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Consortia of Plant-Growth-Promoting Rhizobacteria Isolated from Halophytes Improve Response of Eight Crops to Soil Salinization and Climate Change Conditions

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Abstract: Soil salinization is an environmental problem that adversely affects plant growth and crop productivity worldwide. As an alternative to the conventional approach of breeding salt-tolerant plant cultivars, we explored the use of plant-growth-promoting rhizobacteria (PGPR) from halophytic plants to enhance crop growth under saline conditions. Here, we report the effect of five PGPR consortia from halophytes on the growth of eight (alfalfa, flax, maize, millet, rice, strawberry, sunflower, and wheat) of the crops most commonly produced on salinized soils worldwide. To test the efficiency of halotolerant consortia, we designed a complex environmental matrix simulating future climate-change scenarios, including increased CO_2 levels and temperature. Overall, biofertilizers enhanced growth of most crops with respect to non-inoculated control plants under different CO_2 concentrations (400/700 ppm), temperatures (25/+4 °C), and salinity conditions (0 and 85 mM NaCl). Biofertilizers counteracted the detrimental effect of salinity on crop growth. Specifically, strawberry and rice showed the greatest positive additive response to inoculation in the presence of salt; above-ground biomasses were 35% and 3% greater, respectively, than their respective control grown without salt. Furthermore, depending on the interaction of environmental factors (salinity × CO_2 × temperature) analyzed, the results varied—influencing the most effective biofertilizer determined for each crop now, or in the future. Our findings highlight the importance of conducting studies that consider stress interaction for realistic assessments of the potential of biofertilizers in a climate-changed world.

Keywords: biofertilizer; CO_2; halophilic rhizobacteria; plant biomass; soil salinization; temperature

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1. Introduction

The Food and Agriculture Organization of the United Nations predicts that the world population will have reached almost 10 billion by 2050 [1], leading to a 70% increase in demand for food compared with 2009 [2]. Simultaneously, a rapid change in climate is taking place, and is expected to reduce crop production [3]. Increases in atmospheric CO_2 concentration (c. 760 ppm), temperature (between 2.4 and 4.8 °C), and salinity [4] are

foreseeable in the future environment. Agricultural soils are decreasing by about 1–2% every year in arid and semi-arid zones due to salinization [5]. The costs arising from these changes are potentially enormous, estimated to be USD 12 billion globally per annum, and increasing [6].

Our current highly productive intensive agricultural system was mostly achieved using synthetic nitrogen and phosphorus fertilizers [7]. The dependence of modern agriculture on a massive input of chemical fertilizers has caused deterioration of soil and water quality, rendering soils biologically inert and, occasionally, highly saline—thereby polluting surface and ground water [8]. Thus, it is essential to find inexpensive, environmentally benign, and easy-to-operate options to overcome environmental threats posed by fertilizers [9]. The most suitable alternatives to chemical fertilizers are biofertilizers [10], which have a number of positive effects on agriculture: increasing crop yield at low cost and with ease of access and application, protection of human health (via the food chain), and low impact on the environment [9,11]. The use of biofertilizers is expected to increase in the future, with their market share expected to reach USD 1.66 billion by 2022, with an annual growth rate of 13.2% over the period of 2015–2022 [12].

A biofertilizer is any microbial biostimulant (bacterial or fungal) applied to plants to improve plant nutrition, abiotic stress tolerance, and/or crop quality [11]. Specifically, we focus on plant-growth-promoting rhizobacteria (PGPR), which colonize plant roots and the surrounding rhizosphere. The current PGPR-based biofertilizer market represents about 5% of the total chemical fertilizer market for agricultural practices [12].

The mechanisms by which PGPR are known to promote plant growth are: facilitation of nutrient acquisition; atmospheric nitrogen fixation; phosphorus solubilization; siderophore production for iron uptake; modulation of plant growth hormones, such as production of auxins for root development (the most common being indole-3-acetic acid, IAA); bacterial synthesis of the enzyme ACC deaminase (cleaves ethylene, which is crucial in response to stress); and the production of plant regulators involved in the cell cycle and several developmental processes (cytokinins, gibberellins, salicylic acid, abscisic acid, brassinosteroids, and jasmonates) [13]. However, not always does a single microorganism elicit all of these responses, so the use of plant-growth-promoting consortia, instead of a single strain, is of current research interest [11,13].

Agricultural uses of PGPR are constrained by the variable responses of plant species and cultivars and by environmental conditions, including climate, weather, and soil characteristics, such as high salinity [11,14]. For example, Upadhyay et al. [15] found that PGPR did not demonstrate plant-growth-promoting traits with increasing salinity. In order to overcome this limitation, it is necessary to increase the selection of plant-growth-promoting (PGP) strains with biological activities adapted to specific agronomic situations. In this way, the rhizosphere of halophytic plants serves as a reservoir for various groups of halotolerant PGPR that might improve growth of agricultural crops under salinity stress. This could facilitate the recovery of previously unproductive, marginal lands for cultivation, which is important due to the scarcity of farmland [6]. Despite this, the use of PGPR from halophytic plants as biofertilizers is a relatively unexplored field with a great opportunity for exploitation [16].

Bacteria have, in many studies, been shown to improve production of various crops [9,17], even under conditions of environmental stress [18]. However, reports analyzing the response of PGPR-inoculated plants to interactive abiotic stresses are very scarce. Specifically, analysis of the impact of PGPR inoculants on crops where salinity, temperature, and CO_2 concentration are all changed is an unexplored field, but key to ascertaining the feasibility of using PGPR within the context of climate change.

