Plant and Soil (2005) 270: 223–232
DOI 10.1007/s11104-004-1522-7


# Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere

N. Tejera[1], C. Lluch[1], M.V. Martínez-Toledo[2] & J. González-López[2,*]
[1]*Departamento de Fisiología Vegetal, Facultad de Ciencias, Universidad de Granada, Campus de Fuentenueva s/n, 18071 Granada, Spain.* [2]*Instituto del Agua, Universidad de Granada, 18071 Granada, Spain.* [3]*Corresponding author**

Received 22 January 2004. Accepted in revised form 20 July 2004

*Key words: Azospirillum*, *Azotobacter*, nitrogen fixation, sugarcane

**Abstract**

Bacteria with the ability to grow on nitrogen-free media and with nitrogenase activity under aerobic or microaerobic conditions were isolated from sugarcane roots collected from four different agricultural locations in Granada (Spain). Isolates were Gram negative rods and were identified as *Azotobacter chroococcum* and *Azospirillum brasilense*. Our results suggest that *Azotobacter* isolates do not have a particular affinity for sugarcane rhizospheres and that, on the contrary, *Azospirillum* isolates show specific association and perhaps endophytic colonization of sugarcane. However, obligate endophytes (*Gluconacetobacter diazotrophicus*) were not found in the apoplastic fluid of the stems and macerates extracts of sugarcane tissues with the procedure applied. Population of this microorganism might be in low number in the Spanish sugarcane varieties studied which is also discussed.

## Introduction

Grasses constituted an important source of food all over the world. In particular, grasses such as rice, wheat, maize, sorghum, and sugarcane, currently have much of their N needs supplied by costly mineral fertilizers (Döbereiner et al., 1995; Triplett, 1996). Evidence for biological nitrogen fixation in sugarcane (*Saccharum* spp.) was reported in Brazilian sugarcane varieties. Studies on long-term N-balance and $^{15}N$ isotope dilution technique (Urquiaga et al., 1992) have shown that some sugarcane varieties may actually obtain up to 70% of their N requirements by nitrogen fixation. In this process seems to participate both rhizosphere and endophytic diazotrophs (Baldani et al., 1997). $N_2$ fixing bacteria such as *Enterobacter cloacae*, *Erwinia herbicola*, *Klebsiella pneumoniae*, *Azotobacter vinelandi*, *Paenibacillus polymixa*, *Azospirillum* spp., *Herbaspirillum* spp. and *Gluconacetobacter diazotrophicus* colonise the sugarcane plant and its tissues (Cavalcante and Döbereiner, 1988; Olivares et al., 1996).

*Azotobacter* is able to fix at least 10 mg N per gram of carbohydrate (Becking, 1992). This bacterium is an obligate aerobic, although it can grow under low $pO_2$. The ecological distribution of *Azotobacter* spp. is a complicated subject and is related with diverse factors which determine the presence or absence of this bacterium in an specific soil. It has been shown that the soil characteristics and climate conditions affect the distribution of this microorganism (Döbereiner and Pedrosa, 1987; González-López, 1992); it includes organic matter content, moisture, C/N relation and pH (González-López, 1992).

*Azospirillum* species belong to the facultative endophytic diazotrophs group which colonize the surface and the interior of roots, being this kind of association considered as the starting point of most ongoing BNF programs with non-legume plants worldwide (Baldani et al., 1997). Bacteria are microaerophilic, nitrogen-fixing, Gram-negative rods and often associated with roots of cereals and grasses (Grifoni et al., 1995). However obligate endophytes such as

* FAX No: 34-958-243094.
E-mail: jgl@ugr.es