

Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture¹

YOAV BASHAN²

Department of Agronomy, Ohio State University, Columbus, OH 43210, U.S.A.

AND

HANNA LEVANONY

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

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Key words: *Azospirillum*, bacterial inoculation, beneficial bacteria, plant-bacteria interaction, rhizosphere bacteria, biological nitrogen fixation, plant growth promoting rhizobacteria.

The genus Azospirillum

The first species of *Azospirillum* was isolated by Beijerinck (1925) from N-poor sandy soil in the Netherlands and was originally named *Spirillum lipoferum*. This bacterium was later isolated from soil (Schroder 1932) and from dried seaweed in Indonesia (H. C. Derx, unpublished, 1949, cited in Becking, 1963) and as a phyllosphere bacterium of tropical plants (Becking 1982). J. Döbereiner and J. M. Day (1976. First International Symposium on Nitrogen Fixation, Pullman, WA. Edited by W. E. Newton and C. J. Nyman. Washington State University Press, Pullman, WA. pp. 518-538) isolated the bacterium and were the first to report that it was widely distributed in the rhizosphere of several tropical grasses. Since then, *Azospirillum* has been isolated from the roots of numerous wild and cultivated grasses, cereals, and legumes, and from tropical, subtropical, and temperate soils worldwide (Bally *et al.* 1983; Döbereiner *et al.* 1976; Hill *et al.* 1983; Horemans *et al.* 1988; Kosslak and Bohlool 1983; Ladha *et al.* 1987; Lamm and Neyra 1981; Li and Castellano 1987; Nur *et al.* 1980b; Rao and Venkateswarlu 1982, 1988; Sundaram *et al.* 1988; Tyler *et al.* 1979; Wong *et al.* 1980).

Tarrand *et al.* (1978) proposed *Azospirillum* as the genus and distinguished two species: *Azospirillum brasilense* and *A. Lipo-*

ferum, based on physiological and morphological differences between various strains and on DNA homology experiments (Falk *et al.* 1986). Later, two additional *Azospirillum* species were described: *A. amazonense* (Falk *et al.* 1985; Magalhães *et al.* 1983), isolated from many grasses in the Amazonian area of Brazil, and the salt-tolerant species *A. halopraeferans*, associated exclusively with roots of Kallar grass (Reinhold *et al.* 1987). However, most strains are referred to as either *A. brasilense* or *A. lipoferum* (Krieg and Döbereiner 1986).

Effect of Azospirillum inoculation on plants

Inoculation of plants with *Azospirillum* can result in a significant change in various plant growth parameters, which may or may not affect crop yield. Descriptions of plant responses to inoculation comprise most of the *Azospirillum* literature. The exact mechanisms of action of *Azospirillum* on plants has not yet been fully elucidated.

Most studies of the *Azospirillum*-plant association have been conducted on cereals and grasses (Patriquin *et al.* 1983) and only a few other plant families have been investigated (Bashan *et al.* 1989b, 1989c; Crossman and Hill 1987; Kolb and Martin 1985; Saha *et al.* 1985). The following aboveground plant responses to *Azospirillum* inoculation in cereals and noncereal species were often reported: increases in total plant dry weight, in the amount of N in shoots and grains, and in the total number of tillers, fertile tillers, and ears; earlier heading and flowering time; increased number of spikes and grains per spike; increased grain weight; greater plant height and leaf size; and higher germination rates (Albrecht *et al.* 1981; Baldani and Döbereiner

¹Manuscript No. 191/89 from the Ohio Agricultural Research and Development Center, Ohio State University.

²Author to whom all correspondence should be sent to the following address: Department of Microbiology; Center of Biological Research, La Paz, P.O. Box 128, B.C.S. Mexico 23000.

1980; Bashan 1986a; Bouton and Zuberer 1979; Bouton *et al.* 1979; Cohen *et al.* 1980; Hegazi *et al.* 1983; Kapulnik *et al.* 1981 a; Mertens and Hess 1984; Millet and Feldman 1986; O'Hara *et al.* 1981; Pacovsky *et al.* 1985 b; Schank *et al.* 1981, 1985; Warembourg *et al.* 1987; Yahalom *et al.* 1984). In addition, a marked inoculation effect on development of the root system such as on root length and volume have frequently been observed (discussed later). Visible changes in plant growth attributed to *Azospirillum* inoculation were rarely observed in cereals, especially under field conditions, and only detailed statistical analyses revealed significant increases. However, in a few cases, such an enhancement in vegetative growth of cereals was observed in the field (Kapulnik *et al.* 1983). Visible enhancement in the growth of several vegetable plants has been also recently reported (Bashan *et al.* 1989b).

The effect of *Azospirillum* inoculation on the total yield increase of field-grown plants generally ranged from 10 to 30% (Kapulnik *et al.* 1981 c, 1987; Rao *et al.* 1983; Watanabe and Lin 1984). A few reports indicated extremely higher values, 50-270% over noninoculated controls. Even moderate yield increases (up to 20%) attributed to inoculation with *Azospirillum* are considered commercially valuable to modern agriculture, if obtained consistently. However, the incidence of positive results may not be frequent enough to enable commercialization of the bacterial preparation; negative or no-effect results of inoculation were rarely reported (Albrecht *et al.* 1981; Harris *et al.* 1989; Smith *et al.* 1984a, 1984 b). The reports of very high yield responses were frequently due to low-yielding control plots, resulting from undetermined field conditions (Jagnow 1987). Proper evaluation of "plant yield response" reports is difficult because of lack of statistical analysis of data combined over different growing seasons and locations, i.e., results of different experiments are analysed separately. In addition, many of these experiments could not be repeated properly as a result of the lack of essential technical details describing the methods. Okon (1985) evaluated the worldwide success of *Azospirillum* inoculation and concluded that positive effects on yield were obtained in approximately 65% of all field experiments. Yield increases due to inoculation were reported in 75 % of all experiments using summer cereals and only in 50% of the experiments using spring wheat (Smith *et al.* 1984b; Schank and Smith 1984). Recently, about 70-75% of all pot experiments in cotton and several vegetables resulted in yield increase (Bashan *et al.* 1989b).

Two basic variables that contribute to the complexity of plant yield response to inoculation are the plant cultivars, which often show differential response to inoculation (Bouton *et al.* 1979; Millet *et al.* 1986), and the level of N fertilization. The highest yield increases were obtained when the levels of N fertilization were suboptimal for maximum yield (Kapulnik *et al.* 1981 b; Lau-Wong 1987; Mertens and Hess 1984; O'Hara *et al.* 1987). Therefore, *Azospirillum* inoculation was considered a partial substitute for N fertilization. However, contradictory data (Bashan *et al.* 1989b; Millet and Feldman 1986) showed that yield was increased by inoculation even under high levels of N fertilization and that N can influence the number of bacteria in the rhizosphere (Kolb and Martin 1988).

The crucial problem of most greenhouse and field experiments conducted thus far is the inconsistent plant response to inoculation regardless of the plant species. Results have been erratic and success of a field experiment is never ensured (Patriquin *et al.* 1983). Numerous reports from popular agricultural literature and commercial companies indicate that identi-

cally designed field experiments, conducted simultaneously under similar environmental conditions, have failed to produce the expected similar yield results. Experiments failed to improve repeatability of yield response through agrotechnical-management, host-plant, bacterial-strain, or improved-inoculation techniques (Schank and Smith 1984). The development of a reliable and consistent inoculation technology determines whether the future of *Azospirillum* plant interaction will be used only as a biological model for fundamental studies of plant beneficial bacteria associations or will have a significant impact in future agricultural production. The problem may be overcome by a better understanding of the following factors: mode of action of *Azospirillum* in promoting plant growth; root colonization by *Azospirillum*; interaction and competition of *Azospirillum* with other microorganisms in the rhizosphere and the role of the host plant in these interactions; genetics of the bacteria-plant association and subsequent genetic manipulation of the bacteria; activity and survival of *Azospirillum* in the rhizosphere as affected by various environmental factors and including the inconsistency in plant response as a variable parameter in the statistical analysis.

Inoculation effects on root development

The most marked effects of *Azospirillum* inoculation on plants are the various morphological changes in the root system. These changes are directly related to inoculum concentrations: higher than optimal levels had inhibitory effects, while low bacterial doses had no effect. The optimal inoculum level for seeds or seedlings of many cereals, vegetables, and industrial crop plants was 10^5 - 10^6 cfu/mL (Bashan 1986c; Bashan *et al.* 1989b; Kapulnik *et al.* 1985 c; Okon and Kapulnik 1986; Smith *et al.* 1984b), for corn 10^7 cfu/mL (Fallik *et al.* 1988), and for tomato in vitro $>10^8$ cfu/mL (Hadas and Okon 1987). However, an inoculum concentration of 10^8 - 10^{10} cfu/mL usually inhibited root development (Bashan 1986c, 1990; Barbieri *et al.* 1988; Kapulnik *et al.* 1985c; Morgenstern and Okon 1987a). However, these bacterial concentrations have not revealed how many bacterial cells per seed or seedling are required to obtain plant response.

Positive effects of inoculation have been demonstrated on various root parameters, including increase in root length, particularly of the root elongation zone (Kapulnik *et al.* 1985b, 1985c; Kolb and Martin 1985; Levanony and Bashan 1989b; Sarig *et al.* 1988), increase in number and length of lateral roots, which increases the root volume (Barbieri *et al.* 1986, 1988; Kolb and Martin 1985; Morgenstern and Okon 1987a; Tien *et al.* 1979; Venkateswarlu and Rao 1983), increases in root dry weight (Hadas and Okon 1987; Kapulnik *et al.* 1981 a; Morgenstern and Okon 1987a; Schank *et al.* 1981; Umali-Garcia *et al.* 1980), increase in the number, density, and early appearance of root hairs (Hadas and Okon 1987; Kapulnik *et al.* 1985 c; Martin and Glatzle 1982; Morgenstern and Okon 1987 a; Umali-Garcia *et al.* 1980; Venkateswarlu and Rao 1983), increase in root surface area (Bashan 1986c; Fallik *et al.* 1988), enhanced cell division in the root meristem (Levanony and Bashan 1989b), changes in cell arrangements in the cortex (Kapulnik *et al.* 1985 c; Lin *et al.* 1983), and stimulation of root exudation (Heulin *et al.* 1987; Lee and Gaskins 1982). However, other studies clearly indicated a decrease in root length, mass, and volume, despite an increase in shoot growth parameters (Kucey 1988a; Murty and Ladha 1988; Reynders and Vlassak 1982) and no apparent change in the cell arrangement in the cortex (Levanony *et al.* 1989).

Such contradicting effects on roots by commonly available *Azospirillum* strains are apparently real since most of these morphological parameters are easily and accurately measured and were adequately analysed by reliable statistical methods. Thus, one can conclude that some other environmental factors, such as plant nutrition and irrigation, and (or) the plant genome may significantly determine the type and the magnitude of effects in any bacterial strain - plant cultivar association.

These remarkable morphological changes in the root system led to the hypothesis (Okon 1985) that improvement in plant growth and, finally, yield increase are due to a general, nonspecific improvement of the root system. A greater root volume, even without a change in its efficiency, could result in greater plant growth. Because numerous parameters are involved in this process and the nonspecific improvement of root growth has not yet been defined, this hypothesis can neither be confirmed nor disproved on the basis of the currently available data.

Root colonization by Azospirillum

Azospirillum can colonize roots externally and internally. In external colonization, the bacteria form mainly small aggregates, although many single cells may also be scattered on the root surface. These externally colonizing bacteria are embedded in the mucigel layer of the root surface (Bashan *et al.* 1986; Berg *et al.* 1979; Murty and Ladha 1987; Schank *et al.* 1979; Umali-Garcia *et al.* 1981). Both live and dead roots can be colonized (Bashan *et al.* 1986; Bashan and Levanony 1988a). In internal colonization, *Azospirillum* cells can colonize roots by penetrating into the root intercellular spaces (Patriquin and Döbereiner 1978; Levanony *et al.* 1989; Umali-Garcia *et al.* 1981).

Although *Azospirillum* can externally colonize the entire root system, it tends to preferentially colonize the elongation and root-hair zones (Bashan *et al.* 1986; Okon and Kapulnik 1986). In cereals, colonization is mainly on the root surface and very few bacteria are attached to the root hairs (Bashan and Levanony 1989b; Okon and Kapulnik 1986), whereas in rice, massive root-hair colonization was frequently observed (Murty and Ladha 1987). Penetration of *Azospirillum* into root hairs has been rarely detected and it is assumed that *Azospirillum* does not penetrate into intercellular root spaces through root hairs.

Scanning electron microscopic studies on several plant species have shown that *Azospirillum* cells are connected to the root surface and to each other within the bacterial aggregate by a network of fibrillar material (Bashan *et al.* 1986; Gafni *et al.* 1986; Hadas and Okon 1987; Okon and Kapulnik 1986; Patriquin *et al.* 1983; Umali-Garcia *et al.* 1980). The chemical nature of these fibrils is not fully defined, but there are preliminary indications that the fibrils contain proteinaceous compounds (Bashan and Levanony 1989b). Fibrillar attachment by the bacteria is primarily dependent on active bacterial metabolism; dead bacteria did not attach to roots, but live bacteria attached to dead plant material (Bashan *et al.* 1986; Gafni *et al.* 1986). Root surface attachment is relatively weak; slight rinsing of the roots released most of the bacteria (Bashan *et al.* 1986). Polar attachment of *Azospirillum* cells to roots was demonstrated (Patriquin *et al.* 1983; Whallon *et al.* 1985) and later confirmed (Levanony *et al.* 1989). However, detailed examination of this phenomenon revealed that it comprised only a minority of the cells. Most of the root surface was colonized by bacteria in a horizontal, thermodynamically more stable, position.

Azospirillum attachment to root surfaces occurs rapidly,

within seconds or minutes of bacterial exposure to the plant material. Most of the colonized areas on plant cells were saturated within 2 h after inoculation, varying with the bacterial growth phase and strain (Bashan and Levanony 1988b; Evers *et al.* 1988a, 1988b; Gafni *et al.* 1986; Umali-Garcia *et al.* 1980). Movement of *Azospirillum* along the root surface is minimal owing to formation of multistranded fibrils. These holdfast fibrils ensure vertical bacterial transfer by the growing root tip to deeper soil layers (Bashan and Levanony 1989a). The possibility of bacterial movement through the mucigel without attachment to root surfaces has not yet been explored.

The specific mechanism by which *Azospirillum* attaches itself to the roots remains unknown. Several physiological, environmental, nutritional, and chemical factors enhanced or suppressed *A. brasilense* attachment to the roots (Bashan and Levanony 1989b; Umali-Garcia *et al.* 1980). Lectin binding has been suggested as a possible mechanism (Tabary *et al.* 1984; Umali-Garcia *et al.* 1980) and recently it was speculated that agglutinins may be located in the fibrillar material, helping cell anchorage (Bashan and Levanony 1988b). However, these theories have not been further explored.

