Soil Biology & Biochemistry 103 (2016) 117–130

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Review paper

Azospirillum sp. in current agriculture: From the laboratory to the field

Fabricio Cassán [a, *], Martín Diaz-Zorita [b]

[a] Laboratorio de Fisiología Vegetal y de la Interacción Planta-Microorganismo, Universidad Nacional de Río Cuarto, Ruta 36, Km 601, Campus Universitario, CP 5800, Río Cuarto, Córdoba, Argentina
[b] Technical Development, Monsanto BioAg, Pilar, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form
16 August 2016
Accepted 17 August 2016

Keywords:
Biological-products
PGPR
Phytohormones
Inoculants
Sustainable agriculture

ABSTRACT

Azospirillum sp., one of the best studied genus of plant growth promoting rhizobacteria, are able to colonize hundreds of plant species and improve their growth, development and productivity. Free nitrogen fixation and additive mechanisms related to the ability of *Azospirillum* sp. to produce phytohormones and other related molecules are proposed for explaining the plant growth promotion effects on inoculated plants, mainly under stressing conditions. Under field evaluations, the benefits of *Azospirillum* sp. inoculation, mostly related with plant attributes defined during early growth, can be related with the increase in the root development enlarging the explored soil volume for nutrients and water acquisition. Recently published reports of *Azospirillum* sp. inoculation of dryland crops showed grain yield responses on winter (14.0%) and summer cereals (9.5%) and also on legumes (6.6%). These responses are barely observed under strong stressful growing conditions (i.e. severe droughts, major nutrients limitations, etc.) and are currently obtained 70% of the time because the complex interaction between the modes of action of *Azospirillum* sp. and plants, the methods of inoculation and diverse crop production conditions. The practice of inoculating with selected strains of *Azospirillum* sp. provides a direct contribution increasing crop yields and enhance the efficacy in the use of production resources with extended benefits to the environment. One of the achievements from the research is the commercial use of azospirilla inoculants in approximately 3.5 million ha, mainly cultivated with cereals in South America. However, more coordinated communication programs of its complementary benefits for the development of sustainable crop production practices are still needed.

Contents

* Corresponding author.
E-mail address: fcassan@exa.unrc.edu.ar (F. Cassán).

http://dx.doi.org/10.1016/j.soilbio.2016.08.020
0038-0717/

1. *Azospirillum* sp. as model of plant growth promoting rhizobacteria

Azospirillum sp. is one of the best studied genus of plant growth promoting rhizobacteria at present. This microorganism is able to colonize more than one hundred plant species and significantly improves their growth, development and productivity under field conditions (Bashan and de-Bashan, 2010). One of the principal mechanisms proposed for *Azospirillum* sp. to explain plant growth promotion of inoculated plants, has been related to its ability to produce and metabolize several phytohormones and other plant growth regulation molecules (Tien et al., 1979). From a historical perspective, many studies detailing the beneficial effects of inoculation with beneficial rhizobacteria, especially *Azospirillum* sp., have been undertaken and they describe morphological and physiological changes that occur in inoculated plants. However, in many cases the processes, or the compounds responsible for inducing such responses, have not been unequivocally identified and therefore responses are usually considered within a “black box” model which goes beyond the resulting growth promotion due to the presence of only these organisms or active metabolites in the culture medium or plant tissues.

2. *Azospirillum* sp. and it mechanisms to promote plant growth

First mechanism proposed for explaining the capacity of *Azospirillum* sp. to promote the plant growth was the biological nitrogen fixation (Okon et al., 1983). The evidence used to purpose that mechanism was: (1) inoculation, significantly increases the total nitrogen content in shoots and grains; (2) inoculation, commonly reduces the required doses of nitrogen fertilization for many plant species; (3) inoculation contributes to improve the N balance of plants (Kapulnik et al., 1981). Oppositely, Bashan and Holguin (1997) and Bashan and Levanony (1990) showed that the contribution of nitrogen fixation by *Azospirillum* reached less than 20% of the total N increase in the plant. These findings indicated agronomic significance than initially were expected for this bacterial genus (Kennedy et al., 1997). A decade later, new studies showed there is not unequivocal evidence that nitrogen fixation by *Azospirillum* or other free-living nitrogen fixers plays a significant role in agriculture (For details see Giller and Merckx, 2003).

