Journal of General Microbiology (1986), **132**, 3407–3414. *Printed in Great Britain*

Migration of the Rhizosphere Bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* Towards Wheat Roots in the Soil

By YOAV BASHAN

Department of Plant Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel

(*Received 4 March 1986; revised 2 July 1986*)

Migration of the rhizosphere bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* towards wheat seedlings grown in soil was studied under various environmental conditions. The main factor affecting motility was soil moisture; of secondary importance were the soil type and the duration of plant growth prior to bacterial application. Migration was initiated following a lag of 24 h and was characterized by a bacterial band migrating through the soil towards the plant roots. Migration was significantly stimulated by various wheat genotypes and by synthetic attractants, though they did not differ in the intensity of their effect. It is proposed that these two rhizosphere bacteria can be stimulated to migrate non-specifically towards growing wheat plants.

INTRODUCTION

Certain bacteria of the genus *Azospirillum*, and of the fluorescent *Pseudomonas* groups, are motile (Chet *et al.*, 1973; Tarrand *et al.*, 1978). Interspecific variation involving motile versus non-motile strains as well as variations in the rate of motility are documented (Heulin *et al.*, 1982, 1983; Panopoulos & Schroth, 1974). However, motility of the beneficial associative rhizosphere bacteria, known for their possible contribution to plant growth and yield, particularly in cereals, has received little attention. Relatively few studies, most of them on the genus *Azospirillum*, have been conducted, and all were done *in vitro*. They include aerotaxis of *A. brasilense* towards low oxygen tensions (Barak *et al.*, 1982), chemotaxis of this species towards several amino acids, sugars and organic acids (Barak *et al.*, 1983; Okon *et al.*, 1980; Reinhold *et al.*, 1985) and chemotaxis of *A. lipoferum* towards wheat root exudates and sucrose (Heinrich & Hess, 1985).

Since the latter studies were not conducted under normal bacterial habitat conditions, i.e. soil or the plant rhizosphere, it seemed important to determine whether similar responses occur under soil conditions. The purpose of this study was to examine the motility of associative beneficial *Azospirillum* and *Pseudomonas* spp. in the soil in the presence of their natural attractant – the host plant.

METHODS

Organisms and growth conditions. The rhizosphere bacteria *Azospirillum brasilense* Cd (ATCC 29710) and *Pseudomonas fluorescens* strain 82011 (isolated from roots of wild wheat in Israel) were used. Both strains are known to be plant beneficial bacteria (Bashan, 1986*a*; Okon, 1985; Suslow, 1982). Plant material consisted of common wheat plants, *Triticum aestivum*, cvs. Lachish, Bet Lehem-676, Hazera-18, Chinese Spring, Deganit and Barkai, and the *T. durum* cvs. Inbar and Hazera-870. Bacteria were grown in nutrient broth (Difco) for 24 h at 30 ± 2 °C in a rotary shaker (200 r.p.m.) and were not washed before soil inoculation, to avoid the flagellar damage that preliminary experiments indicated was caused through washing by centrifugation.

Migration assays in the soil. The device shown in Fig. 1 was used in all experiments. It consisted of a large glass dish (170 mm diameter, 90 mm depth) filled with 600–700 g brown-red degrading sand soil (from Rehovot) equilibrated to nearly field capacity with tap water or to several other levels of humidity at approximately 30 mm depth, as described below; acid-purified sand (40–100 mesh, BDH) or heavy soil (Terra rosa soil) were also used. A fine nylon barrier (300 mesh) was placed vertically 20 mm from the dish edge to prevent spreading of plant roots

