# POTENTIAL USE OF THE BACTERIA AZOSPIRILLUM IN ASSOCIATION WITH PRICKLY PEAR CACTUS

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## SUMMARY

The nitrogen fixing bacterium Azospirillum is recognized to synthesize plant growth regulators. This microorganism was found to live associated with prickly pear cactus. Natural Azospirilla populations in Opuntia roots were found to be 11,000 cells per g of fresh root. When Opuntia cladodes were inoculated with a mixture of A. brasilense strains, Azospirilla numbers reached 1 X $10^6$ per g of fresh root. Fresh and dry weights of Opuntia roots were substantially increased by inoculation, 47% and 34.0% respectively over an uninoculated control. Nitrogen percentage and total N content from roots were also increased by inoculation in 21% and 63.0%, respectively, over uninoculated cladodes. Root development enhancement of inoculated Opuntia, may lead to a better nutrient uptake and to an improvement of water status of the plant, a fact of great importance for plants growing under semi-arid or arid conditions.

Soil microorganisms are associated to roots within the root tissue, on the root surface (rhizoplane) and within the soil immediately around the root surface (rhizosphere). Rhizosphere bacteria can lead to beneficial effects on the plant, such as competitive suppression of pathogens, nitrogen fixation, production of growth-regulator substances, etc.

Air is four-fifths nitrogen, and neither man, animals nor higher plants can use atmospheric nitrogen. Then, the nitrogen must be combined with hydrogen (to form ammonia), or with oxygen (to form nitrates) before it can be assimilated. Thus, the absence of this combined element is considered the most common limiting factor of food production. Fortunately, certain bacteria have the ability to convert gaseous nitrogen from the air to combined nitrogen, this process is known as nitrogen fixation.

Growth-regulator substances, such as auxins, are produced by many soil bacteria and fungi. An auxin is an exogenous or endogenous compound which promotes, or inhibits, or otherwise affects growth or some other developmental process in roots (Scott, 1972). The natural auxins are represented by a single compound, indoleacetic acid, which is known to stimulate cell division in the tip of the primary root and in lateral roots, and the vascular cambium.

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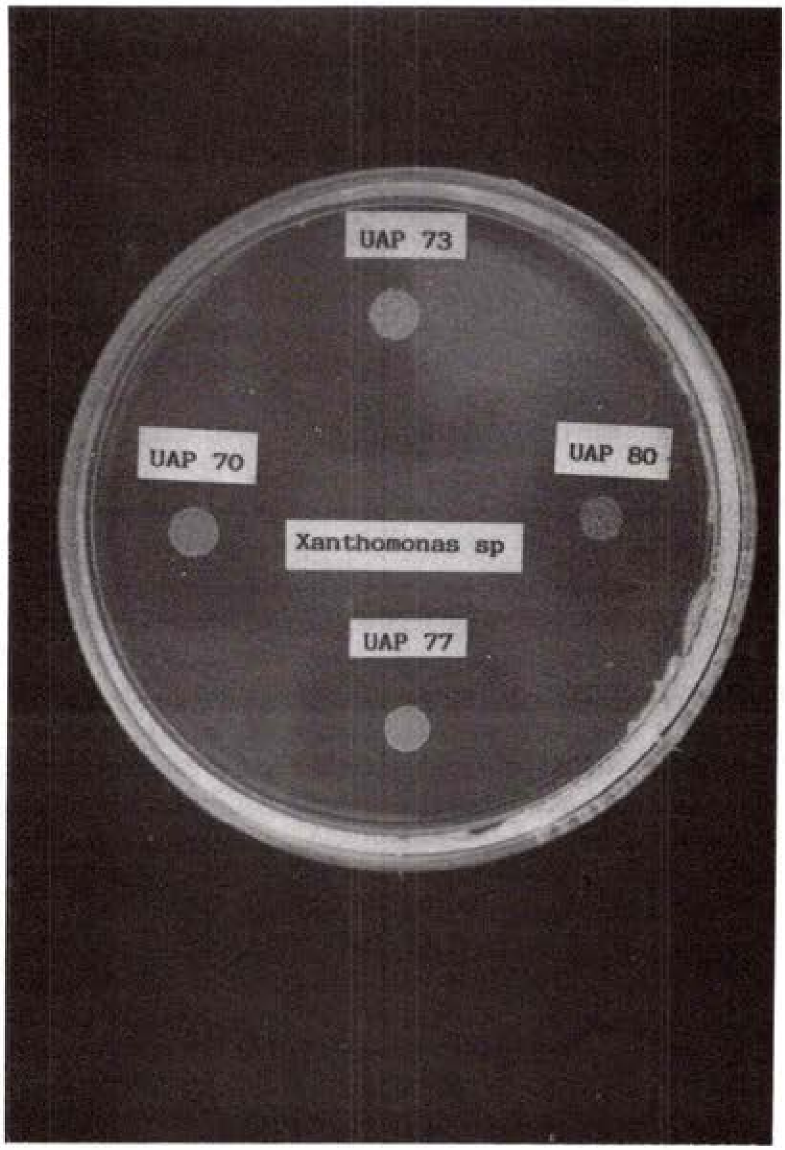
Among the bacteria which colonize roots, increased interest has arisen in bacteria of the genus Azospirillum, because its ability to fix $N_2$ (Kapulnik et al. 1985; Dobereiner and Day, 1976), and to produce plant growth substances such as auxins and cytokinins (Hartman et al. 1983; Tien et al. 1979). One other ability of this microorganism was recently found in my laboratory (data not published); some Azospirillum strains in culture media show competitive suppression against plant pathogenic bacteria, such as Xanthomonas sp, Erwinia species, Agrobacterium tumefaciens, etc. (Fig.1). This bacterium has been isolated from rhizosphere (Caballero-Mellado and Valdes, 1983; Dobereiner and Day, 1976) and roots (Tyler et al. 1979; Wong and Stenberg, 1979) of forage grasses and cereals, from roots of various non-gramineous plants (Shawky, 1989; Kosslak and Bohlool, 1983) and from roots of cactaceous plants, such as Stenocereus pruinosus, S. stellatus and species of Opuntia.

