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Plant Growth-Promoting Rhizobacteria Modulate the Concentration of Bioactive Compounds in Tomato Fruits

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Abstract: *Background*: The application of microorganisms as bioestimulants in order to increase the yield and/or quality of agricultural products is becoming a widely used practice in many countries. In this work, five plant growth-promoting rhizobacteria (PGPR), isolated from cultivated rice paddy soils, were selected for their plant growth-promoting capacities (e.g., auxin synthesis, chitinase activity, phosphate solubilisation and siderophores production). Two different tomato cultivars were inoculated, Tres Cantos and cherry. Plants were grown under greenhouse conditions and different phenotypic characteristics were analysed at the time of harvesting. *Results*: Tres Cantos plants inoculated with PGPR produced less biomass but larger fruits. However, the photosynthetic rate was barely affected. Several antioxidant activities were upregulated in these plants, and no oxidative damage in terms of lipid peroxidation was observed. Finally, ripe fruits accumulated less sugar but, interestingly, more lycopene. By contrast, inoculation of cherry plants with PGPR had no effect on biomass, although photosynthesis was slightly affected, and the productivity was similar to the control plants. In addition, antioxidant activities were downregulated and a higher lipid peroxidation was detected. However, neither sugar nor lycopene accumulation was altered. *Conclusion*: These results support the use of microorganisms isolated from agricultural soils as interesting tools to manipulate the level of important bioactive molecules in plants. However, this effect seems to be very specific, even at the variety level, and deeper analyses are necessary to assess their use for specific applications.

Keywords: plant growth-promoting rhizobacteria (PGPR); tomato; lycopene; functional food; ROS; bioactive compounds

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1. Introduction

In nature, all plant organs are colonised in some way by fungi, actinomycetes, protozoa, algae but, above all, bacteria [1]. Plant growth-promoting rhizobacteria (PGPR) are rhizosphere microorganisms that can exert highly beneficial effects on plant development by direct or indirect mechanisms. The use of microorganisms in agriculture is becoming an important alternative to traditional fertilisation, and many beneficial effects of this application have been described in terms of plant growth, stress tolerance or plant nutrition [1–4].

PGPR and their interactions with plants are starting to be exploited commercially, with increasing examples of its use in agriculture. Among others, the application of PGPR has been investigated in many crops including oat, canola, soy, potato, maize, peas, tomato, lentil, barley and wheat [5]. PGPR are involved in many aspects of the soil ecosystem, making it dynamic for turnover and sustainable for crop production [6]. They colonise plant

roots and enhance plant growth by different mechanisms including phosphate solubilisation [7], nitrogen fixation [8], production of indole-3-acetic acid (IAA) [9], siderophores [10], 1-amino-cyclopropane-1-carboxylate (ACC) deaminase [11], hydrogen cyanate [12], hormones, antibiotics or lytic enzymes [13] and degradation of environmental pollutants [14]. Many PGPR are reported to increase tolerance to stresses such as salinity [15], heavy metals [16] or pathogens and insects [17]. For instance, *Azospirillum brasilense* applied to maize seedlings increased catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) levels in leaves and protected plants from saline stress by inducing the synthesis of salicylic acid (SA) and abscisic acid (ABA) [18]. In a study carried out in tomato (*Lycopersicum sculentum* L.), the use of two different PGPR (i.e., *Pseudomonas aeruginosa* and *Burkholderia gladioli*) diminished the oxidative stress induced by Cd by modulating the antioxidative defence [16]. Although many groups are analysing and suggesting the use of PGPR to improve growth or tolerance to stress in plants, very little is known about the use of these PGPR to change the nutritional value of some crops.

Plants are a source of many bioactive molecules with beneficial effects on human health, and these benefits have begun to be appreciated. Currently, consumers are aware of the functional characteristics of agricultural food products, and more often they choose foods taking into account their benefits for health [19]. In the 1990s, this food from different origins started being called "functional food", applying to whole, fortified, enriched or enhanced foods that provide additional or enhanced health benefits, beyond the provision of essential nutrients, when they are consumed as part of a varied diet [20]. Fruits and vegetables are an important source of healthy elements to the diet such as minerals, both macro- and micronutrients, water-soluble and fat-soluble vitamins and a wide variety of phytochemicals. The most well-known phytochemicals from vegetables are polyphenols, carotenoids, organo-sulfur and seleno-compounds. These bioactive molecules can act, for example, as free radical scavengers or as antimicrobial agents [19]. Many plant foods have been tested for their role in disease prevention and improving health. Some include oat soluble (β-glucan) fibre, soluble fibre from psyllium seed husk, soy protein and sterol- and stanol-ester-fortified margarine, all of which have been clinically proved to reduce total and LDL cholesterol [20].

Throughout the last decades, many studies have proved the beneficial effects of tomato and tomato-derived products in the diet including a lower cardiovascular and cancer risk, mainly prostate cancer [19,21]. Tomato contains phytochemicals with antioxidative, antiproliferative, anti-carcinogenic, anti-tumorigenic, anti-inflammatory, anti-mutagenic and anti-atherogenic properties [22]. Among others, tomato is an important source of bioactive molecules such as phenolic compounds (phenolic acids and flavonoids), vitamins (ascorbic acid and vitamin A) and glycoalkaloids (tomatine). However, the major classes of these bioactive compounds in tomatoes are carotenoids [19], synthesised in leaves, flowers and fruits. In leaves, lutein is the main carotenoid, acting as a photoprotector molecule, whereas in flowers, xanthophyll, violaxanthin and neoxanthin confer the typical yellow colour to flowers. In ripe tomato fruits, lycopene is the major carotenoid, causing the characteristic red colour and the special antioxidant properties of tomatoes [19,21]. Lycopene is a powerful antioxidant found in some red foods such as tomatoes and their products, guava, watermelon, papaya and cherry [23]. Tomatoes provide nearly 85% of total lycopene in the human diet [24], and this carotenoid seems to be responsible for most of the health benefits of this fruit in the diet [21].