Azospirillum is capable of internal colonization of the intercellular spaces of the cortex (Levanony *et al.* 1989; Patriquin and Döbereiner 1978; Whallon *et al.* 1985). Claims of vascular colonization by a specific *A. lipoferum* strain detected by light microscopy (Patriquin and Döbereiner 1978) have not been confirmed by electron microscopy when strain *A. brasilense* Cd was used (Levanony *et al.* 1989). The internal bacterial population comprised most of the total root population of *Azospirillum* in wheat (Bashan *et al.* 1986), whereas in pearl millet, most of the *Azospirillum* population was concentrated on the root surface (Matthews *et al.* 1983). Internal root colonization does not always occur. In Kallar grass, massive *Azospirillum* surface colonization was detected, but an internal population was absent (Reinhold *et al.* 1986), and in sugarcane callus, *Azospirillum* colonized only the callus surface (Berg *et al.* 1979; Vasil *et al.* 1979).

Internal and external root colonization are apparently not associated with any morphological or structural changes in the cortex cells in wheat (Levanony *et al.* 1989), although changes in the outer shape of roots in other plant species were reported (Bashan *et al.* 1989b; Morgenstern and Okon 1987a). It was further noted that inoculation may alter cell arrangement in the cortex of wheat and *Setaria* roots (Kapulnik *et al.* 1985 c; Okon *et al.* 1983). This observation was based on limited microscopic observations and should be further studied, especially since this alteration has not been confirmed by a later study using the same *A. brasilense* strain (Levanony *et al.* 1989).

The mode of *Azospirillum* penetration into the intercellular spaces is unknown. Theories suggested so far have attributed it to (i) bacterial invasion via disrupted cortical tissues where branches of lateral roots emerged from the main roots (Patriquin and Döbereiner 1978; Matthews *et al.* 1983; Umali-Garcia *et al.* 1980, 1981), (ii) invasion through lysed root hairs and mechanical injuries occurring during plant growth, and (iii) direct penetration through the middle lamella following pectinolytic activity once the bacteria gain entry at splits of the epidermis-coated lateral root emergence (Umali-Garcia *et al.* 1980). Strains of this genus are known to produce some pectinases *in vitro* (Okon and Kapulnik 1986; Plazinski and Rolfe 1985a; Tien *et al.* 1981) and can inhibit the pectolytic activity of roots (Goldman and Langenbach 1987).

In conclusion, the mode of root colonization by *Azospirillum*

TABLE 1. Proposed mode of action of *Azospirillum* in plants

(A) Nitrogen fixation	
Evidence for	Evidence against
Accumulation of N compounds in inoculated plants	Nif ⁺ strains and Nif ⁻ mutants caused similar effects on plants
Bacterium has N ₂ -fixation ability	Positive effect on plants under conditions preventing N ₂ fixation
Strong nitrogenase activity in inoculated plants	Negligible nitrogenase activity in responding plants
Normal plant growth after inoculation with little or no N added	Amounts of fixed N ₂ are not sufficient to explain increase in plant yield
Incorporation of 51.8% of plant N from N ₂ following inoculation	
(B) Plant hormones	
Experimental evidence for	No experimental evidence for
Production of several plant hormones by the bacteria <i>in vitro</i>	Effect of hormone mutants
Mimic of plant response towards inoculation by hormone application	Change in hormone balance in the roots
Hormone overproducing mutants caused more pronounced effect on plant growth	Relation between hormonal effects and production of an improved plant
Indication that inoculation changes the amount of hormones in the plant	
(C) General improvement in plant growth and mineral uptake	
Experimental evidence for	No experimental evidence for
Enhancement and accumulation of minerals in plant foliage	Effect of mineral uptake mutants
Enzymatic activities related to ion transport in plant foliage	Enzymatic activities related to mineral and water uptake in the roots
Improvement of many growth parameters	Enhancement of mineral uptake by most strains
Improvement of water uptake	
Partial substitution of N fertilization	
Increase in proton efflux of inoculated plants	
(D) Bacterial nitrate reductase	
Experimental evidence for	No experimental evidence for
The bacteria have an active enzyme	Differential changes between activities in root and foliage
NR ⁻ mutants are less effective on plants than NR ⁺ wild type	Interaction with N ₂ fixation
NR ⁻ mutants have N ₂ -fixation ability	
NR ⁻ mutants cause nitrate accumulation in the foliage	

may vary, depending on the bacterial strain, plant species, environmental conditions, and other unidentified factors. The interaction between all these variables creates different degrees and patterns of root colonization, different population sizes, and different colonization sites. The main colonization sites, for most plant species studied, are the elongation and root-hair zones. Root surface colonization supported by fibrillar anchoring is one of the proposed features of *Azospirillum* root colonization. Internal root colonization has been demonstrated only in a few plant species and is presumably unique to certain plant-*Azospirillum* interactions.

Proposed mode of action of Azospirillum on plant growth

The principal mechanism by which *Azospirillum* enhances plant growth is undetermined. However, several possible modes of action have been proposed (Table 1): N₂ fixation, which contributes N to the plant; hormonal effects, which alter plant metabolism and growth; general improvement in the growth of the entire root system possibly related, but as yet unproven, to hormonal changes, resulting in enhanced mineral and water uptake; and bacterial nitrate reductase activity in roots, which increases nitrate accumulation in inoculated plants.

Nitrogen fixation by *Azospirillum*

All wild-type *Azospirillum* strains fix atmospheric nitrogen efficiently either as free-living bacteria or in association with plants and participate in several transformations in the nitrogen cycle (Heulin *et al.* 1989; Hurek *et al.* 1988; Tarrand *et al.* 1978). Following inoculation, there is an increase in the total N of shoots and grains of inoculated plants (Baldani *et al.* 1983, 1987; Boddey *et al.* 1986; Cohen *et al.* 1980; Hegazi *et al.* 1983; Kapulnik *et al.* 1981 a, 1983, 1985b; Mertens and Hess 1984; Nur *et al.* 1980a; O'Hara *et al.* 1981; Pacovsky *et al.* 1985b; Rennie *et al.* 1983; Schank *et al.* 1981; Wani *et al.* 1985; Warembourg *et al.* 1987; Yahalom *et al.* 1984). Therefore, N₂ fixation was naturally the first major mechanism of action suggested for the enhancement of plant growth by *Azospirillum*. Incorporation of atmospheric nitrogen into the host plant by *Azospirillum* was evaluated mainly by the acetylene reduction assay (which simulates N₂ fixation) (Van Berkum and Bohlool 1980). However, conclusive proof that plants derive some of their N from the atmosphere came from the use of isotopic ¹⁵N₂ incorporation and ¹⁵N-dilution techniques. This subject has recently been adequately reviewed (Boddey 1987; Boddey and Döbereiner 1988).

Evidence that N₂ fixation contributes to the N balance of plants is based on the common observation of an increase in the nitrogenase activity within inoculated roots (Berg *et al.* 1980; Cohen *et al.* 1980; Hegazi *et al.* 1983; Hess 1982; Kapulnik *et al.* 1981 b; Okon *et al.* 1983; Rao and Rajamamohan Rao 1983; Yahalom *et al.* 1984). This well-documented enzymatic activity is of sufficient magnitude to account for the increase in total N yield of inoculated plants if all the fixed N is incorporated into the plants (Sarig *et al.* 1984; Mertens and Hess 1984). Thus, on one hand, inoculation of wheat and maize has indicated that 5-10% (Kucey 1988a) and up to 18% (Rennie 1980; Rennie *et al.* 1983; Rennie and Thomas 1987) of the plant N was derived from N₂ fixation. In addition, inoculated plants grew normally with only a partial amount of the N fertilizer usually required for such growth (Kapulnik *et al.* 1981 b; Millet and Feldman 1986; Nur *et al.* 1980a). On the other hand, studies have shown low or even negligible nitrogenase activity in plants positively responding to inoculation (Kapulnik *et al.* 1985a; Lethbridge and Davidson 1983; Venkateswarlu and Rao 1983). Furthermore, of all the N fixed by the bacteria, less than 5% was incorporated into the host plants (Eskew *et al.* 1981; Okon *et al.* 1983). These amounts of fixed N are insufficient to explain total increases in N content of inoculated plants. Finally, high N fertilization levels, which inhibit N₂ fixation, did not eliminate the plant response to inoculation (Avivi and Feldman 1982; Bashan *et al.* 1989b, 1989c; Kapulnik *et al.* 1981b, 1982, 1983; Mertens and Hess 1984; Millet and Feldman 1986; Pal and Malik 1981; Rai and Gaur 1982; Reynders and Vlassak 1982).

The ultimate control to distinguish the contribution via N₂ fixation from other effects of bacterial inoculation is to use Nif mutants incapable of fixing N₂ but otherwise isogenic in respect to parental strains. Inoculation of cereals with Nif mutants caused the same effects as the parental strains (Barbieri *et al.* 1986; Morgenstern and Okon 1987a; O'Hara *et al.* 1981). Recently, tomato seedlings responded to inoculation with the site-directed Nif mutant of *A. brasilense* Cd in a manner similar to their response to the wild type (Bashan *et al.* 1989c). This indicates that the plant response was caused by factors other than N₂ fixation. Nevertheless, the possibility remains that N₂ fixation contributes to the plant small amounts of N,

which may be important in critical stages of plant development, such as the reproductive and the tillering stages. In conclusion, N₂ fixation occurs in many *Azospirillum* associations; the basic question in each inoculation system remains as to how much N is contributed to the plant by the bacteria and under what growth conditions, a factor that is highly variable and erratic.

Hormonal effects of *Azospirillum* on plants

Many *Azospirillum* strains produce several plant hormones in liquid culture. The major hormone produced is indole-3-acetic acid (IAA) (Barbieri *et al.* 1986; Fallik *et al.* 1989; Hartmann *et al.* 1983; Jain and Patriquin 1985; Kolb and Martin 1985; Ruckdäschel *et al.* 1988; Tien *et al.* 1979; Venkateswarlu and Rao 1983). Other hormones, detected at much lower, but biologically significant levels were indolelactic acid (Tien *et al.* 1979), indole-3-butyric acid (IBA) (Fallik *et al.* 1989), indole-3-ethanol, indole-3-methanol (Crozier *et al.* 1988), unidentified indole compounds (Hartmann *et al.* 1983), several gibberellins (Bottini *et al.* 1989; Tien *et al.* 1979), abscisic acid (ABA) (Kolb and Martin 1985), and cytokinins (Horemans *et al.* 1986; Tien *et al.* 1979).

Plant hormones affect the N₂ fixation capability of *Azospirillum* (Christiansen-Weniger 1988). Applications of external hormones, either synthetic or purified from bacterial culture, to seedlings completely reproduced the effects of *Azospirillum* on root development and morphology (Harari *et al.* 1988; Kucey 1988b; Tien *et al.* 1979; Zimmer and Bothe 1988). In particular, it caused changes in root length (Kolb and Martin 1985; Morgenstern and Okon 1987a), produced more root hairs (Kapulnik *et al.* 1985c; Morgenstern and Okon 1987a) and branching of root hairs (Jain and Patriquin 1984), produced more lateral roots (Barbieri *et al.* 1986; Tien *et al.* 1979), and enhanced the rates of cell division and differentiation in meristematic tissues (Fallik *et al.* 1989). An *Azospirillum* strain and a mutant which overproduced IAA in culture strongly affected plant root morphology (Jain and Patriquin 1985; Kolb and Martin 1985), whereas mutants that failed to produce IAA in culture had no effect on root morphology (Barbieri *et al.* 1986). Inoculation with *Azospirillum* improved the hormonal balance of a hormone-defective mutant of wheat (Inbal and Feldman 1982). Recently, higher amounts of IAA and IBA were identified in inoculated maize roots than in noninoculated plants (Fallik *et al.* 1989).

These results provide indirect evidence for the involvement of *Azospirillum* in hormonal regulation of the plant. However, before it is firmly established that hormonal effects are the principal mechanism by which *Azospirillum* promotes plant growth, the following should be investigated and supported by evidence (i) Do changes in root morphology have a direct effect on growth of a plant having a higher yield, i.e., these changes should not be temporary effects observed exclusively at the seedling stage. Effects on seedling root morphology may fade or disappear when the population of *Azospirillum* in the root sharply declines at mature stages of plant growth. (ii) Irreversible IAA-deficient mutants that are isogenic to the parental strains but are incapable of producing morphological effects on roots need to be produced. (iii) Changes in the hormone balance of soil-grown plants of several plant species must be clearly demonstrated. (iv) Since hormonal effects in plants depend on changes in equilibrium between different hormones, such changes should be demonstrated in inoculated plants.

In conclusion, the study of the hormonal effects of *Azospiril-*

lum is a promising avenue, which should be further explored. However, more direct, rather than circumstantial, evidence is needed before the hypothesis is accepted.

Improvement of root development, mineral uptake, and plant-water relationships by Azospirillum

In addition to increasing (Kapulnik *et al.* 1981 *a*, 1985 *c*) or decreasing (Kucey 1988 *a*) many root parameters, plant inoculation with *Azospirillum* affected many foliage parameters. These changes were directly attributed to positive bacterial effects on mineral uptake by the plant. Enhancement in uptake of NO_3^- , NH_4^+ , PO_4^{2-} , K^+ , Rb^+ , and Fe^{2+} by *Azospirillum* (Barton *et al.* 1986; Jain and Patriquin 1984; Kapulnik *et al.* 1985 *b*; Morgenstern and Okon 1987 *b*; Murty and Ladha 1988; Lin *et al.* 1983; Sarig *et al.* 1988) was proposed to cause an increase in foliar dry matter and accumulation of minerals in stems and leaves. During the plant reproductive period, these minerals could have been transferred to the panicles and spikes and finally resulted in a higher yield.

Increased mineral uptake by plants has been suggested to be due to a general increase in the volume of the root system and not to any specific enhancement of the normal ion uptake mechanism (Morgenstern and Okon 1987 *b*; Murty and Ladha 1988). It has been further suggested that *Azospirillum* inoculation may promote availability of ions in the soil by helping the plant scavenge limiting nutrients (Lin *et al.* 1983); this may explain accumulation of N compounds in the plant without any apparent N_2 fixation. The plant may take up N more efficiently from the limited supply in the soil, resulting in a lower requirement of N fertilization to attain a certain yield. Supporting evidence for increased mineral uptake by inoculated roots is provided by enhancement in proton efflux activity of wheat roots inoculated with *Azospirillum* (Bashan 1990; Bashan *et al.* 1989 *a*). It is well known that proton efflux activity is directly related to the balance of ions in plant roots.

In addition to improved mineral uptake, *Azospirillum* inoculation improved water status in stressed sorghum plants. Inoculated plants were less water stressed, having more water in their foliage, higher leaf water potential, and lower canopy temperature than noninoculated plants. Total extraction of soil moisture by *Azospirillum*-inoculated plants was greater and water was extracted from deeper layers in the soil profile. Therefore, sorghum yield increase in inoculated plants was attributed primarily to improved utilization of soil moisture (Sarig *et al.* 1988).

It is likely that improved mineral and water uptake play a vital role in *Azospirillum*-plant association. However, the descriptive data presented so far have not shown whether these improvements are the cause or the result of other mechanisms such as changes in plant hormonal balance. Furthermore, the wide range of enzymatic activities related to these phenomena were poorly studied and no apparent evaluation of *Azospirillum* mutants deficient in induction of mineral and water uptake by plants has been made. Finally, it should be emphasized that very few strains have been evaluated and it is doubtful if all *Azospirillum* strains possess these abilities. There is evidence that some *A. brasilense* strains failed to improve uptake of several ions but nevertheless improved plant growth (Bashan *et al.* 1990).

