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ORIGINAL PAPER

Functioning of plant-bacterial associations under osmotic stress in vitro

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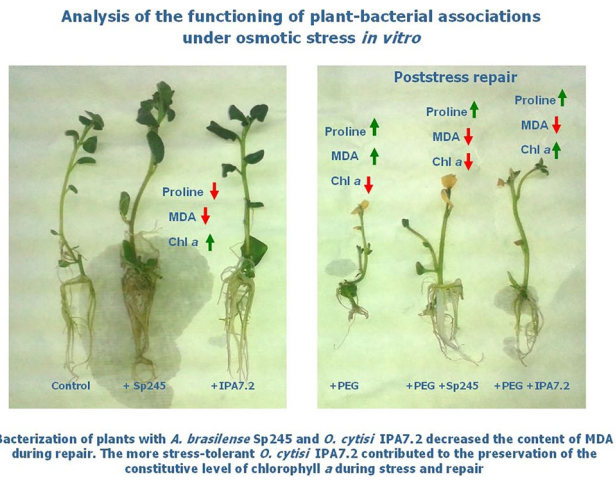
Abstract

The search for effective plant-growth-promoting strains of rhizospheric bacteria that would ensure the resistance of plant-microbial associations to environmental stressors is essential for the design of environmentally friendly agrobiotechnologies. We investigated the interaction of potato (cv. Nevsky) microplants with the plant-growth-promoting bacteria *Azospirillum brasilense* Sp245 and *Ochrobactrum cytisi* IPA7.2 under osmotic stress in vitro. The bacteria improved the physiological and biochemical variables of the microplants, significantly increasing shoot length and root number (1.3-fold, on average). Inoculation also led a more effective recovery of the plants after stress. During repair, inoculation contributed to a decreased leaf content of malonic dialdehyde. With *A. brasilense* Sp245, the decrease was 1.75-fold; with *O. cytisi* IPA7.2, it was 1.4-fold. During repair, the shoot length, node number, and root number of the inoculated plants were greater than the control values by an average of 1.3-fold with *A. brasilense* Sp245 and by an average of 1.6-fold with *O. cytisi* IPA7.2. *O. cytisi* IPA7.2, previously isolated from the potato rhizosphere, protected the physiological and biochemical processes in the plants under stress and repair better than did *A. brasilense* Sp245. Specifically, root weight increased fivefold during repair, as compared to the noninoculated plants, while chlorophyll *a* content remained at the level found in the nonstressed controls. The results indicate that these bacteria can be used as components of biofertilizers. *A. brasilense* Sp245 has favorable prospects for use in temperate latitudes, whereas *O. cytisi* IPA7.2 can be successfully used in saline and drought-stressed environments.

Graphic abstract



Bacterization of plants with *A. brasilense* Sp245 and *O. cytisi* IPA7.2 decreased the content of MDA during repair. The more stress-tolerant *O. cytisi* IPA7.2 contributed to the preservation of the constitutive level of chlorophyll *a* during stress and repair

Keywords Plant cell and tissue culture in vitro · Potato · *Azospirillum brasilense* Sp245 · *Ochrobactrum cytisi* IPA7.2 · Osmotic stress

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Introduction

Drought-induced stress severely affects the growth and yield of cultivated plants. Numerous drought management studies have been conducted around the world in which drought-resistant varieties have been generated by genetic engineering, plant breeding, and the use of growth systems (Cattivelli et al. 2008; Nidumukkala et al. 2019). However, most technologies developed to date take much time to carry out and are expensive.

Recent studies have shown that microorganisms can help plants to cope with drought (Rubin et al. 2017; Mustafa et al. 2019). The plant-stress-reducing microbial inoculation is a cost-effective and environmentally friendly option that can be implemented in a shorter time than the above-mentioned technologies.

Plant-growth-promoting rhizobacteria (PGPR) supply plants with additional mineral and organic nutrients, phytohormones, and available nitrogen (Pii et al. 2015); participate in the competitive bioregulation of the composition of microbial communities in soil; and induce systemic resistance of plants to abiotic and biotic environmental factors (Bashan et al. 2014; Tkachenko et al. 2015; Maksimov and Cherepanova 2018; Mustafa et al. 2019; Veselova et al. 2019).

The proposed PGPR mechanisms that increase drought resistance in plants include production of phytohormones, synthesis of osmolytes to increase cellular osmotic potential, synthesis of 1-aminocyclopropane-1-carboxylate deaminase to lower root ethylene levels, reduction in the content of malonic dialdehyde (MDA) as an indicator of lipid peroxidation, and changes in root morphology for drought resistance (Dimkpa et al. 2009; Forni et al. 2016; Vurukonda et al. 2016). Bacteria also influence the plant content of photosynthetic pigments and the activity of antioxidant enzymes as plant protection systems against oxidative stress (Heidari and Golpayegani 2012; Duo et al. 2018). However, the morphological, physiological, and molecular mechanisms of bacteria-mediated stress resistance in plants and the functioning of bacteria and their plant hosts under osmotic stress remain insufficiently studied.

