Current status of \textit{Azospirillum} inoculation technology: \textit{Azospirillum} as a challenge for agriculture$^1$

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Received July 8, 1989
Accepted March 2, 1990

The genus \textit{Azospirillum}

The first species of \textit{Azospirillum} was isolated by Beijerinck (1925) from N-poor sandy soil in the Netherlands and was originally named \textit{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 This bacterium was later isolated from soil (Schroder 1932) and from dried seaweed in Indonesia (H. C. Derx, unpublished, 1949, cited in Becking, 1963) and as a phyllosphere bacterium of tropical plants (Becking 1982).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, \textit{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 \textit{et al.} 1983; Döbereiner \textit{et al.} 1976; Hill \textit{et al.} 1983; Horemans \textit{et al.} 1988; Kossak and Bohlool 1983; Ladha \textit{et al.} 1987; Lamm and Neyra 1981; Li and Castellano 1987; Nur \textit{et al.} 1980b; Rao and Venkateswarlu 1982, 1988; Sundaram \textit{et al.} 1988; Tyler \textit{et al.} 1979; Wong \textit{et al.} 1980).

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

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

Effect of \textit{Azospirillum} inoculation on plants

Inoculation of plants with \textit{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 \textit{Azospirillum} literature. The exact mechanisms of action of \textit{Azospirillum} on plants has not yet been fully elucidated.

Most studies of the \textit{Azospirillum}-plant association have been conducted on cereals and grasses (Patriquin \textit{et al.} 1983) and only a few other plant families have been investigated (Bashan \textit{et al.} 1989b, 1989c; Crossman and Hill 1987; Kolb and Martin 1985; Saha \textit{et al.} 1985). The following aboveground plant responses to \textit{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 \textit{et al.} 1981; Baldani and Döbereiner.

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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 $10^7$ to $10^8$ cfu/mL (Bashan 1986c; Bashan et al. 1989b; Kapulnik et al. 1985; Okon and Kapulnik 1986; Smith et al. 1984b), for corn $10^5$ cfu/mL (Fallik et al. 1988), and for tomato in vitro $10^5$ to $10^6$ cfu/mL (Hadas and Okon 1987). However, an inoculum concentration of $10^8$ to $10^9$ 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. 1981a; 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. 1985c; Martin and Glatze 1982; Morgenstern and Okon 1987a; 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. 1985c; 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 (Nucey 1988a; Murty and Ladha 1988; Reynolds 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; Eyers 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. lipoforum* strain detected by light microscopy (Patriquin and Döbereiner 1978) have not been confirmed by electron microscopy when strain *A. brasilense* C6 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 (Kapurlik et al. 1983 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 pectinolytic activity of roots (Goldman and Langenbach 1987).

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

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

<table>
<thead>
<tr>
<th>(A) Nitrogen fixation</th>
<th>Evidence for</th>
<th>Evidence against</th>
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<tbody>
<tr>
<td>Accumulation of N compounds in inoculated plants</td>
<td>Nif+ strains and Nif− mutants caused similar effects on plants</td>
<td></td>
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<tr>
<td>Bacterium has N₂-fixation ability</td>
<td>Positive effect on plants under conditions preventing N₂ fixation</td>
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<tr>
<td>Strong nitrogenase activity in inoculated plants</td>
<td>Negligible nitrogenase activity in responding plants</td>
<td></td>
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<tr>
<td>Normal plant growth after inoculation with little or no N added</td>
<td>Amounts of fixed N₂ are not sufficient to explain increase in plant yield</td>
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<td>Incorporation of 58% of plant N from N₂ following inoculation</td>
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<th>(B) Plant hormones</th>
<th>Experimental evidence for</th>
<th>No experimental evidence for</th>
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<tbody>
<tr>
<td>Production of several plant hormones by the bacteria <em>in vitro</em></td>
<td>Effect of hormone mutants</td>
<td>Change in hormone balance in the roots</td>
</tr>
<tr>
<td>Mimic of plant response towards inoculation by hormone application</td>
<td>Relation between hormonal effects and production of an improved plant</td>
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<tr>
<td>Hormone overproducing mutants caused more pronounced effect on plant growth</td>
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<tr>
<td>Indication that inoculation changes the amount of hormones in the plant</td>
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<th>(C) General improvement in plant growth and mineral uptake</th>
<th>Experimental evidence for</th>
<th>No experimental evidence for</th>
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<tr>
<td>Enhancement and accumulation of minerals in plant foliage</td>
<td>Effect of mineral uptake mutants</td>
<td>Enzymatic activities related to mineral and water uptake in the roots</td>
</tr>
<tr>
<td>Enzymatic activities related to ion transformation in plant foliage</td>
<td>Enhancement of mineral uptake by most strains</td>
<td></td>
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<tr>
<td>Improvement of many growth parameters</td>
<td></td>
<td></td>
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<tr>
<td>Improvement of water uptake</td>
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<tr>
<td>Partial substitution of N fertilization</td>
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<td>Increase in proton efflux of inoculated plants</td>
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<th>(D) Bacterial nitrate reductase</th>
<th>Experimental evidence for</th>
<th>No experimental evidence for</th>
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<tr>
<td>The bacteria have an active enzyme</td>
<td>Differential changes between activities in root and foliage</td>
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<tr>
<td>NR− mutants are less effective on plants than NR+ wild type</td>
<td>Interaction with N₂ fixation</td>
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<tr>
<td>NR− mutants have N₂-fixation ability</td>
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<tr>
<td>NR− mutants cause nitrate accumulation in the foliage</td>
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**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, 1985 b; Mertens and Hess 1984; Nur et al. 1980 a; O'Hara et al. 1981; Pacovsky et al. 1985 b; Rennie et al. 1983; Schank et al. 1981; Wani et al. 1985; Warenbourg et al. 1987; Yahalom et al. 1984). Therefore, N 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 N 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 15N incorporation and 15N-dilution techniques. This subject has recently been adequately reviewed (Boddey 1987; Boddey and Döbereiner 1988).