Azospirillum nitrate reductase in plants

An alternative to N_2 fixation as an explanation for N accumulation following *Azospirillum* inoculation of wheat plants only is the bacterial nitrate reductase (NR) theory. NR

activity of wheat leaves was decreased by inoculation with some *Azospirillum* strains. Inoculation of plants with NR⁻ mutants resulted in minimal plant response concomitant with an increase in leaf NR compared with inoculation with the parental NR⁺ strain (Ferreira *et al.* 1987). Inoculation of field-grown plants with *A. brasilense* Sp-245 and its NR⁻ mutant confirmed that the mutant was significantly less effective in increasing yield than the parental strain (Boddey and Döbereiner 1988). These phenomena indicate that the effect of some *Azospirillum* strains on plants is not solely via N_2 fixation (both the parental and the mutant strains have this ability) but rather due to an increase in nitrate assimilation. The parental strain aided nitrate reduction in the roots and thus decreased nitrate translocation to the leaves, while inoculation with the NR⁻ mutant caused direct translocation and reduction of nitrate in the plant foliage.

This theory might partially explain the observation of increased N accumulation in shoots because the unaffected N_2 fixation ability may also contribute N to the plants in addition to NR activity.

Specificity and variability in Azospirillum

One of the most controversial questions concerning the *Azospirillum* association is that of plant-strain specificity as it affects plant growth. Specific differences between responses of C_3 and C_4 plants was suggested; *A. lipoferum* was the predominant species colonizing C_4 plants and *A. brasilense* was the predominant species associated with C_3 plants in tropical zones (Baldani and Döbereiner 1980; Baldani *et al.* 1986). Similar host plant preference was found in temperate zones (Hahtela *et al.* 1981; Lamm and Neyra 1981). When the bacterial species was inoculated onto the respective plant species, success was more frequent when the proper plant bacterial species combination was used (Baldani *et al.* 1983, 1987; Pereira *et al.* 1988; Reynders and Vlassak 1982). Different morphogenetic effects on wheat root hairs have been attributed to different strains (Jain and Patriquin 1984; Patriquin *et al.* 1983). Specificity can occur at the plant cultivar level; only a few of many tested plant cultivars responded to inoculation with a given strain of *Azospirillum* (Bouton *et al.* 1979; Millet *et al.* 1986; Wani *et al.* 1985). On the other hand, studies with *A. brasilense* Cd/Sp7, the type strain for *A. brasilense* commonly used as a reference, indicated no plant specificity. This strain positively affected many winter and summer cereals, vegetables, and industrial crop plants (Bashan *et al.* 1989 *b*; Kapulnik *et al.* 1981 *c*, 1983; Smith *et al.* 1984 *b*; Yahalom *et al.* 1984).

The main difficulties in assessing *Azospirillum* specificity are (i) lack of comparison tests for strains obtained from different sources on certain host plants and (ii) the limited number of strains tested thus far. Furthermore, there are indications that specificity is not at the bacterial species level but rather at the strain level. A quick screening test to assess plant - bacterial strain association is essential to predict successful plant-bacteria combinations prior to testing in the field.

In conclusion, like *Rhizobium* strain selection, the importance of *Azospirillum* strain selection is a crucial consideration in determining specificity. Numerous strains must be isolated from different origins, characterized, and tested under various environmental conditions for each plant species.

Interaction of Azospirillum with other soil-rhizosphere microflora

After application to the soil, *Azospirillum* cells must adapt quickly to the ever-changing conditions of the plant rhizo-

sphere, including frequent changes in nutrient availability and interaction with indigenous microorganisms competing for those nutrients. These interactions could be antagonistic or synergistic or a predator-prey type, where *Azospirillum* cells possibly serve as available prey for the always nutrient-deficient micro- and macro-fauna.

The most studied microbial interaction of *Azospirillum* involves *Rhizobium*. On one hand, greenhouse and field trials have shown that a simultaneous inoculation of *Azospirillum* and *Rhizobium* or addition of *Azospirillum* to naturally rhizobia colonized legumes resulted in increased N₂ fixation, greater number of nodules, and eventually a yield increase (Iruthayathas *et al.* 1983; Rai 1983; Sarig *et al.* 1986). On the other hand, laboratory studies of mixed inoculation resulted in conflicting data; *A. brasilense* applied before *Rhizobium* increased nodule formation in the non-root-hair zone, even when *Rhizobium* was applied at sub-nodule-formation concentration. This increased susceptibility of legumes to *Rhizobium* infection was attributed to a stimulating effect of hormones excreted by *Azospirillum*. These hormones induced formation of a larger number of epidermal cells that differentiated into root hairs, favored for primary infections by *Rhizobium* (Schmidt *et al.* 1988; Yahalom *et al.* 1987). However, negative effects of mixed inoculation that prevent clover nodulation occurred especially when *Azospirillum* cells outnumbered *Rhizobium* cells in the mixture (Plazinski and Rolfe 1985b, 1985c, 1985d). Apparently, root-hair colonization by *Azospirillum* blocked infection sites of *Rhizobium*. These contradicting reports indicate that the exact interaction between *Azospirillum* and *Rhizobium* should be first defined before any conclusions can be drawn.

In contrast to the unclear relations that prevail between *Azospirillum* and *Rhizobium*, synergistic effects of mixed inoculation of *Azospirillum* and vesicular-arbuscular (VA) mycorrhizal fungi resulted in significant increase in growth and P content of plants. This dual inoculation could completely replace application of N and P fertilizers (Barea *et al.* 1983; Pacovsky 1988; Pacovsky *et al.* 1985 a; Subba Rao *et al.* 1985a, 1985b) and enhance infection of plants by mycorrhizal fungi (Barea *et al.* 1983). However, the shortage of data obtained thus far can only encourage further research on this interaction since the basic nature of the interaction among bacteria, fungi, and plant has yet to be described.

A different synergism resulting from *Azospirillum* inoculation is enhancement of straw decomposition by cellulolytic bacteria. Mixed bacterial cultures either in liquid culture or in soil were more efficient in decomposing straw than cellulolytic bacteria alone. The microbial interaction also enhanced N₂ fixation by *Azospirillum*. Cells of both species were found in close proximity in the straw, facilitating the mutually beneficial association (Halsall and Gibson 1985, 1986; Halsall and Goodchild 1986; Halsall *et al.* 1985; Markus and Kramer 1988). Such synergism could provide an indirect explanation for the highest known yield increase by *Azospirillum*, which occurred in soil amended with straw (Hegazi 1988; Hegazi *et al.* 1983). Nevertheless, similar to *Azospirillum* interaction with VA mycorrhizal fungi, this interaction should be first defined in order to evaluate its potential.

The above interactions represent only a minute fraction of *Azospirillum* relationships with other rhizosphere microorganisms. The interactions with common, indigenous, predominant species of pseudomonads, azospirilla, and *Bacillus* populations (10⁶-10⁹ cfu/g) have been scarcely studied. *Azospirillum* failed to serve as a biocontrol agent (Hadas and Okon 1987) and

application of *Pseudomonas* sp., which outnumbered *Azospirillum*, inhibited its beneficial effect on plants (Fallik *et al.* 1988). Many azospirilla produced bacteriocins, which surprisingly also inhibited other *Azospirillum* species (Oliviera and Drozdowicz 1981, 1987; Skorupska *et al.* 1985). These unidentified bacteriocins disappeared after application to several soils but persisted in a soil low in organic matter and clay minerals (Oliveira and Drozdowicz 1988). The ecological significance of *Azospirillum* bacteriocins is unknown.

In vitro antagonistic effects on *Azospirillum* by some soil microorganisms, especially streptomycetes and fungi, have been shown (Drozdowicz and Ferreira Santos 1987; Kulinska and Drozdowicz 1983; Zuberer and Roch 1982). Many strains of *Azospirillum* are highly resistant to a wide range of antibiotics (Bashan and Levanony 1985; Baldani *et al.* 1986; Döbereiner and Baldani 1979; Horemans *et al.* 1987) and isolation of antibiotic-resistant *Azospirillum* strains is a simple task. Nevertheless, the effect of antibiotic-producing microorganisms on *Azospirillum* in the soil has not been studied. Taking advantage of the natural antibiotic resistance of *Azospirillum*, improvement of root colonization by *Azospirillum* and subsequent plant yield increase in wheat plants grown in pots was obtained by temporarily depressing the competing natural microflora, using inhibiting substances to which *Azospirillum* is resistant. Although this inhibition lasted only for a few weeks, it gave a significant advantage to *Azospirillum* cells in root colonization (Bashan 1986a). Obviously, this practice cannot be directly adapted to the field as a vast amount of soil, naturally occupied by the plant root system, would have to be treated, a practice which would entail soil pollution and high cost. However, this approach should be further evaluated using cheap licence agrochemicals.

The size of *Azospirillum* populations has been estimated as 1-10% of the total rhizosphere population (Okon 1985). Routine counts of *Azospirillum* in the rhizosphere indicate that this evaluation is an overestimate. In plants growing in soil, *Azospirillum* populations are relatively small; the highest population was recorded in summer cereals in Brazil (10⁶-10⁸ cfu/g (Baldani *et al.* 1983)). Usually *Azospirillum* populations are much smaller, reaching an average population size of 10³-10⁶ cfu/g in wheat (Balandreau 1986; Bastian and Wolowelsky 1987; Bastian *et al.* 1987; Negi *et al.* 1987). On several occasions an even smaller rhizosphere population was reported (Albrecht *et al.* 1983; Harris *et al.* 1989; Smith *et al.* 1984b). Therefore, a more accurate estimation of *Azospirillum* populations should be within the range of 0.001-1 % of the total rhizosphere population (O'Hara *et al.* 1981).

There are a relatively large number of studies related to the microbial interactions of *Azospirillum*. However, the data are highly fragmented, spread on numerous different aspects, and are of a descriptive nature. All these factors make it unreliable to predict the most important interactions. Because *Azospirillum* accounts for only a small fraction of the rhizosphere population, many other bacterial species (Balandreau 1986) should affect *Azospirillum* in the rhizosphere and should be considered when *Azospirillum* is applied to soil.

Azospirillum as a competitor in the rhizosphere.

A current analysis of the intensive studies on physiological and biochemical properties of *Azospirillum* revealed that this genus has no unique or distinguishing feature compared with other rhizosphere bacterial genera (Pedrosa 1988); yet single species or even individual strains of *Azospirillum* possess

characteristics that are normally found only in several different bacterial species. This creates an organism that is capable of successfully competing in many plant species rhizospheres despite the overwhelming population of indigenous rhizosphere microflora and that can colonize plant roots, under field conditions, from an extremely low bacterial inoculum level (10^6 cfu/m²).

By definition, all wild-type *Azospirillum* are N₂ fixers, which can fully support their N requirements (Tarrand *et al.* 1978). Since their nitrogenase is not protected from oxygen, they must fix nitrogen under microaerophilic conditions (Hartmann and Hurek 1988) and some strains exhibit strong aerotactic response (Barak *et al.* 1982a, 1982b; Das and Mishra 1984; Del Gallo *et al.* 1988; Hurek *et al.* 1987; Okon *et al.* 1980; Reiner and Okon 1986). *Azospirillum* is nutritionally versatile. Many alternative metabolic pathways allow it to consume a wide variety of organic acids, sugars, and amino acids available in the rhizosphere from plant and microbial sources (Okon 1982). Nearly all strains are highly motile, both in vitro and in the soil, and they possess significant chemotactic ability towards an array of compounds (Barak *et al.* 1983; Bashan 1986d; Bashan and Levanony 1987; Heinrich and Hess 1985; Mandimba *et al.* 1986; Okon *et al.* 1980; Reinhold *et al.* 1985, 1988b). In normal growth, *Azospirillum* accumulates large amounts of the storage material poly- β -hydroxybutyrate, which can be stored for use later or during long-term survival (Berg *et al.* 1979; Levanony *et al.* 1989; Tal and Okon 1985). Under stress conditions the bacteria are capable of cyst and floc or aggregate formation, both of which significantly improve survival (Bleakley *et al.* 1988; Lamm and Neyra 1981; Madi *et al.* 1988; Murray and Moyles 1987; Sadasivan and Neyra 1985, 1987). Two species, *A. amazonense* and *A. halopraeferans*, have resistance to acids, or to salt and high temperatures (Hartmann 1988; Reinhold *et al.* 1988a), which improves *Azospirillum* survival in the rhizosphere. Unlike the limited survival capacity of *Azospirillum* in the soil, all studies on *Azospirillum* survival in its natural microecological niche concluded that it survives for prolonged periods of time. Only the size of the population varied. *Azospirillum* strains survived in roots during the winter season under temperate conditions but at low levels (De Coninck *et al.* 1988; Harris *et al.* 1989; Horemans *et al.* 1988). Survival in roots was shown during the entire growth season of cereals (Bashan *et al.* 1987; Bashan and Levanony 1987; Jagnow 1982) and rice (Nayak *et al.* 1986). Inability of *Azospirillum* populations to recover to original size after winter has been shown only under temperate conditions (Harris *et al.* 1989; Horemans *et al.* 1988). The ecological importance of this long-term survival ability has yet to be determined; whether *Azospirillum* can reestablish its population to the extent that it can colonize the next season's crop is not known. Long-term survival and reinoculation ability are obvious obstacles to commercial exploitation of *Azospirillum* technology.

In contrast to some superior features, *Azospirillum* is parasitically colonized by *Bdellovibrio* sp. in soil (Germida 1987) and serves as a prey for soil protozoa. In conclusion, *Azospirillum* has a potential as a successful competitor as well as a good survivor in the rhizosphere. The various phases of *Azospirillum* in the rhizosphere are shown in Fig. 1.

Interaction of *Azospirillum* with soil particles

Inoculation of plants with *Azospirillum* is usually done by applying the bacteria to the soil near the germinating seedling (Bashan 1986b; Okon and Hadar 1987). During this process

the bacterium is exposed to natural physical forces and interactions that prevail between soil bacteria and soil particles. To overcome these barriers and colonize plant roots, *Azospirillum* cells must create a substantial physical force to allow movement through the soil (Bashan 1986d; Bashan and Levanony 1987). Few studies have addressed the interaction of *Azospirillum* with soil particles. *Azospirillum* cells were usually irreversibly adsorbed by the upper fraction of the soil profile in a charge-charge interaction mainly with clays and organic matter. Soil physical and chemical conditions such as pH, flooding, dry regime, and availability of bacterial chemoattractants greatly affect adsorption of *Azospirillum* to soil to different extents (Bashan and Levanony 1988c; Horemans *et al.* 1988). Attachment of *Azospirillum* to pure sand, which lacked clays and organic matter, was weak and accomplished by a network of protein bridges produced between the bacteria cell and the quartz particles. Protein-bridge formation was mainly controlled by nutrient availability (Bashan and Levanony 1988d).

