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ORIGINAL PAPER

Stenotrophomonas rhizophila DSM14405^T promotes plant growth probably by altering fungal communities in the rhizosphere

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Abstract *Stenotrophomonas rhizophila* DSM14405^T is of high biotechnological interest as plant growth stimulator, especially for salinated conditions. The objective of this study was to determine the effect of plant species (cotton, tomato, and sweet pepper) on colonisation and plant growth promotion of this beneficial bacterium in gnotobiotic systems and in non-sterile soil. All plant structures (leaves, stems, and roots) were densely colonised by DSM14405^T reaching up to 10^9 cells g^{-1} fresh weight; under gnotobiotic conditions the abundances were 4–5 orders of magnitude higher than in non-sterile soil. Under non-sterile conditions and ambient humidity, tomato shoots were more densely colonised than shoots of sweet pepper and cotton. *S. rhizophila* DSM14405^T was shown to grow endophytically and colonise the vicinity of root hairs of tomato. Plant growth promotion was particularly apparent in tomato. In general, the impact of plant species on colonisation and plant growth promotion was more pronounced in soil than under gnotobiotic conditions and likely due to the control of diseases and deleterious microorganisms. *S. rhizophila* DSM14405^T was shown to control diseases in sweet pepper and in cotton. Molecular profiling via single strand conformation polymorphism of internal transcribed spacers and 16S rRNA genes (PCR-single strand conformational polymorphism (SSCP)) revealed that *S. rhizophila* DSM14405^T strongly affected fungal, but not bacterial communities in the rhizosphere of tomato and sweet pepper. Major SSCP bands related to uncultured fungi and *Candida subhashii*, disappeared in tomato rhizosphere after *Stenotrophomonas* treatment. This suggests an indirect, species-specific plant growth promotion effect of *S. rhizophila* via the elimination of deleterious rhizosphere organisms.

Keywords Plant growth promoting bacteria · *Stenotrophomonas* · Rhizosphere communities · Fungal communities · Sweet pepper · Tomato · Cotton

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Introduction

With increasing efforts to develop sustainable agriculture, the use of microbial inocula for promotion of crop growth and for the biological control of plant diseases becomes ever more attractive to reduce the current dependency of agriculture on fossil fuels and environmentally harmful synthetic pesticides. A particular challenge limiting plant production in arid areas is soil salinity, increased by inappropriate irrigation techniques (Tanji and Kielen 2002; FAO 2005). Soil salinisation does not only damage crops directly, but also renders the plants more susceptible to soil-borne pathogens adapted to these conditions (Howell et al. 1994). The exploration of bacterial inocula for relief of salt stress and plant growth promotion in saline soils has just started, but first reports are promising (Mo et al. 2006; Egamberdiyeva et al. 2008; Nadeem et al. 2010).

Bacteria of the genus *Stenotrophomonas* are of increasing biotechnological interest due to their ubiquitous occurrence and versatility (Ryan et al. 2009). Their plant growth-promoting properties and their antagonistic behaviour

against soil-borne plant pathogens are well-documented (Berg et al. 1994; Dunne et al. 1998; Ryan et al. 2009), but development for commercial application of *Stenotrophomonas maltophilia*, the most intensively studied species, has been hampered by its potential as opportunistic human pathogen in immune-suppressed patients (Berg et al. 2005a; Hagemann et al. 2006). Within a broad range of isolates of environmental and clinical origin, classified at the time as *S. maltophilia*, a distinguished genomovar consisting only of environmental isolates could be separated (Minkwitz and Berg 2001), and further characterisation led to their description as a separate species, *Stenotrophomonas rhizophila* (Wolf et al. 2002). Unlike *S maltophilia*, *S. rhizophila* does not have human pathogenic traits (Ribbeck-Busch et al. 2005; Hagemann et al. 2006). The species has a lower temperature optimum than *S. maltophilia* (Wolf et al. 2002), and is therefore safe to use. The synthesis of an additional osmolyte, glucosyl glycerol, confers a greater degree of salt resistance in vitro (Hagemann et al. 2008) and makes it an ideal candidate for application in saline soil conditions. In vitro, isolates of this species produce fungal cell wall degrading enzymes, siderophores (Minkwitz and Berg 2001) and volatile antifungal compounds (Kai et al. 2007); antifungal activity against soil-borne plant pathogens has been demonstrated (Minkwitz and Berg 2001). Also, production of the plant growth hormone indol-acetic acid (IAA), and direct growth promotion of strawberry plants in vitro has been shown (Suckstorff and Berg 2003). These studies indicate the potential of *S. rhizophila* to directly promote plant growth as well as to inhibit plant pathogens. Growth of a wide variety of crops was strongly promoted in the saline soil of Uzbekistan by *S. rhizophila* strain DSM14405^T (=e-p10^T and =P69^T) (Egamberdieva et al. 2011); these promising results raise the question whether the observed effects were due to direct plant growth promotion by the strain or to the suppression of deleterious flora and soil-borne pathogens in the rhizosphere. No studies on the survival and population distribution on different plant species have been done.

The aim of this study was to assess the influence of the plant species on root colonisation and plant growth promotion of *S. rhizophila* DSM14405^T. Comparison of plant growth promotion under sterile (gnotobiotic) and non-sterile conditions should shed light on the question whether direct or indirect effects (elimination of deleterious rhizosphere flora) contributed to the plant growth promoting effect of this beneficial bacterium *in planta*. To assess the influence of the strain on native rhizosphere microbial communities, single strand conformational polymorphism (SSCP) profiles of amplified 16S rRNA genes, and internal transcribed spacers (ITS) from the rhizosphere with and without presence of *S. rhizophila* DSM14405^T were compared.

Materials and methods

Plant seeds and bacterial strains

Tomato seeds (*Solanum lycopersicum* cv. 'Avicenne', cv. 'Petto 86'), sweet pepper (*Capsicum annuum* cv. 'Zdorové'), and cotton seeds (*Gossypium* sp., non-specified cultivar) were obtained from Dilfuza Egamberdiyeva (Centre of Agroecology, Tashkent State University of Agriculture, Uzbekistan). Two sweet pepper varieties ('Californian Wonder', 'Chinese Giant') were purchased from www.bobby-seeds.com (Floveg GmbH; Rheinbach-Wormersdorf, Germany). Unless stated otherwise, the sweet pepper cv. 'Zdorovje' and the tomato cv. 'Avicenne' were used in all experiments. Seeds of cotton and sweet pepper cv. 'Californian Wonder' were partially infected with endophytic seed-borne pathogens which could not be eliminated by the seed surface sterilisation procedure described below; in sweet pepper, the pathogens could be identified as *Alternaria* sp.

The bacterial isolate *S. rhizophila* DSM14405^T was isolated from oilseed rape (Minkwitz and Berg 2001; Wolf et al. 2002) and originated from the stock culture collections of the Institute of Environmental Biotechnology, Graz University of Technology, Graz, Austria.

