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Special Issue Article

The *Azospirillum brasilense* type VI secretion system promotes cell aggregation, biocontrol protection against phytopathogens and attachment to the microalgae *Chlorella sorokiniana*

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Summary

The plant-growth-promoting bacterium *Azospirillum brasilense* is able to associate with the microalgae *Chlorella sorokiniana*. Attachment of *A. brasilense* increases the metabolic performances of the microalgae. Recent genome analyses have revealed that the *A. brasilense* Az39 genome contains two complete sets of genes encoding type VI secretion systems (T6SS), including the T6SS1 that is induced by the indole-3-acetic acid (IAA) phytohormone. The T6SS is a multiprotein machine, widespread in Gram-negative bacteria, that delivers protein effectors in both prokaryotic and eukaryotic cells. Here we show that the *A. brasilense* T6SS is required for *Chlorella-Azospirillum* synthetic mutualism. Our data demonstrate that the T6SS is an important determinant to promote production of lipids, carbohydrates and photosynthetic pigments by the microalgae. We further show that this is likely due to the role of the T6SS during the attachment stage and for the production of IAA phytohormones. Finally, we demonstrate that the *A. brasilense* T6SS provides antagonistic activities against a number of plant pathogens such as *Agrobacterium*, *Pectobacterium*, *Dickeya* and *Ralstonia* species *in vitro*, suggesting that, in addition to promoting growth, *A. brasilense* might confer T6SS-dependent bio-control protection to microalgae and plants against bacterial pathogens.

Received 16 February, 2021; revised 25 August, 2021; accepted 28 August, 2021. *For correspondence. E-mail luz@bashanfoundation.org; legonzal04@cibnor.mx; Tel: (+1) 256 307 1963; (+52) 612 123 8484, ext. 3419, 3420. †Anahí Coniglio, and Edgar Amavizca contributed equally to this study. ‡Deceased. §This study is dedicated to the memory of Dr. Yoav Bashan, a leading figure in the field of Plant Growth-Promoting Bacteria (PGPB) for environmental purposes, and founder of the Bashan Institute of Science, USA. Prof. Bashan passed away during the edition of the manuscript.

Introduction

Azospirillum spp. is one of the best-characterized genera of plant growth-promoting bacteria (PGPB) and can colonize more than 130 plant species in 37 families (Pereg *et al*., 2016). The association of *Azospirillum* spp. with these plants significantly improves growth, development, and in many cases, yield under field conditions (Cassan *et al*., 2020). There is no single mechanism involved in promoting plant growth with *Azospirillum*, but rather a combination of mechanisms in different cases of inoculation. These mechanisms work together or in tandem, and the phenomenon is commonly known as 'multiple mechanism theory' (Bashan and de-Bashan, 2010). The production of indole-3-acetic acid (IAA) by *Azospirillum* spp. is one of the main mechanisms proposed for the effect of