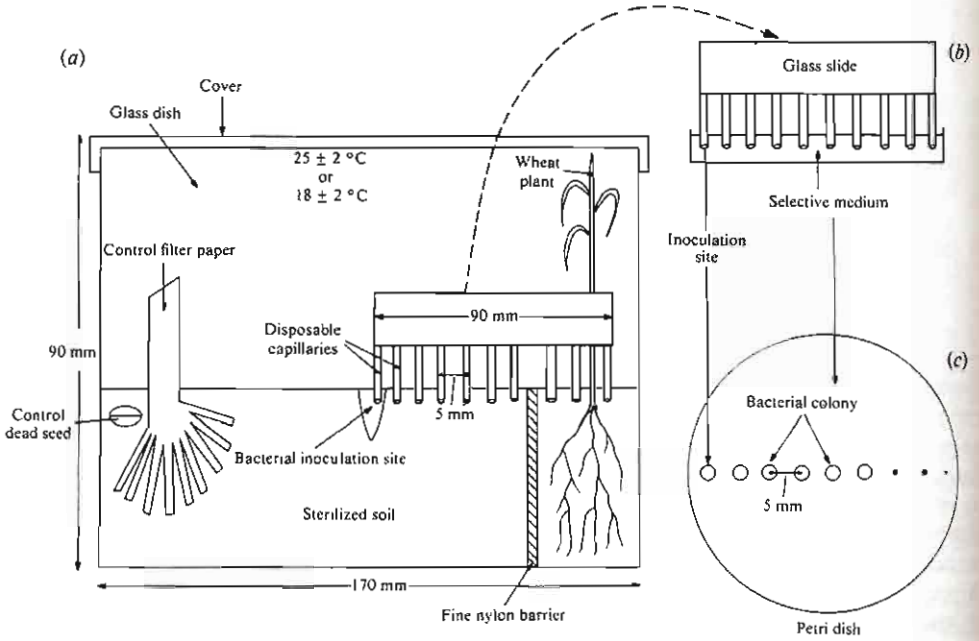

Fig. 1. Schematic representation of (*a*) the apparatus used for determining bacterial migration in soil, (*b*) sampling procedure, and (*c*) measurement of distances migrated.

throughout the dish. The dish was sterilized either by γ-irradiation (25 kGy) using a ^{60}Co source, or by autoclaving three times, each for 1 h at 1·2 atm, with 24 h intervals at room temperature between each sterilization.

Wheat seeds were surface disinfected with 1% (v/v) NaOCl under vacuum for 5 min (the vacuum was released abruptly to ensure disinfection), then washed five times with sterile tap water. The seeds were imbibed for 3–4 h, put on sterile filter paper for an additional 48 h, and then germinating seedlings of approximately equal size were sown (six seedlings per dish, 0·5 cm depth) at the edge of the dish, distal to the nylon barrier.

The dishes were transferred to a growth chamber (Conviron model EF7H, Controlled Environments, Canada) at 22 ± 1 °C for 4 d, and then inoculated by placing 0·5 ml bacterial culture, 10^9 c.f.u. ml^{-1} at the centre and resealed. At various intervals, soil samples were removed by a disposable sterilized sampler, consisting of a microscope slide glass with 0·5 mm diameter capillaries attached to it every 5 mm (the last three capillaries representing the plant site). The capillaries were placed in the soil (3–5 mm depth) and the retrieved samples were placed on agar medium [selective BL medium for *A. brasilense* (Bashan & Levanony, 1985) or King-B medium for *P. fluorescens*: the bacterial strains were tested separately]. The plates were incubated at 30 ± 2 °C and 25 ± 2 °C in the above media for 10 d and 1 d, respectively, until colony formation was visible. The distance of the bacterial migration in the soil was calculated from the site of application to the furthermost colony; when bacteria reached the root system the migration distance was 90 mm. Total bacterial counts (in bands, for both *A. brasilense* and *P. fluorescens*) were done by the plate count method on King-B medium.

Each plate contained seedlings on one side and controls on the opposite side, the latter consisting of dead seeds (killed by γ-irradiation), filter paper or non-planted soil. Before each soil inoculation, bacteria were tested for their motility by light microscopy. Their identity was routinely verified by the enzyme-linked immunosorbent assay (ELISA) for *A. brasilense* Cd (Levanony *et al.*, 1985) and by typical fluorescent colony formation for *P. fluorescens*. The bacterial growth media remaining at inoculation time were tested for serving as attractants and were discarded.

Determination and production of various levels of soil humidity. To obtain soil near field capacity, 5 kg of soil was flooded with tap water and placed on a delicate cheese-cloth, and the excess water was allowed to drain at ambient temperature overnight. The soil was then carefully packed into the glass dishes. To obtain specific levels of humidity, the soil was dried in a forced draught oven at 45 °C for 24 h, and then the desired amount of water was added. The soil was lightly mixed and packed into the glass dishes.