Surface sterilisation and pregermination of seeds

In experiments in gnotobiotic systems, tomato and sweet pepper seeds where surface sterilised according the protocol of Götz et al. (2006). For sweet pepper, the concentration of NaOCl was lowered from 12 to 2.5 %. Cotton seeds were delinted in concentrated H_2SO_4 according to Da Silva et al. (2006). After neutralising in 10 % Na_2CO_3, they were washed in water for 30 min and surface sterilised following the protocol of Gould and Magallenes-Cedeno (1998). Surface-sterilised seeds were imprinted on Luria Bertani agar (Roth GmbH+Co KG; Karlruhe, Germany) to check for sterility and then placed in Petri dishes between two sterile, moistened filter papers; the Petri dishes were sealed with Parafilm. Seeds were pre-germinated for 2–3 days at a temperature of 30°C (cotton and tomato) or 2–7 days at a temperature ~20°C (sweet pepper). The surface sterilisation procedure did not kill pathogens present inside the seeds.

Treatment of seeds and plants with *S. rhizophila* DSM14405^T

S. rhizophila DSM14405^T was grown over night in Luria Bertani broth (Roth GmbH+Co KG) or Nutrient Broth II (SIFIN; Berlin, Germany) for 22 h. Of the rifampicin, 100 μg ml^{-1} (50×stock solution in dimethyl sulfoxide) was added to the medium for the rifampicin-resistant mutants. Bacterial cultures were centrifuged (5,000×*g*), the

cells washed once in 0.85 % NaCl and finally resuspended in an equivalent volume of 0.85 % NaCl (OD_{600nm}=1.2, 200 μl suspension in 96-well microplates). In gnotobiotic test systems (plant agar), 10 μl suspension was dripped onto both the root and the hypocotyl of freshly transplanted plantlets; the suspension was applied undiluted (10^8 cells plant^{-1}) or diluted (10^4 cells plant^{-1}). Ten thousand cells per plant had been shown to be the optimal dose for plant growth promotion of strawberry in such systems (Suckstorff and Berg 2003).

When seeds were sown into soil directly after soaking, the bacterial suspension was diluted 100-fold (10^5 cells seed^{-1} in tomato and 10^7 cells seed^{-1} in cotton). For biopriming, the seeds were soaked in the undiluted bacterial suspension for 5 h, according to Müller and Berg (2008), in order to compensate for losses occurring during seed drying. Primed seeds where then dried in the laminar flow cabinet for additional 9–10 h, and stored at room temperature. Seeds for the control treatments were soaked or primed with sterile 0.85 % NaCl in exactly the same way. Populations in seeds were determined by grinding 10 seeds (or five in cotton) in 0.85 % NaCl in Whirlpack® bags (Carl Roth GmBH) and plating of serial dilutions.

Plant experiments under gnotobiotic conditions

Seedlings were grown under gnotobiotic conditions as described by Suckstorff and Berg (2003), with following modifications. Medium consisted of 6 % plant agar (Duchefa Biochemie BV, Haarlem, Netherlands) with 1/10×Gamborg salts B5 (0.31 gL^{-1}; Duchefa) in demineralised water. In tentative small-scale experiments, tap water was used instead of 1/10×Gamborg medium and in order to test salt resistance, NaCl was added at 0.8 and 1.5 %. Plants were either grown in culture tubes 'de Vit' (Duchefa) in 15 ml medium (6 cm medium height) or in 50 ml plastic centrifuge tubes in 40 ml medium (cotton). Aseptically pre-germinated seedlings were planted into the medium. Lids were removed from the 50-ml centrifuge tubes to allow light access and plant growth and they were placed in autoclaved plastic beakers (19.2 mm diameter, 21 cm height; FUERST GmbH; Hallerndorf, Germany) which were closed with a lid. Incubation was at 20−25°C at a 16/8 h day/night cycle. After 5 weeks growth time, the number of side roots (assessed as number of root tips), the root length, and number of leaves were determined in gnotobiotic systems (plant agar), as described for strawberry in Suckstorff and Berg (2003). In addition, shoot length was measured to detect a possible effect of IAA production by *S. rhizophila* DSM14405^T. If seed-borne diseases were apparent, they incidence was assessed according to following scale: 0=plant healthy, 1=plant diseased, 2=plant killed. One plant constituted one replicate in the assessments of plant growth promotion; 45–52 replicate plants per treatment were assessed. They were then grouped and pooled into three replicates consisting of ~15 plants each which were dissected into roots, stems, and leaves to determine population sizes of *S. rhizophila* DSM14405^T on plants.

Plant experiments in soil

Peat-based potting compost was used as substrate in all experiments under non-sterile conditions (Profi-Substrat, Grammoflor GmbH+Co KG 'Topf Pikier M+Ton+FE', Vechta, Germany; 250 g moist mass; gravimetric water content (GWC)=1.3). This substrate is raised bog peat, amended with clay (90 gL^{-1}) and iron (50 mg FE L^{-1}), nitrogen content is 50–300 mg N L^{-1}, phosphorus 35–130 mg P L^{-1}, potassium 60–330 mg K L^{-1}; pH ($CaCl_2$)=5.8, and salt content <1.5 g/L (manufacturers specifications). This compost was filled into transparent plastic beakers (195 mm diameter, 210 mm height; FUERST). Added to the soil were 200 ml demineralised H_2O to adjust the soil to a GWC (dry weight based) of 3.0−3.5, i.e. 75 % of the resulting substrate wet weight was soil water. Seeds were soaked in bacterial suspension (see above) or 0.85 % NaCl and sown into the compost (10 seeds per pot). Alternatively, bio-primed seeds were used. The rifampicin-resistant mutant RifR5 was employed in experiments on phytosphere colonisation. Lids of the microcosms were removed as soon as the cotton plants touched them (~2 weeks); ambient humidity was 25 %. The plants were incubated at 20−25°C at a 16 h/8 h day/night cycle for 4 weeks. Three replicates of two plants were harvested per treatment; two experiments, one with bio-primed seeds, and another one with seeds soaked in bacterial suspension, were performed. In the experiment on plant growth promotion, seeds primed with *S. rhizophila* DSM14405^T wild type were used. In order to observe more pronounced effects of treatment with *S. rhizophila* DSM14405^T, plants were grown until they had developed inflorescences. After 35 days growth, each was planted into a separate pot and grown for further 53 days, resulting in a total growth time of 87 days. Twenty replicate single pots (7 cm diameter) with one plant were set up per treatment; each plant constituted one replicate in the assessments of plant growth promotion. Plants were fertilised with liquid fertiliser (SUBSTRAL Pflanzennahrung; N/P/K 6:3:6, with trace elements Cu, Fe, Mn, Mo, Zn; Scotts Celarflor Handelsgesellschaft, Salzburg, Austria). Shoot height and number of secondary leaves, and number of shoots and number of both inflorescences and single flower buds were recorded.