Fig. 1. Inhibition zones formed by siderophore-like activity surrounding Azospirillum brasilense colonies.



Roots from cactaceae species mentioned above, growing under arid conditions from Mexico were collected to assess the occurrence of Azospirilla (Mascarua-Esparza et al. 1988). That study showed that Azospirilla numbers were dependent of the cactaceae species, oscillating from 11,000 to 70,000 per g of fresh root. When cladodes from Opuntia were inoculated with different strains of Azospirillum brasilense [strain UAP 154, isolated from rhizoplane of maize (Paredes-Cardona et al. 1988) and strain UAP 02, isolated from rhizosphere soil of S. pruinosus (Mascarua-Esparza et al. 1988)], under greenhouse conditions in pots, Azospirilla populations oscillated from 1 X $10^6$ per g of fresh root after 43 days. These results suggest the possibility to increase Azospirilla populations in roots of Opuntia to obtain some beneficial effects on plant growth, as occur with forage grass and cereals (Boddey, et al. 1986; Kapulnik et al. 1985; Pacovsky et al. 1985).

On the other hand, inoculation of cladodes with pure cells of A. brasilense significantly enhanced root development (Figs. 2a, 2b) over uninoculated controls. Fresh and dry weights of roots were substantially increased by inoculation in 47.0% and 34.0% respectively, over controls (Table 1). Similar results have been obtained with different inoculated plants (Pacovsky et al. 1985; Venkateswarlu and Rao, 1983). However, the increments obtained in both fresh and dry weights of Opuntia roots, I believe, may be higher. Maize plants, when inoculated, increased the root mass between 22-118% (dry weight) compared to the uninoculated controls; dry weight of leaves was also increased (10 to 91%) and total nitrogen content up to 101%, depending of the inoculated Azospirillum strain (Table 2). According to these data, it is obvious that higher increases can be obtained by selecting the inoculated strain, since Azospirillum strains differ in their effectiveness significantly. It would be desirable to assess the response of Opuntia to inoculation with different strains of Azospirillum to select more effective strains. In addition, to obtain positive effects, it is necessary to find compatible partners (plant-bacteria) of a certain environment (Saric et al. 1987).