It is obvious that in a plant-bacterium association, both partners are affected by stress (Forni et al. 2016; García et al. 2017). In this context, it is important to select drought-resistant strains and use them as inocula to promote plant growth and protect stressed plants. Further, research on the functioning of plant-microbial associations should be carried out in vitro under controlled conditions. This allows elimination of unforeseen effects, which are inevitable when plants are grown in vivo, and makes it possible to examine only the target factors in the systems under study.

The aim of this work was to investigate the effect of associative microflora on plant resistance to osmotic stress in vitro. Specifically, this work was addressed to the effect of inoculation of potato (cv. Nevsky) microplants with the plant-growth-promoting rhizobacteria *A. brasilense* Sp245 and *O. cytisi* IPA7.2 on the physiological and biochemical variables of the plants during stress and poststress recovery.

Materials and methods

Plant material

We used potato (*Solanum tuberosum* L. cv. Nevsky) microplants from the in vitro potato microplant collection of the Agronomy Faculty of Vavilov Agrarian University, Saratov, Russia. The microplants in the collection had been grown from apical meristems. Cultivar Nevsky is very tolerant of different climates and produces high yields (Glaz et al. 2019).

Characterization of bacteria

The strains used as inocula came from the IBPPM RAS Collection of Rhizosphere Microorganisms (https://collection.ibppm.ru/). *A. brasilense* Sp245 (IBPPM 219) is a facultative endophyte (Assmus et al. 1995) that promotes the growth of a wide range of cultivated plants (Bashan et al. 2014) and is moderately salt-tolerant (bacterial growth stops at 300 mM NaCl) (Yevstigneyeva et al. 2016). *O. cytisi* IPA7.2 (IBPPM 544, RCAM04481) is a natural potato associate isolated from the rhizosphere of potato (cv. Nevsky) (Burygin et al. 2017). It promotes potato growth and is halotolerant (bacterial growth stops at 750 mM NaCl) (Burygin et al. 2019).

Experimental plan

In vitro-grown microplants were separated into microcuttings with one leaf and one lateral bud, placed in a test tube containing a hormone-free liquid Murashige–Skoog medium (MS) (Murashige and Skoog 1962), and grown under the following conditions: temperature, 24 °C; humidity, 60%; light intensity, 60 $\mu Mm^{-2}\ s^{-1}$; day length, 16 h. On day 10 after the microplants were separated into microcuttings, the nutrient medium received a suspension of 10^6 bacterial cells mL^{-1}. After 5 days of inoculation, the medium with bacteria was replaced with the MS medium containing 25 g L^{-1} of polyethylene glycol (PEG; MW 6000), which corresponded to an osmotic pressure in the growth medium of − 0.3 MPa. The osmotic pressure in the nutrient medium was measured with an Osmomat 030 cryoscopic osmometer (Gonotec GmbH, Germany). After 7 days, the medium

with PEG was replaced with a PEG-free MS medium to investigate poststress repair. For study of the influence of bacteria and water deficiency on plants, four experimental treatments were used: control without bacteria or PEG (C1); two PEG-free treatments with *O. cytisi* IPA7.2 (C2 + IPA7.2) and *A. brasilense* Sp245 (C2 + Sp245); a PEG-free treatment with bacteria (C3); and two treatments with *O. cytisi* IPA7.2 (E + IPA7.2), *A. brasilense* Sp245 (E + Sp245), and PEG. Plant state was assessed by measuring the morphological–physiological variables of the microplants, free proline content, MDA content, and chlorophyll *a* (Chl *a*) content in leaves on day 7 of stress and on day 7 of poststress repair.

Microbiological test

The viability of *A. brasilense* Sp245 and *O. cytisi* IPA7.2 associated with the roots of 22- and 29-day-old potato plants was determined by a method modified from that of Zvyagintsev (1991), with account taken of the bacterial colonization of root segments. Segments about 10 mm in length were cut from different zones of adventitious roots from the experimental and control plants and were placed on a malate–salt medium (Döbereiner and Day 1976) containing 1.5% agar. The samples were cultivated in a thermostat at 35 °C for 3 days. After that, the bacteria that had grown around the root segments were collected for immunodiffusion analysis.

Immunodiffusion

Double immunodiffusion in agarose gel was done by the standard technique of Ouchterlony and Nilsson (1978). Strain-specific antibodies against *A. brasilense* Sp245 were raised as described by Matora et al. (1998), and those against *O. cytisi* IPA7.2 were raised as described by Burygin et al. (2019). For bacterial outer membrane preparations, the cells were washed in phosphate-buffered saline, sedimented by centrifugation, and treated with an extraction buffer (pH 8.5) of 0.1 M Tris–HCl, 10 mM sodium ethylenediaminetetraacetate (EDTA), 0.1 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100 at room temperature for 30 min. The amount of EDTA was 0.05 mM g^{-1} of wet cells. The extract was freed from the cells by centrifugation, and precipitation was run on 69 cm glass plates in 1% agarose gel prepared with phosphate-buffered saline. The experimental results were evaluated after 18–20 h. The plates were dried, stained with Coomassie Brilliant Blue R-250, and destained in an aqueous solution of 45% ethanol and 10% acetic acid.

