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ORIGINAL PAPER

# Bacteria able to control foot and root rot and to promote growth of cucumber in salinated soils

**Dilfuza Egamberdieva · Zulfiya Kucharova · Kakhramon Davranov · Gabriele Berg · Natasha Makarova · Tatyana Azarova · Vladimir Chebotar · Igor Tikhonovich · Faina Kamilova · Shamil Z. Validov · Ben Lugtenberg**

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**Abstract** The aim of the present work was to test known bacterial plant growth-promoting strains for their ability to promote cucumber plant growth in salinated soil and to improve cucumber fruit yield by protecting these plants against soil-borne pathogens. Fifty-two plant-beneficial bacterial strains were evaluated for their ability to protect plants against cucumber foot and root rot after bacterization of the seeds and infestation of salinated soil with the isolated *Fusarium solani* pathogen. Based on the results of initial screenings, five efficient strains were selected, namely *Serratia plymuthica* RR-2-5-10, *Stenotrophomonas rhizophila* e-p10, *Pseudomonas fluorescens* SPB2145, *Pseudomonas extremorientalis* TSAU20, and *P. fluorescens* PCL1751. All five strains are salt tolerant since they grow well in a medium to which 3% NaCl was added. Infestation of the soil with *F. solani* resulted in an increase of the percentage of diseased plants from 17 to 54. Priming of seedlings with the five selected bacterial strains reduced this proportion to as low as 10%. In addition, in the absence of an added pathogen, all five strains showed a significant stimulatory effect on cucumber plant growth, increasing the dry weight of whole cucumber plants up to 62% in comparison to the non-bacterized control. The strains also increased cucumber fruit yield in greenhouse varying from 9% to 32%. We conclude that seed priming with the selected microbes is a very promising approach for improving horticulture in salinated soils. Moreover, allochthonous strains isolated from non-salinated soil, from a moderate or even cold climate, and from other plants than cucumber, functioned as well as autochthonous strains as cucumber-beneficial bacteria in salinated Uzbek soils. These results show that these plant-beneficial strains are robust and they

D. Egamberdieva · Z. Kucharova · K. Davranov
Faculty of Biology and Soil Sciences,
National University of Uzbekistan,
100174 Tashkent, Uzbekistan

D. Egamberdieva · F. Kamilova · S. Z. Validov · B. Lugtenberg
Institute of Biology, Sylvius Laboratory, Leiden University,
Leiden, The Netherlands

G. Berg
Institute of Environmental Biotechnology,
Graz University of Technology,
8010 Graz, Austria

N. Makarova · T. Azarova · I. Tikhonovich
All-Russian Research Institute for Agricultural Microbiology (ARRIAM),
Pushkin,
Saint Petersburg, Russia

V. Chebotar
Bisolvi Inter,
Podbelsky Shosse, 3, Pushkin 8,
196608 Saint Petersburg, Russia

F. Kamilova
Koppert Biological Systems,
Veilingweg 14, P.O. Box 155 2650 AD Berkel en Rodenrijs,
The Netherlands

S. Z. Validov
Federal State Institution "Federal Centre of Toxicology Radiation and Biological Security",
Nauchnyj gorodok 2, 420075 Kazan, Tatarstan, Russia

D. Egamberdieva (✉)
Department of Biotechnology and Microbiology,
Faculty of Biology, National University of Uzbekistan,
Vuzgorodok,
100174 Tashkent, Uzbekistan
e-mail: egamberdieva@yahoo.com

strongly suggest they can also be used successfully in case the climate gets warmer and the soils will become more salinated. Finally, the mechanisms by which they may exert their plant-beneficial action are discussed.

**Keywords** Biological control · Cucumber · Cucumber foot and root rot · *Fusarium solani* · Plant growth promotion · Rhizobacteria

## Introduction

Soil salinization is reducing the area that can be used for agriculture by 1–2% every year, hitting hardest in the arid and semi-arid regions (FAO 2002). Uzbekistan, located in Central Asia, is an example of a country in which soil salinity is a major concern in that it results in degradation of agricultural land (Shirokova et al. 2000; Egamberdieva et al. 2008). In this country, most crops are cultivated on agricultural land in which the soil salinity level varies from 0.5% to 1%. Crop plants do not grow if the salt level is 2% or higher. As a result of soil salinization, plants are under saline or water unbalance stress and become more vulnerable to diseases, often caused by pathogenic fungi.