Recovery of *S. rhizophila* DSM14405^T RifR5 from plants

Plants were grouped into three replicate samples. Each replicate consisted of ~15 plants in gnotobiotic experiments,

three plants (first experiment done with bioprimed seeds in soil) or two plants (second experiment done with soaked seeds in soil). Plants were dissected into fractions of roots, stems, and leaves. From root samples of plants grown in non-sterile soil, a small proportion of each replicate was set aside for rhizosphere-DNA isolation in the first experiment done with bioprimed seeds (see above). For recovery of the epiphytic plant fraction, 20 ml (or, if necessary, up to 45 ml) of 0.85 % NaCl were added to the plant sections, and samples were vortexed and then sonicated for 3 min at a strength of 100 (Elma Transsonic Digital, Schalltec GmbH, Mörfelden-Walldorf, Germany). Serial dilutions were plated on LB Agar+ 100 ppm rifampicin. The plant parts were then surface sterilised by immersion into 2.5 % NaOCl for 5 min, rinsed three times with sterile water and imprinted on Luria Bertani agar to check sterility. They were subsequently placed in Whirlpack® bags (Karlsruhe, Germany) and crushed with pestle and mortar in 5 ml 0.85 % NaCl. Serial dilutions of the extract where plated to quantify the endophytic population. Non-treated control plants were also prepared in the same way to confirm the identity of the recovered bacteria as *S. rhizophila* DSM14405^T. Although some rifampcin-resistant soil bacteria could be observed in the controls, they differed in morphology from the typical yellow colonies of *S. rhizophila* DSM14405^T which were only detectable in preparations from treated plants. The detection limit of this method was 10^3 cells per gram freshweight.

Fluorescence in situ hybridisation and confocal laser scanning microscopy

Sample preparation from gnotobiotic plants for fluorescent in situ hybridisation (FISH) was carried out as described in Daims et al. (2005). Samples were fixed in 4 % paraformaldehyde and stored in a 1:1 mixture of phosphate buffered saline and 96 % ethanol. FISH was performed according to the protocol of Cardinale et al. (2008). Samples were hybridised first with the probe Gam42a in hybridisation buffer containing 40 % formamide and an unlabelled Bet42a probe was applied as competitor to ensure specificity (Manz et al. 1992). Both probes hybridise at the positions 1027–1043 of the 23S-rRNA gene of beta-proteobacteria or gamma-proteobacteria, respectively. Samples were then hybridised with probes EUB338, EUB338II, and EUB338III, which bind to the nucleotides 338–355 of the eubacterial 16S rRNA gene (Amann et al. 1990; Daims et al. 1999); for this, hybridisation buffer containing 10 % formamide was used. Hybridisations were performed at 41°C for 120 min and followed by a washing step with appropriate washing buffer at 42°C for 15 min. Samples were visualised with a Leica TCS solid-phase extraction confocal laser scanning microscope (Leica Microsystems GmbH, Mannheim, Germany). An appropriate number of 0.8–1.0 μm depth optical slices were applied to visualise the sections of roots, shoots, and leaves (confocal stacks). Up to five scans per optical slice were averaged to improve the image resolution and to reduce noise. Three-dimensional models consisting of isosurfaces were obtained from the confocal stacks with the software Imaris 7.0 (Bitplane, Zürich, Switzerland).

Microbial fingerprints performed by single-strand conformation polymorphism of 16S rRNA and ITS genes

DNA from rhizosphere samples of tomato and sweet pepper was isolated using the BIO 101 FastDNA® spin kit according to the manufacturer's instructions (Qbiogene, Carlsbad, CA, USA). Samples were ribolysed 2×20 s at speed 6.5 and cooled on ice for at least 5 min between these three steps (Ribolyser Fast Prep™, Qbiogene). The DNA was finally eluted in 100 μl H_2O. The ITS region of total fungi was amplified in a nested PCR with the primers ITS1f (5′ TCC GTA GGT GAA CCT GCG G 3′) and ITS4r (5′ TCC TCC GCT TAT TGA TAT GC 3′) in the first round, ITS1f and ITS2rP (5′ GCT GCG TTC TTC ATC GAT GC 3) in the second round (White et al. 1990). Ascomycete DNA was amplified using the primers ITS1F (=ITS1A, 5′ CTT GGT CAT TTA GAG GAA GTA A 3′) and ITSA4 (5′ CGC CGT TAC TGG GGC AAT CCC TG 3′) in the first round, ITS1F and ITS2rP (5′ GCT GCG TTC TTC ATC GAT GC 3′) in the second round (Larena et al. 1999). The 16S rRNA genes were amplified from the total eubacterial DNA with primers Unibac-II-515f (5′ GTG CCA GCA GCC GC 3′) and Unibac-II-927rP (5′ CCC GTC AAT TYM TTT GAG TT′ 3; Berg et al. 2005b). Gammaproteobacterial 16S rRNA genes was amplifed using the primers γ-prot 595f and γ-prot 871rP (Mühling et al. 2008). For amplification with ITS primers in the first round, Phusion TAQ and buffer (Finnzymes Oy, Espoo, Finland) were used. For all other PCRs, the TAQ & Go system (Qbiogene) was employed. PCR preparations and PCR conditions were as described in Fürnkranz et al. (2009), except that 10 μg bovine serum albumin was added to the PCR mixture for bacterial DNA to protect the TAQ polymerase against inhibitors present in DNA isolated from soil; for amplification of eubacterial DNA with TAQ & Go, additional $MgCl_2$ (up to 3 mM) was added. PCR products were cleaned using the WIZARD® SV Gel and PCR clean-up system (Promega GmbH, Mannheim, Germany), made single stranded by digestions with λ-exonuclease and then snapped on ice. SSCP was performed in a TGGE apparatus (Biometra, Göttingen, Germany) according to Schwieger and Tebbe (1998). Fungal PCR products and bacterial PCR products were loaded in 9 and 10 % acrylamide gels, respectively. Gels were run at 400 V and 26°C for 17 h (fungal DNA) or 26 h (bacterial DNA). The gels were silver stained as described by Bassam et al. (1991), and scanned at 600 dpi resolution. Scanned images were

converted to 8-bit monochrome .tif files for analysis. Selected bands were excised, crushed, and eluted in 40 ml of sterile water overnight. One millilitre of supernatant was then used for re-amplification with respective primers. PCR products were purified with the GENECLEAN Turbo kit (BIO101 Systems; Qbiogene) and sequenced using the BigDye Terminator Ready Reaction kit (Applied Biosystems, Norwalk, CT, USA) and an ABI 310 automated sequencer (Applied Biosystems). The GenBank database (NCBI–BLASTN; Altschul et al. 1997) was used to retrieve closely related sequences and to calculate percentage similarity. GenBank accession numbers of submitted sequences are JQ260863–JQ260868.

For statistical analysis of data (analysis of variance (ANOVA) and Tukey's test) the statistical package SPSS (SPSS Inc., Chicago, IL, USA) was used. This package also allowed the analysis of root colonisation data with a mixed ANOVA model with plant section as within-subject factor and all other factors as independent factors.