Production of beads containing attractants. Glycine (10^{-2} M) and/or aspartic acid (10^{-2} M), dissolved in distilled water, were mixed with 2% (w/v) sodium alginate (BDH). Beads formed according to Bashan (1986*b*) were lyophilized to dryness and maintained in sealed glass containers with silica gel until use. Five beads were put at the edge of each migration cell in place of the wheat seedlings. Controls included alginate beads with no attractants.

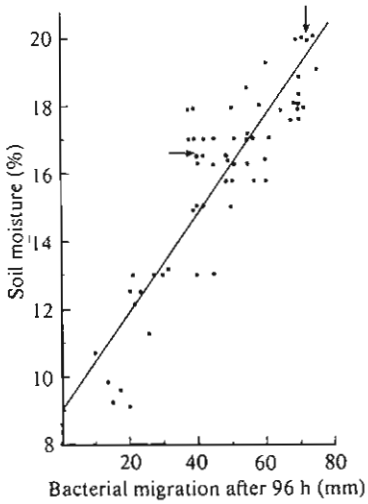

Fig. 2. Relation between motility of *A. brasilense* Cd towards wheat roots (cv. Hazera-18) and the moisture content of brown-red degrading sand soil of Rehovot. The vertical arrow indicates the beginning of soil flooding; the horizontal arrow indicates field capacity of this soil. $y = 0{\cdot}143x + 9{\cdot}01$; $r = 0{\cdot}91$, significant at $P \leqslant 0{\cdot}05$. Each point represents the mean of two determinations; data were pooled from five different experiments.

Statistical analysis. Two dishes served as a replicate and all experiments were done in triplicate and repeated at least three times. Unless otherwise noted results are from a representative experiment in each case. Significance is given by $P \leqslant 0{\cdot}05$ or by standard error.

RESULTS

Effect of soil moisture on bacterial motility. Migration of *A. brasilense* in the soil towards growing wheat seedlings of cv. Hazera-18 was measured at several levels of soil moisture. Data for migration distance, 96 h after inoculation, were combined from five different experiments. Direct linear and significant correlation was obtained between migration distance and soil moisture (Fig. 2). The highest migration rate was obtained in light soil at near field capacity (16%, v/w, water) or above. At 20% moisture, this type of soil tended to be flooded, thus facilitating motility of bacteria in the free water film, even in the absence of seedlings. Similar trends of migration in soil were found for *P. fluorescens*.

Effect of soil type on bacterial migration. The two rhizosphere bacteria were inoculated separately into three soil types: the sand and the brown-red degrading sand soil of Rehovot (both light soils), and the heavy Terra rosa soil. Migration towards wheat seedlings of cv. Hazera-18 was recorded daily (Fig. 3). For both bacteria, migration decreased with increasing soil weight. *P. fluorescens* had a slightly higher rate of migration in the three soil types than *A. brasilense*. For both bacterial strains, the migration rate increased several fold with time: from an average of 6 mm in the first 24 h to 35 mm from 48 to 72 h after inoculation of *P. fluorescens*, in the extreme case (Fig. 3). The reasons for this increase are unknown.

Effect on bacterial migration of duration of plant growth in the soil prior to bacterial inoculation. P. fluorescens was inoculated into the dishes either at planting time or 1, 2 or 3 d later. A linear relationship ($y = 13{\cdot}5x + 11$; $r = 0{\cdot}97$, significant at $P \leqslant 0{\cdot}05$) existed between time of planting and bacterial migration 72 h after inoculation, motility being at its highest rate in dishes containing plants grown for 3 d before inoculation. The slight decrease in soil moisture due to plant growth (from 17·35% at the beginning of the experiment to 16·53% at the end) did not affect migration.