Cucumber is an important vegetable in many countries. Its production worldwide in 2007 was 4.46 million tons, of which Uzbekistan produced 195,300 tons (FAOstat 2007). Cucumber production in Uzbekistan is limited by soil salinization and disease caused by soil-borne pathogens such as *Fusarium*, *Pythium*, *Rhizoctonia*, and *Verticillium* (Zitter et al. 1996; Roberts et al. 2005).

One of the possible measures to improve crop health is to use salt-tolerant bacterial inoculants which can control diseases (biological control agents) and/or promote plant growth (plant growth-promoting rhizobacteria) (Thomashow and Weller 1996; Lugtenberg and Kamilova 2004; Mayak et al. 2004; Haas and Defago 2005; Egamberdieva et al. 2008; Lugtenberg and Kamilova 2009). Indeed, Egamberdieva et al. (2008) were able to isolate salt-tolerant rhizobacteria with high rhizosphere competence from wheat grown in salinated Uzbek soils. However, many of these bacteria appeared to be potential or facultative human and/or plant pathogens (Egamberdieva et al. 2008), which cannot be applied in the field (Berg et al. 2005a).

The aims of our work were (a) to identify the major cucumber pathogen present in salinated Uzbek soil, (b) to select, from 52 established beneficial strains from our instituted, the most suitable ones for the reduction of cucumber foot and root rot (CFRR) and for plant growth stimulation, (c) to test these strains in a commercial greenhouse for improvement of fruit yield, and (d) to evaluate bacterial traits which could be responsible for the mechanisms of beneficial action.

## Materials and methods

### Microorganisms

*Serratia plymuthica* strain RR2-5-10 (Berg et al. 2005b) and *Serratia rhizophila* e-p10 (Wolf et al. 2002) are from the culture collection of Graz University of Technology, Graz, Austria. The strains were isolated from the rhizosphere of oilseed rape growing in weakly loamy sand near Rostock (Germany). Strain *Pseudomonas fluorescens* SPB2145, which was isolated from the roots of a diploid (wild-type) wheat plant growing in dernopodzolic soil in North Western Russia (Kravchenko et al. 2002), is from the Institute of Agricultural Microbiology, ARRIAM, Saint-Petersburg-Pushkin, Russia. *P. fluorescens* biocontrol strain PCL1751, isolated from the rhizosphere of a tomato plant grown in the commercial greenhouse in Uzbekistan (Kamilova et al. 2005), is a strain which controls tomato foot and root rot through the mechanism "competition for nutrients and niches". It is from the collection of the Institute of Biology, Leiden University, The Netherlands. Strain *Pseudomonas extremorientalis* TSAU20 is from the culture collection of the Department of Microbiology and Biotechnology, National University of Uzbekistan, Tashkent, Uzbekistan (Egamberdieva and Kucharova 2009). This strain was isolated from the rhizosphere of wheat grown in salinated Uzbek soil after using the enrichment procedure for the isolation of enhanced root tip colonizers described by Kamilova et al. (2005).

The fungal pathogens *Fusarium oxysporum* f. sp. *radicis–lycopersici* (Forl), *Fusarium culmorum*, *Gaeumannomyces graminis* pv. *tritici* (Ggt), *Alternaria alternata*, *Botrytis cinerea*, and the oomycete *Pythium ultimum* are from Leiden University, Institute of Biology, The Netherlands. *F. oxysporum* f. sp. *radicis–cucumerinum* (Forc) V03-2g was isolated from diseased cucumber plants at ARRIAM, Saint Petersburg-Pushkin, Russia.

### Isolation, purification, and identification of Uzbek pathogenic fungi

*Fusarium solani* was isolated in the present study from diseased cucumber plants grown in salinated Uzbek soil. These plants showed typical *Fusarium* foot and root rot symptoms. Six cucumber foot and root rot pathogens were isolated from different plants. To this end, small pieces of tissue from the diseased plants were plated on potato dextrose agar (PDA) and incubated at 28°C in the dark for 5 days. A single microconidial culture was prepared from each isolate. Pathogenicity of these *F. solani* isolates was tested by sowing cucumber seeds in soil mixed with spores of the pathogen ($3.0 \times 10^7$ spores/kg soil). After 5 weeks, a piece of diseased root of a sick plant was removed and

plated on a PDA plate followed by incubation for 5 days to isolate the pathogen. The procedure of diseasing a plant followed by spore isolation was repeated twice. All six isolates were morphologically indistinguishable and caused indistinguishable disease symptoms.