In this work, we analysed the effect on growth, antioxidant activities, sugar content and concentration of the bioactive molecule lycopene of five PGPR inoculated in two different tomato cultivars: Tres Cantos and Cherry. We discuss the application of microorganisms to modify the level of bioactive molecules in plants in order to improve their use as functional foods.

2. Materials and Methods

2.1. Isolation of Culturable Rhizospheric Bacteria

Rice rhizosphere samples were obtained randomly from cultivated rice fields located in the marshlands of the Guadalquivir River in Seville, southern Spain.

Freshly collected rice roots were washed in sterile NaCl 0.9% with shaking for one hour at 28 °C to isolate rhizospheric bacteria. Bacterial samples were serially diluted with sterile saline solution and plated in triplicates onto tryptone soy agar (TSA) [25] supplemented with cycloheximide (100 μg mL^{-1}) to inhibit fungal growth. Plates were incubated for 48 h at 28 °C, and then colonies with different morphologies and pigmentation were isolated, purified in TSA plates, and stored at −80 °C until use.

2.2. Plant Growth-Promoting Activities

Production of IAA was colorimetrically determined according to [26]. Briefly, liquid bacterial cultures were grown at 28 °C with agitation for 24 h. After centrifugation at 13,000 rpm, 200 μL of supernatants were mixed with 4 volumes of Salkowski reagent (0.5M ferric chloride in 36.75% sulphuric acid) and incubated at room temperature for 20 min. Colour changes were measured at 535 nm in a GENESYS 20 spectrophotometer (ThermoFisher Scientific, San José, CA, USA). Pure indole-3-acetic acid (Merck, Darmstadt, Germany) was used as the standard for quantification. At least two biological replicates with at least three technical replicates were performed for each bacterial strain.

ACC deaminase activity was quantified following the protocol described in [27]. For the detection of chitinase activity, bacteria were grown in minimal medium with colloidal chitin as a unique carbon and energy source. A halo around the colony appeared in those colonies able to degrade chitin.

Cellulolytic activity was assayed as described in [28]. After 4–5 days of growth at 28 °C in CMC medium, agar plates were revealed by covering the whole surface of the plate with Congo red at 0.1% (w/v) and incubating at room temperature for 20–30 min. Then, Congo red was discarded, and the plates were washed three times for 5 min at room temperature with 0.1 M NaCl. A yellow halo around the colony appeared in those bacteria with cellulolytic activity.

To detect amylolytic activity [29], bacterial isolates were grown in starch agar for 3–4 days at 28 °C. Then, plates were revealed with Lugol (Merck, Darmstadt, Germany), which turned the medium darker. The presence of an orange halo around the colony indicated degradation of amylase.

Proteolytic activity was assayed as described in [30]. Bacteria with this activity were detected by the presence of a halo of protein degradation around the colony.

Ureolytic activity was detected according to [31]. Bacteria able to degrade urea were detected by a colour change (yellow to pink) in the growth medium due to the increase in pH.

To evaluate phosphate solubilisation, microorganisms were grown in the National Botanical Research Institute's phosphate growth medium (NBRIP) [32]. Those bacteria able to solubilize phosphate formed a halo around the colony. For potassium solubilisation, microorganisms were grown in Pikovskaya growth medium [33]. When a bacterium was able to solubilize potassium, the purple colour of the medium turned yellow around the colony.

The production of siderophore-type iron-binding compounds was determined using the CAS assay [34]. Briefly, 10 cm diameter Petri dishes were filled with 30 mL of an appropriate solid medium for each strain. Once solidified, the medium was cut into two halves. One of them was replaced with CAS-blue agar. The halves with the growth medium were inoculated with the isolated bacterial strains. Plates were incubated in the dark at 28 °C for 21 days and monitored every 7 days, measuring the advance of the colour-change front in the CAS-blue agar. Those bacteria that induced the formation of a halo larger than 10 mm in 7 days were considered siderophores producers.

2.3. Identification of Bacteria

For the identification of rhizobacteria, DNA was extracted using the miniprep PureLink® Genomic DNA (ThermoFisher Scientific, San José, California, USA) following the instructions of the manufacturer. The 16S rDNA fragment was amplified using the universal primers pA and pH′ (5′-AGAGTTTGATCCTGGCTCAG-3′ and 5′-AAGGAGGTGATCCAG CCGCA-3′, respectively) following reaction conditions previously described [35]. The specific PCR products (approximately 1500 bp) were separated by electrophoresis, purified from the gels and sequenced to classify microorganisms at the genus level.

2.4. Inoculation and Growth Conditions of Tomato Plants

Tomato seeds were surface sterilised in ethanol for 1 min and then incubated for 5 min in 5% (*v*/*v*) sodium hypochlorite. Finally, seeds were washed with sterile water and pre-germinated in 10% water–agar for two days at 25 °C in complete darkness. Once germinated, plants were transferred to 2 L pots (1 plant per pot) filled with vermiculite and watered with Hoagland 0.5× nutrient solution. For inoculated plants, twelve days after plants were transferred to pots, they were root inoculated with 1 mL of bacterial cultures at an OD of approximately 10^5 bacteria mL^{-1}. Plants were grown under controlled greenhouse conditions from February to June at the "Servicio de Invernadero de la Universidad de Sevilla" (Seville, Spain) and watered with Hoagland 0.5× solution weekly until harvesting.