Most *Azospirillum* strains are rhizosphere bacteria; however, numerous strains occur in the soil. *Azospirillum* was originally isolated from sandy soil (Beijerinck 1925); however, bacteria resembling *Azospirillum* occur only in alluvial and not in sandy soils in Somali (Favilli *et al.* 1988). Low levels of *Azospirillum* persist in many Belgian fields regardless of soil texture (De Coninck *et al.* 1988; Horemans *et al.* 1988), whereas high indigenous levels of *Azospirillum* are common in tropical soils (Döbereiner 1988; Döbereiner *et al.* 1976; Patriquin *et al.* 1983). *Azospirillum* inocula survived poorly in soil in the absence of plants both in the field and in the greenhouse, and almost disappeared after less than 15 days (Albrecht *et al.* 1983; Bashan and Levanony 1987, 1988c; Horemans *et al.* 1988; Smith *et al.* 1984b). Nevertheless, a fraction of the population survived in the soil over prolonged periods if stress conditions did not prevail (Hegazi 1988; Gemida 1986; Jagnow 1982; Sadasivan *et al.* 1986a; Schank *et al.* 1985).

In conclusion, although *Azospirillum* is a typical rhizosphere bacterium and no important bacterial growth phase in the soil per se has yet been found, it shares some of its soil adsorption features with other common soil bacteria. Despite its instant adsorption to soil particles upon inoculation, *Azospirillum* can overcome it by its self-motility through the soil to its target plant (Bashan 1986d).

Genetics and immunology of *Azospirillum*

Genetic analysis of *Azospirillum* has been restricted mainly to the nitrogenase structural genes (*nifHDK*) (Bazzicalupo *et al.* 1987; Bozouklian and Elmerich 1987; Fischer *et al.* 1986; Singh and Klingmüller 1986). These genes are the only *nif* genes that show homology to the *Klebsiella pneumoniae nif* genes. This homology facilitated cloning and characterization of these genes (Fahsold *et al.* 1985; Jara *et al.* 1983; Quiviger *et al.* 1982; Singh and Klingmüller 1985). On the other hand, very little is known about *Azospirillum* genetics as related to its association with plants (Elmerich 1984). The main difficulties in *Azospirillum* genetic analysis are shortage of *Azospirillum* mutants, together with the lack of an indigenous genetic recombination system (conjugation or transduction). To overcome this latter barrier, many studies have used the advantage that *Azospirillum* harbors large cryptic plasmids (Pampaluna *et al.* 1988; Skorupska *et al.* 1985). Although there is no uniformity between plasmid patterns in different strains (Elmerich and Franche 1982; Fani *et al.* 1986; Singh and Wenzel 1982), these plasmids are assumed to encode function for plant-bacterial associations like those in *Rhizobium*.

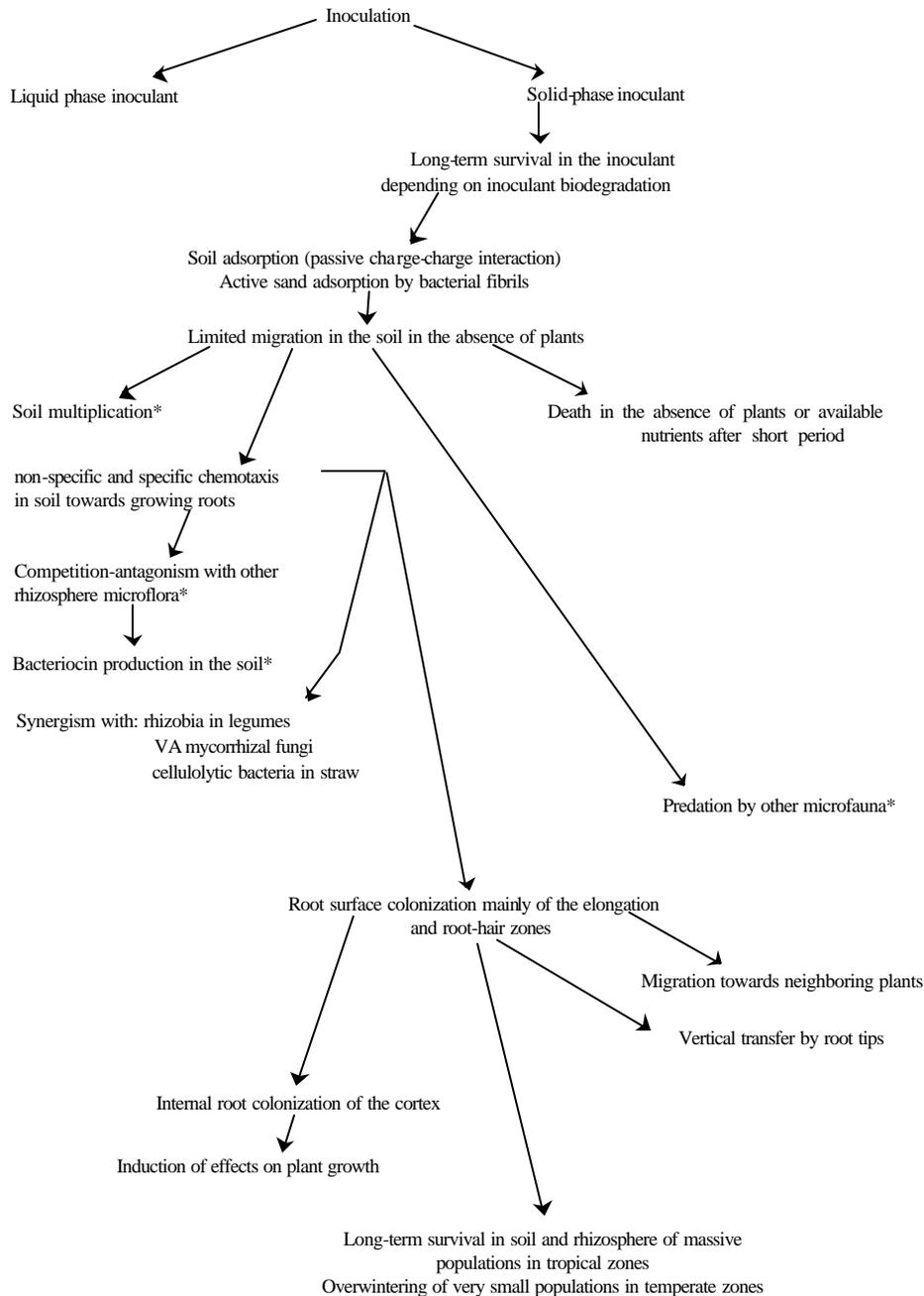


FIG. 1. Phases of *Azospirillum* in the rhizosphere. *, indications only.

An alternative approach to study the association was use of site-directed transposon mutagenesis (Abdel-Salam and Klingmüller 1987; El-Khawas and Klingmüller 1988; Singh 1982; Singh and Klingmüller 1986; Vanstoekem *et al.* 1987, 1988). Many Nifmutants of *Azospirillum* have been isolated by different methods (Barbieri *et al.* 1986; de Araujo *et al.* 1988; Jara *et al.* 1983; Pedrosa and Yates 1984; Singh and Klingmüller 1986, 1988; Vanstoekem *et al.* 1987). Nif mutants induced by site-directed mutagenesis can yield conclusive results regarding nitrogenase participation in *Azospirillum* associations. As a result of the *nif* deletion such mutants cannot revert. Recently, such a Nif deletion mutant of *A. brasilense*

Cd has been shown to have positive effects on tomato seedlings similar to those of the wild-type strain, indicating no contribution of nitrogenase activity to this association (Bashan *et al.* 1989c).

Different genetic techniques showed homology to *nod* (nodulation) and *hsc* (specificity) of *Rhizobium* and to *Agrobacterium* chromosomal virulence genes in *Azospirillum*. However, it is premature to conclude that these genes are functioning in *Azospirillum* (Elmerich and Franche 1982; Elmerich *et al.* 1987; Fogher *et al.* 1985; Galimand *et al.* 1988; Piana *et al.* 1988; Waelkens *et al.* 1987). IAA-overproducing and IAA-deficient mutants have been isolated (Abdel-Salam and Kling-

müller 1988; Barbieri *et al.* 1986, 1988; Hartmann *et al.* 1983), but their role in the plant-bacteria association has not yet been determined.

In conclusion, transposon mutagenesis has been shown to be a powerful tool in genetic analysis of *Azospirillum* since it enables the generation of large number of transposon-tagged mutants differing in different genes. However, genetic understanding of *Azospirillum* is not yet sufficient to effect major improvement in *Azospirillum* inoculation technology.

Immunological study of *Azospirillum* is one of the most neglected areas in *Azospirillum* research. A few studies have been conducted using polyclonal antibodies. Very little is known about *Azospirillum* antigens. Specific antigens for *A. brasilense* Cd were found in its exopolysaccharide and flagella (Levanony and Bashan 1989a), and different antigens were found in the different flagella types (Hall and Kreig 1984). Different species of this genus share common antigens and, therefore, extensive cross-reaction occurs among many strains (Dazzo and Milam 1976; De-Polli *et al.* 1980; Koslak and Bohlool 1983).

These studies reveal that specific polyclonal antibodies can be successfully used in rhizosphere studies for detection and enumeration of *Azospirillum*. *Azospirillum* cells were qualitatively identified in the rhizosphere by a fluorescent antibody technique (Schank *et al.* 1979), peroxidase-antiperoxidase labelling (Matthews *et al.* 1983), and by several other immunotechniques (Ladha *et al.* 1982). *Azospirillum brasilense* Cd has been specifically quantified in the rhizosphere of several cereals by employing indirect and competition enzyme-linked immunosorbent assays (ELISA; Levanony *et al.* 1987) and by an assay using avidin-biotin complex incorporated into the ELISA procedures (Levanony and Bashan 1990). This strain was further identified in the cortex of wheat roots, using an immuno-gold labelling technique (Levanony *et al.* 1989). The accuracy and usefulness of the immuno-techniques proved to be an essential practical tool in rhizosphere research despite the lack of basic immunological knowledge about *Azospirillum*.

Agrotechnical aspects: inoculants and interaction with pesticides

An important objective of understanding the *Azospirillum* system is commercial application of the bacteria in agricultural systems of both modern and developing countries. Astonishingly, very little has been published concerning the agrotechnical aspects of this system, i.e., potential effects of inoculation with *Azospirillum* in conjunction with different chemical compounds applied in a commercial field. Presumably, this type of data, which was expected to be developed by research and development companies, has not been published.

Even after establishing the best *Azospirillum*-plant combination, the problems of successful application in commercial crop production still prevail. Bacteria should reach the root even if the root system is widely spread; bacterial inoculation should be at the precise time needed by the plant (Bashan 1986c); inoculation techniques should be practical, economical, and easy to accomplish for the farmer; the formulated product should deliver sufficient inoculum to the plant, must be competitive with existing commercial standards, and must possess a long shelf life.

To date, only a few different methods are used to inoculate with *Azospirillum*. The simplest inoculation method is by application of bacteria in liquid suspension either directly to the soil or to the seeds. This technique was used in numerous

greenhouse and field experiments (Albrecht *et al.* 1981; Fallik *et al.* 1988; Millet and Feldman 1986; Reynders and Vlassak 1982; Smith *et al.* 1984) but is inadequate because *Azospirillum* survives poorly in soil in the absence of a carrier. More reliable procedures use various organic carriers (Okon 1985; Sadasivam *et al.* 1986b). The best yield results have been obtained from peat suspensions dripped into the sowing furrow or by spreading granular peat inoculant at the time of sowing (Okon and Hadar 1987). These practical inoculants cannot provide some of the requirements of a good inoculant owing to uncontrolled bacterial release and several technical difficulties (Bashan 1986b), and they thus probably result in inconsistent yield results. A different approach using encapsulated freeze-dried bacteria in dry alginate beads overcomes some of the problems of liquid and peat inoculants and fulfills many of the requirements for a good practical inoculant. It is dry, synthetic, simple to use, uniform, biodegradable by soil microorganisms, and nontoxic in nature, contains a large uniform bacterial population, provides for the slow release of the bacteria for long periods, and may be produced on a large scale (Bashan 1986b; Bashan *et al.* 1987). Development of advanced inoculants is a most important task in future application of *Azospirillum*.

Agrochemicals, especially pesticides, may exhibit undesirable side effects on nontarget microorganisms in the environment. Research in this area has been largely neglected. The only information available is from experiments conducted *in vitro* where herbicides had some effect on nitrogenase activity (Haahtela *et al.* 1988; Jagnow *et al.* 1979) and on *Azospirillum* growth (Gadkari 1987, 1988). Incorporation of insecticides into the growth medium caused either cell disruption of *Azospirillum* and formation of cyst-like bacteria (Mano *et al.* 1988) or increase in N₂ fixation and IAA production (Jena *et al.* 1987). Applied research in *Azospirillum* technology must focus on this area.

Concluding remarks and future prospects

Azospirillum was initially tested for agronomic exploitation more than a decade ago as a result of two basic features: (i) its ability to fix atmospheric N and (ii) its intimate association with roots of cereals and grasses. Although no special morphological structure was ever found in or on inoculated roots, increase in N content of inoculated plants as well as reported yield increases ranging from 10 to 200% above noninoculated plants defined *Azospirillum*-cereal interaction as equivalent to *Rhizobium-legume* association. This prompted numerous field inoculation attempts, mostly performed between 1978 and 1984. The large amount of data from these worldwide field experiments, combined with laboratory and greenhouse experiments on the bacterial mode of action, led to several surprises. Despite the optimistic initial results, *Azospirillum* inoculation in the field proved to be inconsistent and unpredictable; experimental results were difficult to repeat even if experiments were performed in an identical fashion. Initial estimates that 50-70% of all field experiments worldwide were successful are probably an overestimation since many failures were never reported, especially when they were performed by commercial companies. These inconsistencies led to a great reduction in field experimentation with *Azospirillum*. Very few field studies are published annually. Current estimations of future success, optimistic at the present level of knowledge, are that inoculation of crops with *Azospirillum* can result in an average 10-15% yield increase in fertilized areas and up to 20% under less-developed agricultural practices, or semi-arid conditions resem-

bling Israeli soils and climate (Okon 1985; Okon and Hadar 1987), with possibly a high percentage of inconsistency.

Complementary to the inconsistency in yield results, N₂ fixation in general was regarded to be of little significance concerning the contribution of *Azospirillum* to plant growth. Despite a few studies consistently demonstrating a direct significant contribution from N₂ fixation, most studies, carried out worldwide under diverse climatic and soil conditions, showed only scarce assimilation, if any, of the N fixed by *Azospirillum* to the plant. Recent experiments that employed Nif⁻ *Azospirillum* showed beneficial effects on plant growth similar to the wild-type strain (Nif⁺), indicating an alternative mechanism(s) involved in this association.

Several mechanisms of plant-microbe interaction may participate in the association and affect plant growth, including N₂ fixation, hormonal effects, general improvement in root growth resulting in improved mineral and water uptake, and activity of bacterial nitrate reductase in the root system. However, there are no quantitative data to support the notion that one of these mechanisms can be solely responsible for induction of such changes in the plant. Therefore, an additive hypothesis is more appropriate to describe the effect of *Azospirillum* on plants: probably more than one mechanism participates in the association. They operate simultaneously or in succession. The sum of their activities, when induced under the proper environmental conditions, results in the observed changes in plant growth. This hypothesis may also explain the frequent failure of inoculation to yield consistent results. Presumably one or more mechanisms are inactive or only partially active, thus maximal benefit expected from the association is rarely achieved. This hypothesis, therefore, may define *Azospirillum* as "plant growth promoting rhizobacteria" (PGPR) and not as previously defined, an "associative nitrogen fixer."