In this study, we tested the effect of five bacterial biofertilizers from halophytes on the growth of eight crops, including those most relevant in terms of world production [19], under a complex environmental matrix characterized by salinity (0 and 85 mM NaCl) and variations in atmospheric CO_2 concentration and air temperature (400 ppm and 25/14 °C and 700 ppm +4 °C). Our goal was to demonstrate the potential of halophytes as a source

of PGPR for the formulation of biofertilizers as the climate changes. We hypothesized that consortia of plant-growth-promoting bacteria isolated from halophytes could empower crop growth against soil salinization and climate change.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

Seeds of maize (*Zea mays* var. DKC6980), wheat (*Triticum durum* var. Senatore Cappelli), sunflower (*Helianthus annuus* var. LG54.85), millet (*Panicum miliaceum* var. White), alfalfa (*Medicago sativa* var. WL656HQ), and flax (*Linum usitatissimum* var. Brown) were pre-hydrated for a minimum of 8 h. Pre-hydrated seeds were placed in trays with perlite, previously moistened with distilled water and incubated at 21/25 °C, 40–60% relative humidity, and natural daylight 250–1000 µmol m^{-2} s^{-1} light flux in a glasshouse (University of Seville Glasshouse General Services) for a week. Perlite was not sterilized because, as we confirmed (unpublished data), it is a relatively inert material concerning bacterial viability. Seedlings were then transferred separately into 1.5 L plastic pots (one plant per pot) filled with organic commercial substrate (Gramoflor GmbH & Co. KG., Vechta, Germany) and sand mixture (3:1). The mixture was previously sterilized at 100 °C for 18 h.

Cold-stored bare-rooted strawberry plants (*Fragaria vesca* var. Fortuna) with one well-developed crown of diameter 8 to 10 mm were planted in 1.5 L plastic pots filled with sand and organic commercial substrate (Gramoflor GmbH und Co. KG.) mixture (4:1). The mixture was previously sterilized at 100 °C for 18 h. The sterility of the substrate was confirmed by mixing multiple samples, randomly selected from different bags, with sterile physiological saline solution and plating onto tryptone soya agar (TSA). No growth was observed after 3 days of incubation at 28 °C.

Rice seeds (*Oryza sativa* var. Puntal) were surface-disinfected by washing first with distilled water and then with 0.5% (*w*/*v*) calcium hypochlorite for 20 min. The seeds were then thoroughly rinsed with sterilized tap water and germinated on a wet filter paper. After 7 d, seedlings were suspended in 4 L closed tanks containing at least 20 plants, in +N BG110 medium [20].

Plants in pots (n = 240 per crop) and closed tanks (n = 24) were kept at 400 ppm CO_2 with a diurnal regime of 16 h of light at 25 °C and 8 h of darkness at 14 °C, 50% relative humidity (80% for rice), and 300 µmol m^{-2} s^{-1} light flux in controlled environment chambers (Aralab/Fitoclima 18.000 EH, Lisbon, Portugal). After 15 days (5 days for rice) of growth, the different treatments (see below) were established. Both before and after treatment, pots were watered daily with 100 mL of distilled water. Strawberry plants were watered with 100 mL of 20% Hoagland's solution, since they were grown on a highly sandy substrate with a low cation-exchange capacity (CEC) and were therefore characterized by low nutrient retention capacity [21].

2.2. Rhizobacteria Selection

Five bacterial biofertilizers which had been isolated, identified, and described in previous works (Table 1) were used in this experiment. They were composed of rhizobacteria originally isolated from the rhizospheres of five different halophytes, commonly inhabiting salt marshes in southwestern Spain. Rhizobacteria that compose Biofertilizer 1 (strains SDT3, SDT13, and SDT14) were isolated from *Spartina densiflora* [22] (*S. densiflora* is now *Sporobolus montevidensis* (Arechav.) P.M.Peterson & Saarela, but we have retained the use of *Spartina* according to Bortolus et al.) [23]. Rhizobacteria in Biofertilizer 2 (strains RA1, RA15, and RA18) were isolated from the rhizosphere of *Allenrolfea occidentalis* [24]. Rhizobacteria in Biofertilizer 3 (strains SMT38, SMT48, and SMT51) came from *Spartina maritima* [25] (now *Sporobolus maritimus* (Curtis) P.M.Peterson & Saarela) and those in Biofertilizer 4 (strains HPJ2, HPJ15, and HPJ50) from *Atriplex portulacoides* [16]. Finally, rhizobacteria in Biofertilizer 5 (strains SRT1, SRT8, and SRT15) were isolated from the rhizosphere of *Salicornia europaea* [16]. We used plant-growth-promoting consortia, instead of a single strain, because different PGPR may have different modes of action for

promoting plant growth that could be additive or synergistic in a microbial mix [13]. To avoid antagonistic interactions between bacteria, it is easier to prepare biofertilizers from a single halophyte than to try to combine bacteria with similar properties from a number of separate halophytes. For rhizobacterial isolation, soil closely adhering to roots was mixed with sterilized saline solution, sediment was allowed to settle, and the sediment was plated onto Tryptone-Soya-Agar-rich bacterial medium (TSA) bacterial media in Petri dishes in order to obtain as many bacterial species as possible [25]. Purity of the isolates was assessed by re-plating and Gram staining. Then, identification of pure isolates was performed by 16S rDNA sequencing by StabVida (Portugal), providing genus and species for each rhizobacterial strain obtained. Next, plant-growth-promoting (PGP) properties were screened for by performing biochemical tests to determine the isolates' capacities for phosphate solubilization, production of siderophores and biofilms, as well as for IAA hormone and ACC deaminase enzyme synthesis (for more details see [16,22,24,25]). Finally, for each halophyte, the three rhizobacterial strains with the best PGP properties (maximum activities of different properties) were selected to become a bacterial consortium and a biofertilizer. The plant-growth-promoting rhizobacteria (PGPR) selected as biofertilizers to be used in our experiment can be seen in Table 1.

Table 1. Plant-growth-promoting rhizobacterial (PGPR) traits for consortia used in this study.