Plant shoots and mature fruits were harvested, weighted and frozen separately at −40 °C until use. For dry weight measurements, 20 g of shoots were dried for 48 h at 70 °C, cooled and reweighed.

2.5. Enzyme Extraction and Analysis

Protein extracts for enzymatic activities were obtained by grinding 0.2 g fresh weight of leaf tissue in 1 mL of extraction buffer containing: 0.1 M Tris–HCl pH 7.5, 20% (by vol.) glycerol, 1 mM EDTA, 10 mM $MgCl_2$ and 14 mM β-mercaptoethanol. The homogenate was centrifuged at 15,000× *g* for 2 min, and the supernatant was filtered through a Sephadex G-25 [36].

Enzymatic activities were reported as units per mg of protein [34] using BSA (bovine serum albumin) as a standard. The ascorbate peroxidase activity (APX, EC: 1.11.1.11) was determined by monitoring the rate of H_2O_2-dependent oxidation of ascorbate (coefficient of extinction of 2.8 mM^{-1} cm^{-1}) in absorbance at 290 nm for 120 s [35]. The reaction mixture consisted of 50 mM HEPES-NaOH buffer (pH 7.6), 0.2 mM ascorbate, 5 mM H_2O_2 and leaf and root extract, 50 and 100 mL, respectively. Catalase activity (CAT, EC 1.11.1.6) was evaluated as described in [36], estimating the absorbance reduction at 240 nm for 120 s, as the result of H_2O_2 utilisation (extinction coefficient of 39.58 m^{-1} cm^{-1}). The reaction mixture contained 60 mM potassium phosphate buffer (pH 7.0), 5 mM H_2O_2 and leaf and root extract, 50 and 100 mL, respectively.

Lipid peroxidation was performed as described in [37], with the trichloroacetic acid (TCA) and thiobarbituric acid (TBA) determining malondialdehyde (MDA) as the end product of lipid peroxidation. This method corrects the interference generated by other compounds, and the MDA is reported as nmol gFW^{-1}. For MDA determination, 0.1 g of leaf tissue were ground in 2 mL ethanol 80% (*v*/*v*). The homogenate was centrifuged at 12,000 rpm for 5 min, and 0.3 mL of the supernatant was incubated with 0.7 mL of TCA 20% (*w*/*v*) (solution A) or solution A plus TCB 0.65% (*w*/*v*) (solution B) for 30 min at 95 °C. After cooling down, samples were centrifuged at 12,000 rpm for 5 min, and the supernatant was used to measure the absorbance at 440, 532 and 600 nm in a spectrophotometer. To calculate MDA concentration, the following formulas were applied:

(1) (A_{532} solution B − A_{600} solution B) − (A_{532} solution A − A_{600} solution A) = **M1**;
(2) (A_{440} solution B − A_{600} solution B) 0.0571 = **M2**;
(3) ((**M1** − **M2**)/157000) 10^6 = nmol MDA mL^{-1} = **M3**;
(4) **M3**/0.015 = nmol MDA gFW^{-1}.

2.6. Gas Exchange Measurements

Net photosynthesis in mature leaves was measured using a portable photosynthesis analyser LI-6400 (LI-COR Biosciences, Lincoln, NE, USA) at a CO_2 concentration of 400 ppm and 350 µmol photons m^{-2} s^{-1} PAR.

2.7. Brix Degrees Measurement

Brix degrees (°Bx) were measured in ground tomatoes by refractometry at 20 °C.

2.8. Lycopene Extraction and Measurement

The lycopene extraction method was adapted from [38]. Fresh tomatoes were finely ground to a puree. Ten grams were poured on a solvent mixture composed of 15 mg of butylated hydroxytoluene (BHT), 7.5 mL of acetone, 7.5 mL of ethanol, 15 mL of hexane and 450 µL of dichloromethane and then sonicated for 30 min, taking care to keep it at a low temperature using a bath of water–ice. Then, the sample was centrifuged at 30,000× *g* for 10 min at 20 °C. The upper layer was removed and dissolved in hexane up to a 10% (*v*/*v*). The absorbance of the hexane (upper) layer was measured in a 1 cm path length quartz cuvette at 503 nm versus a blank of hexane, using a molar extinction coefficient of 17.2×10^4/M/cm, according to [39]. Lycopene amounts are expressed in mg/100 gFW. All analyses were carried out twice.

2.9. Statistical Analysis

All data were analysed by ANOVA, and means were compared by Duncan's multiple range test. A p-value < 0.05 was considered to be statistically significant. All analyses were conducted using SPSS Statistics 25 (IBM). The graphs were produced using SigmaPlot software (Systat Software Inc., San José, CA, USA).

3. Results

3.1. Plant Growth-Promoting Rhizobacteria Isolation and Selection

Aerobic culturable bacteria from rhizosphere soil samples were isolated in TSA medium and identified based on colony morphology and pigmentation. These bacteria showed many plant growth-promoting activities such as chitinase, cellulase, amylolytic, protease, urease and ACC deaminase activities, production of IAA and siderophores as well as phosphate and potassium solubilisation. Based on these plant growth-promoting activities assayed, five bacteria were selected (i.e., SIS001, SIS105, SIS213, SIS221 and SIS303) and identified at the genus level by amplification and sequencing of the *16S* mRNA gene (Table 1). Thus, SIS001 and SIS221 belonged to the genus *Pantoea*, SIS105 and SIS303 to *Pseudomonas*, and SIS213 was a *Bacillus* strain.

