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ORIGINAL PAPER

Antarctic rhizobacteria improve salt tolerance and physiological performance of the Antarctic vascular plants

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Abstract

The two native Antarctic vascular plants, *Deschampsia antarctica* and *Colobanthus quitensis*, are mostly restricted to coastal habitats where they are often exposed to sea spray with high levels of salinity. Most of the studies regarding the ability of *C. quitensis* and *D. antarctica* to cope with abiotic stress have been focused on their physiological adaptations to tolerate cold stress, but little is known about their tolerance to salinity. We investigated whether rhizospheric bacteria associated to *D. antarctica* and *C. quitensis* improve the ability of Antarctic plants to tolerate salt stress. Salt tolerance was assayed in rhizospheric bacteria, and also their effects on the ecophysiological performance (photochemical efficiency of PSII, growth, and survival) of both plants were assessed under salt stress. A total of eight bacterial rhizospheric strains capable of growing at 4 °C were isolated. The strains isolated from *D. antarctica* showed higher levels of salt tolerance than those strains isolated from *C. quitensis*. The ecophysiological performance of *C. quitensis* and *D. antarctica* was significantly increased when plants were inoculated with rhizospheric bacteria. Our results suggest that rhizospheric bacteria improve the ability of both plants to tolerate salinity stress with positive effects on the adaptation and survival of vascular plants to current conditions in Antarctic ecosystem.

Keywords Salt tolerance · Antarctica · Plant growth-promoting rhizobacteria · *Colobanthus quitensis* · *Deschampsia antarctica*

Introduction

As obligate sessile organisms, plants have evolved a series of adaptive strategies to sense and respond to changing environmental conditions (Osakabe et al. 2013). Therefore, the performance and survival of plants depends on their ability to sense and adjust their physiology to tolerate the impacts of different biotic (such as herbivory and pathogens) and abiotic (such as drought, heat or salinity) stresses (Bohnert et al. 1995; Hirt 2009; Osakabe et al. 2013; El Sayed et al. 2014). In addition to these intrinsic capabilities to adjust their physiology, the ability of plants to cope with harsh

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environmental conditions often depends on their mutualistic interactions with beneficial root-associated microbes such as mycorrhizal fungi (Liu et al. 2016; Pedranzani et al. 2016) root endophytes (Molina-Montenegro et al. 2015, 2016; Azad and Kaminskyj 2016), and rhizosphere bacteria (de Zelicourt et al. 2013; Wang et al. 2016).

The Antarctic continent is among the most stressful environments on Earth for plant life (Convey 2008; Convey et al. 2014; Pointing et al. 2015) with their establishment and survival limited by conditions such as low temperatures, desiccation, wind abrasion, high radiation, and low water and nutrient availability (Alberdi et al. 2002; Robinson et al. 2003; Wasley 2006). Only two native vascular plants are present in the Antarctic ecosystem, *Deschampsia antarctica* and *Colobanthus quitensis* (Moore 1970). Both species occasionally cooccur, with *D. antarctica* usually being more abundant (Holtom and Greene 1967; Fowbert and Smith 1994). These species are widely distributed in Antarctic but they are a minor component of antarctic vegetation, which is dominated by cryptogams and are usually encountered as scattered colonies rather than dense closed communities (Smith 1984, 2003). Several studies have pointed the capacity of these species to tolerate low temperatures and avoid photoinhibition in the presence of energy dissipation mechanisms and ultrastructural adaptations (Pérez-Torres et al. 2004; Acuña-Rodríguez et al. 2017). On the other hand, it has been indicated that spatial distribution and physiological performance in these plants could be limited by low temperatures and nutritional status (Cannone et al. 2016; Molina-Montenegro et al. 2016). Nevertheless, it has been demonstrated that *D. antarctica* has the ability to avoid N limitation from slow N mineralization in the soil by directly using N from free amino acids and short-chain peptides present in the soil (Hill et al. 2011). Although several mechanisms and strategies have been indicated for both species to cope with Antarctic environment, little is known about the role that rhizospheric bacteria (hereafter rhizobacteria) might play on the tolerance of Antarctic plants to abiotic stress.

The majority of studies regarding the abilities of *C. quitensis* and *D. antarctica* to cope with stressful abiotic conditions have been focused on their physiological adaptations (e.g., Xiong et al. 1999, 2000; Pérez-Torres et al. 2004; Chew et al. 2012; Lee et al. 2013). While *D. antarctica* has a wide distribution including ice-free coastal areas near seashores in which it is continuously exposed to sea spray, *C. quitensis* is restricted to less-saline microsites (Alberdi et al. 2002; Olave-Concha et al. 2005; Rhuland and Krna 2010). The distribution area of *C. quitensis* encompasses Mexico, the highland regions of Ecuador, Bolivia, Perú, and Chile (close to 2500 m.a.s.l.). It also presents on Tierra del Fuego, Falkland Islands, South Georgia, the South Orkney Islands, the South Shetland Islands, as well as along the west coast of the Antarctic Peninsula with adjacent archipelagos (Moore 1970). Only in the southern distribution, when *C. quitensis* is near of coast, in contact with salt water in estuary or receiving marine spray, we have observed *C. quitensis* growing together with *D. antarctica* or *Sarcocornia fruticose*, but seldom alone (Pers. Obs.). While *D. antarctica* could be suggested as a salt-tolerant plant due to its physiological and anatomical adaptations such as increased productions of dehydrin-like proteins (Olave-Concha et al. 2005), osmoregulants (Rhuland and Krna 2010), and sodium exudation through leaf trichomes (Tapia-Valdebenito et al. 2016), no study has documented any mechanisms for salt stress tolerance in *C. quitensis*.