		PGPR Properties						
Consortium Number	**Bacterial Strains**	**N Fixation**	**P Solubilization**	**Siderophores Production**	**IAA Production (mg/mL)**	**Biofilm Production**	**ACC Deaminase**	**Reference**
1	*Pseudomonas composti* SDT3	-	+	+	-	-	-	[22]
	Aeromonas aquariorum SDT13	-	+	+	3.40	-	-	
	Bacillus thuringiensis SDT14	+	-	+	-	-	-	
2	*Vibrio kanaloae* RA1	+	-	+	0.63	-	-	[24]
	Pseudoalteromonas prydzensis RA15	+	-	+	1.58	-	-	
	Staphylococcus warneri RA18	-	-	-	2.84	-	-	
3	*Bacillus methylotrophicus* SMT38	+	-	+	-	+	-	[25]
	Bacillus aryabhattai SMT48	+	+	+	3.25	-	-	
	Bacillus licheniformis SMT51	+	+	+	1.06	+	-	
4	*Vibrio spartinae* HPJ2	+	+	+	4.12	+	+	[16]
	Marinobacter sediminum HPJ15	-	-	+	15.41	-	-	
	Vibrio parahaemolyticus HPJ50	+	+	+	7.48	+	-	
5	*Vibrio neocaledonicus* SRT1	+	+	+	5.65	+	-	[16]
	Thalassospira australica SRT8	-	-	-	-	+-	+	
	Pseudarthrobacter oxydans SRT15	+	+	-	20.99	-	-	

The references from which the data were obtained are indicated in the table.

To prepare the suspensions used as biofertilizers, selected bacteria were inoculated in individual 250 mL Erlenmeyer flasks containing 50 mL of tryptic soy broth (TSB) and incubated overnight with continuous gentle shaking at 28 °C. Then, cultures were centrifuged at 7000 rpm for 5 min, the supernatant discarded, and pellets washed with sterilized tap water. Cultures were centrifuged a second time and pellets resuspended in sterilized tap water to reach an OD_{600} of approximately 1.0 in order to produce a uniform bacterial concentration of all the strains. Bacterial suspensions were mixed to produce the five final inoculant suspensions, as follows: strains SDT3, SDT13, and SDT14 were mixed to obtain Biofertilizer 1; strains RA1, RA15, and RA18 for Biofertilizer 2; strains SMT38, SMT48, and SMT51 for Biofertilizer 3; strains HPJ2, HPJ15, and HPJ50 for Biofertilizer 4; and strains SRT1, SRT8, and SRT15 were mixed in Biofertilizer 5. For plant inoculation, every 1.5 L pot was watered with 20 mL of the inoculant suspensions. In the case of rice, 30 mL of inoculant suspension was added to each 4 L tank to achieve a final bacterial concentration of 10^5 CFU/mL (estimating that a suspension of OD_{600} 1 corresponds to approximately 10^8 CFU/mL).

2.3. Treatments and Growth Analysis

Twenty-four different treatments were set up (n = 10 per treatment; n = 20 for rice): six biofertilization treatments (5 rhizobacteria consortia + non-inoculated control), two salinity concentrations (0 and 85 mM NaCl), and two CO_2–temperature combinations (400 ppm CO_2 at 25/14 °C (16/8 h), and 700 ppm CO_2 at 29/18 °C (16/8 h)).

The salinity treatment was imposed by immersing the pots in tanks with 6 L of solution, at 0 or 85 mM NaCl, for 25 min. In the case of rice, the appropriate concentration of salt was added to the culture medium. Atmospheric CO_2 concentrations in chambers were continuously recorded by CO_2 sensors (Aralab, Lisbon, Portugal) and maintained by supplying pure CO_2 from a compressed gas cylinder (Air Liquide, B50 35 K). The rhizobacterial inoculation was carried out the day after establishing the environmental treatments (salinity, CO_2, and temperature). After 20 d of growth in the different treatments, maize and rice plants were harvested. All other crops were harvested after 30 d, and all plants were divided into roots and shoots. Dry mass was determined after drying samples at 80 °C for 48 h.

2.4. Delayed Fluorescence Measurements

Leaves from all crops were placed in a black cardboard box and maintained in darkness (n = 3) for 10 min. Delayed fluorescence was detected using a plant-imaging system (NightShade LB 985, Berthold Technologies, Bad Wildbad, Germany) equipped with a CCD camera. Leaves were illuminated for 20 s with light supplied from far red (730 nm), red (660 nm), green (565 nm), and blue (470 nm) LED panels at 2, 105, 40, and 110 μmol m^{-2} s^{-1}, respectively. Immediately after switching off the LEDs, delayed fluorescence was measured, and the recorded intensities of light were converted to counts per second (cps) [26].

2.5. Statistical Analysis

Statistical analysis was performed using SPSS 19.0 statistical program (SPSS Inc., Chicago, IL, USA). Data were analyzed using analysis of variance (ANOVA). Duncan's test was applied to establish the significance between biofertilization treatments ($p < 0.05$).

3. Results

3.1. Crop Growth and Delayed Fluorescence Measurements

Overall, biofertilizers had a positive effect on the growth of the crops. Figure 1 shows how biofertilizers enhanced growth of inoculated maize, alfalfa, and sunflower plants, with respect to non-inoculated control plants, under different CO_2 concentrations and temperatures (400/700 ppm and 25/+4 °C, respectively) and salinity conditions (0 and 85 mM NaCl; Figure 1, for details see below). The most suitable biofertilizer for each crop (for which the highest growth was obtained) changed depending on the CO_2/temperature and salinity treatment (Table 2). Bacterial consortium 4 was by far the least represented in Table 2, even though it showed more PGP properties than Biofertilizers 1 and 2 (Table 1). Bacterial consortia 1 and 3 were the most represented in Table 2.

The beneficial effect of biofertilizers was also detected at a physiological level. Delayed fluorescence emission of sunflower leaves was higher in some of the inoculated plants, indicating lower stress caused by salinity, independent of temperature and CO_2 concentration (Figure 1C,D).

3.1.1. Biofertilizers from Halophytes in the Current Atmosphere

One biofertilizer improved the growth of all the crops studied except wheat, in terms of both above- and below-ground biomass production (Figure 2A). The increases recorded in above-ground biomasses, with respect to the non-inoculated control, ranged from 4% for rice to 54% for strawberry, and, for below-ground biomass, from 2% for wheat to 60% for sunflower (Figure 2A).