Table 1. Plant growth-promoting activities of the five selected isolated bacterial strains.

		Plant Growth-Promoting Activity									
Isolate	**RNA 16S**	**Chitinase Activity**	**Cellulase Activity**	**Amylolytic Activity**	**Protease Activity**	**Ureolytic Activity**	**ACC Deaminase**	**Phosphate Solubilisation**	**Potassium Solubilisation**	**Siderophore Production**	**IAA Production**
SIS001	*Pantoea* sp.	-	+	-	-	-	-	-	+	+	6 mg/L
SIS105	*Pseudomonas* sp.	-	-	+	-	-	+	+	+	+	10 mg/L
SIS213	*Bacillus* sp.	+	+	-	+	-	-	-	-	-	10 mg/L
SIS221	*Pantoea* sp.	-	+	-	-	-	-	+	+	-	8 mg/L
SIS303	*Pseudomonas* sp.	-	+	+	-	-	-	+	+	+	13 mg/L

-: The bacterium did not possess the activity or produce the compound. +: Production of the compound or high enzyme activity.

All the selected bacteria showed IAA production in concentrations ranging from 6 (SIS001) to 13 mg/mL (SIS303). This plant hormone can improve plant nutrition by increasing root development and the ability to explore more space and, therefore, capture more nutrients [40]. Other interesting PGP capacities included the production of siderophores that presented in SIS001, SIS105 and SIS303, or the solubilisation of the potassium capacity of the strains SIS001, SIS221 and SIS303. The strains SIS105 and SIS221 had the ability to solubilise phosphate. Furthermore, *Pseudomonas* sp. SIS105 presented

ACC deaminase activity, which is related to the protection of plants against different stress conditions [41,42] and can increase the yield of some plants under abiotic conditions [11]. On the other hand, *Bacillus* sp. SIS213 presented chitinase, cellulase and protease activities and may have an important role in triggering defensive responses in plants [43,44]. Moreover, SIS303 presented amylolytic activity. None of the selected bacteria presented ureolytic activity.

3.2. Tomato Responses to Inoculation with Selected PGPR

Four months after inoculation with the five selected strains, plants and ripe fruits were harvested for analysis. Inoculated tomato Tres Cantos plants were smaller and accumulated less biomass in terms of dry weight with all the PGPR used in this study (Figure 1A, left). Nevertheless, the number of fruits was similar for all treatments or even higher with SIS303 (Figure 1A, middle). More interestingly, the weight per fruit (Figure 1A, right) was higher with all the treatments, especially with SIS221 and SIS303. On the contrary, cherry plants did not show the same behaviour in response to inoculation with PGPR, and no changes in terms of the dry weight, number or weight of the fruits (Figure 1B) were observed, indicating a high specificity in the response of the plants to the application of rhizobacteria.