Evidently, N 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; Hass 1982; Kapulnik et al. 1981 b; Okon et al. 1983; Rao and Rajamahal 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 Hass 1984). Thus, on one hand, inoculation of wheat and maize has indicated that 5-10% (Kucey 1986 b) and up to 18% (Rennie 1980; Rennie et al. 1983; Rennie and Thomas 1987) of the total N derived from N 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. 1980 a). On the other hand, studies have shown low or even negligible nitrogenase activity in plants positively responding to inoculation (Kapulnik et al. 1985 a; 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 N fixation, did not eliminate the plant response to inoculation (Avivi and Feldman 1982; Bashan et al. 1989 b, 1989 c; Kapulnik et al. 1981 b, 1982, 1983; Mertens and Hess 1984; Millet and Feldman 1986; Pal and Malik 1981; Rai and Gaur 1982; Reyners and Vlassak 1982).

The ultimate control to distinguish the contribution via N fixation from other effects of bacterial inoculation is to use Nif mutants incapable of fixing N 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 1987 a; 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. 1989 c). This indicates that the plant response was caused by factors other than N 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), absicic acid (ABA) (Kolb and Martin 1985), and cytokinins (Horemans et al. 1986; Tien et al. 1979).

Plant hormones affect the N 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 plant root development and morphology (Harari et al. 1988; Kucey 1988 b; Tien et al. 1979; Zimmer and Bothe 1988). In particular, it caused changes in root length (Kolb and Martin 1985; Morgenstern and Okon 1987 a), produced more root hairs (Kapulnik et al. 1985 c; Morgenstern and Okon 1987 a) 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 of 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 uninoculated 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 tobe 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 Azospirillum
Wheat roots inoculated with Azospirillum

Supporting evidence for increased mineral uptake by inoculated plants can be transferred to the panicles and spikes and resulted in a higher yield. Inoculation improved water status in stressed sorghum plants.

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. It has been further suggested thatAzospirillum inoculation may promote availability of ions in the soil by helping the plant scavenge limiting nutrients. This may explain accumulation of N compounds in the plant without any apparent N₂ 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. 1989a). 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.

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 N₂ 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 NR² 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 N₂ 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 NR² 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 N₂ 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 C₃ and C₄ plants was suggested; A. lipoferum was the predominant species colonizing G plants and A. brasilense was the predominant species associated with C₄ plants in tropical zones (Baldani and Döbereiner 1980; Baldani et al. 1986). Similar host plant preference was found in temperate zones (Haathtela 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. 1989b; Kapulnik et al. 1981c, 1983; Smith et al. 1984b; 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 (Iruthyathas 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 (Pflanzinski 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 (10⁶-10⁸ 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).

*Azospirillum* produced bacteriocins, which surprisingly also inhibited other *Azospirillum* species (Oliveira 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; Zubler and Roch 1982). Many strains of *Azospirillum* are highly resistant to a wide range of antibiotics (Bashan and Levanony 1985; Baldani et al. 1986; Dombreiner 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 (10⁵-10⁶ 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 et al. 1986; Bastian 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 (10^3 cfu/m^2).

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 Levannony 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; Levannony et al. 1989; Tal and Okon 1985). Under stress conditions the bacteria are capable of cyst and lloc or aggregate formation, both of which significantly improve survival (Blekley et al. 1988; Lamm and Neyra 1981; Madi et al. 1988; Murray and Moyle 1987; Sadasivan and Neyra 1985, 1987). Two species, *A. amazonense* and *A. halopraefers*, 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 Levannony 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 1986d; 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 Levannony 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 chemotactic agents greatly affect adsorption of *Azospirillum* to soil to different extents (Bashan and Levannony 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 Levannony 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 Somalia (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; Patruquin 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 Levannony 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 (Pampalona 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*. 
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 hsn (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; Waekens et al. 1987). IAA-overproducing and IAA-deficient mutants have been isolated (Abdel-Salam and Klingmüller 1987; El-Khawas and Klingmüller 1988; Singh 1982; Singh and Klingmüller 1986; Vanstoekem et al. 1987, 1988).
muller 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; Kossak 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 (Ladh et al. 1982). Azospirillum brasilense Cd has been specifically quantified in the rhizosphere of several cereals by employing indirect and competition enzyme-linked immunosorbert 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 1988c); 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; Fullik et al. 1988; Millie 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; Sadasivan 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 (Haathela 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 morphologica 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-
Complementary to the inconsistency in yield results, N 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 N 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 N 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-

*Science in * and *Nature*.

**Acknowledgements**

This study is dedicated to the memory of the late Mr. Avner Bashan, who encouraged *Azospirillum* research. The authors thank Professor F. B. Dazzo, Michigan State University, East Lansing, U.S.A.; Dr. M. Singh, Gesellschaft für Biotechnologische Forschung, Braunschweig, Federal Republic of Germany; Professor W. Klingmüller, Bayreuth Universität, Federal Republic of Germany; Professor G. Jagnow, Institute of Soil Biology, Braunschweig, Federal Republic of Germany; Dr. J. Döbereiner, EMBRAPA, Rio de Janeiro, Brazil; Dr. S. K. Harrison, Ohio State University, Columbus, U.S.A.; and Mr. Y. Avivi, Weizmann Institute of Science, Rehovot, Israel, for their criticism and evaluation of the manuscript. The authors thank the many scientists who provided manuscripts before publication. Salary to Y. B. was provided by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center, Ohio State University.


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