Genetic analysis of *Azospirillum* is lacking for nearly all the genes relevant to plant-bacteria interaction and to bacterial functions as well, except for the nitrogenase structural genes. Therefore, it is premature to foresee in the near future a major genetic manipulation that will create genetically engineered *Azospirillum* with superb capabilities in the rhizosphere. Thus, the conventional approach of screening plant-strain interaction may be a more useful tool. Although many strains of *Azospirillum* have been isolated worldwide and stored, only a few are comprehensively described. Very few field-tested isolates of *Azospirillum* are available and it is far behind strain availability of *Rhizobium*. Since *Azospirillum* is widely distributed, exploitation of yet undiscovered strains may be a useful source of new and more beneficial associations.

The ultimate test for even the most beneficial isolate is its ability to survive and to colonize plant roots successfully in the presence of larger numbers of other indigenous rhizosphere microorganisms. The study of interactions between *Azospirillum* and its natural competitors is in its infancy. This obstacle must be overcome, along with development of more effective inoculation methods, before *Azospirillum* can be used reliably as a beneficial inoculant for agriculture.

In conclusion, despite the vast amount of data accumulated on *Azospirillum* during the last 15 years, our knowledge of this association system is too limited to ensure a consistent positive interaction between the bacteria and the plant, let alone commercial exploitation of *Azospirillum* technology. It is suggested that the direction in which *Azospirillum* research should proceed, to gain the full potential of this association, is towards more basic understanding of the underlying funda-

mental components of the system and less towards full-scale field experiments. We assume that this approach will be the best in ultimately harnessing *Azospirillum* activity for the benefit of mankind.

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ABDEL-SALAM, M. S., and KLINGMÜLLER, W. 1987. Transposon Tn5 mutagenesis in *Azospirillum lipoferum*: isolation of indoleacetic acid mutants. *Mol. Gen. Genet.* **210**: 165-170.

——— 1988. Isolation of transposon induced auxin-negative mutations in *Azospirillum lipoferum*. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag Berlin, Heidelberg, pp. 40-48.

ALBRECHT, S. L., OKON, Y., LONNQUIST, L., and BURRIS, R. H. 1981. Nitrogen fixation by corn-*Azospirillum* associations in a temperate climate. *Crop Sci.* **21**: 301-306.

ALBRECHT, S. L., GASKINS, M. H., MILAM, J. R., SCHANK, S. C., and SMITH, R. L. 1983. Ecological factors affecting survival and activity of *Azospirillum* in the rhizosphere. In *Azospirillum*. II. *Experientia Suppl.* **48**: 138-148.

AVIVI, Y., and FELDMAN, M. 1982. The response of wheat to bacteria of the genus *Azospirillum*. *Isr. J. Bot.* **32**: 237-245.

BALANDREAU, J. 1986. Ecological factors and adaptive processes in N₂-fixing bacterial populations of the plant environment. *Plant Soil*, **90**: 73-92.

BALDANI, V. L. D., and DÖBEREINER, J. 1980. Host-plant specificity in the infection of cereals with *Azospirillum* spp. *Soil Biol. Biochem.* **12**: 433-439.

BALDANI, V. L. D., BALDANI, J. I., and DÖBEREINER, J. 1983. Effects of *Azospirillum* inoculation on root infection and nitrogen incorporation in wheat. *Can. J. Microbiol.* **29**: 924-929.

BALDANI, V. L. D., ALVAREZ, M. A. DE B., BALDANI, J. I., and DÖBEREINER, J. 1986. Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in roots of field grown wheat and sorghum. *Plant Soil*. **90**: 35-46.

BALDANI, V. L. D., BALDANI, J. I., and DÖBEREINER, J. 1987. Inoculation of field-grown wheat (*Triticum aestivum*) with *Azospirillum* spp. in Brazil. *Biol. Fertil. Soils*, **4**: 37-40.

BALLY, R., THOMAS-BAUZON, D., HEULIN, T., and BALANDREAU, J. 1983. Determination of the most frequent N₂-fixing bacteria in a rice rhizosphere. *Can. J. Microbiol.* **29**: 881-887.

BARAK, R., NUR, I., OKON, Y., and HENIS, Y. 1982a. Aerotactic response of *Azospirillum brasilense* J. *Bact.* **152**: 643-649.

——— 1982b. Tactic responses of *Azospirillum brasilense* towards oxygen and organic compounds. *Isr. J. Bot.* **31**: 229-236.

BARAK, R., NUR, I., and OKON, Y. 1983. Detection of chemotaxis in *Azospirillum brasilense*. *J. Appl. Bacteriol.* **54**: 399-403.

BARBIERI, P., ZANELLI, T., GALLI, E., and ZANETTI, G. 1986. Wheat inoculation with *Azospirillum brasilense* 56 and some mutants altered in nitrogen fixation and indole-3-acetic acid production. *FEMS Microbiol. Lett.* **36**: 87-90.

- BARBIERI, P., BERNARDI, A., GALLI, E., and ZANETTI, G. 1988. Effects of inoculation with different strains of *Azospirillum brasilense* on wheat roots development. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 181-188.
- BAREA, J. M., BONIS, A. F., and OLIVARES, A. 1983. Interactions between *Azospirillum* and VA mycorrhiza and their effects on growth and nutrition of maize and ryegrass. Soil Biol. Biochem. **15**: 705-709.
- BARTON, L. L., JOHNSON, G. V., and ORBOCK MILLER, S. 1986. The effect of *Azospirillum brasilense* on iron absorption and translocation by sorghum. J. Plant Nutr. **9**: 557-565.
- BASHAN, Y. 1986a. Enhancement of wheat roots colonization and plant development by *Azospirillum brasilense* Cd. following temporary depression of the rhizosphere microflora. Appl. Environ. Microbiol. **51**: 1067-1071.
- 1986b. Alginate beads as synthetic inoculant carriers for the slow release of bacteria that affect plant growth. Appl. Environ. Microbiol. **51**: 1089-1098.
- 1986c. Significance of timing and level of inoculation with rhizosphere bacteria on wheat plants. Soil Biol. Biochem. **18**: 297-301.
- 1986d. Migration of the rhizosphere bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* towards wheat roots in the soil. J. Gen. Microbiol. **132**: 3407-3414.
- 1990. Short exposure to *Azospirillum brasilense* Cd inoculation enhanced proton efflux of intact wheat roots. Can. J. Microbiol. **36**: 419-425.
- BASHAN, Y., and LEVANONY, H. 1985. An improved selection technique and medium for the isolation and enumeration of *Azospirillum brasilense* Cd. Can. J. Microbiol. **31**: 947-952.
- 1987. Horizontal and vertical movement of *Azospirillum brasilense* Cd in the soil and along the rhizosphere of wheat and weeds in controlled and field environments. J. Gen. Microbiol. **133**: 3473-3480.
- 1988a. Interaction between *Azospirillum brasilense* Cd and wheat root cells during early stages of root colonization. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 166-173.
- 1988b. Migration, colonization and adsorption of *Azospirillum brasilense* to wheat roots. In Lectins-biology, biochemistry, clinical biochemistry. Edited by T. C. Bog-Hansen and D. L. J. Freed. Vol. 6. Sigma Chemical Co., St. Louis, MO. pp. 69-84.
- 1988c. Adsorption of the rhizosphere bacterium *Azospirillum brasilense* Cd to soil, sand and peat particles. J. Gen. Microbiol. **134**: 1811-1820.
- 1988d. Active attachment of *Azospirillum brasilense* Cd to quartz sand and to a light-textured soil by protein bridging. J. Gen. Microbiol. **134**: 2269-2279.
- 1989a. Wheat root tips as a vector for passive vertical transfer of *Azospirillum brasilense* Cd. J. Gen. Microbiol. **135**: 2899-2908.
- 1989b. Factors affecting adsorption of *Azospirillum brasilense* Cd to root hairs as compared with root surface of wheat. Can. J. Microbiol. **35**: 936-944.
- BASHAN, Y., and WOLOWELSKY, J. 1987. Soil samplers for quantifying microorganisms. Soil Sci. **143**: 132-138.
- BASHAN, Y., LEVANONY, H., and KLEIN, E. 1986. Evidence for a weak active external adsorption of *Azospirillum brasilense* Cd to wheat roots. J. Gen. Microbiol. **132**: 3069-3073.
- BASHAN, Y., LEVANONY, H., and ZIV-VECHT, O. 1987. The fate of field-inoculated *Azospirillum brasilense* Cd in wheat rhizosphere during the growing season. Can. J. Microbiol. **33**: 1074-1079.
- BASHAN, Y., LEVANONY, H., and MITIKU, G. 1989a. Changes in proton efflux of intact wheat roots induced by *Azospirillum brasilense* Cd. Can. J. Microbiol. **35**: 691-697.
- BASHAN, Y., REAM, Y., LEVANONY, H., and SADE, A. 1989b. Nonspecific responses in plant growth, yield, and root colonization of noncereal crop plants to inoculation with *Azospirillum brasilense* Cd. Can. J. Bot. **67**: 1317-1324.
- BASHAN, Y., SINGH, M., and LEVANONY, H. 1989c. Contribution of *Azospirillum brasilense* Cd to growth of tomato seedlings is not through nitrogen fixation. Can. J. Bot. **67**: 2429-2434.
- BASHAN, Y., HARRISON, S. K., and WHITMOYER, R. E. 1990. Enhanced growth of wheat and soybean plants inoculated with *Azospirillum brasilense* is not necessarily due to general enhancement of mineral uptake. Appl. Environ. Microbiol. **56**: 769-775.
- BAZZICALUPO, M., FANI, R., GALLORI, E., TURBANTI, L., and POLSINELLI, M. 1987. Cloning of the histidine, pyrimidine and cysteine genes of *Azospirillum brasilense* expression of pyrimidine and three clustered histidine genes in *Escherichia coli*. Mol. Gen. Genet. **206**: 76-80.
- BECKING, J. H. 1963. Fixation of molecular nitrogen by an aerobic *Vibrio* or *Spirillum*. Antonie van Leeuwenhoek. J. Microbiol. Serol. **29**: 326.
- 1982. *Azospirillum lipoferum*-a reappraisal. In *Azospirillum*. Genetics, physiology, ecology. Edited by W. Klingmüller. Birkh Auser Verlag, Basel. pp. 130-149.
- BEIJERINCK, M. W. 1925. Über ein *Spirillum*, welches freien Stickstoff binden kann? Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Abt. 2, **63**: 353-359.
- BERG, R. H., VASIL, V., and VASIL, I. K. 1979. The biology of *Azospirillum-sugarcane* association. II. Ultrastructure. Protoplasma **101**: 143-163.
- BERG, R. H., TYLER, M. E., NOVICK, N. J., VASIL, V., and VASIL, I. K. 1980. Biology of *Azospirillum-sugarcane* association: enhancement of nitrogenase activity. Appl. Environ. Microbiol. **39**: 642-649.
- BLEAKLEY, B. H., GASKINS, M. H., HUBBELL, D. H., and ZAM, S. G. 1988. Floc formation by *Azospirillum lipoferum* grown on poly- α -hydroxybutyrate. Appl. Environ. Microbiol. **54**: 2986-2995.
- BODDEY, R. M. 1987. Methods for quantification of nitrogen fixation associated with Gramineae. CRC Crit. Rev. Plant Sci. **6**: 209-266.
- BODDEY, R. M., and DÖBEREINER, J. 1988. Nitrogen fixation associated with grasses and cereals: recent results and perspectives for future research. Plant Soil, **108**: 53-65.
- BODDEY, R. M., BALDANI, V. L. D., BALDANI, J. I., and DÖBEREINER, J. 1986. Effect of inoculation of *Azospirillum* spp. on nitrogen accumulation by field-grown wheat. Plant Soil, **95**: 109-121.
- BOTTINI, R., FULCHIERI, M., PEARCE, D., and PHARIS, R. P. 1989. Identification of gibberellins A₁, A₃, and iso-A₃ in cultures of *Azospirillum lipoferum*. Plant Physiol. **90**: 45-47.
- BOUTON, J. H., and ZUBERER, D. A. 1979. Response of *Panicum maximum* Jacq. to inoculation with *Azospirillum brasilense*. Plant Soil, **52**: 585-590.
- BOUTON, J. H., SMITH, R. L., SCHANK, S. C., BURTON, G. W., TYLER, M. E., LITTELL, R. C., GALLAHER, N. R., and QUESENBERY, K. H. 1979. Response of pearl millet inbreds and hybrids to inoculation with *Azospirillum brasilense* Cd. Crop Sci. **19**: 12-16.
- BOZOUKLIAN, H., and ELMERICH, C. 1986. Nucleotide sequence of the *Azospirillum brasilense* Sp7 glutamine synthetase structural gene. Biochimie **68**: 1181-1187.
- CHRISTIANSEN-WENIGER, C. 1988. An influence of plant growth substances on growth and nitrogenase activity from *Azospirillum brasilense* Cd. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp.141-149.
- COHEN, E., OKON, Y., KIGEL, J., NUR, I., and HENIS, Y. 1980. Increase in dry weight and total nitrogen content in *Zea mays* and *Setaria italica* associated with nitrogen-fixing *Azospirillum* spp. Plant Physiol. **66**: 746-749.
- CROSSMAN, S. M., and HILL, W. A. 1987. Inoculation of sweet potato with *Azospirillum*. HortScience, **22**: 420-422.
- CROZIER, A., ARRUDA, P., JASMIN, J. M., MONTEIRO, A. M., and SANDBERG, G. 1988. Analysis of indole-3-acetic acid and related indoles in culture medium from *Azospirillum lipoferum* and *Azospirillum brasilense* Cd. Appl. Environ. Microbiol. **54**: 2833-2837.
- DAS, A., and MISHRA, A. K. 1984. Aerotolerant growth in *Azospirillum brasilense* Cd induced by dehydroxyphenyl iron-binding compound. Cuff. Microbiol. **11**: 313-316.