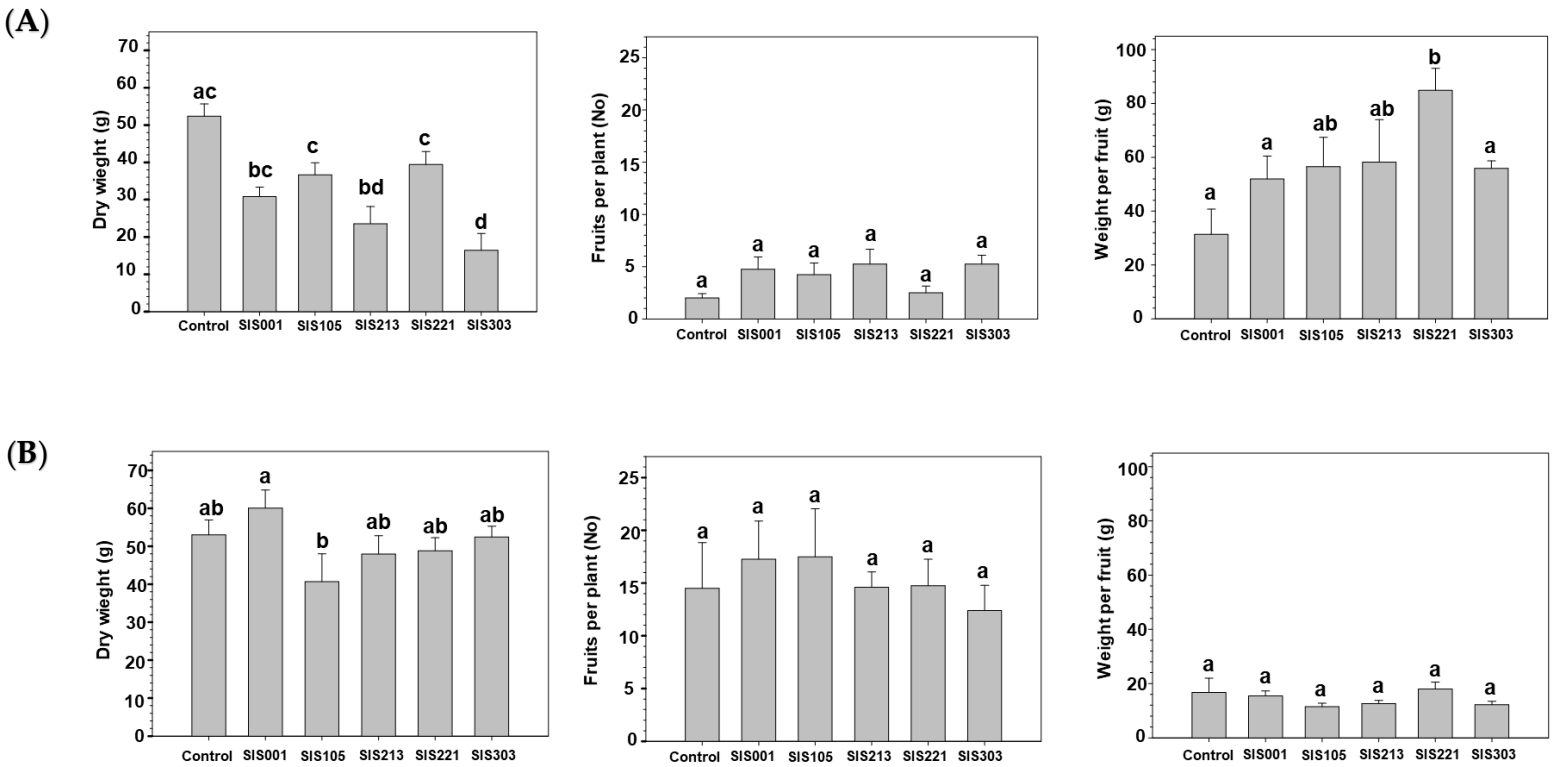

Figure 1. Effect of rhizobacteria inoculation on biometry of (**A**) Tres Cantos and (**B**) cherry plants. Data are the means ± SE of 3 replicates. Values marked with different letters within each panel are significantly different according to Duncan's multiple range test, $p < 0.05$. Treated plants were harvested 4 months after inoculation of rhizobacteria, and the dry weight of the shoot (**left**), number of fruits per plant (**middle**) and weight of every fruit (**right**) was measured.

Most biotic and abiotic stresses promote the generation of reactive oxygen species (ROS) in plants leading to lipid peroxidation, disruption of membrane integrity, inhibition of various enzymatic activities and nuclear damages [16]. Plants try to overcome this oxidative stress by synthetizing antioxidant enzymes related to the Asada–Halliwell pathway. PGPR can induce detoxification mechanisms by improving the antioxidative defence system of plants, which results in higher productivity and tolerance to stress. We measured the activity of the antioxidant enzymes catalase (CAT) and ascorbate peroxidase (APX) as well as the levels of malondialdehyde (MDA) as an indicator for lipid peroxidation. The effect of inoculation with PGPR in Tres Cantos plants was different depending on the bacterial strain used. Thus, SIS001, SIS213 and SIS303 slightly diminished CAT activity, whereas SIS221 had an opposite effect (Figure 2A, left). Interestingly, SIS213 and SIS221 had an inverted effect on APX activity, i.e., higher activity with SIS213 and lower with SIS221 (Figure 2A, middle). Finally, none of the PGPR used in this work had any effect on MDA levels (Figure 2A, right). In cherry plants, CAT activity slightly decreased with SIS105 and SIS213 (Figure 2B, left). APX activity was more affected by PGPR (Figure 2B, middle). Consequently, MDA levels were much higher, especially with SIS101 and SIS105 (Figure 2B, right).

Net photosynthesis was measured in mature leaves at different times after inoculation with PGPR (1, 2 and 3 months). In all conditions assayed, CO_2 assimilation diminished with age, even in uninoculated control plants (Figure 3). In Tres Cantos plants, only SIS105 and SIS221 reduced slightly but significantly net photosynthesis. However, the other treatments did not affect CO_2 assimilation (Figure 3A). In cherry plants, all treatments reduced photosynthesis 1 month after inoculation, but only SIS213 maintained the decreasing assimilation at 2 and 3 months (Figure 3B).

Brix degrees (°Bx) is used to measure sugar content in a solution and is commonly used to assess the ripening of fruits like grape, strawberry or tomato. One °Bx means 1 g of sugar per 100 g of fruit [45]. All treatments slightly diminished °Bx in Tres Cantos (Figure 4A) but had no effect on cherry fruits (Figure 4B). Interestingly, the inoculation of Tres Cantos with PGPR reduced biomass accumulation (Figure 1A, left), photosynthesis (Figure 3A) and Brix degrees (Figure 4A), although the number and weight of fruits were not negatively affected (Figure 1A, middle and right). These effects were not observed in cherry plants.

Finally, we analysed the effect of PGPR in the more important bioactive molecule accumulated in tomato fruits: lycopene. Again, the effect of the inoculation of tomato plants with rhizobacteria had different consequences depending on the cultivar. In Tres Cantos plants, the levels of this carotenoid increased dramatically in all the treatments, achieving up to 10 times more lycopene with SIS213 bacteria (0.8 ± 0.1 vs. 10.6 ± 0.2 mg/100 gFW) (Figure 5A). On the contrary, no significant effect of rhizobacteria on lycopene levels was observed in cherry fruits (Figure 5B).