Prior to identification, fungi were grown on sterile filter paper placed on PDA agar. The filter paper containing the fungal hyphae was collected and ground in liquid nitrogen. DNA was isolated from pulverized fungal biomass using the Nucleon Phytopure kit (Amersham Biosciences GmbH, Freiburg, Germany). For the identification of the fungal isolates, the mtSSU rDNA sequences of two strains were analyzed. Their fragments of mtSSU rDNA were amplified using MS1 and MS2 primers (Zeng et al. 2003) and sequenced by ServiceXS (Leiden, The Netherlands). The sequences of the fragments were compared with those in GenBank using the BLAST system. The sequences of the two analyzed strains showed 99% similarity with the mtSSU rDNA sequence from *F. solani* f. sp. *glycines* isolate 1-potato (GenBank accession no. AF125026). Therefore, the isolates can be referred to as *F. solani* on the basis of their mtSSU rDNA fragment comparison. One of these *F. solani* isolates was used for further studies.

## Salt tolerance

In order to determine the salt tolerance of the bacterial strains, they were cultured in KB medium supplemented with 1–4% NaCl (w/v). The growth rate was determined spectrophotometrically at an optical density of 600 nm at regular time intervals for 27 h.

## Bacterial control of CFRR in pots

Approximately one third of a 7-day-old PDA Petri dish culture of *F. solani* was homogenized and used to inoculate 200 ml of Chapek–Dox medium in a 1-l Erlenmeyer flask. After growth for 3 days at 28°C under aeration (110 rpm), the fungal material was poured over sterile glass wool to remove the mycelium and the filtrate, containing the spores, was adjusted to a concentration of $5 \times 10^6$ spores/ml. For soil infestation, spores were mixed thoroughly with salinated soil to $3.0 \times 10^7$ spores/kg soil. The cucumber seeds of cultivar Simbal (Cebeco Seeds, The Netherlands) were sterilized by immersion in 70% ethanol for 5 min and subsequently in 0.1% $HgCl_2$ for 1 min, washed several times with sterile water, and allowed to germinate for 4 days at room temperature. Subsequently, they were coated with bacteria by soaking them in a suspension of $1 \times 10^8$ CFU/ml bacteria in sterile PBS buffer (Leeman et al. 1995), whereas control seeds were soaked in sterile PBS buffer, both for 15 min. Seeds were dried in a sterile air stream.

The soil used for pot experiments was selected from a deep tillage (0–40 cm) irrigated agricultural field affected by salinity from the Sayhunobod district (41°00′N, 64°00′E), Syr-Darya Province, in the north-east of Uzbekistan. The field has an EC value of 659 mS $m^{-1}$ soil. Soils with an EC greater than 400 mS $m^{-1}$ soil are considered saline. The soil surface horizon is calcareous saline, and the deeper levels are mild alkaline in nature. The soil contains 43 ± 9 g sand/kg, 708 ± 12 g silt/kg, and 25 ± 13 g clay/kg. A high concentration of Ca, K, and Na associated with $CO_3$ and Cl reflects a dominance of carbonate- and chloride-associated salts. The salts that moved towards the surface evidently have higher Na, $CO_3$, and Cl contents, thereby increasing the salinity of the soil. The organic matter content of the soil is 0.694%; total C, 2.506%; total N, 0.091%; Ca, 63.5 g/kg; Mg, 20.7 g/kg; K, 6.2 g/kg; P, 1.2 g/kg; Cl, 0.1 g/kg; Na, 0.7 g/kg, and the pH is 8.0 (Egamberdiyeva et al. 2007).

One seed was sown per plastic pot (9 cm diameter; 15 cm deep), each containing 300 g of saline soil, at a depth of approximately 1.5 cm. Each treatment contained four groups of 12 plants. The plants were grown under open natural conditions at 21–24°C and were watered when necessary. The number of diseased plants was determined when 50–70% of the plants in the control without bacteria were diseased, usually 4 weeks after sowing. Plants were removed from the soil, washed, and the plant roots were examined for foot and root rot symptoms as indicated by browning and lesions. Roots without any disease symptoms were classified as healthy.

## Plant growth promotion in pots

The effect of the bacterial strains on growth of cucumber was measured in plastic pots containing 300 g of the salinated soil mentioned above. The cucumber seeds were sterilized, allowed to germinate, and coated as described previously. The inoculation treatments were set up in a randomized design with 10 replications. The cucumber plants were grown under open natural conditions and temperatures ranged between 28°C and 32°C during the day and between 12°C and 14°C at night. After 4 weeks of growth, the dry weight of the whole plants was determined.