Colony-forming-unit (CFU) counting

For plants aged 22 and 29 days, CFU were counted after plating 0.1 mL of the culture medium on the malate–salt medium.

Measurement of free proline in leaves

Free proline was measured with the Bates method by the formation of a colored product with acidic ninhydrin during heating (Bates et al. 1973). Absorbance was measured at 520 nm in a 1-cm-path-length cuvette on a Specord 250 spectrophotometer (Analytik, Jena, Germany).

Measurement of MDA in leaves

MDA was measured spectrophotometrically by the formation of a colored complex with thiobarbituric acid during heating (Titov and Talanova 2013).

Determination of Chl a in leaves

For determining the leaf content of Chl *a*, we used one leaf from three plants in each treatment. The first normally formed leaf from the apical bud was analyzed. After the leaves were weighed, they were placed in dimethyl sulfoxide at a ratio of 1 mg of leaves to 20 µL of dimethyl sulfoxide. The samples were heated in a water bath at 60 °C for 30 min. Chl *a* content was determined on a Tecan Spark 10 M microplate reader (Tecan, Austria). Twenty-five µl of the sample was placed into each well of a 348-well plate, and the absorbance was measured at the corresponding wavelengths. The absolute values of the pigment concentrations in solutions were found by determining the conversion factor on a Specord 250 spectrophotometer (Analytik, Jena, Germany) in a 1-cm-path-length cuvette by using the ratio between the absorbance values in the range 400–750 nm. The conversion factor was 4.35. The concentration of Chl *a* (µg mL^{-1}) was calculated by the following formula (Wellburn 1994): $C_a = 4.35(12.19A_{664} – 3.45A_{647})$, where A_{664} is the absorbance at 664 nm and A_{647} is the absorbance at 647 nm.

Statistics

Results were processed by two-way ANOVA, with the calculation of the least significant difference (LSD) and with multiple comparisons by Duncan's multiple range test at a significance level of 95% ($P \leq 0.05$), which enabled us to estimate the significance of the differences between the treatments. In the tables and diagrams, treatments significantly different in Duncan's test are marked with different Latin letters. Data on the physiological and morphological variables of the plants were obtained in three independent experiments, and those on the biochemical variables (Chl *a*, MDA, and proline) were obtained in two independent experiments.

Results

Plant-bacterium associations under optimal growth conditions

The two-way ANOVA showed that the bacteria promoted the growth of potato microplants in vitro (Tables 1 and 2: factor A). This promotion was manifested as increased length of shoots and increased numbers of nodes and adventitious roots on days 22 and 29. Under conditions optimal for plant growth, *O. cytisi* IPA7.2 promoted a 1.3-fold increase in shoot length and root number and a 1.5-fold increase in leaf weight, as compared with noninoculated plants (Tables 1 and 2). At a statistically significant level, the growth-promoting effect of *A. brasilense* Sp245 was manifested as a 1.4-fold increase in the number of roots on day 22 and as a 1.3-fold increase in the length of shoots on day 29 of growth. The leaf content of proline and MDA did not increase under optimal conditions and did not depend on the presence of bacteria in the growth medium (Figs. 1a, b and 2a, b). On day 22 of growth under optimal conditions, the highest leaf content of Chl *a* was in the plants inoculated with *A. brasilense* Sp245 (Fig. 3a). Under optimal growth conditions, both *A. brasilense* Sp245 and *O. cytisi* IPA7.2 were detected in the MS medium after 10 and 17 days of plant inoculation (Fig. 4a, b). The number of *O. cytisi* IPA7.2 in the MS medium gradually increased to 10^9 cell mL^{-1}, in contrast to that of *A. brasilense* Sp245, which remained the same (10^6 cell mL^{-1}) throughout the experiment.

Functioning of plant-bacterial associations under osmotic stress (– 0.3 MPa, 7 days of stress)

The choice of the strength and duration of stress was based on preliminary experiments investigating the effect of osmotic stress on potato microplants. Those experiments had shown that at 5% (m/v) PEG, the plants recovered very poorly (data not shown). Here we used 2.5% (m/v) PEG, because at this concentration, potato microplants both experienced stress and restored their growth after the stress was removed.

The presence of PEG inhibited all the physiological and morphological variables analyzed (Tables 1 and 2: factor B). However, inoculation helped to mitigate the stress. In the

Table 1 Effect of *A. brasilense* Sp245 and *O. cytisi* IPA7.2 on the physiological and morphological variables of potato microplants grown in vitro under optimal conditions and under osmotic stress (− 0.3 MPa, 7 days)