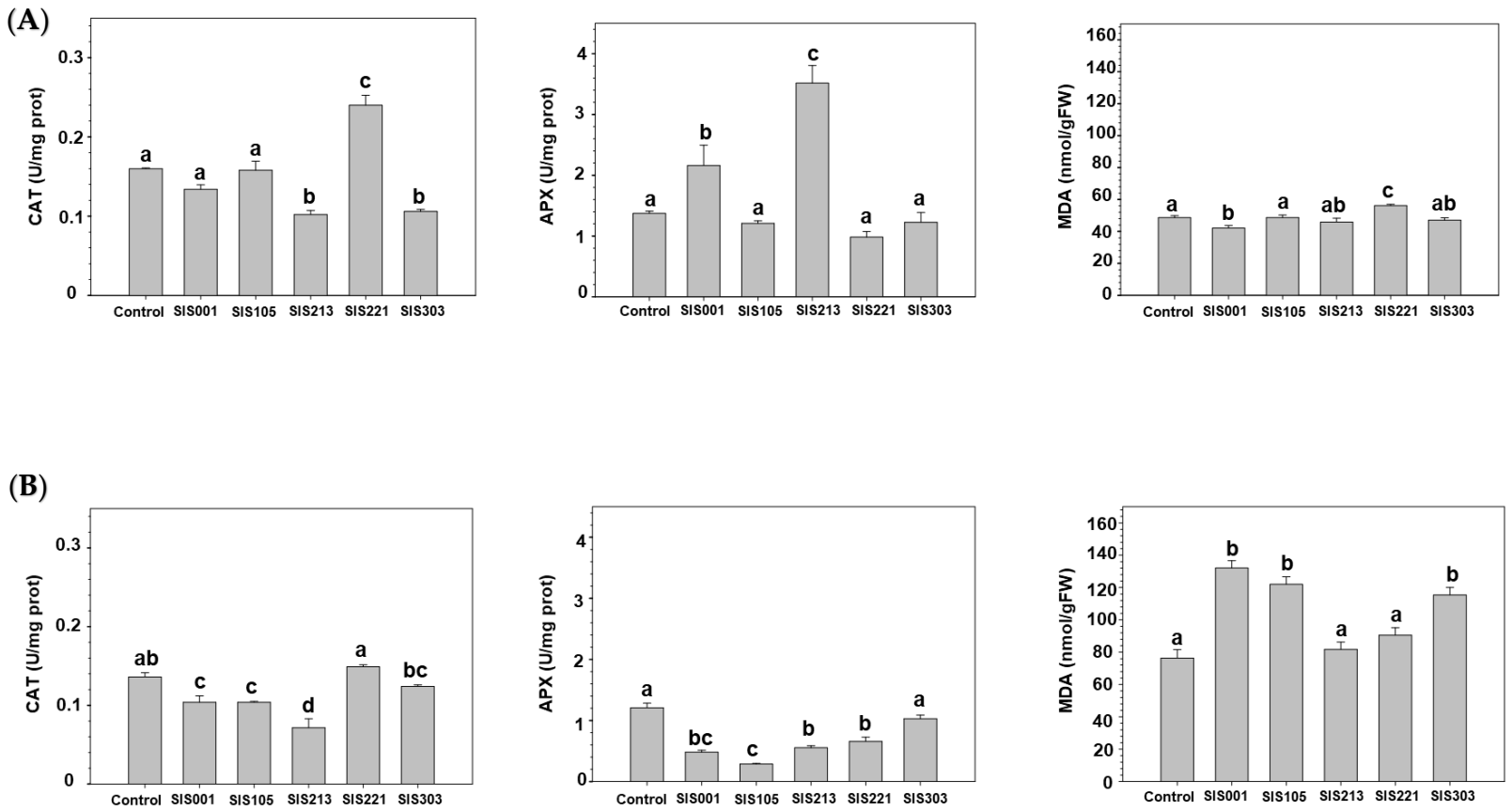

Figure 2. Effect of rhizobacteria inoculation on CAT, APX and MDA levels of (**A**) Tres Cantos and (**B**) cherry plants. Antioxidant activities CAT (**left**) and APX (**middle**), and MDA as an indicator for lipid peroxidation (**right**) were measured in leaves of tomato plants harvested 4 months after of inoculation. Data are the means ± SE of 3 replicates. Values marked with different letters within each panel are significantly different according to Duncan's multiple range test, $p < 0.05$.