The negative effects induced by salinity on plant growth include ion toxicity, osmotic stress, oxidative stress, and nutrient deficiency (reviewed in Zhu 2007; Chakrabarty et al. 2016). It has been well documented that plant rhizobacteria can enhance plant salt stress tolerance through different mechanisms which includes exudation of a wide range of chemical compounds such as ACC (1-aminocyclopropane1-carboxylate) deaminase, IAA (indole acetic acids), or through phosphate solubilization (de Zelicourt and Al-Yousif 2013; Ahemad and Kibret 2014; Glick 2014; Wang et al. 2016). For instance, Bano and Fatima (2009) found that the salt tolerance by *Zea mays* is enhanced upon coinoculation with *Rhizobium* and *Pseudomonas.* Similar beneficial effects of rhizobacteria on plant performance have been found in crop species such as tomato, wheat, maize, or cotton (Mayak et al. 2004; Bano and Fatima 2009; Rajput et al. 2013; Egamberdieva et al. 2015) under low water availability and salt stress conditions.

Despite the recognized interest in plant rhizobacteria, there are still some gaps in knowledge regarding the relationship between rhizobacteria and Antarctic plants. Since it is observed that *C. quitensis* grows in coastal areas in association with *D. antarctica*, a plant reported as salt tolerant, we predict that the presence of antarctic rhizobacteria improves the tolerance to salt stress, and these positive effects are expected to be stronger on *C. quitensis* than in the salt-tolerant *D. antarctica*. The main goals of the present study were (1) to determine whether rhizobacteria isolated from *D. antarctica* and *C. quitensis* can improve the ability of Antarctic plants to tolerate the salt stress, (2) to evaluate if the presence of rhizobacteria can improve the ecophysiological performance in the Antarctic vascular plants, and (3) to determine whether rhizobacterial strains isolated from the antarctic plant can confer cross-positive effects to another antarctic plant species. Specifically, we carried out a screening to determine the tolerance of rhizobacteria to salt concentration and evaluated their impacts on the photochemical efficiency (Fv/Fm), relative growth, and survival of the two vascular Antarctic plants under salt stress.

Materials and methods

Study site, plant material, and rhizospheric soils

Rhizospheric soil associated to plants was collected in Livingston Island (62°37′S; 60°27′W) during the 2014–2015 growing season. Rhizospheric soil (~ 50 g) samples associated to five *C. quitensis* and five *D. antarctica* individuals growing at 10 m far away from seashore (3 m a.s.l) were carefully extracted, collected and stored in sterile hermetic bags, and transported to laboratory at 4 °C. In this site, both plant species grow under the permanent influence of marine spray. Samples of soil taken from the study site along a gradient from the shoreline to foothill were used to determine the electrical conductivity (EC) in the study site. Salt concentration of the soil was estimated by measuring EC from a solution of 1:5 (soil: water), after being dipped for 1 h using EC meter (ExStik II, Extech Instruments Corporation, USA). The area selected to collect samples of roots and rhizospheric soil showed a mean of EC = 254.8 µs, while in the area close to shoreline, the EC was = 647.2 µs and in the foothill area, the mean of EC was = 30.4 µs.

Rhizobacteria isolation and identification

Fine specimens of soil particles (≤ 2 mm) tightly adhered to the roots (rhizospheric soil) were carefully separated from the roots and used for bacterial culture. One gram of rhizospheric soil was diluted in of 10 ml sterile physiological solution. This solution was diluted in tenfold series and plated in triplicate onto Luria–Bertani (LB) and Reasoner's 2A agar (R2A) media (Oxoid) and incubated at 4 °C for 7 days. The colonies present in solid media were purified by repeated plating to obtain individual colonies (Ørskov 1922). The purified strains were grown at 4 °C under select conditions to evaluate their tolerance to salt. Pure strains were frozen in 25% glycerol at − 80 °C for later molecular identification.

The rhizobacteria cells were resuspended in 50 µl of sterile TE (10 mM Tris/HCl, pH 8, 1 mM EDTA) in 1.5 ml tubes and incubated at 95 °C for 10 min. Two µl of supernatant containing the released DNA was used for PCR colony method. The PCR protocol began with an initial 5-min denaturation step at 94 °C followed by 36 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s min, and final extension at 72 °C for 1 min. The protocol was concluded with an additional 5-min of final extension at 72 °C. Molecular identification of isolates was performed by partial sequencing of the 16S rRNA gene obtained using universal primers 27F and 1492R (Brosius et al. 1978). PCR products were checked by electrophoresis in 1% agarose gel-visualizing bands with 1.5 Kb molecular weight. The amplicons were purified using EZNA gel extraction kit (Omega Bio-Tek). The purified amplicons were sequenced in both senses by Macrogen Inc (Seoul, Korea). The sequences of each strain were analyzed with BLAST (Basic Local Alignment Search Tool, database nucleotide collection nr/nt) using Megablast to determine the percentage of maximal identity, i.e., the nearest neighbor with the sequences available in the global database. The accessions of the sequences were deposited in the GenBank database (see Table 1).