Results

Survival of *S. rhizophila* DSM14405^T on seeds

Soaking of seeds in bacterial suspensions and direct sowing yielded start populations of ~10^5 CFU seed^{-1} (10^7 cells seed^{-1} in the larger cotton seeds). Biopriming of tomato, sweet pepper, and cotton seeds resulted in populations of 10^3–10^6 CFU seed^{-1}, irrespective whether the wild type or the rifampicin-resistant mutant RifR5 was used. Populations remained stable for 30–45 days. However, after 4 months storage at room temperature, no *S. rhizophila* DSM14405^T could be detected in bio-primed seeds with dilution-plating methods except for one batch of cottonseeds; there, low numbers of the rifampicin-resistant mutant DSM14405^T RifR5 (10^2 CFU seed^{-1}) could be re-isolated. After 4 months, the cells had either died or had entered the viable-but-non-culturable state.

Colonisation of different plant species by *S. rhizophila* DSM14405^T

In gnotobiotic systems, there was a significant effect of plant section and an interaction between plant species and plant section (roots, stems, and leaves), but no significant effect of plant species itself, except for leaves, where cell numbers on sweet pepper were significantly lower than on cotton (Fig. 1, left side). *S. rhizophila* DSM14405^T established high populations of 10^8–10^9 cells g^{-1} fresh weight on the roots and stems of all host plants from diverse families (Fig. 1). Populations on the leaves were lower (10^7–10^8 cells g^{-1} fresh weight). Population distribution on cotton was different; here the stems bore the lowest proportion of

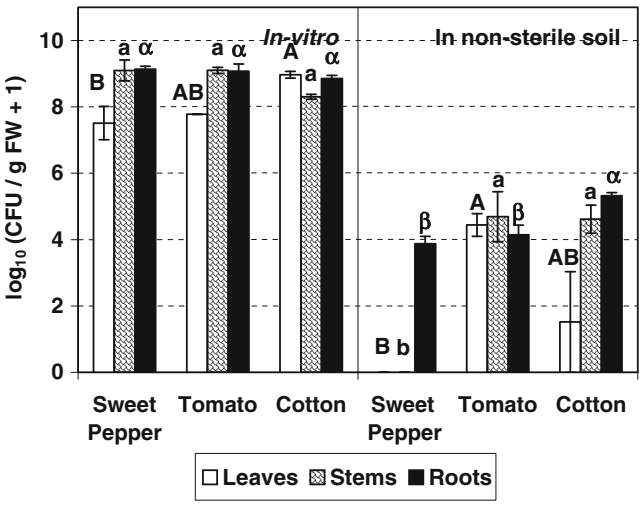

Fig. 1 Colonisation of different plant species (tomato, sweet pepper, and cotton) in vitro and in non-sterile soil by *S. rhizophila* DSM14405^T RifR5. Plants were either grown in Gamborg agar medium (in vitro) or in commercial potting compost (Grammoflor 'Profi Substrat') and harvested after 4 weeks growth. Tomato variety 'Avicenne' and sweet pepper varieties 'Californian Wonder' (in vitro) and 'Zdorové' in non-sterile soil were used. Soil moisture was kept at field capacity. Plantlets in vitro were treated with 10^4 cells after planting; for the experiment in soil, seeds were soaked in suspension of *S. rhizophila* DSM14405^T RifR5 (see 'Materials and methods' for details), resulting in ~10^5 cells seed^{-1} (10^7 cells seed^{-1} in cotton), respectively. Different letters indicate significantly different means at $p<0.05$ (Tukey's test); note that comparison were made only within the categories 'in vitro' and 'non-sterile soil, and within the plant region (root, shoot, leaves), respectively. There was a significant effect of plant species ($p<0.001$ in soil, not significant in vitro), plant section ($p=0.01$ in soil, $p<0.001$ in vitro) and a significant interaction between plant species and plant section ($p<0.03$)

DSM14405^T population (Fig. 1, left side). Application of a higher initial dose (10^8 cells plant^{-1}) did not result in higher cell densities on the plants. This indicates that the carrying capacity of the host rather than the application dose determines the final population density.

Colonisation of the phytosphere in non-sterile soil was examined in two experiments, the first one with bio-primed seeds (initial population 10^3–10^6 CFU seed^{-1}, presented in Fig. 1) and the second one with seeds soaked in suspension before sowing (initial population ~10^5 CFU seed^{-1}), respectively. Both experiments yielded similar results. Plant species and plant section affected population density significantly ($p<0.001$ and $p=0.01$, respectively) and also affected the shape and distribution of *S. rhizophila* populations, which was apparent in a significant interaction between plant species and plant section ($p<0.03$; Fig. 1, right side). Population density of *S. rhizophila* DSM14405^T RifR5 in the rhizosphere was uniformly high in all three plant species (~10^4–10^5 CFU g^{-1} root fresh weight) albeit 4–5 orders of magnitude lower than in gnotobiotic in vitro systems (Fig. 1). The differences between the plant species

became apparent in the above-ground sections. In tomato (cv. 'Avicenne'), populations tended to increase from bottom to top with the highest population density on the leaves (Fig. 1, right side). Cotton and sweet pepper (cv. 'Zdorové') showed the opposite trend with highest population density in the rhizosphere. This was particularly pronounced in sweet pepper, where population density declined sharply down to the to the detection limit of 10^2–10^3 cells per gram plant fresh weight in all aboveground plant parts (Fig. 1). Colonisation of two other sweet pepper varieties ('Californian Wonder', 'Chinese Giant') was not significantly different from that on the variety shown here ('Zdorové'). On cotton, stems were colonised but a significant decline occurred on the leaves (Fig. 1).

No endophytic *S. rhizophila* DSM14405^T could be isolated from surface-sterilised plant parts, neither in vitro plants nor in plants grown in non-sterile soil, although the exposure time and concentration of the surface sterilant NaOCl had already been reduced to the minimum necessary for successful surface sterilisation.

However, light micrographs of *S. rhizophila* on gnotobiotic tomato roots indicated a colonisation around and possibly also inside the root hairs (Fig. 2a) and FISH–confocal laser scanning microscopy of root-associated bacteria (stained with the bacterial EUB338MIX FISH probe) revealed that *S. rhizophila* DSM14405^T colonised the inside of tomato roots (Fig. 2b and c).

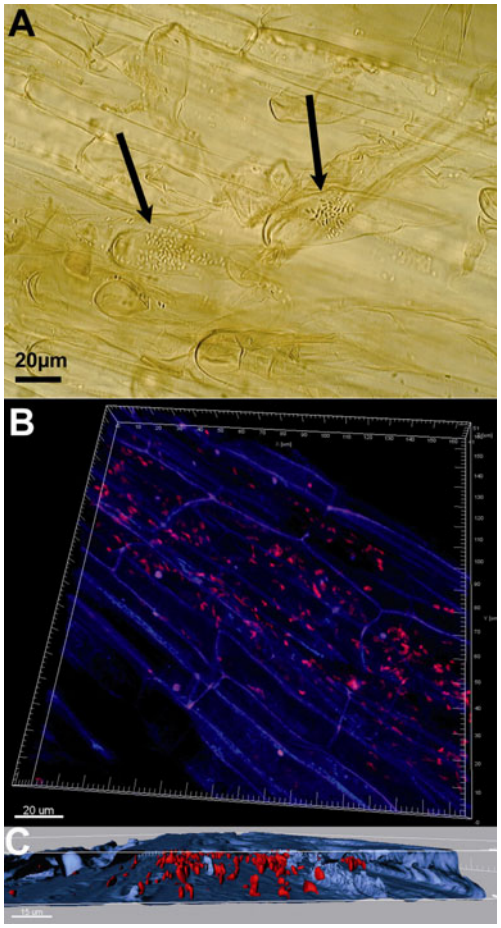

Fig. 2 Cells of *S. rhizophila* DSM14405^T in tomato roots (gnotobiotic in vitro plants, cv. 'Avicenne'). **a** Light micrograph viewing on top of the root surface with root hairs growing towards the viewer; the bacterial cells form colonies between and possibly within the root hairs (*arrows*). **b** Volume rendering of a confocal laser scanning microscopy Z-stack showing FISH stained bacterial cells (red signal, probe EUB338MIX) within the roots of tomato (*blue*/*pink* signal). **c** Cut plane of the isosurface reconstruction of panel B (*red* bacteria, *blue* root tissue) showing endophytic colonies of *S. rhizophila* DSM14405^T