It has been suggested that plant growth substances, such as auxins, produced by Azospirillum are responsible for the observed increase in plant growth (Okon et al. 1983; O'Hara et al. 1981). We have reported that our strains, those inoculated to Opuntia, A. brasilense UAP 02 and UAP 154 produced in vitro 77 and 54 $\mu$g/ml of indole-acetic acid respectively (Mascarua-Esparza et al. 1988; Paredes-Cardona et al. 1988). The increase of root size is important to improve the nutrient uptake from the soil or from fertilizers, because of the larger root absorbing surface (Tien et al. 1979). This fact may also be of great importance due to the better utilization of soil moisture (Sarig et al. 1988), especially for plants growing under semi-arid or arid conditions, where soil moisture is available for a very short period (Sarig et al. 1984).

Fig. 2a. Root system of Opuntia sp inoculated with a mixture of A. brasilense UAP 02 and UAP 154 strains (right); uninoculated control (left).

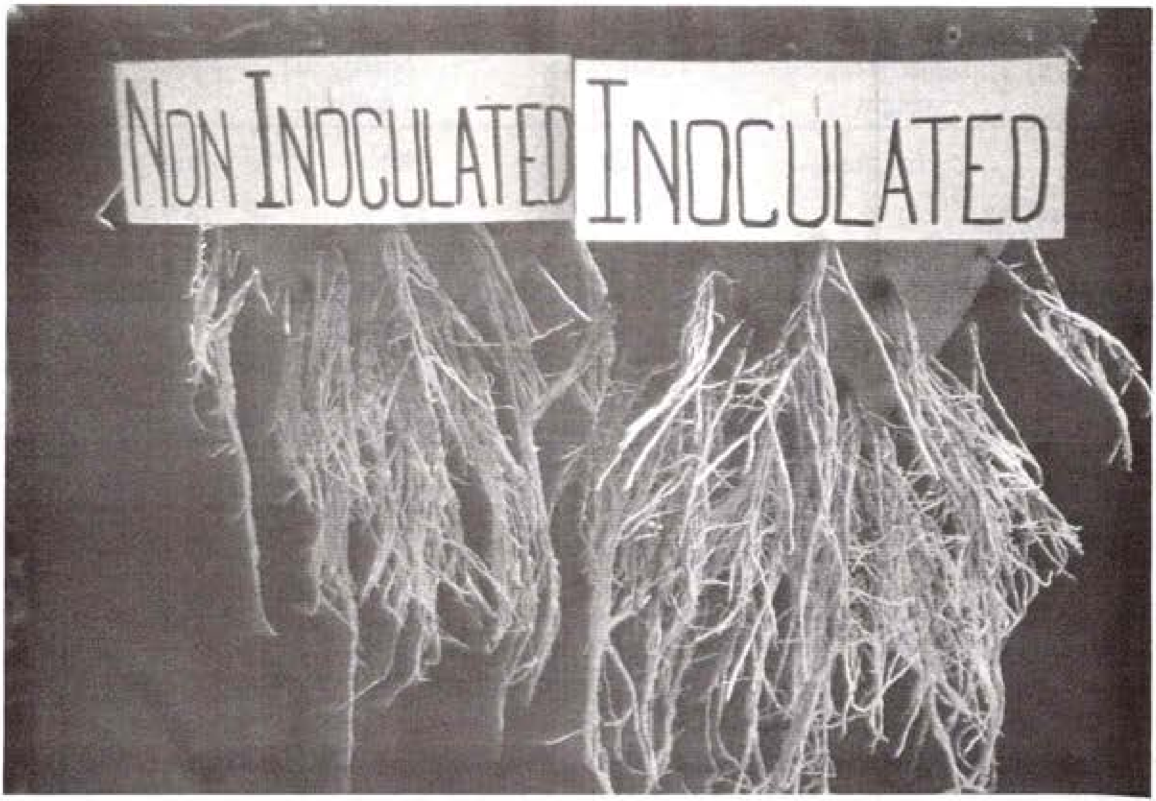



Fig. 2b. Root system of Opuntia sp (photographed in water) inoculated with a mixture of A. brasilense UAP 02 and UAP 154 strains (right); uninoculated control (left).