Evaluation of rhizobacteria salt tolerance

The tolerance of the isolated rhizobacteria to different salt concentrations was evaluated in vitro. The isolated rhizobacteria strains able to grow at 4 °C were cultured in 400 ml liquid medium, LB, and R2A, for 3 days. The liquid culture was adjusted to $OD_{620} = 0.001$. Then 100 µl of bacterial liquid culture was used to prepare serial dilutions (1:10). 10 µl each of the 3rd and 5th dilutions were spread out on Petri dishes with solid LB and R2A. The tolerance of the isolated strains was evaluated using four salt concentrations: NaCl control, ~ 171 mM, low NaCl ~ 428 mM, medium NaCl ~ 856 mM and, high NaCl ~ 1.7 M. Three plates per NaCl treatment were inoculated with each isolated bacterial

Table 1 Bacterial strains isolated from rhizospheric soil associated to *D. antarctica* (Da) and *C. quitensis* (Cq) present in Antarctica (Robert Island; 62°25′S/59°28′W)

Strain code	Genbank Accession	Nearest neighbor	16S rRNA similarity (%)	Maximum NaCl tolerance (mM)
Cq1	KX268490	*Rhodococcus* sp.	99	–
Cq2	KX268491	*Enterobacter* sp.	100	428
Cq5	KX268495	*Devosia* sp.	99	–
Cq7	KX268496	*Burkholderia* sp.	100	–
Da1	KX268492	*Arthrobacter* sp.	100	856
Da2	KX268493	*Planococcus* sp.	99	856
Da3	KX268494	*Arthrobacter* sp.	100	856
Da4	KX268497	*Arthrobacter* sp.	100	856

Code, genebank accession, nearest neighbor, 16S rRNA similarities and maximum percentage of salt tolerance are indicated for each isolated bacteria

strain (100 µl, OD = 0.01). Although only the rhizobacterial strains capable of growth at 4º C were used, all plates were incubated at 15 °C for 7 days in order to accelerate the growth. After this period, we counted the total number of colony-forming units (CFUs) (Thawatchai et al. 2008). Afterward, the most salt-resistant bacteria were used in the subsequent assays.

Assessing the effect of rhizobacteria on the ecophysiological performance of Antarctic plants

To evaluate the effects of the isolated rhizobacteria strains on the ecophysiological performances (photochemical efficiency of PSII, growth, and survival) of the two vascular plants *C. quitensis* and *D. antarctica*, under salt stress, we performed two experiments under controlled conditions in growth chambers. Photochemical efficiency of PSII (Fv/Fm; where Fv = [Fm − F0], Fm = maximum fluorescence yield, and F_0 = minimum fluorescence yield) (Maxwell and Johnson 2000) was estimated using a pulse-modulated-amplitude fluorimeter (FMS 2, Hansatech, Instrument Ltd, and Norfolk, UK). We used Fv/Fm as a response variable because it has been well correlated with plant fitness (Molina-Montenegro et al. 2013) and suggested as a good proxy of the physiological status and stress tolerance of a plant (Hasanuzzaman et al. 2017). To compare the photochemical efficiencies between treatments, a group of leaves from each individual was dark-adapted for 30 min (to obtain open PSII centers) using a black box (30 × 20 × 15 cm) to ensure maximum photochemical efficiency.

To obtain free-rhizobacteria individuals (B-individuals), roots were treated with a wide spectrum antibiotic (rifampicin 50 µg/ml) (Mbah and Wakil 2012). This antibiotic was applied twice to the soil during irrigation (on days 0 and 15) to assure the elimination of bacteria. To verify the absence of bacteria, a small sterile brush was rubbed on the roots and then plated on LB or R2A solid medium. To obtain B+ plants, we initially treated plant roots with antibiotics and reinoculated them with each bacterial strain. A total of 120 plants (60 of *C. quitensis* and 60 of *D. antarctica*) were used in this experiment. All plants were grown in previously sterilized soil (2 h at 140 °C). To test the effect of each bacterial strain (3 strains), 20 individuals per plant species were randomly chosen and assigned either to the reinoculation treatment (B+, $n = 10$) or to the control treatment (B−, $n = 10$).

In the first experiment, we evaluated the effect of reinoculation on Fv/Fm, growth, and survival in *C. quitensis* and *D. antarctica* individuals with the most-tolerant rhizobacteria strains isolated from each plant species (Cq2 and Da3, respectively), under medium salt condition (856 mM). All rhizobacteria used in this assays were cultured in Laura Bertani broth (LB) (Gibco®). Later, they were mixed on an orbital shaker at a speed of 120 rpm and incubated at 10 °C for 72 h. The incubated broth cultures were then centrifuged for 15 min at 3000 rpm. Pelleted cells were suspended in sterile distilled water, and the optical density of the cells was adjusted to about 10^8 cells ml^{-1} (OD ~ 660 = 0.08) (Bhuvaneswari et al. 1980) and approximately, 2 ml of inoculum was injected into the rhizosphere, according the procedure describe by Hadi and Bano (2010). The photochemical efficiencies and survival of both *C. quitensis* and *D. antarctica* individuals were evaluated weekly, over 8 weeks, and their growth was assessed as the difference between total fresh biomass values at the start and at the end of experimental time. For each plant species, a total of 40 individuals were randomly assigned to four treatments: without rhizobacteria and without salt addition (B−S−, $n = 10$), without rhizobacteria and with salt addition (B−S+, $n = 10$), inoculation with bacteria and without salt addition (B+S−, $n = 10$), and inoculation with rhizobacteria and salt addition(B+S+, $n = 10$). Plants without rhizobacteria were obtained by means of antibiotics as indicated above.