Other mechanisms of plant growth promotion have been studied and proposed for this microbial genus, such as phytohormone and/or siderophore production, phosphate solubilization (Puente et al., 2004), biocontrol of phytopathogens (Bashan and de-Bashan, 2010) and protection of plants against stress like soil salinity or toxic compounds (Creus et al., 1997). Tien et al. (1979) were the first to suggest that *Azospirillum* sp. could enhance plant growth by auxins and particularly indole-3-acetic acid (IAA) production, and subsequent studies showed the capacity of this genus to produce several other phytohormones and plant growth regulators. Although many mechanisms have been described to explain the plant growth promotion by *Azospirillum* sp., one single mechanism is mostly not responsible for the full effect. *Azospirillum* sp. modes of action could be better explained by the “additive hypothesis” which allows explaining the plant growth promoting effects due to inoculation. This hypothesis was suggested more than 20 years ago (Bashan and Levanony, 1990) and considers multiple mechanisms rather than one mechanism participating in the successful association of *Azospirillum* with plants.

Today, after more than eight decades of studies, we know that these rhizobacteria have been correlated with the production of auxins (Prinsen et al., 1993), cytokinins (Tien et al., 1979), gibberellins (Bottini et al., 1989), ethylene (Strzelczyk et al., 1994), and other plant growth regulators, such as abscisic acid (Cohen et al., 2008), nitric oxide (Creus et al., 2005) and polyamines like spermidine, spermine and the diamine cadaverine (Cassán et al., 2009). Several of the biologically active plant regulators produced by *Azospirillum* sp. are summarized and ranked according their effects on plants in Table 1 (adapted from Cassán et al., 2014).

In the case of the most important groups of plant hormones produced by *Azospirillum* sp. such auxins (IAA), cytokinins (Z) and gibberellins (GA_3), their concentration is lower at early exponential growth phase, but increases during the exponential and/or stationary growth phase, because these compounds are continuously accumulated in the culture medium according to a batch fermentation model (Ona et al., 2003; Cassán et al., 2009a, 2010). Considering this fact, under industrial production of inoculants, the bacterial growth and their capacity to accumulate these metabolites in culture medium should alter the behaviour of the bio-product and its capacity to promote, in a short term way, the plant growth according to the concentration and composition of those phytohormones in the formulation. In this sense, Okon (1982) reported that after seed inoculation the number of viable cells of *Azospirillum* declines very rapidly. So, the short term benefits of inoculation are not strictly related to the presence of the bacterial cells in the inoculant, but are at least in part related to the presence and concentration of phytohormones. So, the final model to explain the positive effects of inoculation with *Azospirillum* sp. a phytohormones-producer plant growth promoting rhizobacteria (PGPR) are summarized and illustrated in Fig. 1.

Table 1
Overview of biologically active plant growth regulators produced *in vitro* by *Azospirillum* sp. based on their class (i.e. biologically active molecules identified from *Azospirillum* sp. liquid cultures by unequivocal methodology like HPLC or, GC-MS) and its hierarchy (i.e. related to the importance of the phytohormonal role in its interaction with the plant considered by the authors based on available evidence) (adapted from Cassán et al., 2014). IAA: indole-3-acetic acid; PAA: phenylacetic acid; IBA: indole-3-butyric acid; NO: nitric oxide; iP: isopentenyl adenine; iPr: isopentenyl adenine riboside; Z: zeatin; t-Zr: trans zeatin riboside; GA3,1, gibberellins n; Et: Ethylene; ABA: abscisic acid; Cad: cadaverine; Spm: spermine; Spd: spermidine; Put: putrescine.

Class	Hierarchy	Molecules	References
Auxins	1st	IAA, PAA, IBA	Prinsen et al. (1993) Martínez-Morales et al. (2003) Somers et al. (2005)
Gibberellins	4th	GA_3, GA_1	Bottini et al. (1989) Piccoli and Bottini (1996)
Cytokinins	3rd	iP, iPr, Z, *t*-Zr	Horemans et al. (1986) Esquivel-Cote et al. (2010)
Ethylene	5th	Et	Strzelczyk et al., 1994
ABA	6th	ABA	Kolb and Martin (1985)
Nitric oxide	2nd	NO	Creus et al. (2005)
Polyamines	7th	Cad, Spm, Spd, Put	Cassán et al. (2009a) Thuler et al. (2003)

2.1. *Effects of Azospirillum sp. on rhizobia-legume symbiosis*

Root nodulating bacteria induce nodule formation on legume