The preferences of the "tip" bacteria, which can be considered the first colonizers of the root, indicate that specific exudates play a part in the root-colonization process.

In conclusion, the present study showed certain differences between typical "tip" and "base" bacteria, but

the results should be interpreted with great care. By definition, the characterization of bacterial communities as performed in the present study is selective and might be biased. Colony-forming units obtained by plate counts do not represent the total numbers of bacteria. Moreover, most groups formed in the cluster analysis, and also the typical sets of characteristics, were common to bacteria isolated from the tip as well as from the base of the roots. Nevertheless, the present results may provide a basis for further studies on the mechanisms of root colonization of both native and introduced bacteria, since one of the key processes of colonization is the use of specific substrates produced by the plants.

References

Bowen GD, Rovira AD (1973) Are modelling approaches useful in rhizosphere biology? Bull Ecol Res Commun (Stockholm) 17:443–450

Deacon JW, Lewis SJ (1982) Natural senescence of the root cortex of spring wheat in relation to susceptibility to common root rot (*Cochliobolus sativus*) and growth of a free-living nitrogen fixing bacterium. Plant and Soil 66:13–20

Debette J, Blondeau R (1980) Presence de *Pseudomonas maltophilia* dans la rhizosphere de quelques plantes cultivées. Can J Microbiol 26:460–463

Gehlen M, Trampisch HJ, Dott W (1985) Physiological characterization of heterotrophic bacterial communities from selected aquatic environments. Microb Ecol 11:205–219

Gerhardt P (1981) (ed. in chief) Manual of methods for general bacteriology. American Society for Microbiology, Washington DC

Gould WD, Hagedorn MK, Bardinelli TR, Zablotowicz RM (1985) New selective media for enumeration and recovery of fluorescent pseodomonads from various habitats. Appl Environ Microbiol 49:28–32

Gower JC (1974) Maximal predictive classification. Biometrics 30:634–654

Harrigan WF, McCance ME (1966) Laboratory methods in microbiology. Academic Press, London

Hill MO (1979) Twinspan: A Fortran program for arranging multivariate data in an ordered two-way table by classification of the individuals and attributes. Section of Ecology and Systematics, Cornell University, Ithaca, New York

Katznelson H, Bose B (1959) Metabolic activity and phosphate-dissolving capability of bacterial isolates from wheat roots, rhizosphere, and non-rhizosphere soil. Can J Microbiol 5:79–85

King EO, Ward MK, Raney DE (1954) Two simple methods for the determination of pyocyanin and fluorescin. J Lab Clin Med 44: 301–304

Kleeberger A, Castoph H, Klingmuller W (1983) The rhizosphere microflora of wheat and barley with special reference to gram-negative bacteria. Arch Microbiol 136:306–311

Lambert B, Leyns F, Van Rooyen L, Gossele F, Papon Y, Swings J (1987) Rhizobacteria of maize and their antifungal activities. Appl Environ Microbiol 59:1866–1871

McCullagh P, Nelder JA (1983) Generalized linear models. Chapman and Hall, London

McDougall BM, Rovira AD (1970) Sites of exudation of 14C-labelled compounds from wheat roots. New Phytol 69:999–1003

Miller HJ, Henken G, Van Veen JA (1989) Variation and composition of bacterial populations in the rhizospheres of maize, wheat and grass cultivars. Can J Microbiol 35:656–660

Miller HJ, Liljeroth E, Henken G, van Veen JA (1990) Fluctuations in the fluorescent pseudomonad and actinomycete populations of rhizosphere and rhizoplane during the growth of spring wheat. Can J Microbiol 36:254–258

Neal JL, Larson RI, Atkinson TG (1973) Changes in rhizosphere populations of selected physiological groups of bacteria related to substitution of specific pairs of chromosomes in spring wheat. Plant and Soil 39:209–212

Newman EI, Watson A (1977) Microbial abundance in the rhizosphere: A computer model. Plant and Soil 48:17–56

Olsson S (1987) Effects of root exudation on growth of bacteria and fungal pathogens in the rhizosphere. PhD dissertation, Lund University, Sweden

Olsson S, Bååth E, Söderström B (1987) Growth of *Verticillium dahlie* Kleb. and of bacteria along the roots of rape (*Brassica napus* L.) seedlings. Can J Microbiol 33:916–919

Rovira AD (1973) Zones of exudation along plant roots and spatial distribution of microorganisms in the rhizosphere. Pestic Sci 4:361–366

Rovira AD, Newman EI, Bowen HJ, Campbell R (1974) Quantitative assessment of the rhizosphere microflora by direct microscopy. Soil Biol Biochem 6:211–216

Scher FM, Ziegle JS, Kloepper JW (1984) A method for assessing the root-colonizing capacity of bacteria on maize. Can J Microbiol 30:151–157

Sierra G (1957) A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek J Microbiol Serol 23:15–22

Tarand JJ, Krieg NR, Döbereiner J (1978) A taxonomic study of the *Siprillum lipoferum* group with description of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* comb. nov. and *Azospirillum brasiliense* sp. nov. Can J Microbiol 24:967–980

Turner SM, Newman EI, Campbell R (1985) Microbial population of ryegrass root surfaces: Influence of nitrogen and phosphorus supply. Soil Biol Biochem 17:711–715

Van Elsas JD, Dijkstra AF, Goevert JM, Van Veen JA (1986) Survival of *Pseudomonas fluorescens* and *Bacillus subtilis* introduced into two soils of different texture in field microplots. FEMS Microb Ecol 38:151–160

Van Vuurde JWL, Schippers B (1980) Bacterial colonization of seminal wheat roots. Soil Biol Biochem 12:559–565