## Plant growth promotion and fruit yield in the greenhouse

The salinated soil of the greenhouse in the province of Tashkent that was used in 2007 has an EC value of 560 mS $m^{-1}$ soil and the soil is a calcareous serozem with 2.4% organic matter, N 0.1%, P 1.34%, K 7.1%. The pH is 7.8. To investigate the effects of seed inoculation with the five selected bacterial strains on the growth of the plants and on the yield of cucumber fruit, experiments were conducted in both 2007 and 2008. The soil used in 2008 was similar to the one used in 2007 and had an EC value of 520 mS $m^{-1}$ soil.

For cultivation of the bacteria, 750-ml flasks containing 100 ml of KB medium for *Pseudomonas*, and corn-molasses medium for *Stenotrophomonas* and *Serratia*, were inoculated with 5 ml of a bacterial suspension and grown in a shaker (220 rpm) at 28°C for 48 h. These flasks were used for inoculation of 5-l bottles, each containing 1 l of growth medium. After cultivation in a shaker (220 rpm) at 28°C for 48 h, the cell suspensions were aseptically transferred into sterile 10-l canisters and stored at +5°C.

The cucumber seeds were sterilized, allowed to germinate, and coated as described previously. Coated and uncoated seeds were sown in pots (one plant per pot), which contained a mixture of soil and biohumus (2:1, v/v). Seedlings were first kept in a small greenhouse under semi-controlled environmental conditions (temperature fluctuating between 15 and 21°C). When seedlings had reached the two- to four-leaf stage, they were transplanted to a non-heated greenhouse in six rows in each experimental plot (3 m × 2.5 m). Intra-row spacing was 50 cm and rows were 60 cm apart. There were four replicate plots per treatment (total 12 treatments), and the experiment was set up as a randomized complete block design. Weeds were removed by hand and plots were irrigated after visual inspection of plants. The plant height and total yield were determined. The first experiment was conducted from 01.04.2007 to 26.06.2007. The temperature ranged between 24 and 27°C during the day and between 14 and 17°C at night. The second experiment was conducted from 15.03.2008 to 30.05.2008, and the temperatures ranged between 22 and 24°C during the day and between 12 and 16°C at night.

## In vitro screening for traits involved in biocontrol and plant growth promotion

The bacterial isolates were tested in vitro on antagonistic activity against the fungi mentioned above using a plate bioassay with PDA or Czapek–Dox agar (both from Difco Laboratories, Detroit, MI, USA) supplemented with 1.5% NaCl. Fungal strains were grown on agar plates at 28°C for 5 days. Disks containing a fresh culture of the fungus (approximately 5 mm in diameter) were cut out of the edge of the fungal growth and placed in the center of a 9-cm-diameter Petri dish. Bacteria grown on solid LC medium (containing per liter demineralized water—tryptone, 10 g; yeast extract, 5 g; NaCl, 10 g; and agar-agar, 18 g) supplemented with NaCl to a final concentration of 1.5% were streaked on the test plates perpendicular to the fungus. Plates were incubated at 28°C for 7 days until the fungi had covered the control plates without bacteria. Antifungal activity was recorded as the width of the zone of growth inhibition between the fungus and the test bacterium.

The following tests described in this paragraph were carried out in the presence of 1.5% added NaCl. Hydrogen cyanide production was detected using cyanide indicator paper (Castric 1975). Lipase activity was detected using the Tween lipase indicator assay (Howe and Ward 1976), protease activity using 5% skimmed milk agar plates (Brown and Foster 1970), and β-glucanase activity using the glucan substrate lichenan in top agar plates (Walsh et al. 1995), and cellulase activity was tested using the substrate carboxymethylcellulose in top agar plates (Hankin and Anognostakis 1977).

To measure auxin production, bacteria were incubated in medium containing (in grams per liter): $KH_2PO_4$, 0.4; $K_2HPO_4$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.2; $(NH_4)_2SO_4$, 1.5; NaCl, 0.1; malic acid, 2.5; yeast extract, 0.5; and L-tryptophane, 0.01. The final pH was 6.8–7.0 (Kravchenko et al. 1994). In case a high salt concentration was needed, NaCl was added to a final concentration of 30 g/l. Bacteria were grown in 100-ml flasks containing 10 ml of medium for 4 days at 28°C on a rotary shaker at 220 rpm. After centrifugation (13,000×*g*) of the cells, supernatant fluids were extracted twice with 20 ml ethyl acetate acidified with HCl to pH 3.0. The resulting extracts were evaporated to dryness under vacuum at 45°C, dissolved in 0.5 ml of methanol, and passed through membrane filters (pore size 0.45 μm). Quantitative analysis of indolyl-3-acetic acid (IAA) were carried out using a JASCO LC-900 series HPLC system (Jasco International Co., Ltd.) equipped with a Rheodyne (Cotati, CA, USA) Model 7125 20-μl valve loop injector, a PU-980 pump, a DG-980-50 degassing module, a LG-980-02 ternary gradient unit, FP-920 FL (fluorescent) detector, and a computer with BORVIN (JMBS Development, Le Fontanil, France) chromatography software connected to the HPLC system via JASCO LC-Net. Indole metabolites were separated with a Waters C18 column (size 250 × 4.6 mm) using an isocratic gradient of water–acetonitrile–acetic acid (83:17:0.2, v/v/v). The elution rate was 0.9 ml/min and the column temperature 33°C. The elution pattern was recorded at $E_x$ = 280 nm and $E_m$ = 350 nm wavelengths. The presence of IAA in the eluents of the analyzed samples was judged by comparison of their retention times with those of commercially available standards. The concentration was judged from the peak area using a Fp-920 detector.