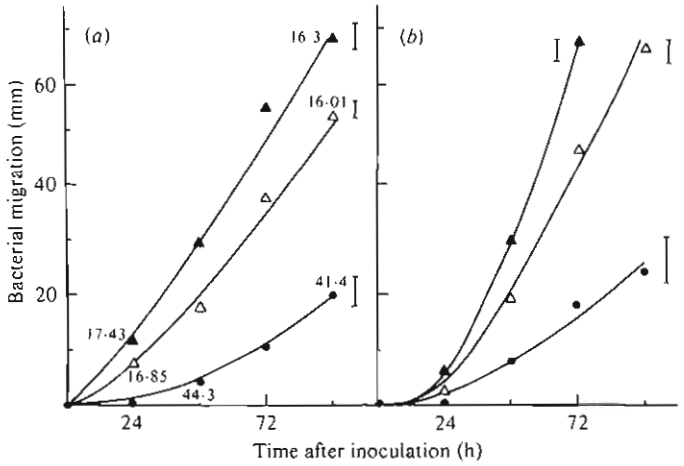

Fig. 3. Motility of (*a*) *A. brasilense* and (*b*) *P. fluorescens* in three types of soil towards wheat roots (cv. Hazera-18). ▲, Sand; △, brown-red degrading sand soil of Rehovot; ●, Terra rosa soil (heavy). The numbers near each line represent soil moisture (%) at the beginning (equivalent to field capacity) and end of the experiment; the bars represent the standard error of the lines.

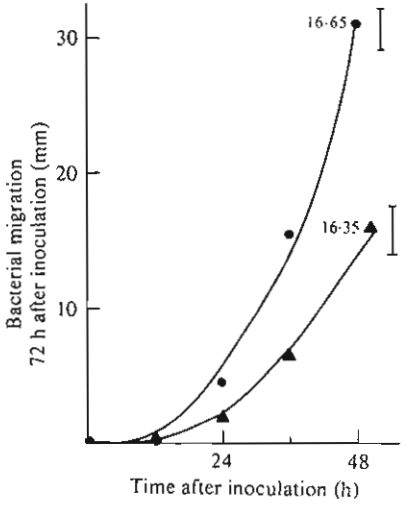

Fig. 4

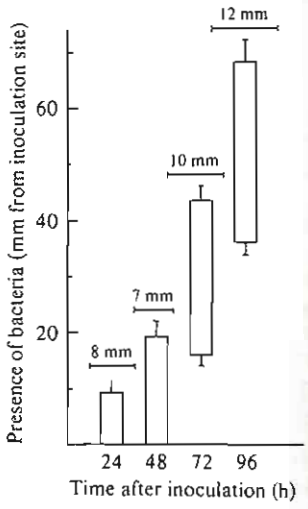

Fig. 5

Fig. 4. Motility of *P. fluorescens* (●) and *A. brasilense* (▲) in the first 48 h after inculation towards wheat roots (cv. Hazera-18) in brown-red degrading sand soil of Rehovot. The numbers near each line represent soil moisture (%) 48 h after inoculation and the bars represent the standard error of the lines.

Fig. 5. Maximal and minimal distance of migration in the soil of *A. brasilense* towards wheat roots (cv. Hazera-18). The vertical bars a represent the standard error of the maximum and minimum (separately); the horizontal bars represent the width of the bacterial band in the soil.

Initiation of bacterial motility. Preliminary observations indicated that motility did not start immediately following inoculation. There was a lag period of 24–36 h for both bacteria before substantial migration towards the growing plants was initiated (Fig. 4). The more motile strain (*P. fluorescens*) had a shorter lag period.

Characterization of bacterial migration in the soil. The minimum and maximum migration of *A. brasilense* in the soil was measured as the greatest distance between colonies developed after sampling on the agar medium. The width of the migration front was measured with an identical

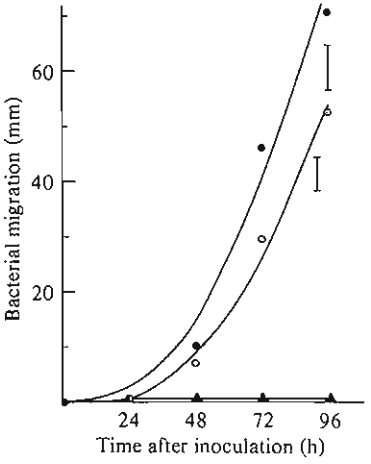

Fig. 6. Migration of *A. brasilense* in the soil towards 10^{-2} M-glycine (●) and 10^{-2} M-aspartic acid (○); ▲, controls (dead seeds or filter paper). The bars represent the standard error of the lines.