In the second experiment, we tested the effect of cross-inoculation by comparing the photochemical efficiencies of *C. quitensis* and *D. antarctica* individuals grown without rhizobacteria (B− treatment) and with the most salt-resistant rhizobacteria (B+ treatment) from *D. antarctica* (Da3; *Arthrobacter* sp.) and from *C. quitensis* (Cq2; *Enterobacter* sp.). Each strain selected was inoculated to the other plant species as well as on themselves, and after 8 weeks the photochemical efficiency in both plant species with and without the own rhizobacteria strain as well as with the strain from another plant species were compared.

All experiments were performed in automatic air-cooled growth chambers (model: LTJ300LY; Tianyi Cool, China) where temperature was set at 4 °C, with a photosynthetically active radiation (PAR) of 260 µmol $m^{-2}s^{-1}$ and an 18/6 h light/dark period. Since treatments can be affected by the characteristics of the growth chamber (see Potvin and Tardif 1988), individuals from different treatments were randomly moved within growth chambers every week. Plants were maintained in plastic pots (300 cc) filled with sterilized commercial soil (perlite–commercial substrate–sand; 1:1:1). At the end of each month, the growth chambers were switched off, cleaned, and individuals were transferred between chambers, and growth conditions were reestablished.

Statistical analyses

The differences in the growths of the different isolated strains, measured as the mean number of CFUs, in response to different treatments of salt concentration (control, low, medium, and high concentration of NaCl) were evaluated using one-way ANOVA and Tukey HSD ($\alpha = 0.05$) as an a posteriori test.

In experiment 1, differences in photochemical efficiency (Fv/Fm) of plants inoculated (B+) and noninoculated with bacteria (B−) and with (+S) and without (S−) salt along time were evaluated with repeated measures ANOVA, using treatments as the independent variable and the Fv/Fm as response variable. Survival percentage was evaluated weekly and estimated by means of the Kaplan–Meier method, and statistical differences were assessed by the Cox–Mantel test (see Fox 1993). Differences in total fresh biomass values were compared using one-way ANOVA and Tukey HSD test. These analyses were performed independently for each plant species. In experiment 2, the effects of inoculation with salt-tolerant strains on photochemical efficiency of photosystem II (Fv/Fm) were also evaluated using one-way ANOVA and Tukey HSD test. This analysis was performed independently for each plant species. For all the ANOVAs, the assumptions of normality and homogeneity of variances were evaluated using Shapiro-Wilks and Bartlett tests, respectively (Sokal and Rohlf 1995).

Results

Identification of isolated strains

A total of eight rhizobacteria strains capable of growing at more than 400 mM of NaCl—four associated to *C. quitensis* and four associated to *D. antarctica*—were isolated. All of them showed pigmented colonies and regular borders in solid medium. PCR products ranged from 1329 to 1448 bp, covering almost full-length of 16S rRNA gene sequence for all strains. The sequences were deposited in GenBank, and their accession numbers are shown in Table 1. The strains isolated from *C. quitensis* belonged to four genera (*Rhodococcus*, *Enterobacter*, *Devosia*, and *Burkholderia*), and the strains associated to *D. antarctica* belonged to two genera (*Arthorbacter* and *Planococcus*) (Table 1).

Evaluation of rhizobacteria salt tolerance

The strains isolated from *D. antarctica* showed significantly higher salt tolerance than those strains isolated from *C. quitensis* rhizhosphere (Table 2). While all strains isolated from rhizospheric soil of *D. antarctica* maintained between 71 and 100% of growth at 856 mMof NaCl, only one rhizosperic bacterial strain isolated from soil associated to *C. quitensis* (Cq2: *Entherobacter* sp.) was able to maintain 100% of growth at 428 mM of NaCl (Table 2).

Table 2 Mean percentages of colony-forming units (CFUs) of eight rhizobacteria strains isolated from *Colobanthus quitensis* (Cq) and *Deschampsia antarctica* (Da) grown on different NaCl concentrations (2.5, 5, and 10%)

Strain	NaCl concentration		
	428 mM	856 mM	1.7 M
Colobanthus quitensis			
Rhodococcus sp. (Cq1)	6.0 (±0.4)[a]	0.0[b]	0.0[b]
Enterobacter sp. (Cq2)	98.6 (±35.2)[a]	0.0[b]	0.0[b]
Devosia sp. (Cq5)	0.0	0.0	0.0
Burkholderia sp. (Cq 7)	0.0	0.0	0.0
Deschampsia antarctica			
Arthrobacter sp. (Da1)	99.9 (±0.1)[a]	99.0 (±0.1)[a]	0.0[b]
Planococcus sp. (Da2)	72.8 (±3.1)[a]	71.3 (±6.0)[a]	0.0[b]
Arthrobacter sp. (Da3)	98.5 (±0.1)[a]	97.9 (±0.1)[a]	0.0[b]
Arthrobacter sp. (Da4)	98.2 (±0.1)[a]	99.8 (±0.01)[a]	0.0[b]