In order to measure growth on 1-aminocyclopropane-carboxylate (ACC) as the sole N source, bacterial isolates were incubated in BM minimal medium (Lugtenberg et al. 1999) supplemented with 1.5% NaCl and also supplemented with 3.0 mM of either ACC (Sigma Chemical Co., St. Louis, MO, USA) (to test ACC utilization) or of $(NH_4)_2SO_4$ (positive control) as the sole N source or without added N source (negative control).

## Statistical procedures

Data were tested for statistical significance using the analysis of variance package included in Microsoft Excel

98. Comparisons were done using Student's *t* test. Mean comparisons were conducted using a least significant difference (LSD) test ($P = 0.05$).

## Results

Fifty-two bacterial strains, previously isolated in our institutes from various plants and shown to be able to control several plant diseases caused by fungi in non-salinated soils, were evaluated twice for their ability to promote plant growth and to control CFRR in preliminary experiments in salinated soil. Even without addition of a pathogen, a substantial fraction of the plants were diseased (results not shown). Based on the results of the experiments, five efficient strains, *S. plymuthica* RR2-5-10, *S. rhizophila* e-p10, *P. fluorescens* SPB2145, *P. extremorientalis* TSAU20, and *P. fluorescens* PCL1751, were selected for more detailed investigations.

To test their salt tolerance, these bacterial strains were grown on KB medium supplemented with final concentrations of 0 to 4% NaCl. From the resulting growth curves, it was concluded that all strains grow well in the presence of up to 3% NaCl.

The five selected strains were screened for their ability to suppress CFRR caused by the identified *F. solani* isolate (Table 1). Seventeen percent of the plants which had grown in soil to which no *F. solani* spores had been added were diseased, whereas in the presence of the pathogenic fungus 54% of the plants had disease symptoms (Table 1). All selected bacterial isolates, with the exception of *S. plymuthica* strain RR2510, showed statistically significant disease reduction in comparison to the *Fusarium*-infected control plants (Table 1). Strain *S. rhizophila* e-p10 performed best.

**Table 1** Control of cucumber foot and root rot in salinated soil by selected bacterial isolates

| Treatments[a] | *F. solani* | Diseased plants (% ± SD) |
|---|---|---|
| Positive control | − | 17 ± 6.8 |
| Negative control | + | 54 ± 10.7 |
| *S. plymuthica* RR2-5-10 | + | 42 ± 6.8 |
| *S. rhizophila* e-p10 | + | 10 ± 7.9* |
| *P. fluorescens* SPB2145 | + | 29 ± 8.3* |
| *P. extremorientalis* TSAU20 | + | 19 ± 7.9* |
| *P. fluorescens* PCL1751 | + | 21 ± 10.7* |

*$P < 0.05$, significantly different from the negative control

[a] Bacteria were coated on pre-germinated cucumber seeds, and plants were grown under open natural conditions in pots containing salinated soil (EC value 659 mS $m^{-1}$) infested with *F. solani* spores ($3.0 \times 10^7$ spores/kg), except for the positive control in which no spores were added to the soil

The five selected bacterial strains were screened for their cucumber growth stimulating abilities using pots containing salinated soil. All bacterial strains increased the dry weight of whole cucumber plants in a statistically significant way in comparison with the untreated control (Table 2). The best performer was strain *S. plymuthica* RR2-5-10, which increased the dry weight by 62%.

To test the effects of bacterization on plant growth in salinated soil under practical horticultural conditions, experiments were conducted in a commercial greenhouse in the years 2007 and 2008, using the five selected strains. Also under these conditions, which are representative for commercial cucumber production in Uzbekistan, all five tested bacterial strains caused statistically significant increases in plant height in both years (Table 3). More importantly, in 2007 the bacterial treatments increased the yield of cucumber fruit up to 32% compared to the uninoculated control plants. In this year, only the effect of *S. rhizophila* strain e-p10 was not statistically significant. In 2008, the cucumber fruit yields from bacterized plants were significantly larger for all five tested strains than from control plants (Table 3). Over the 2 years, strain *P. extremorientalis* TSAU20 was the best performer.