Table 1. *Numbers of A. brasilense in various zones of the bacterial band migrating in sterile soil towards wheat roots*

Distance (mm) from the furthermost band migrating towards plant*	Number of *A. brasilense* in soil†		
	After 24 h	After 72 h	After 96 h
0–5	$2{\cdot}1 \pm 0{\cdot}3 \times 10^8$	$3{\cdot}7 \pm 0{\cdot}6 \times 10^8$	$4{\cdot}4 \pm 0{\cdot}5 \times 10^8$
10–15	$2{\cdot}4 \pm 0{\cdot}5 \times 10^8$	$8{\cdot}4 \pm 0{\cdot}9 \times 10^6$	$8{\cdot}7 \pm 1{\cdot}1 \times 10^6$
40	ND	0‡	0‡

ND, Not determined.

* All the soil was sampled at the distances indicated, to the dish bottom. Width of sampling was as shown in Fig. 5.

† Counted by the plate count method on BL medium (Bashan & Levanony, 1985).

‡ Verified by ELISA (Levanony *et al.*, 1985).

sampler placed on the soil at the expected front of bacterial migration. The numbers of *A. brasilense* in the soil were determined in various areas of bacterial migration. Migration in soil was characterized by a bacterial band, with nearly all bacteria migrating towards the plants. No bacteria could be detected in a given soil area 48 h after the bacterial population had migrated from this site. *A. brasilense* numbers slightly increased in the soil during the experiment, but these differences were not significant (Table 1). No measurable bacterial death was detected in areas free of bacteria, using the ELISA technique, which is capable of quantifying dead as well as live bacteria. The size of the band varied and slightly increased as it reached the area closer to the plant (Fig. 5).

Migration of A. brasilense in the soil towards synthetic attractants. A. brasilense migrated through the soil towards glycine and aspartic acid in a similar manner as towards living wheat plants (Fig. 6).

Migration of A. brasilense towards various wheat cultivars. Migration towards eight wheat cultivars was tested. Excluding variation between experiments due to differences in soil moisture, generally speaking, bacterial migration was higher towards wheat seedlings than towards non-planted soil (Fig. 7). No significant difference was detected between the wheat cultivars (compare results from single experiments). The possibility that the bacteria were attracted by degrading seeds or by a water sink created by the plants was tested and excluded (Fig. 7).

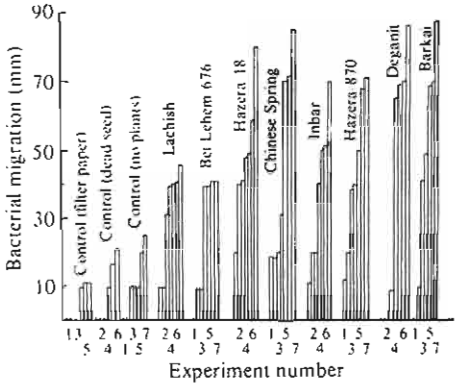

Fig. 7. Migration of *A. brasilense* towards various wheat cultivars after 96 h. The data are from seven different experiments. Columns with the same number are from the same experiment.

DISCUSSION

Motility is one of the major taxonomic properties of the genus *Azospirillum* and many species of *Pseudomonas* (see Krieg, 1984; Tarrand *et al.*, 1978). Attraction and migration of beneficial rhizosphere bacteria towards their respective host plants provide an important ecological advantage for these bacteria. Additionally, species of rhizosphere bacteria with poor survival in soil must reach the root environment in order to survive (Chet & Mitchell, 1976; Rovira, 1969). The ability of associative beneficial rhizosphere bacteria such as *Azospirillum* and *Pseudomonas* to attain a significant population on the host root system is a prerequisite for their effects on plant growth (Bashan, 1986*a*; Suslow, 1982). Although there is great interest in manipulating specific beneficial bacteria into the rhizosphere, little is known about the processes determining the rhizosphere population size, and the migration of these bacteria in the soil (Bashan, 1986*a*; Bennett & Lynch, 1981*a*, *b*; Bowen, 1979; Foster & Bowen, 1982; Lynch, 1981; Rovira *et al.*, 1983; Suslow, 1982).

The present study demonstrated the migration of two rhizosphere beneficial bactera in the soil towards living wheat plants or towards synthetic attractants known to be produced *in vivo* by them (Rovira, 1969). Substances released from degrading seeds during germination, and the water sink produced by the plant during its growth, failed to attract the bacteria. The nylon barrier which prevented the spread of plant roots throughout the dish provided further evidence that bacterial migration occurred through the soil.