Table 1. Effect of inoculation with a mixture of Azospirillum brasilense UAP 02 and UAP 154 on Opuntia

| Treatment | Root fresh weight | Root dry weight | Nitrogen content | Total N in roots |
|---|---|---|---|---|
| | (g/plant) | (g/plant) | (%) | (mg/plant) |
| Uninoculated | 8.18 | 1.48 | 1.13 | 16.72 |
| Inoculated | 12.06 (47) | 1.99 (34) | 1.37 (21) | 27.26 (63) |

Data are mean from five replicates

Numbers in the parentheses indicate percent increase over uninoculated control.

*The Kjeldhal digestion of samples was carried out three times for each of the five replicates, numbers represent the mean values.

Cladodes inoculated and uninoculated controls were grown 43 days in pots containing 4.0 Kg of a sandy soil; they were irrigated with distilled water at day 0 (400 ml), day 12 (750 ml), and day 25 (1250 ml). The light/dark period was 12/12 h. Day/night temperature and relative humidity were 37-40°C/40% and 18-20°C/50%, respectively.

Table 2. Effect of inoculation with Azospirillum brasilense strains on the growth of maize.

| Strain | Dry weight* Roots | Increment (%) | Dry weight* Aerial part | Total N (mg/plant) Aerial part | Increment (%) |
|---|---|---|---|---|---|
| UAP 151 | 1.75±0.30 a | 116.0 | 2.16±0.19 a | 46.54±4.8 a,b | 86.16 |
| UAP 46 | 1.77±0.16 a | 118.5 | 1.81±0.28 a,b | 40.36±6.6 b | 61.44 |
| UAP 41 | 1.49±0.36 b | 84.0 | 1.75±0.03 b | 38.77±5.6 b | 55.08 |
| UAP 154 | 1.46±0.31 b | 80.0 | 1.72±0.25 b | 50.34±8.4 a | 101.36 |
| UAP 38 | 1.08±0.14 e,d | 33.3 | 1.65±0.07 b,c | 33.41±2.4 c | 33.64 |
| SP 245 | 1.59±0.10 a,b | 96.3 | 1.62±0.10 b,c | 46.81±1.6 a | 87.24 |
| SP 7 | 1.42±0.28 b | 75.0 | 1.50±0.16 c | 34.40±4.3 b,c | 37.60 |
| UAP 30 | 0.99±0.17 e,d | 22.0 | 1.40±0.19 c,d | 30.25±4.0 d,e | 21.00 |
| UAP 42 | 1.17±0.03 d | 38.0 | 1.36±0.32 c,d | 27.90±6.5 d,e | 11.60 |
| UAP 35 | 1.20±0.48 c,d | 48.0 | 1.25±0.10 d | 27.33±2.9 d,e | 9.32 |
| Control | 0.81±0.13 e | ---- | 1.13±0.25 e | 25.00±7.1 e | ---- |

*Expressed in g/plant

Data represent the mean values of eight plants

Data with standard deviation of the mean followed by the same letter within each column do not differ at $P \leq 0.05$, using Student t-test.

Source: Adapted from Paredes-Cardona, E. et al. 1988. Rev. Lat-amer. Microbiol. 30.351-355

Content of nitrogen in the roots of inoculated Opuntia was increased both percent (21%) as well as the total amount of nitrogen (63%), compared to the uninoculated control plants. Similar results were obtained with inoculated sorghum growing in pots (Pacovsky et al. 1985). Probably the very high increases in N percent and total N content from roots of inoculated Opuntia were probably partially due to the $N_2$ fixation by the Azospirilla. $^{15}N$ studies will be necessary to verify this possibility. Working with $^{15}N$ Christiansen-Weninger et al. (1985), reported that 25.9% of the nitrogen in the roots of sorghum was derived from associated biological nitrogen fixation. Although the mechanism by which the nitrogen content from roots of Opuntia was increased has not been defined, these results show the potential use of Azospirillum inoculation on prickly pear cactus.

In other words, inoculation of prickly pear cactus with Azospirillum leads to an increase of the nitrogen content and particularly to an increase of the root development, which may lead to better nutrient uptake and to an improvement of the water status of the plant. These benefits from inoculation with selected strains of Azospirillum could accomplish a better growth rate of the cactus. More evaluations of Azospirillum inoculation on prickly pear cactus must be carried out, particularly on the cladodes production and nitrogen content.

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