In order to get a possible clue about the mechanism(s) behind biocontrol (Table 1), bacterial plant growth stimulation (Tables 2 and 3), and fruit yield (Table 3), traits possibly involved in biocontrol and plant growth stimulation were tested. Strains *S. plymuthica* RR2-5-10, *S. rhizophila* e-p10, and *P. fluorescens* strain SPB2145 showed in vitro antagonistic activity against the *F. solani* cucumber pathogen as well as against at least five of the seven other plant pathogenic fungi (Table 4). Other biocontrol traits such as production of HCN and protease as well as the plant growth promotion traits production of auxin and utilization of ACC were also present (Table 4). Strain *P. fluorescens* SPB2145 produced a very high level of auxin (Table 4). Strain *P. extremorientalis* TSAU20

**Table 2** Effect of selected plant growth-promoting bacteria on dry weight of whole cucumber plants growing in salinated soil (EC level 659 mS $m^{-1}$)

| Bacterial strain | Dry weight[a] | Percentage[b] |
|---|---|---|
| None | 0.224 | 100 |
| *S. plymuthica* RR2-5-10 | 0.364 | 162* |
| *S. rhizophila* e-p10 | 0.300 | 133* |
| *P. fluorescens* SPB2145 | 0.320 | 141* |
| *P. extremorientalis* TSAU20 | 0.290 | 129* |
| *P. fluorescens* PCL1751 | 0.325 | 145* |

*$P < 0.05$, significantly different from the control

[a] Expressed as grams per plant

[b] Expressed relative to the control value of 100%

**Table 3** Effect of bacterial strains on cucumber (cv. Simbal) height and fruit yield in two different greenhouse experiments

| Treatment | 2007[a] | | | | 2008[b] | | | |
|---|---|---|---|---|---|---|---|---|
| | Plant height (cm) | % | Fruit yield (kg/m$^2$) | % | Plant height (cm) | % | Fruit yield (kg/m$^2$) | % |
| None | 169 ± 1.8 | 100 | 8.9 ± 0.4 | 100 | 174 ± 7.4 | 100 | 10.3 ± 0.1 | 100 |
| *S. plymuthica* RR2-5-10 | 176 ± 3.1* | 104 | 10.0 ± 0.7* | 112 | 193 ± 7.3* | 111 | 12.8 ± 0.3* | 124 |
| *S. rhizophila* e-p10 | 178 ± 1.4* | 105 | 9.7 ± 0.6 | 109 | 196 ± 7.3* | 113 | 12.4 ± 0.13* | 120 |
| *P. fluorescens* SPB2145 | 177 ± 1.4* | 105 | 10.1 ± 0.5* | 113 | 183 ± 6.3* | 105 | 11.4 ± 0.4* | 111 |
| *P. extremorientalis* TSAU20 | 179 ± 2.3* | 106 | 10.0 ± 0.4* | 112 | 197 ± 6.4* | 113 | 13.6 ± 0.2* | 132 |
| *P. fluorescens* PCL1751 | 175 ± 3.8* | 104 | 10.2 ± 0.6* | 115 | 200 ± 4.6* | 115 | 12.0 ± 0.6* | 117 |

*$P < 0.05$, significantly different from the control

[a] Cucumber seeds were sown on 1.04.2007 and fruits were harvested on 26.06.2007; the temperature range was: day 24–27°C, night 14–17°C. The EC value of the soil was 560 mS m$^{-1}$

[b] Cucumber seeds were sown on 15.03.2008 and the fruits were harvested on 30.05.2008; the temperature range was: day 22–24°C, night 12–16°C. The EC value of the soil was 520 mS m$^{-1}$

inhibits in vitro growth of only *P. ultimum*, whereas strain *P. fluorescens* PCL1751 did not demonstrate antagonistic activity against any of the tested pathogens (Table 4).

## Discussion

Salt stress not only causes a decline in the metabolic activity of plant cells but also results in an increased susceptibility of the plant towards phytopathogens (Kurth et al. 1986; Werner and Finkelstein 1995). Indeed, in this study we have shown (Table 1) that 17% of the cucumber plants grown in salinated Uzbek soil show symptoms of foot and root rot caused by the identified *F. solani* (Mart.) Sacc. This result is consistent with our previous finding that a high percentage of wheat rhizosphere-colonizing bacteria from Uzbek salinated soil are potentially pathogenic (Egamberdieva et al. 2008).