Treatment		Shoot length (cm)	Number of nodes on shoot (pcs)	Number of roots (pcs)	Average root length (cm)	Shoot wet weight (mg)	Leaf wet weight (mg)	Root wet weight (mg)
Designation	Presence (±) of bacteria or PEG							
C1	− bact. − PEG	4.01ab	4.67b	5.17a	3.88bcd	158,13cd	110.56b	159.34bcd
C2	+ bact. Sp245 − PEG	4.81bc	5.25b	7.00c	3.91cd	159.05d	159.89bc	182.68d
	+ bact. IPA7.2 − PEG	5.16c	5.33b	6.67bc	4.13d	155.90bcd	168.51d	175.28cd
C3	− bact. +PEG	3.65a	3.58a	4.17a	3.05a	88.76a	53.11a	78.09a
E	+ bact. Sp245 + PEG	4.70bc	4.50b	4.75a	3.29abc	113.39a	85.88ab	47.86a
	+ bact. IPA7.2 + PEG	4.77bc	4.75b	5.25a	2.96a	101.61a	102.15b	52.42a
$F_{fact.}$		3.546*	5.231*	8.378*	6.138*	7.258*	12.613*	30.074*
$LSD_{0.05}$		0.850	0.781	1.082	0.605	37.304	39.130	36.302
Factor A	− bact. − PEG	3.83a	4.12a	4.67a	3.46	123.45	81.83a	118.72
	E + bact. Sp245	4.75b	4.88b	5.88b	3.60	136.22	122.89b	115.27
	E + bact. IPA7.2	4.96b	5.04b	5.96b	3.54	128.75	135.33d	113.85
	$F_{fact.}$	8.053*	6.261*	7.148*	0.231	0.587	10.162*	0.095
	$LSD_{0.05}$	0.601	0.552	0.765	–	–	27.669	–
Factor B	−PEG	4.66	5.08b	6.28b	3.97b	157.69b	146.32b	172.43b
	+PEG	4.37	4.28a	4.72a	3.10a	101.25a	80.38a	59.46a
	$F_{fact.}$	1.358	12.781*	24.824*	28.301*	34.090*	42.293*	144.225*
	$LSD_{0.05}$	–	0.451	0.624	0.349	21.538	22.59	20.959

Different letters (a, b, c, d) show that values differ significantly at $p \leq 0.05$ according to Duncan's multiple range test. Asterisk indicates $F_{fact} > F_{theor}$

Table 2 Effect *A. brasilense* Sp245 and *O. cytisi* IPA7.2 on the growth variables of potato microplants grown in vitro under optimal conditions and on day 7 of repair after osmotic stress

Treatment		Shoot length (cm)	Number of nodes on shoot (pcs)	Number of roots (pcs)	Average root length (cm)	Shoot wet weight (mg)	Leaf wet weight (mg)	Root wet weight (mg)
Designation	Presence (±) of bacteria or PEG							
C1	− bact. − PEG	4.82b	5.92c	7.08b	3.89b	140.77	80.44	189,03d
C2	+ bact. Sp245 − PEG	6.50c	5.92c	7.75b	4.16b	176.43	146.79	165.58cd
	+ bact. IPA7.2 − PEG	5.74bc	6.75c	9.25c	4.08b	189.11	127.51	186.13d
C3	− bact. +PEG	3.58a	3.58a	4.67a	3.20ab	109.27	76.08	18.11a
E	+ bact. Sp245 + PEG	5.13b	4.75b	6.25b	3.22ab	156.73	125.35	71.84ab
	+ bact. IPA7.2 + PEG	5.83bc	5.92c	7.58b	2.92a	171.34	113.29	93.58b
$F_{fact.}$		8.291*	9.858*	9.473*	3.040*	0.926	1.424	9.783*
$LSD_{0.05}$		0.994	1.012	1.422	0.860	-	-	70.676
Factor A	− bact. − PEG	4.20a	4.75a	5.88a	3.55	125.02	78.26	103.57
	E + bact. Sp245	5.82b	5.33a	7.00b	3.69	166.58	136.07	118.71
	E + bact. IPA7.2	5.79b	6.33b	8.42c	3.50	180.23	120.40	139.85
	$F_{fact.}$	13.853*	10.010*	12.840*	0.219	1.842	3.251	1.321
	$LSD_{0.05}$	0.703	0.716	1.005	–	–	–	–
Factor B	−PEG	5.69b	6.19b	8.03b	4.04b	156.28	118.25	180.24b
	+PEG	4.85a	4.75a	6.17a	3.12a	158.27	104.91	61.18a
	$F_{fact.}$	8.487*	24.428*	20.563*	14.189*	0.007	0.486	42.266*
	$LSD_{0.05}$	0.574	0.584	0.821	0.497	–	–	40.805

Different letters (a, b, c, d) show that values differ significantly at $p \leq 0.05$ according to Duncan's multiple range test. Asterisk indicates $F_{fact} > F_{theor}$

inoculated plants, as compared with noninoculated stressed ones, the length of shoots and the number of nodes increased significantly (1.3-fold) with both bacteria, but leaf weight increased 1.6-fold with *A. brasilense* Sp245 and 1.9-fold with *O. cytisi* IPA7.2 (Table 1). After 7 days of stress, no bacteria were detected in the plant growth medium (Fig. 4a). In the microbiological test, root fragments were overgrown by bacteria only when the plants were inoculated with *O. cytisi* IPA7.2 (Fig. 5a). That the identified bacteria belonged to this strain was determined by immunodiffusion with specific antibodies (Fig. 5c).