Percentage of CFUs showed in each NaCl concentration is relative to the mean percentage recorded in the control treatment (medium without salt). Means labeled with different uppercase letters indicate significant differences in the mean percentages of UFCs between different levels of NaCl concentrations (Tukey HSD tests, $\alpha = 0.05$). Standard errors are shown within parentheses

Assessing the effect of rhizobacteria on the ecophysiological performance of Antarctic plants

Experiment 1

The photochemical efficiency (Fv/Fm) was significantly higher (ANOVA, $F_{3,36} = 1567.6$; $p = 0.001$) in *D. antarctica* individuals when grown associated to the rhizhobacteria and/or only irrigated with water (control), in comparison to those individuals with rhizobacteria plus salt as well as by those with salt and without rhizobacteria (Fig. 1a). Similarly, those individuals of *C. quitensis* that had grown with the rhizobacteria or irrigated with water (control) showed significantly higher Fv/Fm (ANOVA, $F_{3,36} = 1953.5$; $p = 0.001$), followed by those with the presence of salt plus rhizobacteria, and finally by those *C. quitensis* individuals under salt condition and without rhizobacteria (Fig. 1b). In addition, in both *D. antarctica* and *C. quitensis,* those individuals associated with the rhizobacteria maintained higher Fv/Fm along time (ANOVAs, $F_{36,168} = 53$; $p < 0.0001$ and $F_{36,168} = 67$; $p < 0.0001$, for *D. antarctica* and *C. quitensis*, respectively) compared mainly with those individuals treated with salt and without rhizobacteria (Fig. 1a, b).

On the other hand, the accumulated fresh biomass of those *D. antarctica* individuals associated to rhizobacteria was significantly greater (ANOVA, $F_{3,25} = 60.72$; $p = 0.002$), followed by those individuals irrigated with water (control) and individuals with salt plus rhizobacteria,

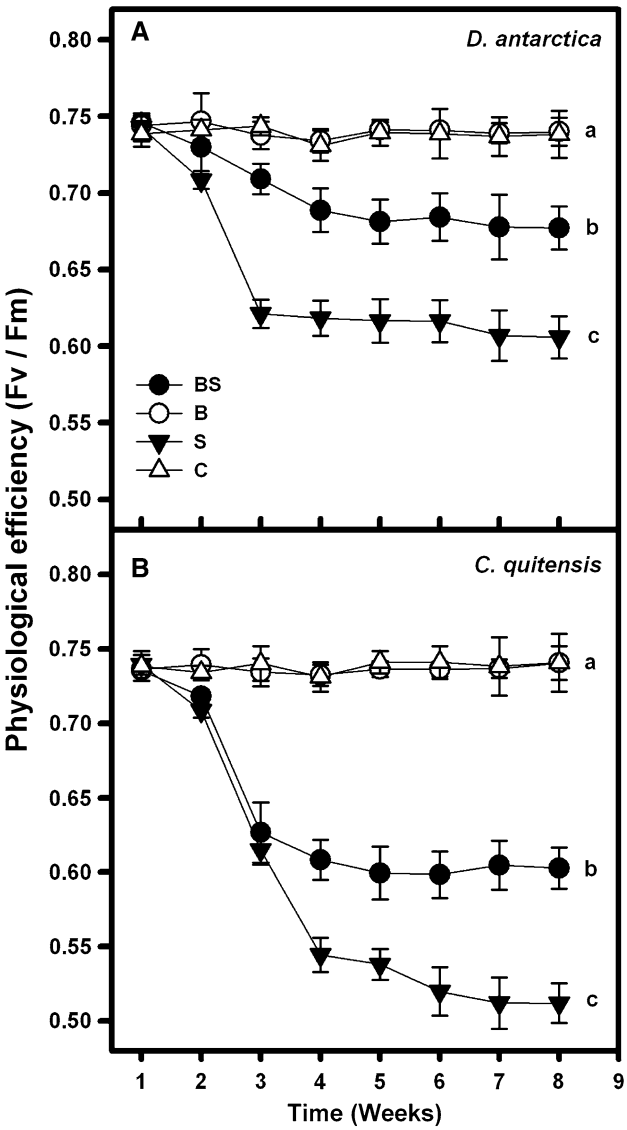

Fig. 1 Physiological Performance: Photochemical efficiencies (Fv/Fm) of photosystem II (PSII) of *Colobanthus quitensis* and *Deschampsia antarctica* individuals exposed to four treatments: with bacteria/salt added (BS, black circles), with bacteria/without salt added (B, open triangle), without bacteria/salt added (S, black square), and without bacteria/without salt added (C, open rhombus). *C. quitensis* was reinoculated with *Enterobacter* sp. (Cq2), and *D. antarctica* was reinoculated with *Arthrobacter* sp. (Da3). Salt addition treatment consisted of 850 mM NaCl. Are showed (±SD). Different letters indicate significant differences (Tukey HSD tests, $\alpha = 0.05$)

and finally by those with salt and without rhizobacteria (Fig. 2a). Similarly, in *C. quitensis* individuals, the accumulated fresh biomass was significantly greater (ANOVA, $F_{3,24} = 49.21$; $p = 0.001$) when grown in the presence of the rhizobacteria or irrigated with water (control), followed by those individuals under salt condition plus the presence of rhizobacteria, and finally by those under salt and without the rhizobacteria (Fig. 2b).