Plant growth promotion of cotton, sweet pepper, and tomato in gnotobiotic systems

In cotton, treatment with low doses of *S. rhizophila* DSM14405^T (10^4 cells plant^{-1}) increased the length of the main root; no other significant direct effects on plant growth could be detected (Table 1). Treatment with *S. rhizophila* DSM14405^T significantly reduced incidence and severity of seed-borne diseases (most likely *Fusarium* spp., Table 1). Application of high doses of *S. rhizophila* DSM14405^T (10^8 cells plant^{-1}) reduced the number and length of side roots in cotton and in tomato (Table 1).

In vitro plants of Tomato (cv. 'Avicenne') were negatively affected by *S. rhizophila* DSM14405^T. A significant reduction in the number of side roots was already observed at the lower initial dose (10^4 cells plant^{-1}); the effect increased with dose (Table 1). The effect on the number of secondary leaves was strongly dose dependent. Low initial doses of *S. rhizophila* DSM14405^T had a positive influence; high doses had a negative effect (Table 1).

S. rhizophila DSM14405^T had no direct plant growth promoting effect on sweet pepper (cv. 'Californian Wonder'). However, a biocontrol effect was observable similar to cotton. Incidence of seed-borne fungal diseases (identified as *Alternaria* spp.) was significantly reduced by the treatment with *S. rhizophila* DSM14405^T (Table 1).

Tentative experiments in tap water instead of 1/10×Gamborg salts and at different salinitites (0.5 % and 1.0 % NaCl) indicated that the plant growth promoting effect of *S. rhizophila* DSM14405^T on tomato and sweet pepper was not increased under nutrient limiting conditions and that the strain did not alleviate salt stress of in vitro plants (data not shown).

Plant growth promotion of cotton, sweet pepper, and tomato in non-sterile soil

In order to allow for more subtle effects of *S. rhizophila* DSM14405^T to become apparent, single plants were grown in 10 cm pots (20 replicate pots per treatment) for a longer

Table 1 Effect of *S. rhizophila* DSM14405^T on sweet pepper (cv. 'Californian wonder'), tomato (cv. 'Avicenne') and cotton (*Gossypium* sp.) in vitro

Plant species	Parameter monitored	Experiment 1 (low dose)[a]			Experiment 2 (high dose)[b]		
		Control	*S. rhizophila* DSM14405^T 10^4 cells plant^{-1}	Significance (direction of the effect)[c]	Control	*S. rhizophila* DSM14405^T 10^8 cells plant^{-1}	Significance (direction of the effect)[c]
Cotton[d]	No. of root tips	50±3.8	48±2.3	ns	14±1.9	8.0±1.6	0.019 (−)
	Length of main root (cm)	nd	nd	nd	1.5±0.18	1.7±0.17	ns
	Length of side roots (cm)	nd	nd	nd	38±5.3	21±6.5	0.056 (−)
	Shoot length (cm)	10.4±0.4	10.8±0.2	ns	5.7±0.61	4.3±0.63	ns
	No. of leaves (healthy)	2.4±0.3	2.8±0.2	ns	nd	nd	nd
	Disease severity[e]	0.7±0.12	0.19±0.07	<0.001 (−)	nd	nd	nd
Tomato (cv. Avicenne)	No root tips	17±1.2	13±0.9	<0.001 (−)	16±2.7	8.0±1.7	0.015 (−)
	Root length (cm)	66±4.2	57±4.0	0.078 (−)	51±7.9	25±6.2	0.017 (−)
	Shoot length (cm)	2.5±0.15	2.5±0.16	ns	3.1±0.26	5.0±1.7	ns
	No. of secondary leaves	3.0±0.1	3.4±0.1	0.029 (+)	3.0±0.31	2.1±0.36	0.056 (−)
Sweet pepper (cv. Californian Wonder)	No root tips	19.9±0.82	19±0.9	ns	nd	nd	
	Length of main root (cm)	3.0±0.13	3.4±0.14	0.058(+)	nd	nd	
	No of secondary leaves	3.8±0.11	3.7±0.11	ns	nd	nd	
	Disease severity[e]	0.21±0.07	0.04±0.04	0.041 (−)	nd	nd	

Plants were grown in plant agar+1/10×Gamborg salts B5 for 5 weeks. Upon transfer to plant agar, sterile seedlings were inoculated with *S. rhizophila* DSM14405^T cells suspended in 0.85 % NaCl. Control plants were inoculated with 0.85 % NaCl only; 16 h/8 h day night cycle; growth temperature was 18–25°C

ns non significant, *nd* not determined

[a] Experiment 1 (low dose) : 35 replicates per treatment

[b] Experiment 2 (high dose): 15 replicates per treatment

[c] Significance assessed by *t* test

[d] Cotton in experiment 2 (higher dose) was assessed at 3 weeks growth time (before development of secondary leaves); all other plants and cotton in experiment 1 were assessed after 5 weeks, when first secondary leaves were developed symptoms, two plants killed by the fungus

[e] Incidence and severity of diseases (re-isolated fungus identified as *Alternaria* sp. in sweet pepper; not identified in cotton); 0=healthy, 1=diseased, 2=killed

period of 51 days. By that time, the sweet pepper plants were already fully developed and in bloom, and the tomatoes had developed their first flower buds. No significant effect of *S. rhizophila* DSM14405^T could be observed in sweet pepper (cv. 'Zdorové'), although the average shoot height, number of leaves and flower buds tended to be higher (Table 2). However, *S. rhizophila* DSM14405^T significantly increased shoot height and shoot weight of tomato (cv. 'Avicenne'). Flower development of tomato was not increased; however, it has to be noted that the harvest was done just after appearance of the first flower buds. In cotton, no plant growth promoting effect was visible and *S. rhizophila* DSM14405^T reduced the number of side buds significantly (Table 2).