After 7 days of stress, the leaf content of proline increased by an order of magnitude in both control plants (C3) and treatments with *A. brasilense* Sp245 (E + Sp245) and *O. cytisi* IPA7.2 (E + IPA7.2) (Fig. 1a). The leaf content of MDA (an indicator of oxidative stress) also increased twofold in noninoculated as well as inoculated plants. That is, the inoculated and noninoculated plants experienced the same degree of oxidative stress (Fig. 2a). During stress, the leaf content of Chl *a* decreased sharply—14.5-fold in the control (C3) plants and 7.7-fold in the *A. brasilense* Sp245-inoculated (E + Sp245) plants. In turn, *O. cytisi* IPA7.2 helped to preserve the constitutive level of green pigments (Fig. 3a).

Functioning of plant-bacterial associations on day 7 of repair after osmotic stress (– 0.3 MPa, 7 days of repair)

On day 7 of poststress repair, we found bacteria in the growth medium in both control and experimental plants (Fig. 4b). As confirmed immunochemically (Fig. 5), root fragments were also being intensely overgrown by bacteria during inoculation with both *A. brasilense* Sp245 and *O. cytisi* IPA7.2. Within 7 days after the stress was removed, the control (C3) plants also lagged behind the inoculated plants in terms of their growth variables during repair. In particular, the bacteria contributed to an increase in the length of the shoot, in the number of nodes on the shoot, and in the number of roots (1.3-fold with *A. brasilense* Sp245 and 1.6-fold with *O. cytisi* IPA7.2. In addition, *O. cytisi* IPA7.2 promoted a fivefold increase in root wet weight during repair, as compared to noninoculated plants (Table 2). On day 7 of poststress repair, the leaf content of proline remained high after PEG stress in the control (C3) and in the experiment with *A. brasilense* Sp245 (E + Sp245). The leaf content of proline in the plants inoculated with *O. cytisi* IPA7.2 decreased slightly during repair, which was evident by eye

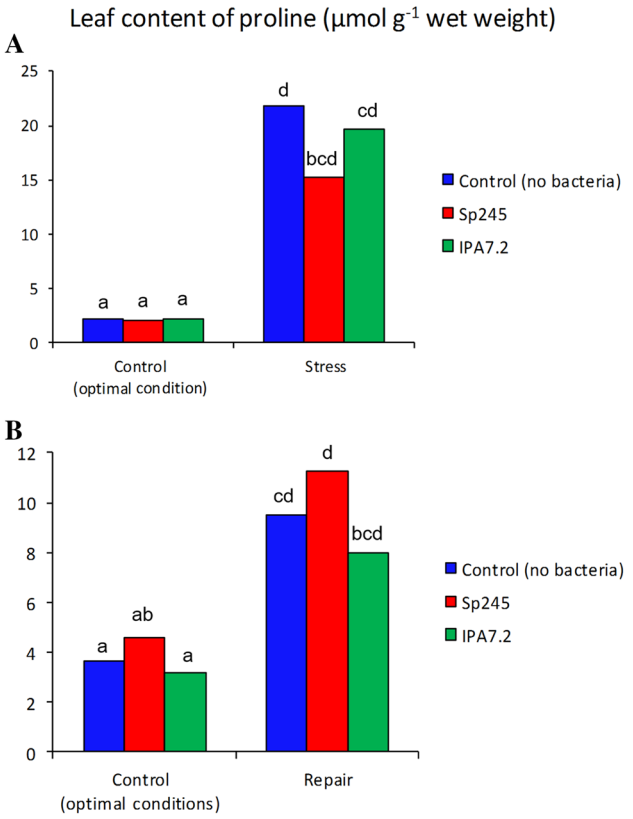

Fig. 1 Effect of *A. brasilense* Sp245 and *O. cytisi* IPA7.2 on the leaf content of proline in Nevsky microplants on day 22 (7 days of stress) (**a**) and on day 29 of growth (7 days of repair) (**b**). At the stress stage, $LSD_{0.05} = 9.238$; at the repair stage, $LSD_{0.05} = 3.505$. For a significance level $P \leq 0.05$, the data bars marked with different letters differ significantly