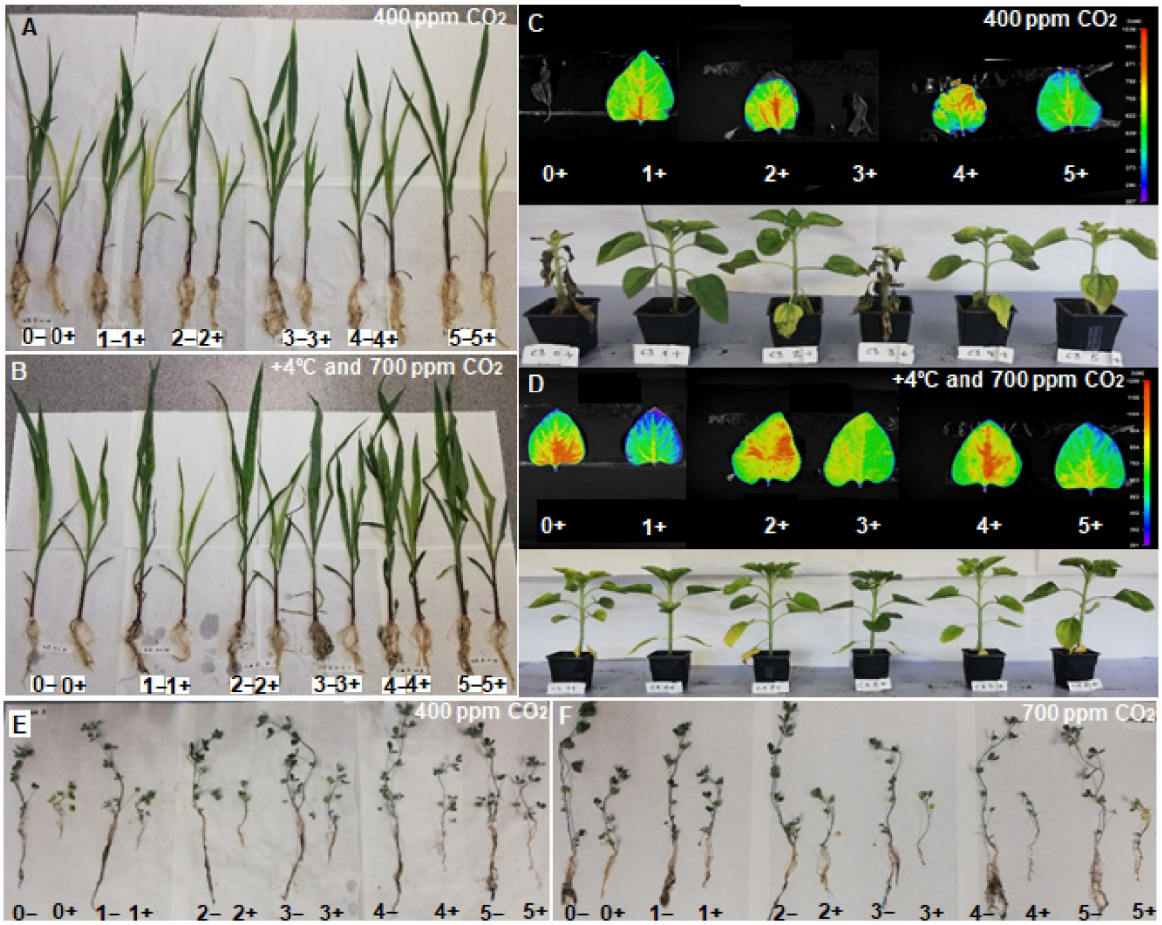

Figure 1. Effects of biofertilizers on the growth of different crops. (**A**) Maize plants inoculated with rhizobacteria consortia, numbered from 1 to 5, grown at 400 ppm CO_2 and (**B**) at +4 °C and 700 ppm CO_2, with (+) and without (−) 85 mM NaCl. Number 0 was used for non-inoculated controls. (**C**) Sunflower plants grown at 400 ppm CO_2 and (**D**) at +4 °C and 700 ppm CO_2 with (+) 85 mM NaCl. In the top of panels C and D, there are photographs taken by the plant-imaging system NightShade LB 985 (leaves of the same age were used, taken in the middle of the stem). The color scale mirrors the detected counts per second (cps) of delayed fluorescence emission in leaves: the redder, the less stressed the leaf is. The absence of color indicates that the leaf was dead. (**E**) Alfalfa plants grown at 400 ppm CO_2 and (**F**) at +4 °C and 700 ppm CO_2, with (+) and without (−) 85 mM NaCl.

Table 2. Biofertilizers that generated the highest production of above-ground biomass for each crop under the different treatment conditions.

	400 ppm CO_2		+4 °C and 700 ppm CO_2	
Crop	**0 mM NaCl**	**85 mM NaCl**	**0 mM NaCl**	**85 mM NaCl**
Maize	3 (2, 5) ***	**4** **	1 ***	**4** ^{ns}
Rice	1 (3) ***	2 (1) ***	1 (3) **	3 (1) ***
Wheat	**1** ^{ns}	**1** ^{ns}	1 (5) **	**3 (1, 4, 5)** *
Sunflower	4 (1, 2) **	1 ***	2^{ns}	2***
Millet	2 (3, 5) *	2 (5) **	5 (1, 2, 3) *	2 ^{ns}
Strawberry	1 (2, 3, 4) *	3 ***	3 (5) ***	3 ***
Alfalfa	3 ***	5 ***	**2** ^{ns}	2 (1) *
Flax	3 (1) ***	5 ***	**1 (3, 5)** ***	**3 (5)** *

The numbers in the table correspond to the biofertilizers tested (1–5). The biofertilizers that did not show significant differences (Duncan's test) with respect to those that produced the greatest growth are indicated in parentheses. Note: ^{ns} $p > 0.05$, * $p < 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$ for Duncan's test. Biofertilizers that did not enhance growth compared with non-inoculated controls appear in bold.