Effect of *S. rhizophila* DSM14405^T on the native rhizosphere flora of tomato and sweet pepper

Plant growth promotion of tomato by *S. rhizophila* DSM14405^T was more apparent in non-sterile soil and a suppressive effect on seed-borne pathogens was observed in vitro; this indicates a more indirect effect via the suppression of pathogens and deleterious microbes rather than a direct plant growth promotion effect. Therefore, the influence of *S. rhizophila* DSM 14405^T on the community profiles of selected microbial taxa of the rhizosphere in the rhizosphere of tomato and sweet pepper was investigated. Total fungi and *Ascomycetes* were chosen because these groups contain many plant pathogens; as *S. rhizophila* DSM14405^T

Table 2 Effect of *S. rhizophila* DSM14405^T on sweet pepper (cv. 'Zdorovjé'), tomato (cv. 'Avicenne') and cotton (*Gossypium* sp.) in non-sterile soil

Plant species	Parameter monitored	Treatment[a]		
		Control	*S. rhizophila* DSM14405^T Bioprimed (□10^4 CFU seed^{-1})	Significance (direction of the effect)
Tomato (cv. Avicenne)	Shoot length (cm)	30.5±1.5	35±1.4	0.028 (+)[b]
	No. of secondary leaves	9±0.6	11±0.6	0.07 (+)
	No. of inflorescences	1.2±0.16	1.8±0.5	ns
	No. of single flowers	3±0.6	3.7±0.7	ns
Sweet pepper (cv. Zdorové)	Shoot length (cm)	19±1.0	20.5±0.7	ns
	No. of shoots	3.1±0.34	2.9±0.26	ns
	No. of secondary leaves	22±1.4	24±1.1	ns
	No. of flowers	6±0.8	8±1.0	ns
Cotton	Shoot length (cm)	28±0.9	28±1.0	ns
	No. of secondary leaves	7.6±0.42	7.3±0.34	ns
	No. of side buds	2.9±0.48	1.25±0.33	0.009 (−)

Plants were grown in potting compost (Grammoflor 'Profi Substrat' Grammoflor Co & KG Vechta, Germany) for 51 days. Seeds were bioprimed with *S. rhizophila* DSM14405^T cells suspended in 0.85 % NaCl (see text for details). Controls were primed with 0.85 % NaCl only; 16/8 h day/night cycle, growth temperature was 18–25°C

ns non significant

[a] Twenty replicate plants per treatment × plant species

[b] Significance assessed by *t* test

belongs to the *Gammaproteobacteria*, within the bacteria, it appears likely that these groups are also affected by competition effects; therefore profiles these groups were also compared.

Profiles of *Ascomycetes* appeared largely similar irrespective of plant species and treatment (Fig. 3, right gel). A few minor exclusive bands appeared in the rhizosphere of tomato and sweet pepper plants treated with *S. rhizophila* DSM14405^T (Fig. 3, bands B_{asco} 1 and B_{asco} 2). Sequencing of those bands showed a close relationship to fungi of the genus *Cladosporium* (Table 3). Cluster analysis did not reveal a significant influence of *S. rhizophila* DSM14405^T on the total *Ascomycetes* profile (data not shown).

Striking differences were visible in the total fungal community of tomato (Fig. 3 left gel). Major bands almost disappeared in the treatment with *S. rhizophila* DSM14405^T (Fig. 3, bands B1 B3, B4 and B5); some weaker bands also appeared less pronounced or less frequent in treatments with *S. rhizophila* (B2, B7, B8). With around 80 % similarity, the sequences represented by the major bands were distantly related to those of *Mortierella* or other yet uncultured fungi, from soil or forest habitats (B2 and B3, respectively; Table 3), or even to ITS sequences of the green algae *Scenedesmus* (Johnson et al. 2007) or soil ciliates (B4 and B1, respectively; Table 3). Most remarkably, the sequence obtained from band B5 showed a very close relationship (96 %) to the human pathogen *Candida subhashii* (Adam et al. 2009; Table 3). Another dominant band, B6, appeared to increase due to the treatment with *S. rhizophila* DSM14405^T; unexpectedly, it proved to be closely related to *Dictyochloris* sequences (*Chlorophyceae*; Shoup and Lewis 2003) and only more distantly related to other yet uncultured soil fungi (82 % similarity; Table 3). Sequences related to *Ascomycetes* isolated from mosses and liverworts (Table 3; band B8) could be recovered more frequently in the control samples than in samples treated with *S. rhizophila* DSM14405^T; however, their presence was generally on the border of the detection limit (Fig 3). The influence of *S. rhizophila* DSM14405^T on total fungal communities of sweet pepper was less apparent than in tomato; thus we did not excise and sequence single bands here.

The community profiles of *Gammaproteobacteria* and all bacteria were largely similar, irrespective of plant species and treatment (Fig. 4). Interestingly, a band corresponding to *S. rhizophila* DSM14405^T was only present in total eubacterial profiles, but not in gamma-proteobacterial profiles (Fig. 4).

Discussion

Plant-associated microorganisms fulfil important functions for plant growth and health. Direct plant growth promotion by microbes is based on improved nutrient acquisition and

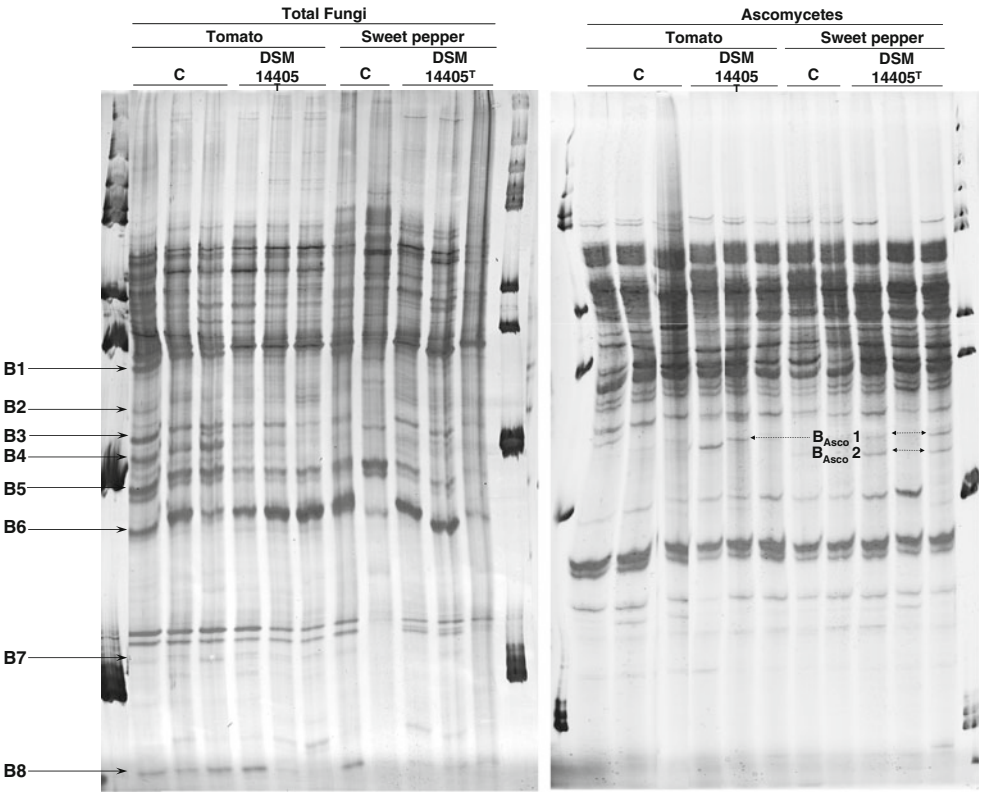

Fig. 3 Impact of *S. rhizophila* DSM14405^T on fungal community profiles in the rhizosphere of sweet pepper and tomato detected by PCR-SSCP. Fungal DNA was detected with ITS1 and ITS2 primers specific for the respective groups. *Arrows* indicate an influence of *S. rhizophila* DSM14405^T on the banding pattern; dotted arrows indicate bands present only in one treatment but not in all replicates; *solid arrows* indicate bands consistently present or significantly stronger only on one treatment