We subsequently tested the suggestion we did in a previous paper (Egamberdieva et al. 2008), namely that the effect of root pathogens can be decreased by out-competing them by priming seeds with beneficial root-colonizing bacteria. All five selected strains showed statistically significant repression of CFRR caused by the isolated pathogen (Table 1). In the absence of an added pathogen, all five strains significantly increased the plant dry weight (Table 2). This effect is not necessary the result of plant growth stimulation by the bacteria, it may also be caused by their biocontrol properties since the natural level of pathogens is high. The five strains significantly increased cucumber fruit yield. To our knowledge, this is the first report illustrating an increase in fruit yield of a major vegetable crop plant growing in salinated soil caused by priming of the seeds.

We have reported previously that the rhizosphere of wheat plants growing in irrigated salinated Uzbek soil is rich in potential human pathogenic bacteria (Egamberdieva et al. 2008). In the present work, we found that *F. solani* is an important plant pathogen and also an opportunistic pathogen for humans (Zhang et al. 2006). These observations can explain that workers in Uzbek agriculture and horticulture in salinated regions of Uzbekistan suffer from diseases (Smith 1991; Crighton et al. 2003). As a remedy, we suggest a way of rhizosphere management which includes the application of enhanced colonizing strains, such as at least some of the five described here. This is likely to decrease the level of pathogens in plant and eventually in soil as well. This will not only result in higher plant yield but also in a healthier environment for farm workers and fruit users.

A surprising result was that, by far, most of the 52 tested bacterial strains could grow with a normal growth rate in the presence of up to 3% added NaCl. This applied for all five selected bacteria of which only two were isolated from plants grown in salinated soil. This result shows that for the application of bacteria in salinated soils there is no strict need to isolate these bacteria from plants grown in salinated soil. These results are consistent with observations showing that the rhizosphere is characterized by changing osmotic conditions, and that its microbial inhabitants can adapt to increased osmolarity, i.e., by producing osmoprotective substances (Miller and Wood 1996). Indeed, the *Stenotrophomonas* strain is known for the production of osmoprotective substances in extraordinarily high amounts, which can contribute to its rhizosphere competence under salinated conditions (Berg et al. 2010). All together, the results show that increased salt levels do not negatively affect the ability of the five selected strains to decrease disease levels. This also implies that salt has no severe influence on their plant growth stimulation and biocontrol traits.

The observation that salinity level of the site of isolation of the beneficial bacterium is not crucial led us to the question whether temperature at which plants are grown and the nature of the host plant itself are important for beneficial effects.

**Table 4** Traits possibly involved in biocontrol and/or plant growth-promoting activity of bacterial strains

| Strain | Antagonistic activity against | | | | | | | | Production of exoenzymes | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *F. solani* | *Forl* | *V03-2g* | *B. cinerea* | *P. ultimum* | *F. culmorum* | *Ggt* | *A. alternata* | HCN | Lipase | Protease | Cellulase | Glucanase | Production of IAA | Growth on ACC |
| *S. plymuthica* RR2-5-10 | + | + | – | + | + | + | + | + | – | – | + | – | – | + | – |
| *S. rhizophila* e-p10 | + | + | + | + | + | + | + | + | – | – | – | – | – | + | + |
| *P. fluorescens* SPB 2145 | + | + | + | + | + | + | – | – | + | – | – | – | – | ++ | , |
| *P. extremorientalis* TSAU20 | – | – | – | – | + | – | – | – | – | – | + | – | – | – | – |
| *P. fluorescens* PCL1751 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – |

All tests were conducted in the presence of 1.5% added NaCl

Both the strain that performed best with respect to fruit yield increase over the 2 years (Table 4), namely *P. extremorientalis* TSAU20 as well as the good performer *P. fluorescens* PCL1751, were isolated from Uzbek soils which become very hot in summer. However, since strains *S. plymuthica* strain RR2-5-10, *S. rhizophila* e-p10, and *P. fluorescens* SPB2145, all isolated from regions with a moderate to cold climate, performed well in Uzbekistan, the temperature of the region of origin is apparently not very crucial for performing well at higher temperatures.

*S. plymuthica* strain RR2-5-10 and *S. rhizophila* e-p10 were isolated from the rhizosphere of oilseed rape, strains *P. fluorescens* SPB2145 and *P. extremorientalis* TSAU20 from the rhizosphere of wheat, and *P. fluorescens* strain PCL1751 was isolated from the rhizosphere of tomato. Since all these five strains performed well on cucumber, the plant of origin is apparently not crucial for good performance in plant growth promotion. Taken together, these results show that these beneficial bacterial strains have a remarkable flexibility with respect to performing under various environmental conditions. We therefore can be rather confident that a climate change, which will be accompanied by increases in temperature and salination levels, will not seriously affect the plant-beneficial ability of many bacterial strains.