Survival percentage along time in *D. antarctica* individuals was significantly higher (Cox–Mantel test = 6.9, $p = 0.0013$) when grown in the presence of rhizobacteria and/or irrigated only with water, followed by those individuals under salt condition and with rhizobacteria, and finally by those individuals under salt condition and in the absence of rhizobacteria (Fig. 3a). Similarly, the survival of *C. quitensis* individuals, with the presence of rhizobacterian and/or irrigated only with water (control), was significantly higher (Cox–Mantel test = 7.2, $p = 0.001$) than those *C. quitensis* individuals grown under salt condition plus rhizobacteria, and finally by those individuals treated with salt and without rhizobacteria (Fig. 3b).

Experiment 2

The inoculation with rhizobacteria strains isolated from *C. quitensis* and *D. antarctica* significantly increased the photochemical efficiencies in both antarctic plant species (Fig. 4). The cross-inoculation with rhizobacteria isolated from *D. antarctica* (Da3; *Arthrobacter* sp.) enhanced the Fv/Fm in *C. quitensis* in 28.3%, followed by the inoculation with a strain isolated from themselves (Cq2; *Enterobacter* sp.) with 15.4% (Fig. 4). Similarly, in *D. antarctica*, the presence of the rhizobacteria strains isolated from themselves (Da3) enhanced the Fv/Fm in 10%, followed by the cross-inoculation with the strains isolated from *C. quitensis* (Cq2) with only 3% (Fig. 4).

Discussion

Our results provide evidence that rhizobacteria associated to the native Antarctic vascular plants *C. quitensis* and *D. antarctica* can tolerate salt stress, this tolerance being greater in those rhizobacteria isolated from *D. antarctica*. In addition, we found that some of these rhizobacteria were able to improve the abilities of both plants to tolerate salinity stress, as assessed by photochemical efficiency, growth, and survival traits.

The most salt-tolerant strains, *Arthrobacter spp*. (Da1, Da3, and Da4) and *Planoccocus* sp. (Da2), were associated to *D. antarctica*. Salt tolerance has been previously described in rhizobacteria of the genera *Arthrobacter* and *Planococcus*. Ganzert et al. (2011) described two salt-tolerant *Arthrobacter* species (*A. livingstonensis* and *A. cryotolerans*) from Antarctic soils of Livingstone Island (South Shetland archipelago), which were able to tolerate up to 1.7 M of NaCl under culture conditions. In the same way, salt tolerance was found by Rajput et al. (2013) in *Planococcus rifietoensis* (up to 300 mM of NaCl) isolated from saline soils of Pakistan, which is, approximately the half of the maximum tolerance found for the *Planococcus* strain

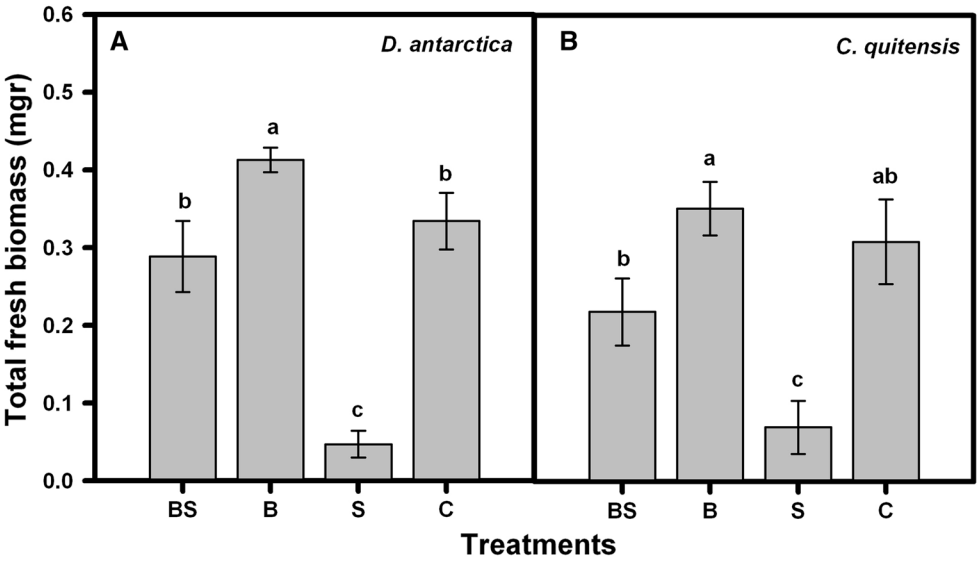

Fig. 2 Fresh Biomass Accumulation: Total fresh biomass accumulation in *Colobanthus quitensis* and *Deschampsia antarctica* individuals exposed to four treatments: with bacteria/salt added (BS), with bacteria/without salt added (B), without bacteria/salt added (S), and without bacteria/without salt added (C). *Colobanthus quitensis* was reinoculated with *Enterobacter* sp. (Cq2), and *Deschampsia antarctica* was reinoculated with *Arthrobacter* sp. (Da3). Salt addition treatment consisted of 850 mM NaCl. Bars are means (±SD). Different letters indicate significant differences (Tukey HSD tests, $\alpha = 0.05$)

isolated in this study (e.g., Da2). On the other hand, *Enterobacter* sp. (Cq2) was the strain with higher salinity tolerance associated to *C. quitensis* (428 mM). Similar levels of salt tolerance have been reported in other *Enterobacter* strains isolated from arid saline soils (Egamberdieva et al. 2014; Habib et al. 2016). In both studies, *Enterobacter* spp. showed plant growth-promoting (PGP) properties increasing plant growths in tomato (*Solanum lycopersicum*; Egamberdieva et al. 2014) and okra (*Abelmoschus esculentus*; Habib et al. 2016) under saline conditions.