Water-saturated soil conditions are known to facilitate the movement of bacteria through the soil (Hamdi, 1971, 1974; Kellerman & Fawcett, 1907; Madsen & Alexander, 1982; McCoy & Hagedorn, 1979; Thornton & Gangulee, 1926). Wallace (1978) proposed that water-facilitated dispersal of bacteria in soil is likely to be limited to only a few centimetres because soil hydraulic conductivity is small relative to the short-term motility rates of flagellated cells. However, Frazier & Fred (1922) observed 17 cm migration of *Rhizobium* in sterilized soils. It is also known that nodulation of legumes sown in partially dry soil is likely to be affected by the failure of the inoculum to migrate away from the seed site (Brockwell *et al.*, 1972; Brockwell & Whalley, 1970). Bitton *et al.* (1974) found that water-mediated movement of *Klebsiella aerogenes* through saturated soil depended on the moisture level of the soil and the surface properties of the bacteria. Griffin & Quail (1968) found that movement of *P. aeruginosa* in soil was restricted at water contents lower than field capacity, and Robson & Loneragan (1970) proposed that water may have been responsible for dispersal of *R. meliloti* in the field. The present study indicated that soil moisture had a dominant role compared to other factors affecting migration. In light soils significant migration compared to negligible movement was brought about by a small increase in the soil moisture percentage. Decrease in soil moisture might eliminate the effects of other factors, such as root exudates and oxygen tension, by physically preventing the migration due to the absence of a continuous film of water.

Bacterial migration towards root exudates was demonstrated for *Rhizobium* attracted to *Cicer arietinum* (Gitte *et al.*, 1978) and *P. syringae* pv. *lachrymans* towards cucumber leaves (Chet *et al.*, 1973). The present study indicated the presence of an unknown mechanism by which bacteria respond to exudates and translate it into motility. This may explain the long delay in the bacterial response between inoculation time and actual migration. However, this interpretation needs further study since the attraction in sterile soil would be expected to be stronger than that in natural soil, since in the former, substances released by the roots are not metabolized by nearby soil micro-organisms.

The migration of most bacterial populations as a massive band towards the plant indicates that the attraction exerted by the plant affects most of the bacterial cells. Such a phenomenon was less obvious in *in vitro* systems in which random motility of part of the population towards nonattractant compounds such as buffers was observed (Barak *et al.*, 1982; Heinrich & Hess, 1985). Since minimal bacterial multiplication, and no bacterial death, were detected in soil, it can be concluded that migration is due to motility of individual cells towards the plant root and not to bacterial cell division.

Soil comprises a series of discontinuous surfaces and water films that restrict bacterial motility, except under extremely moist conditions (Wallace, 1978). The movement of *P. aeruginosa* and *R. trifolii* in sand was found to depend upon the continuity of water pathways, which was in turn dependent upon the pore size distribution (Griffin & Quail, 1968; Hamdi, 1974). The discontinuous thin water film covering small soil particles does not allow direct migration towards the plant in unflooded soil. Thus, the measured migration distance in the present study represents only a minimum value.

The fact that *A. brasilense* migrated to the same extent towards several cultivars of two wheat species indicated that the bacteria were attracted to compound(s) produced by the wheat genotype, rather than to a particular substance produced by a certain cultivar. Such root exudates and their effects on colonization of wheat by rhizosphere bacteria are known (Ayers & Thornton, 1968; Hale & Moore, 1979).

In conclusion, this non-specific migration in soil of two rhizosphere bacteria towards wheat seedlings provides further support to previous *in vitro* studies of aerotaxis and chemotaxis of rhizosphere bacteria.

This paper was written in memory of the late Mr Avner Bashan for his constant encouragement and interest during this research. I thank Miss Dori Filon, Mrs Adi Ravid Ben-Yehuda and Mrs Hanna Levanony for their excellent technical assistance, Mr Y. Avivi for careful criticism of the manuscript and Professor M. Feldman for stimulating discussions.

REFERENCES

Ayers, W. A. & Thornton, R. H. (1968). Exudation of amino acids by intact and damaged roots of wheat and peas. *Plant and Soil* **28**, 193–207.

Barak, R., Nur, I., Okon, Y. & Henis, Y. (1982). Aerotactic response of *Azospirillum brasilense*. *Journal of Bacteriology* **152**, 643–649.