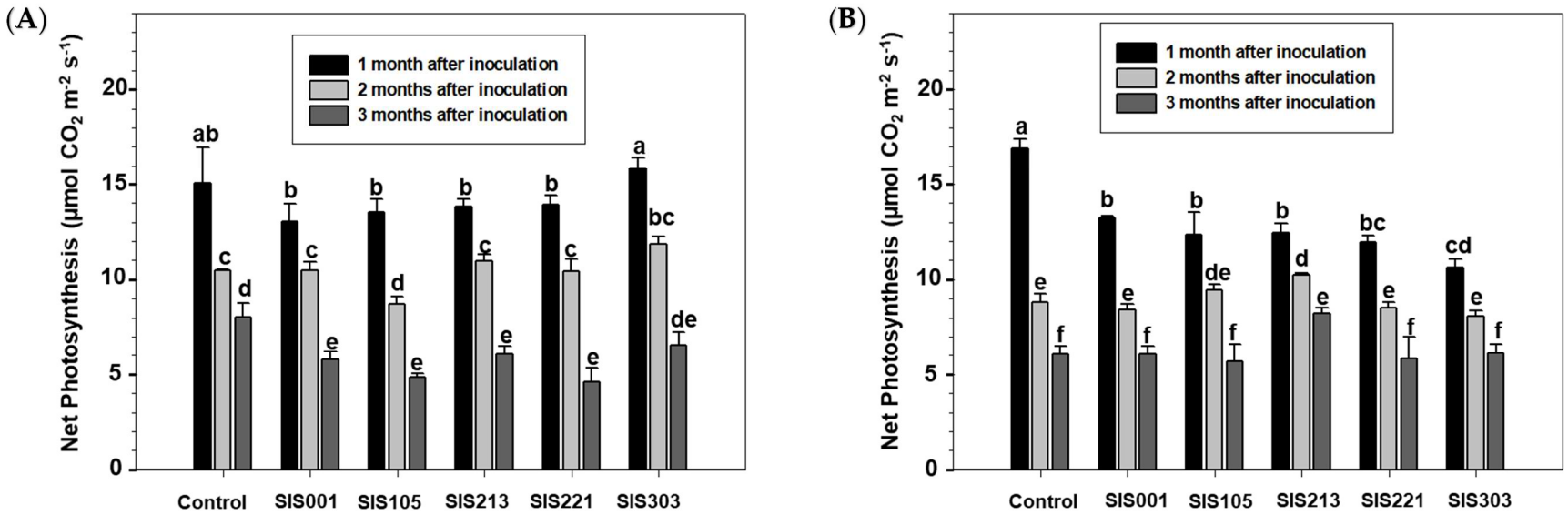

Figure 3. Effect of rhizobacteria inoculation on net photosynthesis of (**A**) Tres Cantos and (**B**) cherry plants. Net CO_2 assimilation was measured in mature leaves at 1, 2 and 3 months after inoculation with PGPR. Data are the means ± SE of 3 replicates. Values marked with different letters within each panel are significantly different according to Duncan's multiple range test, $p < 0.05$.

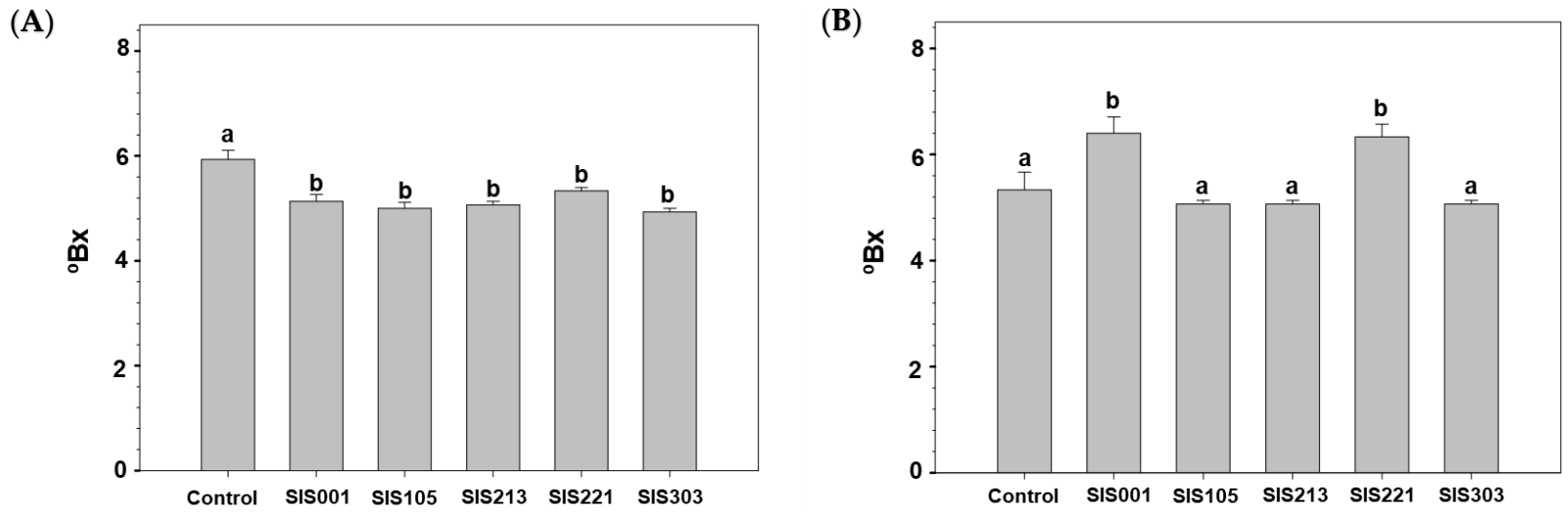

Figure 4. Effect of rhizobacteria inoculation on degrees Brix of (**A**) Tres Cantos and (**B**) cherry plants. The levels of sugars in terms of degrees Brix (°Bx) were measured in juice from tomato fruits after harvesting. Data are the means ± SE of 3 replicates. Values marked with different letters within each panel are significantly different according to Duncan's multiple range test, $p < 0.05$.