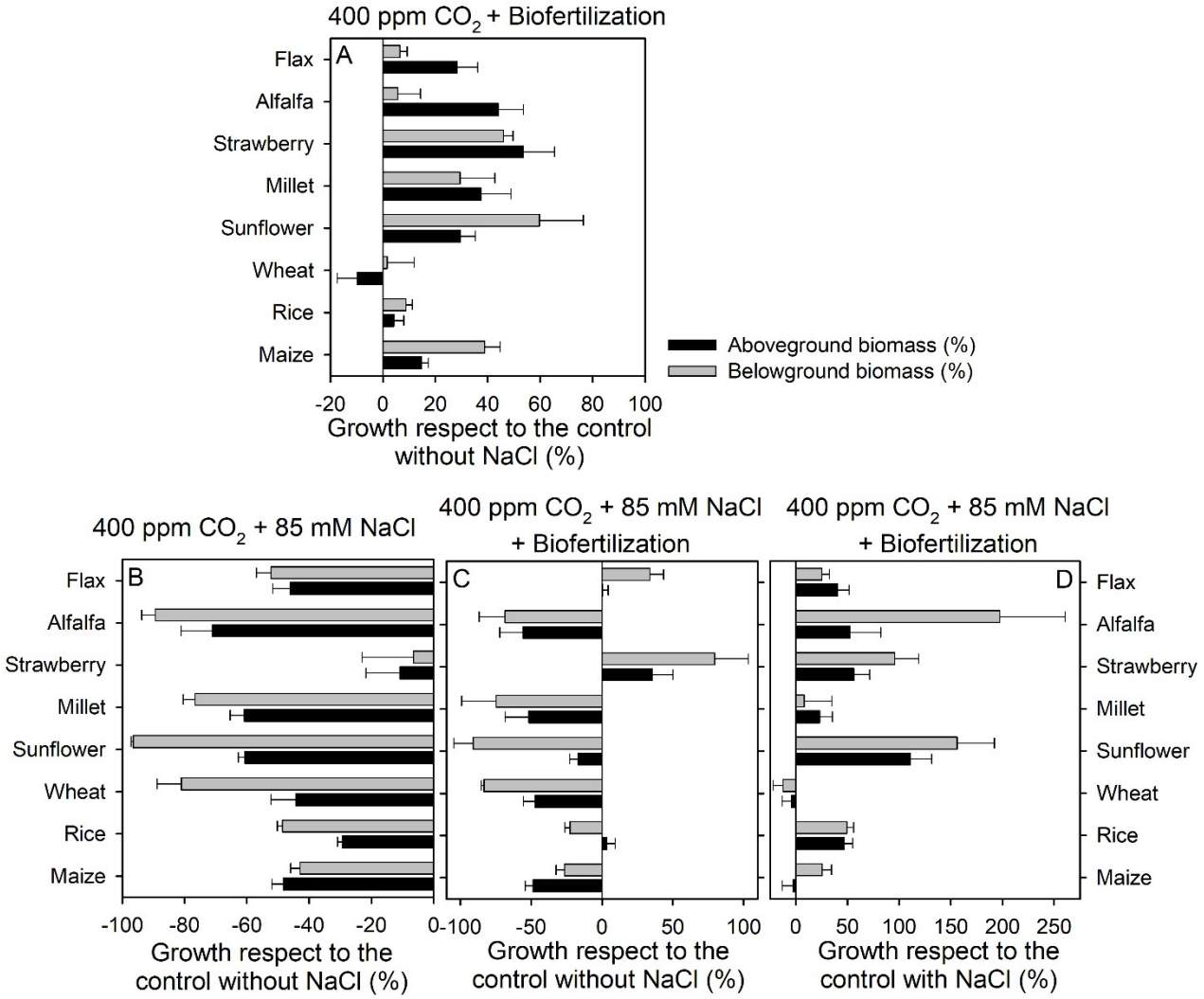

Figure 2. Effect of the most suitable biofertilizer for each crop on its growth at 400 ppm CO_2. (**A**) Percentage change in above- and below-ground biomass production of biofertilized crops with respect to non-inoculated controls. (**B**) Percentage change in above- and below-ground biomass production of non-inoculated and (**C**) inoculated crops, grown with 85 mM NaCl, with respect to non-inoculated controls grown both without and (**D**) with NaCl. Each value represents the mean of ten replicates (twenty for rice) ± SE. Note: the label in the upper part of the graph indicates the treatment that is presented and the label in the lower part indicates with respect to which control treatment the percentage change of biomass was calculated.

3.1.2. Biofertilizers from Halophytes under Salinity in the Present Climate

When crops were treated with salt (85 mM NaCl), growth reductions were observed compared with the control (no salt, Figure 2B), but the addition of biofertilizers counteracted this detrimental effect of salinity (Figure 2C,D). Inoculated crops showed a higher growth than non-inoculated controls exposed to NaCl, except in the cases of maize and wheat. Furthermore, above- and below-ground biomass in strawberry, above-ground biomass in rice, and below-ground biomass in flax—grown with biofertilization—were even higher than in the absence of salt and inoculation. For strawberry in particular, above- and below-ground biomasses were 35% and 80% greater, respectively, than in the control grown without salt (Figure 2C).

3.1.3. Biofertilizers from Halophytes in the Atmosphere of the Future

Crops recorded an increase in their biomass production at 700 ppm CO_2 and air temperature +4 °C (i.e., light/darkness photoperiod (16/8 h): 29/18 °C) except for rice, strawberry (above-ground), and sunflower and maize (below-ground) biomasses (Figure 3A). In the

presence of biofertilizer, the negative effect of high CO_2 and +4 °C on rice and strawberry was reversed and growth in millet and maize was enhanced (Figure 3B). However, inoculation diminished the production of flax and alfalfa biomasses and sunflower and wheat below-ground biomasses at elevated CO_2 (Figure 3C). Biofertilizers 1, 3, and 5 produced the greatest growth of crops at elevated CO_2 and +4 °C (Table 2).