We tried to evaluate on which mechanisms the observed cucumber fruit yield increase can be based. Mechanisms (Bloemberg and Lugtenberg 2001, 2004; Kravchenko et al. 2003, 2004; Haas and Defago 2005; Lugtenberg and Kamilova 2009; Raaijmakers et al. 2009) by which bacteria are able to prevent damage caused by plant pathogens and promote plant growth include antagonism (Thomashow and Weller 1996), induction of systemic resistance (Van Loon 2007), competition for nutrients and niches (Kamilova et al. 2005), mobilization of nutrients (Lifshitz et al. 1987; Lugtenberg et al. 2001), and production of phytohormones (Costacurta and Vanderleyden 1995; Spaepen et al. 2009).

It is known that unfavorable environmental factors such as salinity and drought lead to sharp changes in the balance of phytohormones, associated with a decline in the level of growth-activating hormones such as IAA (Zholkevich and Pustovoytova 1993; Jackson 1997; Sakhabutdinova et al. 2003). We propose that bacteria which are able to produce IAA under saline conditions may supply additional phytohormone to the plant, thus may help stimulate root growth and reverse the growth inhibiting effect of salt stress to a certain extent in both shoot and root growth. Furthermore, plant stress can be reduced by ACC deaminase-producing bacteria which can lower the level of the plant stress hormone ethylene (Okon and Itzigsohn 1995; Glick et al. 1998, 2007; Okon et al. 1998; Dobbelaere et al. 1999; Belimov et al. 2009).

The bacterial strains *S. plymuthica* RR2510, *S. rhizophila* e-p10, and *P. fluorescens* SPB 2145, which produce

auxin, were antagonistic against *F. solani* and at least five of the seven other tested pathogens. Therefore, it is likely that these strains are also able to reduce diseases caused by other pathogens. Since efficient root colonization is the delivery system for antibiotics around the root in case strains control a disease through antibiosis (Chin-A-Woeng et al. 2000), these strains are likely to be also good root colonizers (Kravchenko et al. 2006). So, in addition to a probable role for antibiosis, competition for nutrients and niches is likely to also play a role in the beneficial effects of these three strains. In addition, HCN production may play a role in the activity of strain *P. fluorescens* SPB2145. Furthermore, ACC deaminase might contribute to the beneficial effect caused by *S. rhizophila* ep10.

*P. fluorescens* strain PCL1751 was isolated from the rhizosphere of tomato and was characterized as an enhanced tomato root colonizer which controls tomato foot and root rot through competition for nutrients and niches (Kamilova et al. 2005). Since it is negative for all tested biocontrol and plant growth-promotion traits (Table 4), it is likely that its major mechanism of biocontrol is competition for nutrients and niches. This mechanism can also explain the strain's ability to stimulate plant growth since Kamilova et al. (2006) have shown previously that the nutrients that are utilized by the pathogenic fungus Forl and by beneficial *Pseudomonas* bacteria in the rhizosphere of tomato and cucumber are very similar.

*P. extremorientalis* strain TSAU20 originates from the rhizosphere of wheat plants growing in salinated Uzbek soil. It was isolated as an enhanced wheat root colonizer by the procedure that was also used for strain PCL1751. Therefore, it is likely that competition for nutrients and niches contributes substantially to its beneficial effects shown in Tables 1, 2, and 3. It is active against the oomycete *Pythium* but not antagonistic towards any of the seven fungal pathogens tested (including *F. solani*) (Table 4), suggesting that antagonism does not play a significant role. The strain also secretes protease (Table 4). This enzyme has been implied in the control of diseases caused by nematodes (Siddiqui et al. 2005) but to our knowledge not in those caused by fungi.

The results presented here indicate that plant growth-promoting rhizobacteria (PGPRs) can have a substantial beneficial effect on crop yield in salinated soil. For the future, we suggest the following lines of research: (1) to identify major pathogens present on other crops in salinated soils, such as wheat, cotton, tomato, and sweet pepper; (2) to test the effects of the successful PGPRs as well as of new strains on yields of such crops growing in salinated soils; and (3) to test whether the level of pathogens in the rhizosphere is decreased by the application of PGPRs and, if so, whether this decrease is long lasting. In that case, it may decrease the proposed negative effects of these pathogens on the health of farm workers.

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