- DAZZO, F. B., and MILAM, J. R. 1976. Serological studies of *Spirillum lipoferum*. Soil Crop. Sci. Soc. Fla. Proc. **35**: 122-126.
- DE ARAUJO, E. F., ZAHA, A., SCHRANK, I. S., and SANTOS, D. S. 1988. Characterization of DNA segments adjacent to the *nifHDK* genes of *Azospirillum brasilense* Sp7 by Tn5 site-directed mutagenesis. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 16-25.
- DE CONINCK, K., HOREMANS, S., RANDOMBAGE, S., and VLASSAK, K. 1988. Occurrence and survival of *Azospirillum* spp. in temperate regions. Plant Soil, **110**: 213-218.
- DEL GALLO, M., GRATANI, L., and MORPURGO, G. 1988. Selection at the chemostat of *Azospirillum brasilense* Cd N₂-fixing at high O pressure. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 75-82.
- DE-POLLI, H., BOHLOOL, B. B., and DÖBEREINER, J. 1980. Serological differentiation of *Azospirillum* species belonging to different hostplant specificity groups. Arch. Microbiol. **126**: 217-222.
- DÖBEREINER, J. 1988. Isolation and identification of root associated diazotrophs. Plant Soil, **110**: 207-212.
- DÖBEREINER, J., and BALDANI, V. L. D. 1979. Selective infection of maize roots by streptomycin-resistant *Azospirillum lipoferum* and other bacteria. Can. J. Microbiol. **25**: 1264-1269.
- DÖBEREINER, J., MARRIEL, I. E., and NERY, M. 1976. Ecological distribution of *Spirillum lipoferum* Beijerinck. Can. J. Microbiol. **22**: 1464-1473.
- DROZDOWICZ, A., and FERREIRA SANTOS, G. M. 1987. Nitrogenase activity in mixed cultures of *Azospirillum* with other bacteria. Zentralbl. Mikrobiol. **142**: 487-493.
- EL-KHAWAS, H., and KLINGMÜLLER, W. 1988. A transformable mutant of *Azospirillum brasilense* ATCC 29710. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 64-74.
- ELMERICH, C. 1984. Molecular biology and ecology of diazotrophs associated with nonleguminous plants. Biotechnology, **2**: 967-978.
- ELMERICH, C., and FRANCHE, C. 1982. *Azospirillum* genetics: plasmids, bacteriophages and chromosome mobilization. In *Azospirillum*. Genetics, physiology, ecology. Edited by W. Klingmüller. Birkhäuser Verlag, Basel. pp. 9-17.
- ELMERICH, C., BOZOUKLIN, H., VIEILLE, C., FOGHER, C., PERROUD, B., and PERRIN, A. 1987. *Azospirillum*: genetics of nitrogen fixation and interaction with plants. Trans. R. Soc. London, B **317**: 183-192.
- ESKEW, D. L., EAGLESHAM, A. R. J., and APP, A. A. 1981. Heterotrophic ¹⁵N₂ fixation and distribution of newly fixed nitrogen in a rice-flooded soil system. Plant Physiol. **68**: 48-52.
- EYERS, M., VANDERLEYDEN, J., and VAN GOOL, A. 1988a. Attachment of *Azospirillum* to isolated plant cells. FEMS Microbiol. Lett. **49**:435-439.
- EYERS, M., WAELKENS, F., VANDERLEYDEN, J., and VAN GOOL, A. P. 1988 b. Quantitative measurement of *Azospirillum* plant cell attachment. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 174-180.
- FAHSOLD, R., SINGH, M., and KLINGMÜLLER, W. 1985. Cosmid cloning of the nitrogenase structural genes of *Azospirillum lipoferum*. In *Azospirillum*. III. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 30-40.
- FALK, E. C., DÖBEREINER, J., JOHNSON, J. L., and KRIEG, N. R. 1985. Deoxyribonucleic acid homology of *Azospirillum amazonense* Magalhães *et al.* 1984 and emendation of the description of the genus *Azospirillum*. Int. J. Syst. Bacteriol. **35**: 117-118.
- FALK, E. C., JOHNSON, J. L., BALDANI, V. L. D., DÖBEREINER, J., and KRIEG, N. R. 1986. Deoxyribonucleic and ribonucleic acid homology studies of the genera *Azospirillum* and *Conglomeromonas*. Int. J. Syst. Bacteriol. **36**: 80-95.
- FALLIK, E., OKON, Y., and FISCHER, M. 1988. Growth response of maize roots to *Azospirillum* inoculation: effect of soil organic matter content, number of rhizosphere bacteria and timing of inoculation. Soil Biol. Biochem. **20**: 45-49.
- FALLIK, E., OKON, Y., EPSTEIN, E., GOLDMAN, A., and FISCHER, M. 1989. Identification and quantification of IAA and IBA in *Azospirillum brasilense* -inoculated maize roots. Soil Biol. Biochem. **21**: 147-153.
- FANI, R., BUZZICALUPO, M., COLAMIZ, P., and POLSINELLI, M. 1986. Plasmid transformation of *A. brasilense*. FEMS Microbiol. Lett. **35**: 23-29.
- FAVILLI, F., TRINCI, F., and BALLONI, W. 1988. *Azospirillum* spp ecology of some soils of the Somali republic. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 223-233.
- FERREIRA, M. C. B., FERNANDES, M. S., and DÖBEREINER, J. 1987. Role of *Azospirillum brasilense* nitrate reductase in nitrate assimilation by wheat plants. Biol. Fertil. Soils **4**: 47-53.
- FISCHER, M., LEVY, E., and GELLER, T. 1986. Regulatory mutation that controls *nif* expression and histidine transport in *Azospirillum brasilense* J. Bacteriol. **167**: 423-426.
- FOGHER, C., DUSHA, I., BARBOT, P., and ELMERICH, C. 1985. Heterologous hybridization of *Azospirillum* DNA to *Rhizobium* nod and fix genes. FEMS Microbiol. Lett. **30**: 245-249.
- GADKARI, D. 1987. Influence of the herbicides Arelon, Goltix and Stomp on growth and nitrogenase activity of *Azospirillum lipoferum*. Zentralbl. Mikrobiol. **142**: 587-594.
- _____ 1988. Influence of herbicides on growth and nitrogenase activity of *Azospirillum*. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 150-158.
- GAFNI, R., OKON, Y., KAPULNIK, Y., and FISCHER, M. 1986. Adsorption of *Azospirillum brasilense* to corn roots. Soil Biol. Biochem. **18**: 69-75.
- GALIMAND, M., VIEILLE, C., PERROUD, B., ONYEOCHA, I., and ELMERICH, C. 1988. Advances in genetics of *Azospirillum brasilense* Sp7: use of Tn5 mutagenesis for gene mapping and identification. In *Azospirillum*. IV. Genetics, physiology, ecology, Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 1-9.
- GASKINS, M. H., ALBRECHT, S. L., and HUBBELL, D. H. 1985. Rhizosphere bacteria and their use to increase plant productivity: a review. Agric. Ecosyst. Environ. **12**: 99-116.
- GERMIDA, J. J. 1986. Population dynamics of *Azospirillum brasilense* and its bacteriophage in soil. Plant Soil, **90**: 117-128.
- _____ 1987. Isolation of *Bdellovibrio* spp. that prey on *Azospirillum brasilense* in soil. Can. J. Microbiol. **33**: 459-461.
- GOLDMAN, G. H., and LANGENBACH, T. 1987. Inhibition of maize root pectolytic activity by *Azospirillum* spp. Cienc. Cult. (Sao Paulo), **39**: 647-650.
- HAAHTELA, K., WARTIONAARA, T., SUNDMAN, V., and SKUIJINS, J. 1981. Root-associated N₂ fixation (acetylene reduction) by *Enterobacteriaceae* and *Azospirillum* strains in cold-climate spodosols. Appl. Environ. Microbiol. **41**: 203-206.
- HAAHTELA, K., KILPI, S., and KARL, K. 1988. Effects of phenoxy acid herbicides and glyphosate on nitrogenase activity (acetylene reduction) in root-associated *Azospirillum*, *Enterobacter* and *Klebsiella*. FEMS Microbiol. Ecol. **53**: 123-127.
- HADAS, R., and OKON, Y. 1987. Effect of *Azospirillum brasilense* inoculation on root morphology and respiration in tomato seedlings. Biol. Fertil. Soils, **5**: 241-247.
- HALL, P. G., and KRIEG, N. R. 1984. Application of the indirect immunoperoxidase stain technique to the flagella of *Azospirillum brasilense* Appl. Environ. Microbiol. **47**: 433-435.
- HALSALL, D. M., and GIBSON, A. H. 1985. Cellulose decomposition and associated nitrogen fixation by mixed cultures of *Cellulomonas gelida* and *Azospirillum* species of *Bacillus macerans*. Appl. Environ. Microbiol. **50**: 1021-1026.
- _____ 1986. Comparison of two *cellulomonas* strains and their interaction with *Azospirillum brasilense* in degradation of wheat straw and associated nitrogen fixation. Appl. Environ. Microbiol. **51**:855-861.
- HALSALL, D. M., and GOODCHILD D. J. 1986. Nitrogen fixation associated with development and localization of mixed population of

- Cellulomonas* sp. and *Azospirillum brasilense* grown on cellulose or wheat straw. Appl. Environ. Microbiol. **51**: 849-852.
- HALSALL, D. M., TURNER, G. L., and GIBSON, A. H. 1985. Straw and xylan utilization by pure cultures of nitrogen-fixing *Azospirillum* spp. Appl. Environ. Microbiol. **49**: 423-428.
- HARARI, A., KIGEL, J., and OKON, Y. 1988. Involvement of IAA in the interaction between *Azospirillum brasilense* and *Panicum miliaceum* roots. Plant Soil, **110**: 275-282.
- HARRIS, J. M., LUCAS, J. A., DAVEY, M. R., LET HBRIDGE, G., and POWELL, K. A. 1989. Establishment of *Azospirillum* inoculant in the rhizosphere of winter wheat. Soil Biol. Biochem. **21**: 59-64.
- HARTMANN, A. 1988. Osmoregulatory properties of *Azospirillum* spp. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 122-130.
- HARTMANN, A., and HUREK, T. 1988. Effect of carotenoid overproduction on oxygen tolerance of nitrogen fixation in *Azospirillum brasilense* Sp7. J. Gen. Microbiol. **134**: 2449-2455.
- HARTMANN, A., SINGH, M., and KLINGMÜLLER, W. 1983. Isolation and characterization of *Azospirillum* mutants excreting high amounts of indoleacetic acid. Can. J. Microbiol. **29**: 916-923.
- HEGAZI, N. A. 1988. Modification of soil environment through straw application versus *Azospirillum* spp. inoculation. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 215-222.
- HEGAZI, N. A., MONIB, M., AMER, H. A., and SHOKR, E.-S. 1983. Response of maize plants to inoculation with azospirella and (or) straw amendment in Egypt. Can. J. Microbiol. **29**: 888-894.
- HEINRICH, D., and HESS, D. 1985. Chemotactic attraction of *Azospirillum lipoferum* by wheat roots and characterization of some attractants. Can. J. Microbiol. **31**: 26-31.
- HESS, D. 1982. Induction of nitrogenase activity in *Azospirillum* by wheat. In *Azospirillum*. Genetics, physiology, ecology. Edited by W. Klingmüller. Birkhäuser Verlag, Basel. pp. 69-74.
- HEULIN, T., GUCKERT, A., and BALANDREAU, J. 1987. Simulation of root exudation of rice seedlings by *Azospirillum* strains: carbon budget under gnotobiotic conditions. Biol. Fertil. Soils, **4**: 9-14.
- HEULIN, T., RAHMAN, M., OMAR, A. M. N., RAFIDISON, Z., PIERRAT, J. C., and BALANDREAU, J. 1989. Experimental and mathematical procedures for comparing N₂-fixing efficiencies of rhizosphere diazotrophs. J. Microbiol. Methods, **9**: 163-173.
- HILL, W. A., BACON-HILL, P., CROSSMAN, S. M., and STEVENS, C. 1983. Characterization of N₂-fixing bacteria associated with sweet potato roots. Can. J. Microbiol. **29**: 860-862.
- HOREMANS, S., DE CONINCK, K., NEURAY, J., HERMANS, R., and VLASSAK, K. 1986. Production of plant growth substances by *Azospirillum* sp. and other rhizosphere bacteria. Symbiosis, **2**: 341-346.
- HOREMANS, S., DEMARSIN, S., NEURAY, J., and VLASSAK, K. 1987. Suitability of the BLCR medium for isolating *Azospirillum brasilense*. Can. J. Microbiol. **33**: 806-808.
- HOREMANS, S., DE KONINCK, K., and VLASSAK, K. 1988. Aspects of the ecology of *Azospirillum* sp. in Belgian soils. In *Azospirillum*. IV. Genetics, physiology, ecology, Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 207-214.
- HUREK, T., REINHOLD B., FENDRIK, I., and NIEMANN, E. -G. 1987. Root-zone-specific oxygen tolerance of *Azospirillum* spp. and diazotrophic rods closely associated with Kallar grass. Appl. Environ. Microbiol. **53**: 163-169.
- HUREK, T., REINHOLD, B., NIEMANN, E.G., and FENDRIK, I. 1988. N₂-dependent growth of *Azospirillum* spp. in batch cultures at low concentrations of oxygen. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 115-121.
- INBAL, E., and FELDMAN, M. 1982. The response of a hormonal mutant of common wheat to bacteria of the genus *Azospirillum*. Isr. J. Bot. **31**: 257-263.
- IRUTHAYATHAS, E. E., GUNASEKARAN, S., and VLASSAK, K. 1983. Effect of combined inoculation of *Azospirillum* and *Rhizobium* on nodulation and N₂-fixation of winged bean and soybean. Sci. Hortic. (Amsterdam), **20**: 231-240.
- JAGNOW, G. 1982. Growth and survival of *Azospirillum lipoferum* in soil and rhizosphere as influenced by ecological stress conditions. In *Azospirillum*. Genetics, physiology, ecology. Edited by W. Klingmüller. Birkhäuser Verlag, Basel. pp. 100-107.
- 1987. Inoculation of cereal crops and forage grasses with nitrogen-fixing rhizosphere bacteria: possible causes of success and failure with regard to yield response—a review. Z. Pflanzenzemaehr. Dueng. Bodenkd. **150**: 361-368.
- JAGNOW, G., HEINEMEYER, O., and DRAEGER, S. 1979. Choice of liquid, semisolid, or soil suspension media: an important factor modifying the effect of pesticides on the nitrogenase (C₂H₂) activity of *Clostridium pasteurianum*, *Azotobacter chroococcum* and *spirillum lipoferum* Beijerinck. Ecotoxicol. Environ. Saf. **3**: 152-158.
- JAIN, D. K., and PATRIQUIN, D. G. 1984. Root hair deformation, bacterial attachment, and plant growth in wheat-*Azospirillum* associations. Appl. Environ. Microbiol. **48**: 1208-1213.
- 1985. Characterization of a substance produced by *Azospirillum* which causes branching of wheat root hairs. Can. J. Microbiol. **31**: 206-210.
- JARA, P., QUIVIGER, B., LAURENT, P., and ELMERICH, C. 1983. Isolation and genetic analysis of *Azospirillum brasilense* Nif- mutants. Can. J. Microbiol. **29**: 968-972.
- JENA, P. K., ADHYA, T. K., and RAO, V. R. 1987. Nitrogen fixation and indole acetic acid production by *Azospirillum* sp. as influenced by an insecticide, carbofuran. J. Appl. Bacteriol. **63**: 355-360.
- KAPULNIK, Y., KIGEL, J., OKON, Y., NUR, I., and HENIS, Y. 1981a. Effect of *Azospirillum* inoculation on some growth parameters and N content of wheat, sorghum and panicum. Plant Soil, **61**: 65-70.
- KAPULNIK, Y., OKON, Y., KIGEL, J., NUR, I., and HENIS, Y. 1981 b. Effects of temperature, nitrogen fertilization, and plant age on nitrogen fixation by *Setaria italica* inoculated with *Azospirillum brasilense* (strain Cd). Plant Physiol. **68**: 340-343.
- KAPULNIK, Y., SARIG, S., NUR, I., OKON, Y., KIGEL, J., and HENIS, Y. 1981 c. Yield increases in summer cereal crops of Israeli fields inoculated with *Azospirillum*. Exp. Agric. **17**: 179-187.
- KAPULNIK, Y., SARIG, S., NUR, I., OKON, Y., and HENIS, Y. 1982. The effect of *Azospirillum* inoculation on growth and yield of corn. Isr. J. Bot. **31**: 247-255.
- KAPULNIK, Y., SARIG, S., NUR, I., and OKON, Y. 1983. Effect of *Azospirillum* inoculation on yield of field-grown wheat. Can. J. Microbiol. **29**: 895-899.
- KAPULNIK, Y., FELDMAN, M., OKON, Y., and HENIS, Y. 1985a. Contribution of nitrogen fixed by *Azospirillum* to the N nutrition of spring wheat in Israel. Soil Biol. Biochem. **17**: 509-515.
- KAPULNIK, Y., GAFNI, R., and OKON, Y. 1985b. Effect of *Azospirillum* spp. inoculation on root development and NO₃⁻ uptake in wheat (*Triticum aestivum* cv. Miriam) in hydroponic systems. Can. J. Bot. **63**: 627-631.
- KAPULNIK, Y., OKON, Y., and HEMS, Y. 1985c. Changes in root morphology of wheat caused by *Azospirillum* inoculation. Can. J. Microbiol. **31**: 881-887.
- 1987. Yield response of spring wheat cultivars (*Triticum aestivum* and *T. turgidum*) to inoculation with *Azospirillum brasilense* under field conditions. Biol. Fertil. Soils **4**: 27-35.
- KOLB, W., and MARTIN, P. 1985. Response of plant roots to inoculation with *Azospirillum brasilense* and to application of indoleacetic acid. In *Azospirillum*. III. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 215-221.
- 1988. Influence of nitrogen on the number of N₂-fixing and total bacteria in the rhizosphere. Soil Biol. Biochem. **20**: 221-225.
- KOSSLAK, R. M., and BOHLOOL, B. B. 1983. Prevalence of *Azospirillum* spp. in the rhizosphere of tropical plants. Can. J. Microbiol. **29**: 649-652.
- KOTHARI, S. K., and SARAF, C. S. 1986. Response of green gram (*Vigna radiata* (L.) Wilczek) to bacterial seed inoculation and application of phosphorus fertilizer. J. Agric. Sci. **107**: 463-466.
- KRIEG, N. R., and DÖBEREINER, J. 1986. The genus *Azospirillum*. In Bergey's manual of systematic bacteriology. Edited by N. R. Krieg