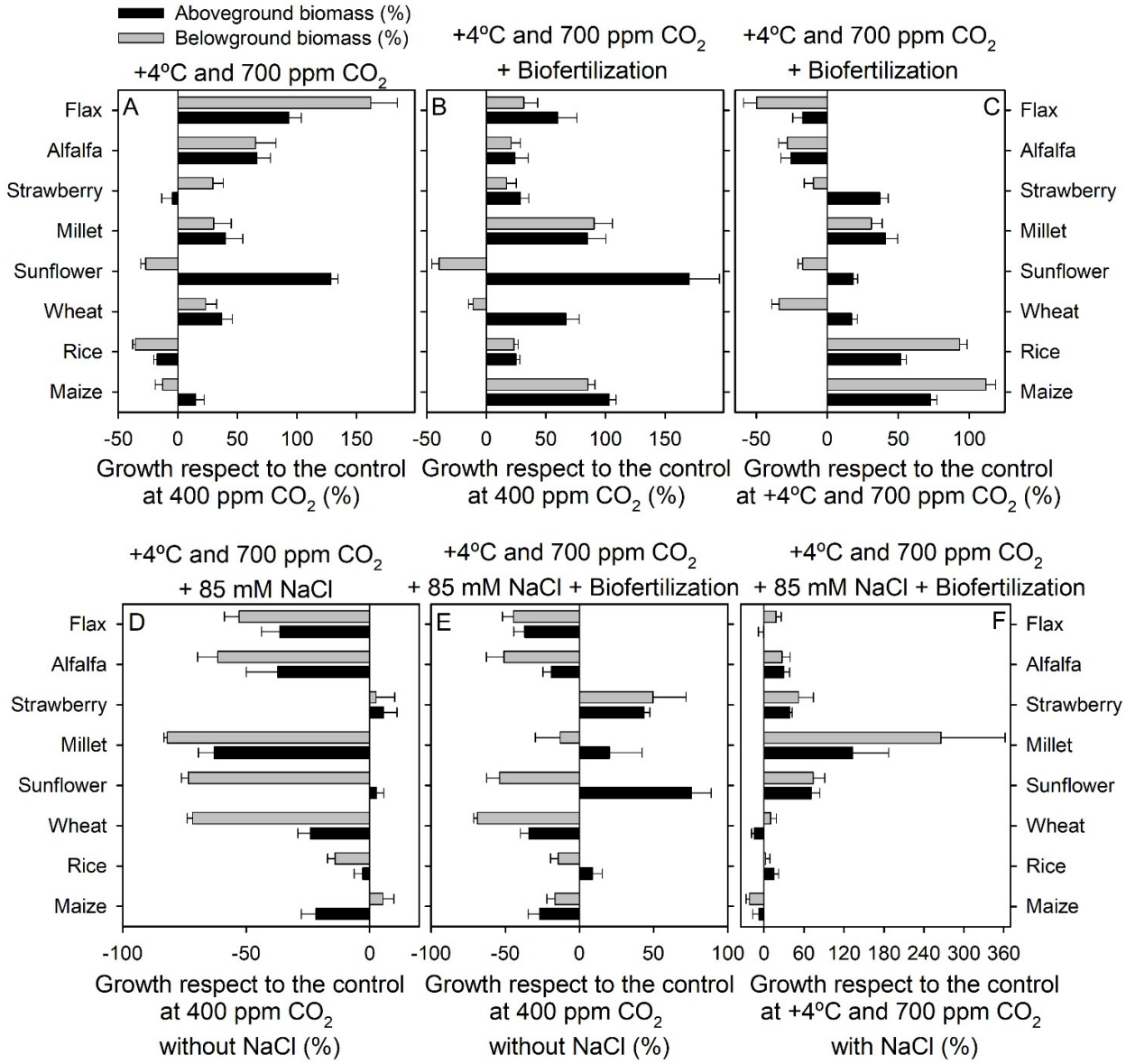

Figure 3. Effect of the most effective biofertilizer for each crop on its growth in the future climate scenario. (**A**) Growth of above- and below-ground biomass production of crops grown at 700 ppm CO_2 and 29/18 °C expressed as a percentage of that at 400 ppm CO_2 and 25/14 °C. (**B**) Growth of above- and below-ground biomass of crops grown with biofertilization at 700 ppm CO_2 and 29/18 °C, expressed as a percentage of biomass produced at 400 ppm CO_2 and 25/14 °C without biofertilizer. (**C**) Growth of above- and below-ground biomass of crops grown with biofertilization at 700 ppm CO_2 and 29/18 °C temperature, expressed as a percentage of biomass produced under the same conditions but without biofertilizer. (**D**) Percentage change in above- and below-ground biomass production of non-inoculated and (**E**) inoculated crops, grown with 85 mM NaCl, with respect to non-inoculated controls grown both at 400 ppm CO_2 without and (**F**) with NaCl at 700 ppm CO_2 and 29/18 °C. Each value represents the mean of ten replicates (twenty for rice) ± SE. Note: the label in the upper part of the graph indicates the treatment that is presented and the label in the lower part indicates with respect to which control treatment the percentage change of biomass was calculated.

3.1.4. Biofertilizers from Halophytes under Salinity in the Future Climate

Salinity negatively affected crop growth at elevated temperature and CO_2, except in the cases of strawberry, sunflower (above-ground), and maize (below-ground) biomasses (6%, 3%, 3%, and 5% more than the control, respectively; Figure 3D). In these cases, the deleterious effect of salt (observed in Figure 2B) was compensated by the high CO_2 and +4 °C. Overall, biofertilization alleviated salinity effects, except for maize and wheat above-ground biomasses (Figure 3E,F). Above-ground biomass of inoculated strawberry, millet, and sunflower increased 38%, 133%, and 71%, respectively, with respect to the control without salt (Figure 3F).