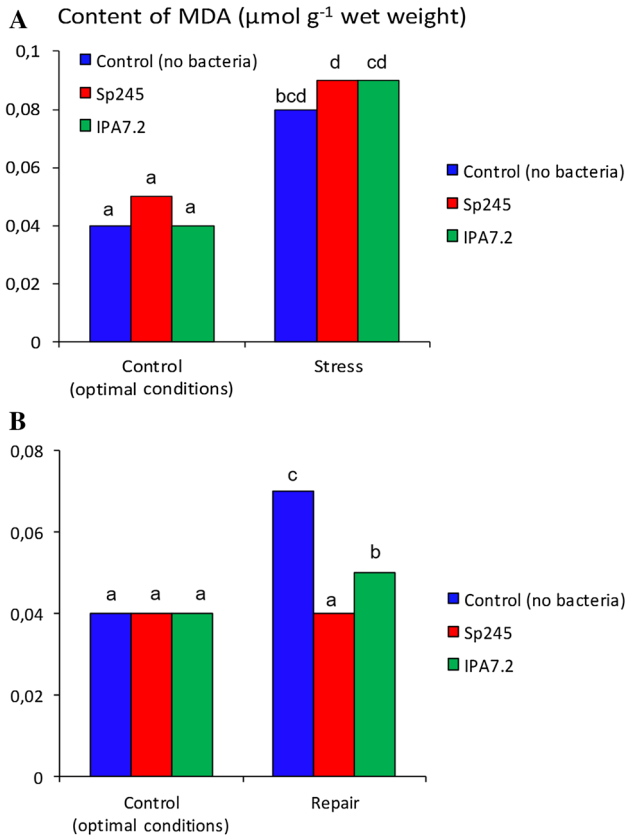

Fig. 2 Effect of *A. brasilense* Sp245 and *O. cytisi* IPA7.2 on the leaf content of MDA in Nevsky microplants on day 22 (7 days of stress) (**a**) and on day 29 of growth (7 days of repair) (**b**). At the stress stage, $LSD_{0.05} = 0.021$; at the repair stage, $LSD_{0.05} = 0.01$. For a significance level $P \leq 0.05$, the data bars marked with different letters differ significantly

as faster restoration of shoot length, node and root numbers, and root weight (Fig. 1b; Table 2). The leaf content of MDA in the inoculated plants after 7 days of repair (E + Sp245 and E + IPA7.2) approached that in the nonstressed ones and was 1.75-fold lower with *A. brasilense* Sp245 and 1.4-fold lower with *O. cytisi* IPA7.2, as compared with noninoculated controls (Fig. 2b). A significant increase in the leaf content of Chl *a* was observed in the plants inoculated with *O. cytisi* IPA7.2, in contrast to those inoculated with azospirilla, in which Chl *a* content did not differ from that in the noninoculated controls (C3) (Fig. 3b).

Discussion

In recent years, the ability of PGPR to increase plant resistance to abiotic stresses, including drought, has been studied widely. Many investigators have shown that PGPR improve plant resistance to abiotic stresses through mechanisms such as production of 1-aminocyclopropane-1-carboxylate deaminase, changing of the plant phytohormone status, induction of the synthesis of plant antioxidant enzymes, improvement of the assimilation of mineral elements, production of extracellular polymeric substances (exopolysaccharides), and induction of resistance genes (Forni et al. 2016; Oskuei et al. 2017; Bandeppa et al. 2018; Etesami and Maheshwari 2018; Martins et al. 2018).

We used two strains of PGPR that differ in salt tolerance: *O. cytisi* IPA7.2 and *A. brasilense* Sp245. Earlier, Yevstigneyeva et al. (2016) and Burygin et al. (2019) showed that *O. cytisi* IPA7.2 can grow with a 2.5-fold higher concentration of NaCl than can *A. brasilense* Sp245. Because the initial responses of the bacteria and their plant hosts to drought and salinity are similar, as they are related to water deficit, we conclude that *O. cytisi* IPA7.2 is more resistant to drought than *A. brasilense* Sp245.

Osmotic stress (− 0.3 MPa, 7 days) disturbed the functioning of the bacteria in the plant growth medium. By calculating the CFU after stress, we did not find any bacteria in the growth medium (Fig. 4a). Only the salt-tolerant strain *O. cytisi* IPA7.2 was detected on the plant

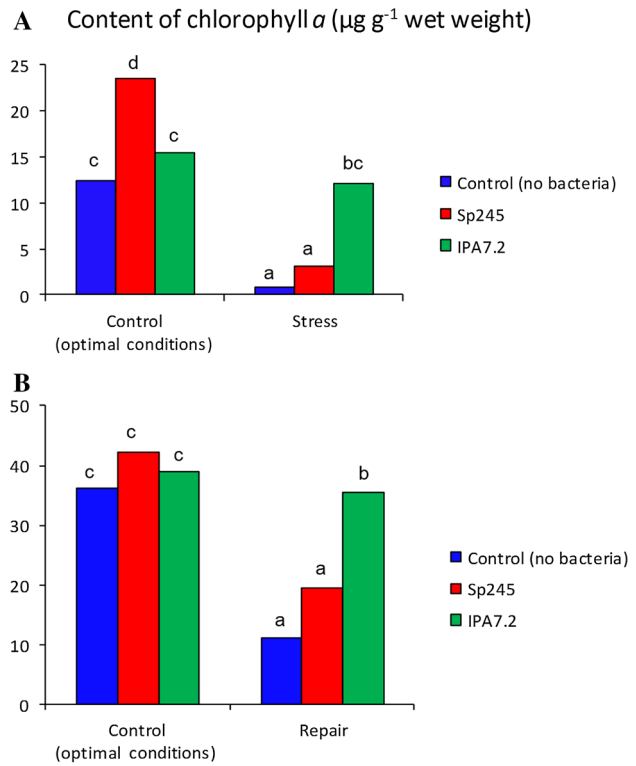

Fig. 3 Effect of *A. brasilense* Sp245 and *O. cytisi* IPA7.2 on the leaf content of Chl *a* in Nevsky microplants on day 22 (7 days of stress) (**a**) and on day 29 of growth (7 days of repair) (**b**). At the stress stage, $LSD_{0.05} = 5.299$; at the repair stage, $LSD_{0.05} = 12.234$. For a significance level $P \leq 0.05$, the data bars marked with different letters differ significantly