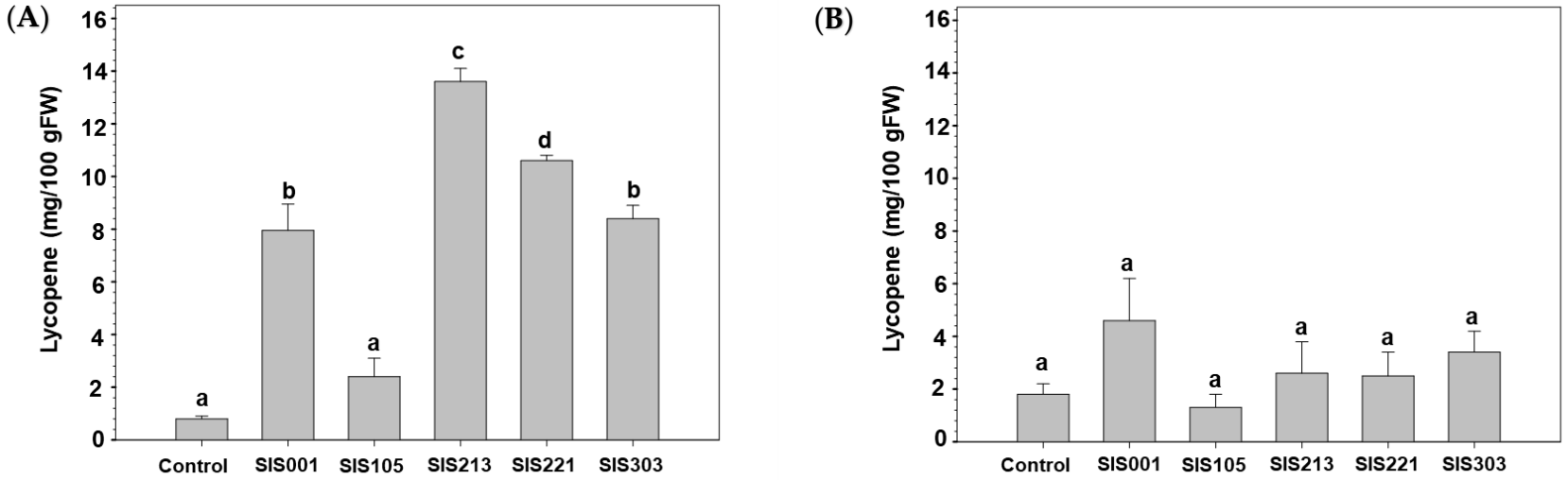

Figure 5. Effect of rhizobacteria inoculation on lycopene levels in (**A**) Tres Cantos and (**B**) cherry fruits. The carotenoid lycopene was measured in ground tomatoes as described in Material and Methods. Data are the means ± SE of 3 replicates. Values marked with different letters within each panel are significantly different according to Duncan's multiple range test, $p < 0.05$.

4. Discussion

Plants must deal with a myriad of different microorganisms in soils, forming a complex community with the so-called phytomicrobiome [46,47]. Some of them can induce beneficial effects on plants; thus, they are referred to as plant growth-promoting rhizobacteria (PGPR). These microorganisms have been used in agriculture since the beginning of the 20th century, with the rhizobial inoculation of legumes. More recently, other PGPR are being used to improve plant production by avoiding or reducing the use of fertilizers for a greener agriculture. Thus, inoculants with strains of *Bacillus*, *Pseudomonas*, *Glomus*, *Acetobacter*, *Azospirillum* and many others have been used with relative success [47]. PGPR are starting to be extensively used in agriculture not only to reach higher crop productivities but also due to the increasing interest of inoculants for improving tolerance against many stresses, both biotic and abiotic [1]. However, there is little information about the capacity of PGPR to change the pattern of bioactive molecules synthesised, which could be interesting not only for human or animal nutrition but also for human health or cosmetics.

Tomato is a popular fruit crop grown and consumed by people all over the world. Consuming tomatoes regularly decreases the risk of chronic degenerative diseases, mainly due to the presence of bioactive molecules in tomato fruits such as carotenoids, particularly lycopene, ascorbic acid, vitamin E and phenol compounds [48]. In the present work, we analysed whether the inoculation of tomato plants with PGPR strains isolated from rice rhizosphere could have some beneficial effects in the accumulation of lycopene, without negatively affecting other parameters such as yield, fruit sugar content or antioxidant activities. Inoculation with any of the five PGPR used in this work had no negative effect on productivity in terms of the number or weight of the fruits in the two tomato varieties used. However, inoculation with SIS221 and SIS303 resulted in larger fruits in the genotype Tres Cantos, with a slightly lower °Bx. Inoculated cherry plants had less antioxidant activity and more MDA accumulation than the cultivar Tres Cantos, in which plant inoculation did not induce lipid peroxidation. Finally, the tomato cultivar Tres Cantos responded positively to inoculation with all of the PGPR in terms of lycopene accumulation in fruits, with levels up to 10-fold higher when inoculated with SIS213. On the contrary, cherry plants did not show a clear response. Lycopene is a strong antioxidant molecule, showing the highest physical quenching rate constant with singlet oxygen [48]. In a study carried out using tomato plants in field trials, moderate salt stress (60 mM NaCl) diminished photosynthesis by closing stomata and increased lycopene content in fruits [49]. In our case, it could be possible that the modulation of antioxidant activities by inoculation with PGPR resulted in an increase in lycopene concentration in Tres Cantos tomato fruits. In another study, however, lycopene levels in tomato fruits were lower when plants were grown under water stress conditions [50]. Interestingly, when this water stress was combined with biotic stress, plants responded by raising the level of carotenoids including lycopene. Therefore, the effect of biotic and abiotic factors in the accumulation of lycopene or other biomolecules depends on many factors and is not a straightforward response. This is demonstrated in our work, where the same treatment on two tomato varieties had different effects on the response of the plant and in the accumulation of bioactive molecules. In a study comparing lycopene level in 18 different tomato genotypes, the values observed ranged between 2.33 and 16.0 mg/100 gFW. Therefore, the levels detected in Tres Cantos or cherry tomato fruits in this work without inoculation with PGPR would belong to the low lycopene production scale. However, after inoculation, the level detected in Tres Cantos fruits for SIS213 or SIS221 would be placed among the highest lycopene levels detected [48]. Thus, inoculation of tomato plants with PGPR could be an easy, cheap and green way to modify bioactive molecules, such as lycopene, to improve the nutritional quality of fruits. In agreement with our results, inoculation of tomato plants with *Priestia megaterium* has recently been described to increase the amount of lycopene and carotene in tomato fruits [51].

Application of PGPR is an interesting strategy to naturally manipulate the level of bioactive molecules in plants. However, it is clear that the mechanisms underlying

the response of plants to this microorganism and other factors are far from being fully understood, and more conscientious studies are required.

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