4. Discussion

Inoculating plants with PGPR can be an effective strategy to stimulate crop growth and improve crop tolerance to abiotic stresses, and is likely to become more frequently used as climate conditions become more extreme. However, no studies on the effect of PGPR on crops (considering the stress interaction (salinity × elevated temperature) and an atmosphere enriched with CO_2) have been carried out. Such studies are necessary for assessment of the role of biofertilizers as an alternative to chemical fertilizers under conditions of climate change. For this reason, we evaluated the effect of five bacterial biofertilizers obtained from halophytes on the growth of maize, rice, wheat, sunflower, millet, strawberry, alfalfa, and flax grown under the interaction of NaCl stress and elevated CO_2 and temperature, reproducing current and future climate scenarios.

In our study, the rhizobacteria isolated from the halophyte *Spartina densiflora* (Biofertilizer 1) showed endophytic colonization of rice roots (see Supplementary Materials Figure S1). However, no differences were found between the effects of Biofertilizer 1 (endophytic in rice) and, for example, Biofertilizer 3 (epiphytic in rice), on crop growth; both generated, to the same extent, the highest production of above-ground biomass of most crops in all different treatments. Most rhizobacteria colonize the root surface but cannot penetrate into the root cortex [27]. Although the presence of bacterial endophytes in plants is variable and, occasionally, transient [28], Pillay and Nowak [29] found that endophytes are often capable of eliciting greater physiological changes than those caused by many surface bacteria. In this regard, Naveed et al. [30] found that maximum photosystem II efficiency of maize, inoculated with an endophytic strain, increased up to 10% compared with uninoculated plants under drought stress, indicating an improvement in the response to stress. We found that the delayed fluorescence emission of sunflower leaves became higher in some of the inoculated plants, especially in the presence of salt, indicating lower stress due to salinity; however, consortium 1 did not enhance this emission compared with other biofertilizers (Figure 1).

The fact that Biofertilizer 1 or 3 stimulated the growth of crops (except wheat) indicates that the effective PGP properties were either atmospheric nitrogen fixation, phosphate solubilization, siderophore production for iron uptake, or IAA synthesis, and not biofilm formation or ACC deaminase activities, as Biofertilizers 1 and 3 do not have these properties (Table 1). IAA enhances root development and, as a result, roots with a larger surface area absorb more water and nutrients from soils and translocate them to various organs of the plants, resulting in increased top growth [9]. We found above-ground biomass of biofertilized alfalfa was 44% higher, while Liu et al. [31] recorded an increase in shoot dry weight of up to 33% for alfalfa inoculated with a PGPR strain from its own rhizosphere, despite previous studies reporting that the influence of PGPR is crop- or niche-specific [32]. Except in the case of alfalfa and flax, for which there are no previous data, we obtained lower growth for the rest of the crops than that recorded in other studies. Naveed et al. [30] observed that an endophyte increased maize shoot and root dry masses up to 48% and 47%, respectively, while these increases were up to 73% and 79% for sunflower and 30% and 31% for rice, respectively, when these crops were inoculated with isolates obtained from their rhizospheres [33,34]. Vestberg et al. [35] found that strawberry inoculated with *Bacillus subtilis* and *Pseudomonas fluorescens* increased shoot dry weight by 73% and

24%, respectively. In contrast, dual inoculation had no effect. For wheat, we found little positive effect of the biofertilizers tested, although *Providencia* sp. AW5, isolated from the rhizosphere of wheat, showed its suitability as an inoculant for this crop [32]. Overall, biofertilizers had a positive effect on the growth of the crops under the current climate conditions (400 ppm CO_2 and air temperature (light/darkness photoperiod 16/8 h): 25/14 °C).

It is important to note that the effect of a biofertilizer on a crop is modulated by the growth conditions and, here, we were particularly interested in the effects of salinity as biofertilizers were prepared from the root systems of halophytes. We recorded 47% and 49% higher above- and below-ground biomass in inoculated rice, while Jha et al. [36] found 8% and 27% increases, respectively, for the same salt concentration as used in our experiments. In the same way, we recorded that above- and below-ground biomasses in inoculated alfalfa were 53% and 197% greater, respectively, than in non-inoculated control plants (Figure 2D), while Ansari et al. [37] found that plant height improved by 15–23%. These percentages were 111% and 156%, respectively, for sunflower. For salt concentrations of about half ours, a previous study found 98% and 112% higher shoot and root biomasses, respectively, for inoculated sunflower [38]. At lower salinity, Karlidag et al. [39] found up to 23% and 22% higher shoot and root weights, respectively, for strawberry inoculated with bacteria isolated from the rhizosphere of plants naturally grown in high salty soils in Turkey, whereas we found 56% and 96% increases, respectively. In the case of above- and below-ground biomass in strawberry, above-ground biomass in rice, and below-ground biomass in flax, we observed a synergistic response of salt and inoculation. This effect was also recorded at elevated CO_2 and +4 °C for above- and below-ground biomass in strawberry and above-ground millet and sunflower. It is clear that the potential of biofertilizers obtained from halophytes was revealed in the presence of salt stress.

Although the five bacterial biofertilizers used in our experiments synthesized IAA, Biofertilizer 5 showed the highest production (21 mg/mL of IAA [16]) and produced the greatest effect on growth in half of the crops in the presence of salt (Table 2). PGPR may improve salt tolerance of crops by altering hormonal root–shoot signaling, so managing IAA production in halophytic and non-halophytic plants by endophytic and rhizospheric bacteria may be an important tool in enhancing salt tolerance [6]. Tiwari et al. [40] demonstrated that inoculation of wheat with IAA-producing salt-tolerant *Halomonas* sp. resulted in a higher IAA content in the rhizosphere of plants grown in saline soil, and raised plant growth. The effect of Biofertilizer 5 may also be attributed to stress relief in the crop through the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, together with bacterial IAA synthesis. Biofertilizers 4 and 5 were the only ones with this activity. As saline stress in plants is partially the result of the plant producing ethylene [41], it is reasonable to conclude that lowering ethylene levels by using bacterial ACC deaminase might provide plants with some protection against this stress [42]. Moreover, certain PGPR strains also have the ability to protect plants from the harmful effects of high Na^+ concentrations in the saline soil environment by producing exopolysaccharides. These compounds reduce Na^+ uptake in the plant by binding Na^+ and also by biofilm formation [18]. Biofertilizers 3, 4, and 5 had a demonstrated capability for biofilm formation.