The consequences of soil salinity on plants include inhibition of seed germination, seedling growth, flowering, and fruit set (Zhu 2002; Sairam and Tyagi 2004; Zhu 2007; Ruiz-Carrasco et al. 2011). In this study, we found evidence suggesting that Antarctic rhizobacteria improve the salt tolerance of both antarctic plants as they improve their photochemical efficiencies (Fv/Fm) as well as the survival and biomass, this last considered a proxy of growth. Specifically, *C. quitensis* and *D. antarctica* plants grown in saline soils and inoculated with rhizobacteria showed respective increases of 8.9 and 8.5% of their Fv/Fm with respect to plants grown in saline soils not inoculated with rhizobateria (Fig. 3). Similarly, those individuals of both antarctic plant species showed significantly greater ecophysiological performance (biomass and survival) when inoculated with rhizobacteria, even under salt condition. As predicted, *C. quitensis* was more benefited than *D. antarctica* when inoculated with some rhizospheric bacteria since the presence of these microorganisms induced a buffered decrease in all ecophysiological parameters compared with the treatment irrigated with salt without rhizobacteria. On the other hand, cross-inoculation improves the photochemical efficiency, being more evident in *C. quitensis* when inoculated with rhizobacteria isolated from *D. antarctica*. Although we found evidence that rhizospheric bacteria can improve salt tolerance in these plant species, field experiments or mimicking natural conditions must be conducted to test if this functional symbiosis could help to cope with antarctic environment and colonization into sites with high salt content.

A growing number of studies have documented positive effects of rhizobacteria of noncultivated plant species. For instance, Egamberdieva et al. (2013) found that root inoculation with *Pseudomonas* strains improves root and shoot growth in *Galega officinalis* under salinity stress. In addition, Qin et al. (2014) found significant increases in *Limonium sinense* growth after inoculation with four salt-tolerant rhizobacteria under saline stress. Similarly, Karthikeyan et al. (2012) found significant increases in plant biomass in *Catharanthus roseus* inoculated with the salt-tolerant *Achromobacter*. Despite the fact that we did not evaluate the putative mechanisms by which the different rhizosphere bacteria improve the tolerance of both vascular plants to salt stress; PGP properties are likely to be involved. Berríos et al. (2013) characterized an Antarctic strain of *Pseudomonas* sp. isolated from rhizospheric soils associated *D. antarctica* with PGP properties. They found that the bacteria are able to solubilize different sources of inorganic phosphate and to promote plant root development. These plants inoculated with rhizobacteria with PGP properties, growing under abiotic stress conditions (PEG 10%, 200 mM NaCl, and 20 mM ZnCl2), showed better growth and capacity to tolerate these stresses better than the noninoculated plants. On the other hand, enhanced mRNA expression levels of the various ROS-scavenging enzymes and higher proline content in tubers induced by PGPR-treated plants contributed to increase the tolerance to salinity. Similarly, Wang et al. (2012) demonstrated that a consortium of three plant growth-promoting rhizobacteria (PGPR) strains (*Bacillus*