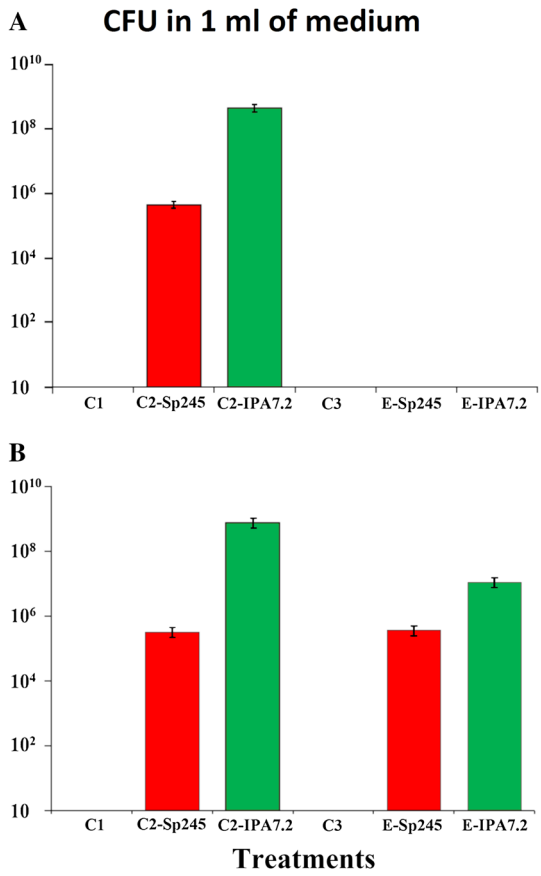

Fig. 4 CFU of *A. brasilense* Sp245 and *O. cytisi* IPA7.2 in MS nutrient medium on day 22 (7 days of stress) (**a**) and on day 29 of growth (7 days of repair) (**b**). Bars indicate standard deviations

roots (Fig. 5a). Apparently, most *A. brasilense* Sp245 cells died on the surface and in the outer layers of the roots as a result of PEG stress, but some of them remained inside and appeared on the root surface during poststress repair (Fig. 5a).

The detection of drought-tolerant rhizobacterial strains and the testing of their ability to protect osmotically stressed plants have been described earlier for *Azospirillum* (García et al. 2017) and *Bacillus* (Bandeppa et al. 2018). Specifically, after being inoculated with *Azospirillum* sp. Az19 (PGPR Collection of IMyZA INTA Castelar), a strain highly tolerant of osmotic and salt stress, maize seedlings endured drought better than did noninoculated controls (García et al. 2017).

Bandeppa et al. (2018) isolated and identified two *Bacillus* strains (*B. cereus* NA D7 and *Bacillus* sp. MR D17) from the rhizosphere of mustard under water deficit conditions. The strains were osmotolerant and promoted plant growth both under optimal conditions and under osmotic stress. Inoculation with these strains enhanced seed germination and seedling fresh weight in osmotically stressed mustard. Both strains are recommended as inoculants for the mitigation of osmotic stress in plants growing in drought-affected regions (Bandeppa et al. 2018).

One cause for the disruption of physiological processes under extreme environmental factors is the intense generation of reactive oxygen species (ROS) such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-), which can lead to oxidative stress in plants (Lipiec et al. 2013). An increased content of MDA, a product of lipid peroxidation in the plant cell membranes, may be an indicator of ROS. In our experiments, all plants experienced oxidative stress (Fig. 2a), as judged by the leaf content of MDA. However, the MDA content in the bacterized plants decreased much faster than in the control, and on day 7, it approached that in the plants grown under optimal conditions (Fig. 2b).

A universal indicator of the functioning of the plant protection system against oxidative stress is the content of proline, which acts as an osmotic agent and as a radical acceptor able to protect cells from ROS (Meise et al. 2018). In this study, the concentration of proline increased in both inoculated and noninoculated stressed plants. These results are consistent with those of other authors (Sziderics