hormonal stimulation (Berg 2009). Diverse mechanisms are involved in suppression of plant pathogens (Mazzola 2002; Weller 2007; Lugtenberg and Kamilova 2009; Barret et al. 2011), which is often indirectly connected with plant growth. Isolates of *S. rhizophila* directly inhibited plant pathogenic fungi in vitro (Wolf et al. 2002); and produced antifungal volatiles (Kai et al. 2007). In this study, plant growth-promoting effects of *S. rhizophila* DSM14405^T were most pronounced in non-sterile soil and only subtle at best in gnotobiotic systems, despite populations being 4–5 orders of magnitude lower in non-sterile soil. Also, plant growth promotion in artificially salinated soil was only observable in native, not in sterilised soils (Berg et al. 2012). Positive effects on in vitro plantlets were largely due to the biocontrol of seed-borne diseases, which were systemically present within the seeds and could not be eliminated via surface sterilisation. This is the first demonstration of the biocontrol efficacy of this strain in cotton and in solanaceous crops. In vitro antagonistic activity of *S. rhizophila* DSM14405^T against the important soil-borne plant pathogens *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, and *Verticillium dahliae* has been demonstrated earlier (Minkwitz and Berg 2001). These observations, together with the proven capability of the strain to produce the antifungal volates β-phyenylethanol, dodecanal, siderophores, and fungal cell wall-degrading enzymes (Kai et al. 2007; Minkwitz and Berg 2001) point towards biocontrol of deleterious microorganisms and pathogens present in the soil substrate being responsible for plant growth promotion by *S. rhizophila* DSM14405^T. This is also likely to be the case in the Uzbek soils as there was a strong presence of soil-borne pathogens (*Fusarium solani* and *V. dahliae*; Egamberdiyeva, personal communication). Consequently, the plant growth promotion by *S. rhizophila* DSM14405^T in these soils was much stronger than observed in our study (Egamberdieva et al. 2011). Based on those results and our findings, we suggest that *S. rhizophila* DSM14405^T promotes plant growth indirectly through shaping the fungal rhizosphere community.

Unexpectedly, none of the fungal sequences in our SSCP profiles visibly affected by *S. rhizophila* DSM14405^T was related to known plant pathogens; many of the closest related fungal sequences were indeed from yet uncharacterised fungi inhabiting pristine environments. They may belong to the so-called deleterious microorganisms or deleterious rhizobacteria, which affect plant growth negatively but do not necessarily parasitize the plant tissue. Their deleterious activities include alterations of the supply of water, ions, and plant growth substances by changing root functions and/or by limiting root growth (Schippers et al. 1987; Kremer and Kennedy 1996) and their effects can be even plant species-specific (Mejri et al. 2010). Furthermore, the activity of *S. rhizophila* against human pathogenic genera like *Candida* (Adam et al. 2009) may have positive implications for food safety. Unexpectedly, some sequences whose relative abundance in the rhizosphere was affected by *S. rhizophila* bore similarity to those of green algae

Table 3 Identification and characteristics of the closest BLAST matches of fungal DNA sequences obtained by SSCP from tomato rhizosphere

Band no.	Similarity (%)	Accession no.	Description	Habitat
B_{Asco} 1	97	EU570258	*Cladosporium sphaerospermum* CPC 14016, *Capnodiales Ascomycota*	Wheat (*Triticum aestivum*)
B_{Asco} 2	97	AM159631	*Cladosporium sphaerospermum* clone K7	Paintings in the castle of Schönbrunn, Vienna
	98	GU721592	Uncultured fungus clone f3Fc57	HVAC filter dust
B5	**100**	**AB663086**	***Candida sp*. NY7122, *Saccharomycetales*, *Ascomycota***	**Soil, Ibaraki, Tsukuba, Japan**
	97	**EU836707**	***Candida subhashii* UAMH 10744**	**Human host, peritoneum**
B2	71	GQ517296 FJ761879.1	Uncultured fungus clones	*Quercus macrocarpa* phyllosphere
		AY354234.1	*Davidiella tassiana* isolate	Betula pendula xylem (live stem), Lithuania
B8	93	AM397675	Uncultured ascomycete clone BuxP4-1	Bryophyte *Buxbaumia aphylla*
	93	AM397689 AM397688.1	Uncultured ascomycete clones	*Bazzania trilobata* (riverbanks) Rhineland-Palatinate, Germany
B7	96	FJ873574	*Cryptococcus sp.* EN14M04 *Filobasidiales, Basidiomycota*	Mushrooms of Thailand
	96	AF042417	*Tremella foliacea* CCJ1396, *Tremellales, Basidiomycota*	–
B3	**81**	**GU997894 GU997756**	**Uncultured *Mortierella* clones, *Mortierellales*, *Zygomycota***	Ectomycorrhiza root tip, *Betula nana*, Toolik Lake, AK, USA
	78	**HQ630296.1, HQ630295.1**	***Mortierella parvispora* strains**	
B4	**80**	**DQ417571**	***Scenedesmus* sp. Pic 6/16 T–1 W (*Chlorophyceae*) *Chlorococcales*Chorophyta**	Picnic Pond, Itasca State Park, MN, USA
	78	**JN660672.1 JN660634**	**Uncultured organism clones ciidir**	Tomato rhizosphere cv. 'Gabriella' Valle de Guasave, Sinaloa, Mexico
B6	**96**	**AF367862, AF367860**	***Dictyochloris* (*Chlorophyceae*), *Chlorococcales, Chorophyta***	–
B1	**89**	**FJ554355.1**	**Uncultured *stichotrichid* clone**	–
	87	AF508762	*Oxytricha granulifera* (soil ciliate)	–

Bold major bands. B1–8 originate from tomato rhizosphere, rthe ascomycete bands B_{asco} 1 and 2 originate from the rhizosphere of sweet pepper. For position of bands, see Fig. 3

(*Chlorophyceae*: *Dictyochloris*, *Scenedesmus*), and the question arises how these phototrophic organisms receive sufficient light for growth in the rhizosphere. Fujita and Nakahara (2006) detected large numbers of viable microalgae, among them *Chlorella* and *Scenedesmus*, in the darkness of subsurface soil and facultative heterotrophy could be demonstrated in *Dictyochloris* when grown in darkness (Parker et al. 1961).