- and J. G. Holt. Vol. 1. Williams and Wilkins Co., Baltimore, MD. pp. 96-104.
- KUCEY, R. M. N. 1988a. Alteration of size of wheat root systems and nitrogen fixation by associative nitrogen-fixing bacteria measured under field conditions. *Can. J. Microbiol.* **34**: 735-739.
- 1988b. Plant growth-altering effects of *Azospirillum brasilense* and *Bacillus C-11-25* on two wheat cultivars. *J. Appl. Bacteriol.* **64**: 187-196.
- KULINSKA, D., and DROZDOWICZ, A. 1983. Occurrence of microorganisms antagonistic to *Azospirillum* spp. *Zentralbl. Microbiol.* **138**: 585-594.
- LADHA, J. K., BARRAQUIO, W. L., and WATANABE, I. 1982. Immunological techniques to identify *Azospirillum* associated with wetland rice. *Can. J. Microbiol.* **28**: 475-478.
- LADHA, J. K., So, R. B., and WATANABE, I. 1987. Composition of *Azospirillum* species associated with wetland rice plant grown in different soils. *Plant Soil*, **102**: 127-129.
- LAMM, R. B., and NEYRA, C. A. 1981. Characterization and cyst production of azospirilla isolated from selected grasses growing in New Jersey and New York. *Can. J. Microbiol.* **27**: 1320-1325.
- LAU-WONG, M. M. 1987. Field testing of the effectiveness of bacterial fertilizer in Nepal. *Agric. Ecosyst. Environ.* **19**: 145-153.
- LEE, K. J., and GASKINS, M. H. 1982. Increased root exudation of ^{14}C -compounds by sorghum seedlings inoculated with nitrogenfixing bacteria. *Plant Soil*, **69**: 391-399.
- LETHBRIDGE, G., and DAVIDSON, M. S. 1983. Root-associated nitrogen-fixing bacteria and their role in the nitrogen nutrition of wheat estimated by ^{15}N isotope dilution. *Soil Biol. Biochem.* **15**: 365-374.
- LEVANONY, H., and BASHAN, Y. 1989a. Localization of specific antigens of *Azospirillum brasilense* Cd in its exopolysaccharide by immuno-gold staining. *Cuff. Microbiol.* **18**: 145-149.
- 1989b. Enhancement of cell division in wheat root tips and growth of root elongation zone induced by *Azospirillum brasilense* Cd. *Can. J. Bot.* **67**: 2213-2216.
- 1990. Avidin-biotin complex incorporation into enzymelinked immunosorbent assay (ABELISA) for improving the detection of *Azospirillum brasilense* Cd. *Cuff. Microbiol.* **20**: 91-94.
- LEVANONY, H., BASHAN, Y., and KAHANA, Z. E. 1987. Enzymelinked immunosorbent assay for specific identification and enumeration of *Azospirillum brasilense* Cd. in cereal roots. *Appl. Environ. Microbiol.* **53**: 358-364.
- LEVANONY, H., BASHAN, Y., ROMANO, B., and KLEIN, E. 1989. Ultrastructural localization and identification of *Azospirillum brasilense* Cd on and within wheat root by immuno-gold labeling. *Plant Soil*, **117**: 207-218.
- Li, C. Y., and CASTELLANO, M. A. 1987. *Azospirillum* isolated from within sporocarps of the mycorrhizal fungi *Hebeloma crustuliniforme*, *Laccaria laccata* and *Rhizopogon vinicolor*. *Trans. Br. Mycol. Soc.* **88**: 563-565.
- LIN, W., OKON, Y., and HARDY, R. W. F. 1983. Enhanced mineral uptake by *Zea mays* and *Sorghum bicolor* roots inoculated with *Azospirillum brasilense*. *Appl. Environ. Microbiol.* **45**: 1775-1779.
- MADI, L., KESSEL, M., SADOVNIK, E., and HENIS, Y. 1988. Electron microscopic studies of aggregation and pellicle formation in *Azospirillum* spp. *Plant Soil*, **109**: 115-121.
- MAGALH&ES, F. M. M., BALDANI, J. I., SOUTO, S. M., KUYKENDALL, J. R., and DÖBEREINER, J. 1983. A new acid tolerant *Azospirillum* species. *An. Acad. Bras. Cienc.* **55**: 417-430.
- MANDIMBA, G., HEULIN, T., BALLY, R., GUCKERT, A., and BALANDREAU, J. 1986. Chemotaxis of free-living nitrogen-fixing bacteria towards maize mulage. *Plant Soil*, **90**: 129-139.
- MANO, D. M. S., MATOS, A. C. M., and LANGENBACH, T. 1988. The effect of Dicofof on morphology, growth and nitrogenase activity of *Azospirillum lipoferum*. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 159-165.
- MARKUS, P., and KRAMER, J. 1988. Importance of nonsymbiotic nitrogen-fixing bacteria in organic fanning systems. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 197-204.
- MARTIN, P., and GLATZLE, A. 1982. Mutual influences of *Azospirillum* spp. and grass seedlings. In *Azospirillum*. Genetics, physiology, ecology. Edited by W. Klingmüller. Birkhäuser Verlag, Basel. pp. 108-120.
- MATTHEWS, S. W., SCHANK, S. C., ALDRICH, H. C., and SMITH, R. L. 1983. Peroxidase-antiperoxidase labeling of *Azospirillum brasilense* in field-grown pearl millet. *Soil Biol. Biochem.* **6**: 699-703.
- MERTENS, T., and HESS, D. 1984. Yield increases in spring wheat (*Triticum aestivum* L.) inoculated with *Azospirillum lipoferum* under greenhouse and field conditions of a temperate region. *Plant Soil*, **82**: 87-99.
- MILLET, E., and FELDMAN, M. 1986. Yield response of a common spring wheat cultivar to inoculation with *Azospirillum brasilense* at various levels of nitrogen fertilization. *Plant Soil*, **80**: 255-259.
- MILLET, E., Avivi, Y., and FELDMAN, M. 1986. Yield response of various wheat genotypes to inoculation with *Azospirillum brasilense*. *Plant Soil*, **80**: 261-266.
- MORGENSTERN, E., and OKON, Y. 1987a. The effect of *Azospirillum brasilense* and auxin on root morphology in seedlings of *Sorghum bicolor* X *Sorghum sudanense*. *Arid Soil Res. Rehabil.* **1**: 115-127.
- 1987b. Promotion of plant growth and NO_3^- and Rb^+ uptake in *Sorghum bicolor* X *Sorghum sudanense* inoculated with *Azospirillum brasilense* - Cd. *Arid Soil Res. Rehabil.* **1**: 211-217.
- MURRAY, R. G. E., and MOYLES, D. 1987. Differentiation of the cell wall of *Azospirillum brasilense*. *Can. J. Microbiol.* **33**: 132-137.
- MURTY, M. G., and LADHA, J. K. 1987. Differential colonization of *Azospirillum lipoferum* on roots of two varieties of rice *Oryza sativa* L.). *Biol. Fertil. Soils*, **4**: 3-7.
- 1988. Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponic conditions. *Plant Soil*, **108**: 281-285.
- KAYAK, D. N., LADHA, J. K., and WATANABE, I. 1986. The fate of marker *Azospirillum lipoferum* inoculated into rice and its effect on growth, yield, and N_2 fixation of plants studied by acetylene reduction, $^{15}\text{N}_2$ feeding and ^{15}N dilution techniques. *Biol. Fertil. Soils*, **2**: 7-14.
- NEGI, M., SADASIVAM, K. V., and TILAK, K. V. B. R. 1987. Establishment of *Azotobacter* and *Azospirillum* in the rhizosphere of barley (*Hordeum vulgare* L.) in organic-amended soils. *Zentralbl. Mikrobiol.* **142**: 149-154.
- NUR, I., OKON, Y., and HEMS, Y. 1980a. An increase in nitrogen content of *Setaria italica* and *Zea mays* inoculated with *Azospirillum*. *Can. J. Microbiol.* **26**: 482-485.
- 1980b. Comparative studies of nitrogen-fixing bacteria associated with grasses in Israel with *Azospirillum brasilense*. *Can. J. Microbiol.* **26**: 714-718.
- O'HARA, G. W., DAVEY, M. R., and LUCAS, J. A. 1981. Effect of inoculation of *Zea mays* with *Azospirillum brasilense* strains under temperate conditions. *Can. J. Microbiol.* **27**: 871-877.
- 1987. Effect of nitrogen on the yield response of *Pennisetum americanum*, *Triticum aestivum* and *Zea mays* to inoculation with *Azospirillum brasilense* under temperate conditions. *Biol. Fertil. Soils*, **4**: 67-72.
- OKON, Y. 1982. *Azospirillum*: physiological properties, mode of association with roots and its application for the benefit of cereal and forage grass crops. *Isr. J. Bot.* **31**: 214-220.
- 1985. *Azospirillum* as a potential inoculant for agriculture. *Trends Biotechnol.* **3**: 223-228.
- OKON, Y., and HADAR, Y. 1987. Microbial inoculants as crop-yield enhancers. *CRC Crit. Rev. Biotechnol.* **6**: 61-85.
- OKON, Y., and KAPULNIK, Y. 1986. Development and function of *Azospirillum*-inoculated roots. *Plant Soil*, **90**: 3-16.
- OKON, Y., CAKMAKCI, L., NUR, I., and CHET, I. 1980. Aerotaxis and chemotaxis of *Azospirillum brasilense*: a note. *Microb. Ecol.* **6**: 277-280.
- OKON, Y., HEYTLER, P. G., and HARDY, R. W. F. 1983. N_2 fixation