Barak, R., Nur, I. & Okon, Y. (1983). Detection of chemotaxis in *Azospirillum brasilense*. *Journal of Applied Bacteriology* **53**, 399–403.

Bashan, Y. (1986*a*). Enhancement of wheat root colonization and plant development by *Azospirillum brasilense* Cd. following temporary depression of the rhizosphere microflora. *Applied and Environmental Microbiology* **51**, 1067–1071.

Bashan, Y. (1986*b*). Alginate beads as synthetic inoculant carriers for the slow release of bacteria that affect plant growth. *Applied and Environmental Microbiology* **51**, 1089–1098.

Bashan, Y. & Levanony, H. (1985). An improved selection technique and medium for the isolation and enumeration of *Azospirillum brasilense*. *Canadian Journal of Microbiology* **31**, 947–952.

Bennett, R. A. & Lynch, J. M. (1981*a*). Colonization potential of bacteria in the rhizosphere. *Current Microbiology* **6**, 137–138.

Bennett, R. A. & Lynch, J. M. (1981*b*). Bacterial growth and development in the rhizosphere of gnotobiotic cereal plants. *Journal of General Microbiology* **125**, 95–102.

Bitton, G., Lahav, N. & Henis, Y. (1974). Movement and retention of *Klebsiella aerogenes* in soil columns. *Plant and Soil* **40**, 373–380.

BOWEN, G. D. (1979). Integrated and experimental approaches to the study of growth of organisms around roots. In *Soil-borne Plant Pathogens*, pp. 209–227. Edited by B. Schippers & W. Games. London: Academic Press.

BROCKWELL, J. & WHALLEY, R. D. B. (1970). Studies on seed pelleting as an aid to legume seed inoculation. 2. Survival of *Rhizobium meliloti* applied to medic seed sown into dry soil. *Australian Journal of Experimental Agriculture and Animal Husbandry* **10**, 455–459.

BROCKWELL, J., BRYANT, W. G. & GAULT, R. R. (1972). Ecological studies of root-nodule bacteria introduced into field environments. 3. Persistence of *Rhizobium trifolii* in association with white clover at high elevations. *Australian Journal of Experimental Agriculture and Animal Husbandry* **12**, 407–413.

CHET, I. & MITCHELL, R. (1976). Ecological aspects of microbial chemotactic behavior. *Annual Review of Microbiology* **30**, 221–240.

CHET, I., ZILBERSTEIN, Y. & HENIS, Y. (1973). Chemotaxis of *Pseudomonas lachrymans* to plant extracts and to water droplets collected from leaf surfaces of resistant and susceptible plants. *Physiological Plant Pathology* **3**, 473–479.

FOSTER, R. C. & BOWEN, G. D. (1982). Plant surfaces and bacterial growth: the rhizosphere and the rhizoplane. In *Phytopathogenic Prokaryotes*, pp. 159–185. Edited by M. S. Mount & G. H. Lacy. London: Academic Press.

FRAZIER, W. C. & FRED, E. B. (1922). Movement of legume bacteria in soil. *Soil Science* **14**, 29–35.

GITTE, R., RAI, P. & PATIL, R. R. (1978). Chemotaxis of *Rhizobium* spp. towards root exudates of *Cicer arietinum*. *Plant and Soil* **50**, 553–556.

GRIFFIN, D. M. & QUAIL, G. (1968). Movement of bacteria in moist particulate systems. *Australian Journal of Biological Science* **21**, 579–582.

HALE, M. G. & MOORE, L. D. (1979). Factors affecting root exudation II: 1970–1978. *Advances in Agronomy* **31**, 93–124.

HAMDI, Y. A. (1971). Soil-water tension and the movement of rhizobia. *Soil Biology and Biochemistry* **3**, 121–126.

HAMDI, Y. A. (1974). Vertical movement of rhizobia in soil. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene, Abteilung Originale* **129**, 373–377.

HARRIS, R. F. (1981). Effect of water potential on microbial growth and activity. In *Water Potential Relations in Soil Microbiology*, pp. 23–96. Madison, Wisconsin: Soil Science Society of America.

HEINRICH, D. & HESS, D. (1985). Chemotactic attraction of *Azospirillum lipoferum* by wheat roots and characterization of some attractants. *Canadian Journal of Microbiology* **31**, 26–31.

