

## SHORT COMMUNICATION

# Evaluation of biological nitrogen fixation in sugarcane associated microorganisms

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Sugarcane is a highly nutrient extractor crop, regarding nitrogen it is generally accepted that sugarcane plants extract between 120 to 270 kg · ha<sup>-1</sup> and the first ratoon between 100 to 170 kg · ha<sup>-1</sup>.<sup>1</sup> These values suppose that without nitrogen fertilization continuous sugarcane harvests may deplete nitrogen reserves in soil no matter how fertile it could be, however that doesn't occur,<sup>2</sup> therefore biological nitrogen fixation (BNF), a natural occurring event, could be responsible for this fact.

BNF is a skill reserved in nature to a few bacterial genera, some of them symbiotic and other plants associated or free living. This biochemical process consists of the atmospherical dinitrogen reduction to ammonia by means of the nitrogenase enzyme complex present in these microorganisms, like that atmospherical dinitrogen enters nitrogen cycle thus becoming an important nitrogen source for the biosynthetic processes of beneficiary crops and also helping soil enhancing, which explains the previous affirmation. In the sugarcane crop, a gramineae, interaction between plants and diazotrophic bacteria occurs throughout association, differently from what takes place in the symbiotic rhizobium-legume interaction. Several diazotrophic bacterial genera have been reported associated with sugarcane at different levels depending on the genus being *Azospirillum* sp. and *Acetobacter diazotrophicus* among the most significant BNF contributors.<sup>1</sup> *Azospirillum*

has been found in the rhizosphere and rhizoplane of sugarcane and other gramineae.<sup>3</sup> *A. diazotrophicus* has been isolated from roots, apical zones, stems and leaves in a great number of sugarcane cultivars<sup>4</sup> but never from soil.<sup>5</sup> Several environmental and genetical factors have influence on the association like the strain-cultivar specificity and the competitive capacity within the microbiota, which exerts influence on the BNF process and its contribution to vegetal nutrition.<sup>6</sup> The detection and quantification of this process in sugarcane endophytes and rhizospherical microorganisms is of the outmost importance to determine its contribution to nitrogen metabolism in gramineae, thus making possible to improve the crop yields by means of application by inoculation of nitrogen fixing efficient strains.

Quantification studies of BNF associated with sugarcane first began in the early 70s using the acetylene reduction assay (ARA).<sup>7</sup> This method because of its sensitivity is used in screenings to detect bacteria with high nitrogen fixation potentials. These studies have been complemented with other techniques such as isotopic dilution (ID) of <sup>15</sup>N and total nitrogen balance in the plant-soil system.<sup>8</sup>

In the laboratory using the ARA technique some *Azospirillum* and *A. diazotrophicus* strains with high nitrogenase activity values were detected in pure culture media. The technical conditions of the chromatographic runs were the

following: gas chromatograph type UNICAM Series 610 with flame ionization detector (FID); crystal packed column 2.7 m x 4.4 mm; packing material Porapak N 80-100 mesh; oven temperatures: injector 100 °C, column 100 °C, detector 150 °C; flows: N<sub>2</sub> 30 mL · min<sup>-1</sup>, H<sub>2</sub> 32 mL · min<sup>-1</sup>, air 320.5 mL · min<sup>-1</sup>; run time 6 min; ethylene retention time 3.25 min; acetylene retention time 4.73 min; injection volume 1 mL.