- by *Azospirillum* brasilense and its incorporation into host *Setaria italica*. Appl. Environ. Microbiol. **46**: 694-697.
- OLIVEIRA, R. G. B., and DROZDOWICZ, A. 1981. Bacteriocins in the genus *Azospirillum*. Rev. Microbiol. **12**: 42-47.
- 1987. Inhibition of producing strains of *Azospirillum lipoferum* by their own bacteriocin. Zentralbl. Mikrobiol. **142**: 387-391.
- 1988. Are *Azospirillum* bacteriocins produced and active in soil? In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 101-108.
- PACOVSKY, R. S. 1988. Influence of inoculation with *Azospirillum brasilense* and *Glomus fasciculatum* on sorghum nutrition. Plant Soil, **110**: 283-287.
- PACOVSKY, R. S., FULLER, G., and PAUL, A. E. 1985 a. Influence of soil on the interactions between endomycorrhizae and *Azospirillum* in Sorghum. Soil Biol. Biochem. **17**: 525-531.
- PACOVSKY, R. S., PAUL, E. A., and BETHLENFALVAY, G. J. 1985b. Nutrition of sorghum plants fertilized with nitrogen or inoculated with *Azospirillum brasilense*. Plant Soil, **85**: 145-148.
- PAL, U. R., and MALIK, H. S. 1981. Contribution of *Azospirillum brasilense* to the nitrogen needs of grain sorghum (*Sorghum bicolor* (L.) Moench) in humid sub-tropics. Plant Soil, **63**: 501-504.
- PAMPALUNA, M., ANTONELLI, M. N., DIANA, L., and FOGHER, C. 1988. Mobilization of cryptic plasmids in *Azospirillum*. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 54-63.
- PATRIQUIN, D. G., and DÖBEREINER, J. 1978. Light microscopy observations of tetrazolium-reducing bacteria in the endorhizosphere of maize and other grasses in Brazil. Can. J. Microbiol. **24**: 734-747.
- PATRIQUIN, D. G., DÖBEREINER, J., and JAIN, D. K. 1983. Sites and processes of association between diazotrophs and grasses. Can. J. Microbiol. **29**: 900-915.
- PEDROSA, F. O. 1988. Physiology, biochemistry, and genetics of *Azospirillum* and other root-associated nitrogen-fixing bacteria. CRC Crit. Rev. Plant Sci. **6**: 345-384.
- PEDROSA, F. O., and YATES, M. G. 1984. Regulation of nitrogen fixation (*nif*) genes of *Azospirillum brasilense* by *nifA* and *ntrC* (*gln*) type gene products. FEMS Microbiol. Lett. **23**: 95-101.
- PEREIRA, J. A. R., CAVALCANTE, V. A., BALDANI, J. I., and DÖBEREINER, J. 1988. Field inoculation of sorghum and rice with *Azospirillum* spp. and *Herbaspirillum seropedicae*. Plant Soil, **110**: 269-274.
- PIANA, L., DELLEDONNE, M., ANTONELLI, M. N., and FOGHER, C. 1988. Root hair deformation induced on maize and medicago by an *Azospirillum* transconjugant containing a *Rhizobium meliloti* modulation region. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 83-91.
- PLAZINKSI, J., and ROLFE, B. G. 1985a. Analysis of the pectolytic activity of *Rhizobium* and *Azospirillum* strains isolated from *Trifolium repens*. J. Plant Physiol. **120**: 181-187.
- 1985 b. *Azospirillum*-*Rhizobium* interaction leading to a plant growth stimulation without nodule formation. Can. J. Microbiol. **31**: 1026-1030.
- 1985c. Influence of *Azospirillum* strains on the nodulation of clovers by *Rhizobium* strains. Appl. Environ. Microbiol. **49**: 984-989.
- 1985d. Interaction of *Azospirillum* and *Rhizobium* strains leading to inhibition of nodulation. Appl. Environ. Microbiol. **49**: 990-993.
- QUIVIGER, B., FRANCHE, C., LUTFALLA, G., RICE, D., HASELKORN, R., and ELMERICH, C. 1982. Cloning of a nitrogen fixation (*nif*) gene cluster of *Azospirillum brasilense*. Biochimie, **64**: 495-502.
- RAI, R. 1983. Efficacy of associative N₂ fixation by streptomycin-resistant mutants of *Azospirillum brasilense* with genotypes of chick pea *Rhizobium* strains. J. Agric. Sci. **100**: 75-80.
- RAI, S. N., and GAUR, A. C. 1982. Nitrogen fixation by *Azospirillum* spp. and effect of *Azospirillum lipoferum* on the yield and Nuptake of wheat crop. Plant Soil, **69**: 233-238.
- RAO, A. V., and VENKATESWARLU, B. 1982. Associative symbiosis of *Azospirillum lipoferum* with dicotyledonous succulent plants of the Indian desert. Can. J. Microbiol. **28**: 778-782.
- 1988. Seeds of graminaceous plants as carriers of *Azospirillum*. Cuff. Sci. **57**: 257-258.
- RAO, J. L. N., and RAJAMAMOHAN RAO, V. R. 1983. Nitrogenase activity in the rice rhizosphere soil as affected by *Azospirillum* inoculation and fertilizer nitrogen under upland conditions. Cuff. Sci. **52**: 686-688.
- RAO, V. R., NAYAK, D. N., CHARYULU, P. B. B. N., and ADHAY, T. K. 1983. Yield responses of rice to root inoculation with *Azospirillum*. J. Agric. Sci. **100**: 689-691.
- REINER, O., and OKON, Y. 1986. Oxygen recognition in aerotactic behaviour of *Azospirillum brasilense* Cd. Can. J. Microbiol. **32**: 829-834.
- REINHOLD, B., HUREK, T., and FENDRIK, I. 1985. Strain-specific chemotaxis of *Azospirillum* spp. J. Bacteriol. **162**: 190-195.
- REINHOLD, B., HUREK, T., NIEMANN, E.-G., and FENDRIK, I. 1986. Close association of *Azospirillum* and diazotrophic rods with different root zones of Kallar grass. Appl. Environ. Microbiol. **52**: 520-526.
- REINHOLD, B., HUREK, T., FENDRIK, I., POT, B., GILLIS, M., KERSTERS, K., THIELEMANS, S., and DE LEY, J. 1987. *Azospirillum halopraeferans* sp. nov., a nitrogen-fixing organism associated with roots of Kallar grass (*Leptochloa fusca* (L.) Kunth.). Int. J. Syst. Bacteriol. **37**: 43-51.
- REINHOLD, B., HUREK, T., BALDANI, I., and DÖBEREINER, J. 1988a. Temperature and salt tolerance of *Azospirillum* spp. from salt-affected soils in Brazil. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 234-241.
- REINHOLD, B., HUREK, T., and FENDRIK, I. 1988b. Plant-bacteria interactions with special emphasis on the Kallar grass association. Plant Soil, **110**: 249-257.
- RENNIE, R. J. 1980. ¹⁵N-isotope dilution as a measure of dinitrogen fixation by *Azospirillum brasilense* associated with maize. Can. J. Bot. **58**: 21-24.
- RENNIE, R. J., and THOMAS, J. B. 1987. ¹⁵N-determined effect of inoculation with N₂ fixing bacteria on nitrogen assimilation in western Canadian wheats. Plant Soil, **100**: 213-223.
- RENNIE, R. J., DEFREITAS, J. R., RUSCHEL, A. P., and VOSE, P. V. 1983. ¹⁵N isotope dilution to quantify dinitrogen (N₂) fixation associated with Canadian and Brazilian wheat. Can. J. Bot. **61**: 1667-1671.
- REYNDERS, L., and VLASSAK, K. 1982. Use of *Azospirillum brasilense* as biofertilizer in intensive wheat cropping. Plant Soil, **66**: 217-223.
- RUCKDÄSCHEL, E., KITTELL, B. L., HELINSKI, D. R., and KLINGMÜLLER, W. 1988. Aromatic amino acid aminotransferases of *Azospirillum lipoferum* and their possible involvement in IAA biosynthesis. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 49-53.
- SADASIVAN, L., and NEYRA, C. A. 1985. Flocculation in *Azospirillum brasilense* and *Azospirillum lipoferum*: exopolysaccharides and cyst formation. J. Bacteriol. **163**: 716-723.
- 1987. Cyst production and brown pigment formation in aging cultures of *Azospirillum brasilense* ATCC 29145. J. Bacteriol. **169**: 1670-1677.
- SADASIVAM, K. V., NEGI, M., and TILAK, K. V. B. R. 1986a. Survival of *Azospirillum brasilense* and *Azotobacter chroococcum* in organic-amended soil-based carriers. Zentralbl. Mikrobiol. **141**: 567-570.
- SADASIVAM, K. V., TYAGI, R. K., and RAMARETHINAM, S. 1986b. Evaluation of some agricultural wastes as carriers for bacterial inoculants. Agric. Wastes, **17**: 310-306.
- SAHA, K. C., SANNIGRAHI, S., and MANDAL, L. N. 1985. Effect of

- inoculation of *Azospirillum lipoferum* on nitrogen fixation in rhizosphere soil, their association with roots, yield and nitrogen uptake by mustard (*Brassica juncea*). Plant Soil, **87**: 273-280.
- SARIG, S., KAPULNIK, Y., NUR, I., and OKON, Y. 1984. Response of non-irrigated *Sorghum bicolor* to *Azospirillum* inoculation. Exp. Agric. **20**: 59-66.
- SARIG, S., KAPULNIK, Y., and OKON, Y. 1986. Effect of *Azospirillum* inoculation on nitrogen fixation and growth of several winter legumes. Plant Soil, **90**: 335-342.
- SARIG, S., BLUM, A., and OKON, Y. 1988. Improvement of the water status and yield of field-grown grain sorghum (*Sorghum bicolor*) by inoculation with *Azospirillum brasilense*. J. Agric. Sci. **110**: 271-277.
- SCHANK, S. C., and SMITH, R. L. 1984. Status and evaluation of associative grass-bacteria N₂-fixing systems in Florida. Proc. Soil Crop Sci. Soc. Fla. **43**: 120-123.
- SCHANK, S. C., SMITH, R. L., WEISER, G. C., ZUBERER, D. A., BOUTON, J. H., QUESENBERRY, K. H., TYLER, M. E., MILAM, J. R., and LITTELL, R. C. 1979. Fluorescent antibody technique to identify *Azospirillum brasilense* associated with roots of grasses. Soil Biol. Biochem. **11**: 287-295.
- SCHANK, S. C., WEIER, K. L., and MACRAE, I. C. 1981. Plant yield and nitrogen content of a digitgrass in response to *Azospirillum* inoculation. Appl. Environ. Microbiol. **41**: 342-345.
- SCHANK, S. C., SMITH, R. L., MILAM, J. R., and LITTELL, R. C. 1985. Testing grass-bacteria combinations for associative N₂ fixation potential. Proc. Soil Crop Sci. Soc. Fla. **45**: 179-184.
- SCHMIDT, W., MARTIN, P., OMAI, S. H., and BANGERTH, F. 1988. Influence of *Azospirillum brasilense* on nodulation of legumes. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 92-100.
- SCHRODER, M. 1932. Die Assimilation des Luftstickstoffs durch einige Bakterien. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. **2**, 85: 177-212.
- SINGH, M. 1982. Transfer of bacteriophage Mu and transposon Tn5 into *Azospirillum*. In *Azospirillum*. Genetics, physiology, ecology. Edited by W. Klingmüller. Birkhäuser Verlag, Basel, pp. 35-43.
- SINGH, M., and KLINGMÜLLER, W. 1985. Problems and prospects of site-directed transposon mutagenesis in *Azospirillum*. In *Azospirillum*. III. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, pp. 20-29.
- 1986. Transposon mutagenesis in *Azospirillum brasilense*: isolation of auxotrophic and Nif mutants and molecular cloning of the mutagenized *nif* DNA. Mol. Gen. Genet. **202**: 136-142.
- 1988. A TO induced *nifA* like mutant of *Azospirillum brasilense*. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 26-31.
- SINGH, M., and WENZEL, W. 1982. Detection and characterization of plasmids in *Azospirillum*. In *Azospirillum*. II. Genetics, physiology, ecology. Edited by W. Klingmüller. Birkhäuser Verlag, Basel, pp. 44-51.
- SKORUPSKA, A., BRZEZINSKA, M., CHOMA, A., KULINSKA, D., and LOKIEWICZ, Z. 1985. Physiological characterization, plasmids and bacteriocinogenicity of *Azospirillum*. Microbios, **44**: 243-251.
- SMITH, R. L., SCHANK, S. C., and LITTELL, R. C. 1984a. The influence of shading on associative N₂ fixation. Plant Soil, **80**: 43-52.
- SMITH, R. L., SCHANK, S. C., MILAM, J. R., and BALTENSBERGER, A. A. 1984b. Responses of *Sorghum* and *Pennisetum* species to the N₂-fixing bacterium *Azospirillum brasilense*. Appl. Environ. Microbiol. **47**: 1331-1336.
- SUBBA RAO, N. S., TILAK, K. V. B. R., and SINGH, C. S. 1985a. Synergistic effect of vesicular-arbuscular mycorrhizas and *Azospirillum brasilense* on the growth of barley in pots. Soil Biol. Biochem. **17**: 119-121.
- 1985b. Effect of combined inoculation of *Azospirillum brasilense* and vesicular-arbuscular mycorrhiza on pearl millet (*Pennisetum americanum*). Plant Soil, **84**: 283-286.
- SUNDARAM, S., ARUNAKUMARI, A., and KLUCAS, R. V. 1988. Characterization of azospirilla isolated from seeds and roots of turf grass. Can. J. Microbiol. **34**: 212-217.
- TABARY, F., BALANDREAU, J., and BOURRILLON, R. 1984. Purification of the rice embryo lectin and its binding to nitrogen-fixing bacteria from the rhizosphere of rice. Biochem. Biophys. Res. Commun. **119**: 549-555.
- TAL, S., and OKON, Y. 1985. Production of the reserve material poly-β-hydroxybutyrate and its function in *Azospirillum brasilense* Cd. Can. J. Microbiol. **31**: 608-613.
- TARRAND, J. J., KRIEG, N. R., and DÖBEREINER, J. 1978. A taxonomic study of the *Spirillum lipoferum* group, with descriptions of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum brasilense* sp. nov. Can. J. Microbiol. **24**: 967-980.
- TIEN, T. M., GASKINS, M. H., and HUBBELL, D. H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. **37**: 1016-1024.
- TIEN, T. M., DIEM, H. G., GASKINS, M. H., and HUBBELL, D. H. 1981. Polygalacturonic acid transeliminase production by *Azospirillum* species. Can. J. Microbiol. **27**: 426-431.
- TYLER, M. E., MILAM, J. R., SMITH, R. L., SCHANK, S. C., and ZUBERER, D. A. 1979. Isolation of *Azospirillum* from diverse geographic regions. Can. J. Microbiol. **25**: 693-697.
- UMALI-GARCIA, M., HUBBELL, D. H., GASKINS, M. H., and DAZZO, F. B. 1980. Association of *Azospirillum* with grass roots. Appl. Environ. Microbiol. **39**: 219-226.
- 1981. Adsorption and mode of entry of *Azospirillum brasilense* to grass roots. In Associative N-fixation. Edited by P. B. Vose and A. P. Ruschel. Vol. 1. CRC Press, Boca Raton, FL, pp. 49-62.
- VAN BERKUM, P., and BOHLOOL, B. B. 1980. Evaluation of nitrogen-fixation by bacteria in association with roots of tropical grasses. Microb. Rev. **44**: 491-517.
- VANSTOCKEM, M., MICHIELS, K., VANDERLEYDEN, J., and VAN GOOL, A. P. 1987. Transposon mutagenesis of *Azospirillum brasilense* and *Azospirillum lipoferum*: physical analysis of Tn5 and Tn5-Mob insertion mutants. Appl. Environ. Microbiol. **53**: 4104-415.
- VANSTOCKEM, M., MILCAMP, A., MICHIELS, K., VANDERLEYDEN, J., and VAN GOOL, A. P. 1988. Tn5-mutagenesis in *Azospirillum brasilense*. In *Azospirillum*. IV. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg, pp. 32-39.
- VASIL, V., VASIL, I. K., ZUBERER, D. A., and HUBBELL, D. H. 1979. The biology of *Azospirillum*-sugarcane association. I. Establishment of the association. Z. Pflanzenphysiol. **95**: 141-147.
- VENKATESWARLU, B., and RAO, A. V. 1983. Response of pearl millet to inoculation with different strains of *Azospirillum brasilense*. Plant Soil, **74**: 379-386.
- WAELEKENS, F., MARIS, M., VERRETH, C., VANDERLEYDEN, J., and VAN GOOL, A. 1987. *Azospirillum* DNA shows homology with *Agrobacterium* chromosomal virulence genes. FEMS Microbiol. Lett. **43**: 241-246.
- WANI, S. P., CHANDRAPALAI, S., and DART, P. J. 1985. Response of pearl millet cultivars to inoculation with nitrogen-fixing bacteria. Exp. Agric. **21**: 175-182.
- WAREMBOURG, F. R., DREESSEN, R., VLASSAK, K., and LAFONT, F. 1987. Peculiar effect of *Azospirillum* inoculation on growth and nitrogen balance of winter wheat (*Triticum aestivum*). Biol. Fertil. Soils, **4**: 55-59.
- WATANABE, I., and LIN, C. 1984. Response of wetland rice to inoculation with *Azospirillum lipoferum* and *Pseudomonas* sp. Soil Sci. Plant Nutr. **30**: 117-124.
- WHALLON, J. H., ACKER, G. F., and EL-KHAWAS, H. 1985. Electron microscopy of young wheat roots inoculated with *Azospirillum*. In

- Azospirillum*. III. Genetics, physiology, ecology. Edited by W. Klingmüller. Springer-Verlag, Berlin, Heidelberg. pp. 223-229.
- WONG, P. P., STENBERG, N. E., and EDGAR, L. 1980. Characterization of a bacterium of the genus *Azospirillum* from cellulolytic nitrogen-fixing mixed cultures. Can. J. Microbiol. **26**: 291-296.
- YAHALOM, E., KAPULNIK, Y., and OKON, Y. 1984. Response of *Setaria italica* to inoculation with *Azospirillum brasilense* as compared to *Azotobacter chroococcum*. Plant Soil, **82**: 77-85.
- YAHALOM, E., OKON, Y., and Dovrat, A. 1987. *Azospirillum* effects on susceptibility to *Rhizobium* nodulation and on nitrogen fixation of several forage legums. Can. J. Microbiol. **33**: 510-514.
- ZIMMER, W., and BOTHE, H. 1988. The phytohormonal interaction between *Azospirillum* and wheat. Plant Soil, **110**: 239-247.
- ZUBERER, D. A., and ROTH, M. 1982. In vitro inhibition of nonsymbiotic nitrogen-fixing bacteria by rhizosphere actinomycetes associated with grasses. Can. J. Microbiol. **28**: 705-709.

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