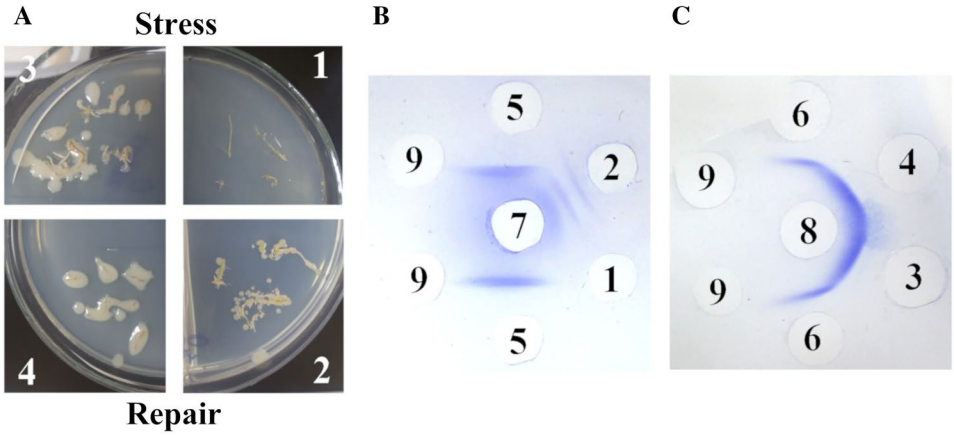

Fig. 5 Detection of *A. brasilense* Sp245 and *O. cytisi* IPA7.2 in the microbiological test (**a**) and in the immunochemical assay (**b**, **c**). **a** Root segments from 22-day-old (7 days of stress) and 29-day-old potato microplants inoculated with *A. brasilense* Sp245 (1, 2) and *O. cytisi* IPA7.2 (3, 4); **b** Outer membrane preparations of *A. brasilense* Sp245 that had grown around the corresponding root segments and under stress (1) and repair (2); **c** Outer membrane preparations of *O. cytisi* IPA7.2 that had grown around the corresponding root segments under stress (3) and repair (4); outer membrane preparation of *A. brasilense* Sp245 cells (5); outer membrane preparation of *O. cytisi* IPA7.2 (6) (positive controls); antibodies to *A. brasilense* Sp245 (7); antibodies to *O. cytisi* IPA7.2 (8); phosphate-buffered saline (9)

et al. 2007; Chakraborty et al. 2013). This suggests that in the bacterized plants, along with proline, other antioxidant protection systems experienced changes in their activity and content. These included both enzymatic (catalase, superoxide dismutase) and nonenzymatic (cysteine, glutathione, ascorbic acid) components that prevent the accumulation of ROS and reduce oxidative damage during stress (Miller et al. 2010). Changes in the activity of the antioxidant enzymes in stressed plants under the effect of growth-promoting rhizobacteria have been shown, in particular, by Naseem and Bano (2014) and Gusain et al. (2015).

Water stress is manifested in many disturbances of the physiological processes in plants, especially in the disruption of photosynthesis. The decrease in the content of green pigments during drought can be explained by the inhibition of chlorophyll synthesis along with the activation of its degradation owing to the accumulation of ROS (Chaves et al. 2009). We found that the leaf content of Chl *a*, an indicator of the efficiency of photosynthesis under stress, decreased sharply in the noninoculated plants and in the plants inoculated with *A. brasilense* Sp245, a bacterium with greater sensitivity to stress. However, the more resistant bacterium *O. cytisi* IPA7.2 helped to preserve the constitutive level of chlorophyll during stress and repair (Fig. 3a, b). It should be noted that under optimal conditions, inoculation with *A. brasilense* Sp245 contributed to a higher leaf chlorophyll content, as compared to inoculation with *O. cytisi* IPA7.2.

The biochemical variables were reflected in changes in the morphological–physiological characteristics of the plants. Bacterization helped to soften the stress on the microplants, resulting in significantly higher values of shoot length, leaf weight, and root number, as compared with the noninoculated plants during stress and repair (Tables 1 and 2).

Conclusion

Under osmotic stress, the interaction in vitro of the plant-growth-promoting rhizobacteria *A. brasilense* Sp245 and *O. cytizi* IPA7.2 with potato microplants protected the plants. When inoculated under optimal conditions, the bacteria increased the physiological and biochemical characteristics of the microplants. Bacterization decreased the leaf content of MDA and increased the plants' physiological and morphological variables, ultimately helping the plants to recover better after stress. Under stress and repair, *O. cytisi* IPA7.2, isolated from the rhizosphere of potato grown in the arid Volga region (Burygin et al. 2017), protected the plants better than did *A. brasilense* Sp245. Specifically, root weight increased with statistical significance during repair, as compared with the noninoculated plants, while Chl *a* content remained at the level found in the nonstressed controls.

In summary, *A. brasilense* Sp245 and *O. cytisi* IPA7.2 not only facilitated the growth of the plants but also improved their resistance to osmotic stress. On the basis of the obtained results, we recommend the use of these bacteria as components of biofertilizers. *A. brasilense* Sp245 has favorable prospects for use in temperate latitudes, whereas *O. cytisi* IPA7.2 can be successfully used in saline and drought-stressed environments. It would also be advisable to make composite biopreparations by using both strains tested here after they are checked for compatibility in vitro and after a possible synergistic effect on plants is elucidated.

Further work should be directed to searching for and identifying other osmotolerant potato rhizobacteria and to making clear the physiological and biochemical mechanisms of bacteria-mediated stress tolerance in plants. Specific points of interest are how the phytohormone status is changed and how antioxidant enzymes are induced in inoculated plants subjected to drought stress.

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