The pure cultures of *Azospirillum* in Nfb<sup>9</sup> and *A. diazotrophicus* in LGI-P<sup>4</sup> media were taken at the tenth hour of logarithmic growing phase and incubated for one hour in a 10 % acetylene atmosphere at 35 °C. The ARA values reported (media of ten replays) (Table 1) in eight *Azospirillum* strains and two *A. diazotrophicus* strains can be considered good in five strains of *Azospirillum* and the two of *A. diazotrophicus* when compared with values obtained from the pattern strains used: *Azospirillum brasiliense* sp7 (ATCC 29145), *Azospirillum lipoferum* sp. Br17 (ATCC 29709) and *A. diazotrophicus* PAL5 (ATCC 49037), all of Brazilian origin. These values constitute a very useful indicator for the selection of efficient strains to be proposed as main components in the formulation of bioproducts to be commercialized and applied in sugarcane with the aim to magnify the BNF process. These results together with other important indicators lead to the selection of the 8-I strain of *Azospirillum*

**Table 1.** Nitrogenase activity of strains measured by ARA.

| <i>Azospirillum</i> sp.           |     |                                                                           |
|-----------------------------------|-----|---------------------------------------------------------------------------|
| Strain                            |     | nmol C <sub>2</sub> H <sub>4</sub> · vial <sup>-1</sup> · h <sup>-1</sup> |
| Sp7 (pattern)                     | (c) | 337                                                                       |
| SpBr17 (pattern)                  | (d) | 292                                                                       |
| Sp70                              | (b) | 380                                                                       |
| Sp69                              | (b) | 370                                                                       |
| SpGI                              | (f) | 70                                                                        |
| Sp8I                              | (c) | 338                                                                       |
| SpQI                              | (f) | 89                                                                        |
| Sp40FA                            | (a) | 390                                                                       |
| Sp79I                             | (c) | 337                                                                       |
| Sp11                              | (c) | 270                                                                       |
| <i>Acetobacter diazotrophicus</i> |     |                                                                           |
| PAL5 (pattern)                    | (c) | 194.40                                                                    |
| PR8                               | (b) | 222.16                                                                    |
| 166                               | (a) | 229.76                                                                    |

*rillum* with which a biofertilizer were developed and inoculation field experiments carried out under different edafoclimatic and phytotechnical management conditions, demonstrating the profitable effect of these inoculations.<sup>10</sup>

#### BIBLIOGRAPHY

1. Urquiaga S., Katia H.S. and Boddey M. Contribution of Nitrogen Fixation to Sugarcane. <sup>15</sup>N and N balance estimate. **Soil. Science, Soc. Am. J.**, **56**, 105, 1992.
2. Sampaio E.V., Salcedo I.M., Victoria R.I. and Trivelin P.C. Redistribution of

the Nitrogen Reserves of <sup>15</sup>N Enriched Stem Cutting and Dinitrogen Fixed 90-days Old Sugarcane. **Plant and Soil**, **108**, 275, 1988.

3. Vose P.B. Development in Non-legume Dinitrogen Fixing Systems. **Canadian Journal of Microbiology**, **29**, 837, 1993.
4. Cavalcante V.A. and Döbereiner J. A New Acid-tolerant Nitrogen-fixing Bacterium Associated with Sugarcane. **Plant and Soil**, **108**, 23, 1988.
5. Döbereiner J. Recent Change in Concepts of Plant-bacteria Interaction: Endophytic N<sub>2</sub> Fixing-bacteria. **Ciencia e Cultura. Journal of the Brazilian Association for the Advancement of Science**, **44**, 310, 1992.
6. Okon Y. and Kapulnik J. Development and Function of *Azospirillum*-inoculated Roots. **Plant and Soil**, **90**, 3, 1986.
7. Dilworth M. Biochim. **Biophys. Acta**, **127**, 285, 1966.
8. Boddey R.M. Methods for the Quantification of Nitrogen Fixation Associated with Gramineae. **CRC Crit Rev. Plant Science**, **6**, 209, 1987.
9. Döbereiner J. Ten Years of *Azospirillum*. **Exp. Suppl.**, **48**, 9, 1983.
10. Roldós J., Pérez J y Casas M.A. Capacidad nitro fijadora de la cepa INICA-8 de *Azospirillum* sp. y su relación con la caña de azúcar. **Revista ATAC**, Vol. 1, 1999.



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