In our model of future atmospheric CO_2 concentrations (700 ppm) and increased air temperature (+4 °C; i.e., light/darkness photoperiod (16/8 h): 29/18 °C), we recorded an increase in crop biomass production in wheat, millet, alfalfa, and flax, and above-ground biomasses in maize and sunflower. In contrast, rice, strawberry (above-ground), and sunflower and maize (below-ground) biomasses did not increase. Kim et al. [43] reported that above-ground dry matter of maize was 7% lower at 750 ppm CO_2 and 31/25 °C than the control (370 ppm and 25/19 °C). Contrarily, Kim et al. [44] found that rice plants grown at 622 ppm CO_2 and 27.3 °C showed 47% and 101% higher shoot and root dry masses, respectively, than those grown at 380 ppm CO_2 and 25.2 °C. Similarly, Cheng et al. [45] recorded that the whole plant dry weight for rice was significantly increased by elevated CO_2 (680 ppm). However, these data, like those of most rice studies, were taken during the

final period of the grain-filling stage. We studied the effect of consortia on the vegetative growth of plants, without waiting for the harvest, since the dimensions of the controlled environment chambers used to simulate climate change conditions did not allow us to keep the crops longer. De Costa et al. [46] noted that the percentage increase in biomass in the elevated CO_2 treatment, as compared with the current concentration, varied depending on the phenological stage of the crop. They recorded lower percentages during the vegetative stage (27% at 363 ppm CO_2 and >30 °C) compared with the grain-filling stage (71%). In agreement with our results, Keutgen et al. [47] found that an increase in the CO_2 level above 600 ppm decreased net CO_2 assimilation and, in consequence, hindered growth of strawberry plants. During the initial vegetative growth, the photosynthetic rates of two varieties of strawberry were also diminished by the interaction between elevated CO_2 and temperature (650 ppm and 30 °C [48]). However, the positive fertilizing effect of the inocula outweighed the detrimental effect of elevated CO_2 (e.g., maize, rice, and wheat above-ground biomasses increased 72%, 52%, and 17% with respect to the control without inoculation). Furthermore, except for strawberry, the increase in growth registered in the biofertilized crops, compared with the controls (at 400 ppm CO_2, and at 4 °C and 700 ppm CO_2, both without biofertilization) was more pronounced at elevated CO_2 and air temperature +4 °C than under the current climate scenario, which indicates that there could be an additive effect between the biofertilizer and the high concentration of CO_2. This effect disappeared in the presence of salt.

Biofertilizers 1, 3, and 5 produced the greatest growth of the crops at elevated CO_2 and +4 °C; the determining properties that they have in common were IAA synthesis and those involved in nutritional improvement. Nonetheless, Biofertilizer 3 was the most effective in the presence of salt (Table 2). It appears that for most of the crops we tested, biofilm formation was a more relevant property to counteract salt stress at elevated CO_2 and +4 °C than activity of ACC deaminase. Marilley et al. [49] provided evidence for a CO_2-induced alteration in the structure of the rhizobacterial populations, suggesting a possible alteration of the plant-growth-promoting rhizobacterial effect; however, the extent to which elevated CO_2 affects microbial interactions in the rhizosphere remains controversial [50]. The plant-mediated effects of atmospheric CO_2 on soil microbial communities are well documented, indicating a dominant, plant-mediated mechanism [50,51]. Elevated atmospheric CO_2 concentration alters plant photosynthetic rate, leading to changes in rhizodeposition and other root activities [49]. Van Veen et al. [52] also suggested quantitative and qualitative changes in rhizodeposition linked to CO_2 enrichment. These changes might be in the composition/availability of chemo-attractants or signal compounds, C:N ratio, or nutrient availability in the rhizosphere [53].

5. Conclusions

There are no studies evaluating the interactive effect of elevated CO_2 concentration and increased temperature with PGPR inoculation on tolerance of crops to salinity stress. This is despite the existence of numerous studies [11–13] proposing PGPR-based biofertilizers (as alternatives to chemical fertilizers) to adapt current agricultural practices to IPPC scenarios and to recover marginal lands that are unproductive on account of their salinity. Sustainable agriculture and food security are great challenges that must be addressed in the context of a future climate change scenario [54]. Quantifying crop growth in response to the interaction of various stressors (including those imposed by elevated CO_2) is critical to carrying out realistic and accurate assessments of crop performance in the context of the climate-change predicted by the IPCC [4].

We can generalize that there is a positive effect of PGPR consortia from halophytes on vegetative growth among agricultural crops, especially under saline stress conditions. Biofertilizers improved growth, regardless of the crop sensitivity to salt. This highlights the potential of halophytes as a source of PGPR for the formulation of wide-scope biofertilizers. Overall, Biofertilizers 1, 3, and 5 were the most suitable for most crops tested, which indicates that the PGP properties presented by the consortia are more important than the origin of

the bacteria, since they come from halophytes that grow in different habitats [25,55,56]. Depending on the interaction of stressors, the PGP properties that promote plant growth will change. This means that the choice of which is, or will be, the most effective biofertilizer for each crop in the present or future will change. For example, for alfalfa in the presence of salt, Biofertilizer 5 was the most effective under current CO_2 and temperature conditions, but Biofertilizers 1 or 2 will be more effective in the atmosphere of the future. Overall, the determining PGP properties in the absence of salt were: atmospheric nitrogen fixation, phosphate solubilization, siderophore production for iron uptake, and IAA synthesis, while in the presence of salt they were: synthesis of the enzyme ACC deaminase and capability for biofilm formation.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11081609/s1, Figure S1: Biofertilizers in the rice roots. (**a**) Micrographs showing the localization of rhizobacterial consortia 1 and 3 (**b**) by confocal microscopy in rice roots. In each panel, merged images from the rhizobacteria fluorescence and bright-field illumination are shown at the top. Fluorescence of both bacteria and plant membranes is shown at the bottom. Brightness and contrast were enhanced to improve visibility. Scale bar, 10 μm.

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