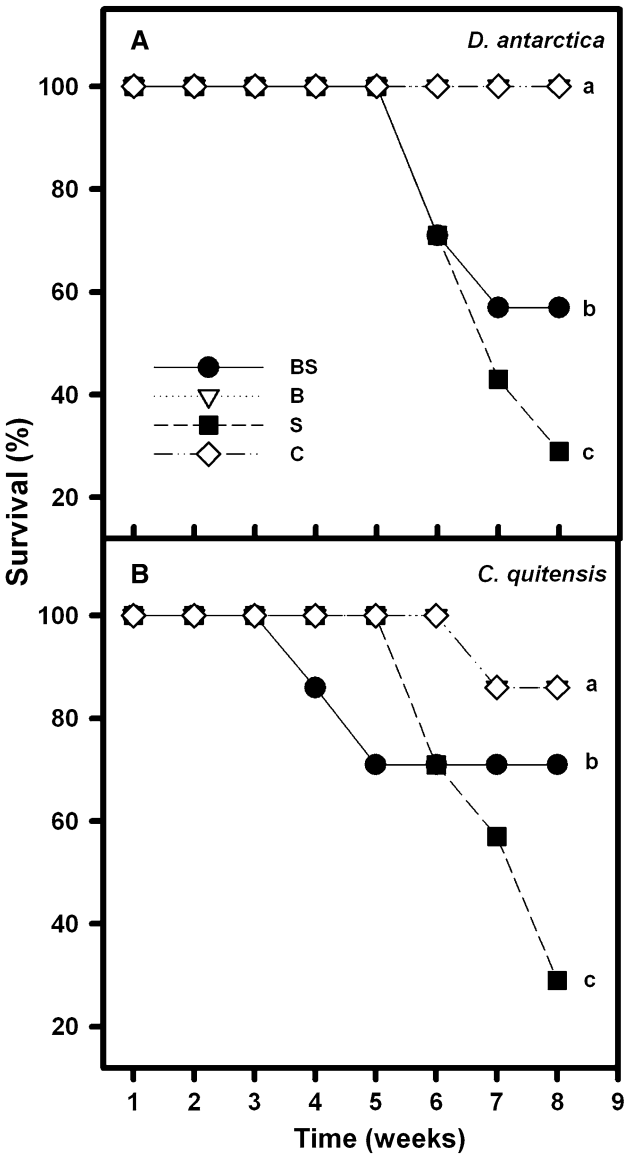

Fig. 3 Survival: Percentages of survival of *Colobanthus quitensis* and *Deschampsia antarctica* individuals exposed to four treatments: with bacteria/salt added (BS, black circles), with bacteria/without salt added (B, open triangle), without bacteria/salt added (S, black square), and without bacteria/without salt added (C, open rhombus). *Colobanthus quitensis* was reinoculated with *Enterobacter* sp. (Cq2) and *Deschampsia antarctica* was reinoculated with *Arthrobacter* sp. (Da3). Salt-addition treatment consisted of 850 mM NaCl. Different letters indicate significant differences (Cox–Mantel tests, $\alpha = 0.05$)

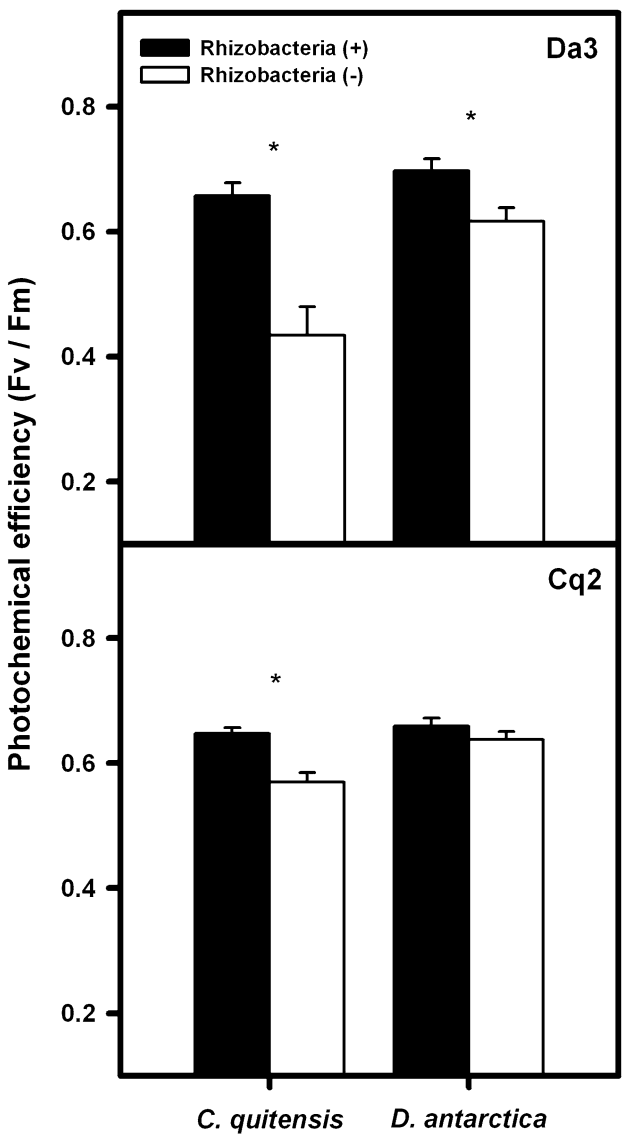

Fig. 4 Photochemical efficiencies (Fv/Fm) of photosystem II (PSII), of *Colobanthus quitensis* and *Deschampsia antarctica* plants growing under increased salinity (850 mM NaCl) either without rhizosphere bacteria (white bars) or reinoculated with rhizospheric bacteria (black bars). *Arthrobacter* sp. (Da3) was isolated from *D. antarctica* and *Enterobacter* sp. (Cq2) was isolated from *C. quitensis*. Bars are means (±SD). Asterisks indicate significant differences, $\alpha = 0.05$)

cereus AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21), could induced systemic tolerance to drought stress in cucumber plants, by protecting plant cells, maintaining photosynthetic efficiency and root vigor. Hence, previous results as well as those found in this study, reinforce the importance of rhizosphere bacteria to improve the growth, ecophysiological performance and development of plants in harsh environments like the Antarctic ecosystem.

Finally, variations of environmental components are widely indicated as a major threat to global food security (Fedoroff et al. 2010; FAO 2014). Climate change will further affect agriculture as the desertification will increase the salt concentration in the soil, reducing the arable lands for different crops (IPCC 2014). Under this scenario, Antarctica offer interesting opportunities to provide bioresources for human use, since the Antarctic terrestrial biota has evolved under a particular combination of environmental stresses like the high level of soil salinity. The rhizobacteria described in

this study are promising candidates to be used for improving salt tolerance. Future studies should address the potential biotechnological applications of the salt-tolerant rhizobacteria described, especially for sustainable production of food crops in saline soils, ensuring food security in a future where drought and salt stress could limit the food supply.

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