HEULIN, T., BALLY, R. & BALANDREAU, J. (1982). Isolation of very efficient N_2-fixing bacteria from the rhizosphere of rice. In *Azospirillum. Experientia Supplementum* **42**, 92–99. Edited by W. Klingmüller. Basel: Birkhäuser Verlag.

HEULIN, T., WEINHARD, P. & BALANDREAU, J. (1983). Motility changes in *Azospirillum lipoferum*. In *Azospirillum* II. *Experientia Supplementum* **48**, 89–94. Edited by W. Klingmüller. Basel: Birkhäuser Verlag.

KELLERMAN, K. F. & FAWCETT, E. H. (1907). Movement of certain bacteria in soils. *Science* **25**, 806.

LEVANONY, H., BASHAN, Y. & KAHANA, Z. E. (1985). Enzyme-linked immunosorbent assay for the identification and enumeration of *Azospirillum brasilense* Cd. *FEMS Symposium on Microbial Communities in Soil, Copenhagen, Denmark*, pp. 77–77a.

LYNCH, J. M. (1981). The rhizosphere. In *Experimental Microbial Ecology*, pp. 1–23. Edited by R. G. Burns & J. H. Slater. Oxford: Blackwell Scientific Publications.

KRIEG, N. R. (editor) (1984). *Bergey's Manual of Systematic Bacteriology*, vol. 1. Ancaster, Ontario: Williams & Wilkins.

MCCOY, E. L. & HAGEDORN, C. (1979). Quantitatively tracing bacterial transport in saturated soil systems. *Water, Air and Soil Pollution* **11**, 467–479.

MADSEN, E. L. & ALEXANDER, M. (1982). Transport of *Rhizobium* and *Pseudomonas* through soil. *Journal of the Soil Science Society of America* **46**, 557–560.

OKON, Y. (1985). *Azospirillum* as a potential inoculant for agriculture. *Trends in Biotechnology* **3**, 223–228.

OKON, Y., CAKMAKCI, L., NUR, I. & CHET, I. (1980). Aerotaxis and chemotaxis of *Azospirillum brasilense*. *Microbial Ecology* **6**, 277–280.

PANOPOULOS, N. J. & SCHROTH, M. N. (1974). Role of flagellar motility in the invasion of bean leaves by *Pseudomonas phaseolicola*. *Phytopathology* **64**, 1389–1397.

REINHOLD, B., HUREK, T. & FENDRIK, I. (1985). Chemotaxis of *Azospirillum* spp. *Journal of Bacteriology* **162**, 190–195.

ROBSON, A. D. & LONERAGAN, J. F. (1970). Nodulation and growth of *Medicago truncatula* on acid soils. II. Colonization of acid soils by *Rhizobium meliloti*. *Australian Journal of Agricultural Research* **21**, 435–445.

ROVIRA, A. D. (1969). Diffusion of carbon compounds away from wheat roots. *Australian Journal of Biological Science* **22**, 1287–1290.

ROVIRA, A. D., BOWEN, G. D. & FOSTER, R. C. (1983). The significance of rhizosphere microflora and mycorhizas in plant nutrition. In *Encyclopedia of Plant Physiology*, New Series, vol. 15A, pp. 61–93. Edited by A. Lauchli & R. L. Bieleski. Berlin: Springer Verlag.

SUSLOW, T. V. (1982). Role of root colonizing bacteria in plant growth. In *Phytopathogenic Prokaryotes*, pp. 187–223. Edited by M. S. Mount & G. H. Lacy. London: Academic Press.

TARRAND, J. J., KRIEG, N. R. & DÖBEREINER, J. (1978). A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and *Azospirillum brasilense* sp. nov. *Canadian Journal of Microbiology* **24**, 967–980.

THORNTON, H. G. & GANGULEE, B. A. (1926). The life cycle of the nodule organism *Bacillus radicicola* in soil and its relation to the infection of the host plant. *Proceedings of the Royal Society* **B99**, 427–451.

WALLACE, H. R. (1978). Dispersal in time and space: soil pathogens. In *Plant Disease: an Advanced Treatise*, vol. 2, pp. 181–202. Edited by J. G. Horsfall & E. B. Cowling. New York: Academic Press.