Plant species as a factor shaping rhizosphere communities is well documented in earlier studies (Berg and Smalla 2009; Hartmann et al. 2009) and differences may exist even between members of the same plant family; three different gramineous hosts shared only about one tenth of all computed protein sequences in their rhizosphere microbiomes (Barret et al. 2011). Here, we could demonstrate that two closely related plant species (both within the family of *Solanaceae*) displayed significant differences in their fungal, but not bacterial rhizosphere communities. Contrarily, an effect of biocontrol agents on microbial and fungal communities that becomes visible in banding patterns generated by SSCP or DGGE analysis is rarely reported; a majority of studies showed no or only subtle short-term changes (Götz et al. 2006; Scherwinski et al. 2008; Berg and Zachow 2011). This profound effect of *S. rhizophila* DSM14405^T on the fungal communities observed in our study is even more noteworthy as the inoculant contributes only a minor proportion to the total rhizosphere flora. *S. rhizophila* DSM14405^T was not detectable in profiles of gammaproteobacteria. Its population density of 10^4–10^5 cells g^{-1} root fresh weight in non-sterile soil is only minute compared to a total of 10^8–10^{11} CFU g^{-1} root fresh weight, which was observed even in saline, mineral soils (Egamberdiyeva et al. 2008). Total populations per plant declined in non-sterile soil with 10^5 cells applied per seed and ~10^4 cells recovered per plant (10^7 applied/10^5 recovered in cotton). This is in remarkable contrast to the strong plant competence in gnotobiotic systems where the population rose by several orders of magnitude in all plant parts. We observed a similar increase in our soil substrate when it was sterilised (unpublished data). This indicates that the strain is probably

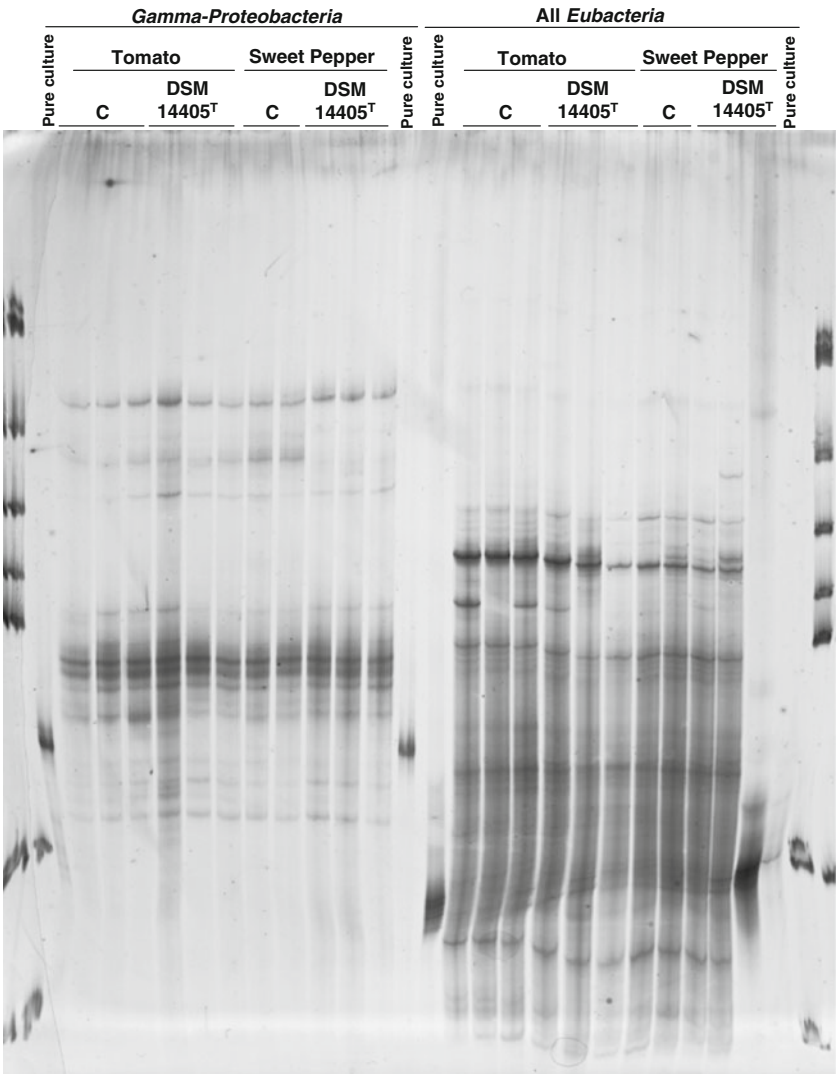

Fig. 4 Impact of *S. rhizophila* DSM14405^T on bacterial community profiles in the rhizosphere of sweet pepper and tomato detected by PCR-SSCP. Gammaproteobacterial communities were detected with primers γ-prot 595f and γ-prot 871rP, eubacterial communities were detected with primers Unibac-II-515f and Unibac-II-927rP. Pure culture= DNA from pure culture of *S. rhizophila* DSM14405^T amplified with respective primers co-separated for control of its presence in environmental profiles

outcompeted by indigenous soil microbes. *S. rhizophila* DSM14405^T may face even stiffer competition from indigenous rhizobacteria in the organic nutrient-rich substrate used here than in the nutrient-poor salinated mineral soils of Usbekistan (calcisols), where it displayed stronger plant growth promoting capability (Egamberdieva et al. 2011). There, populations of indigenous microbes are 1,000-fold lower than in a non-salinated agricultural soil (Ji and Wilson 2002; Egamberdiyeva et al. 2008). The question remains on how *S. rhizophila* DSM14405^T could exert such a strong effect on the fungal communities while representing only such a minute compound in the rhizosphere. Nutrient release from dying surplus inoculum could have favoured indigenous antagonists as nutrients, notably nitrogen, can be a limiting factor in the rhizosphere (Jensen and Nybroe 1999). However, this effect should have been confined to the seed were the inoculum was directly applied, whereas the change in fungal communities occurred in colonised roots; also, we did not apply any additional nutrients or culture medium. It should be borne in mind that population size of applied inocula and their antagonistic activity need not to be correlated. Formulation additives deleterious to the growth of applied inocula (trace elements and carob, respective) increased antagonistic activity of *Pseudomonas fluorescens* and *Pantoea agglomerans*, respectively, whereas additives supportive of their growth (*N* compounds) decreased efficacy (Wiyono et al. 2008; Schmidt et al. 2001). This gives rise to the assumption that adverse conditions, such as increased competition in this case, might induce an increase in antibiotic production of microbial inocula. Furthermore, the gaseous nature of the antifungal substances produced by *S. rhizophila* DSM14405^T (Kai et al. 2007) may ensure a very fast, effective distribution in the soil pores, even at low population densities. On the other hand, synthesis of antifungal compounds in rhizosphere bacteria is largely regulated by quorum sensing and therefore requires a certain population threshold (Müller et al. 2009; Williams 2007). The possibility exists, however, that signal molecules provided by resident bacteria in the rhizosphere may help to reach the threshold necessary for quorum sensing. Interspecies

signalling is recorded in biocontrol pseudomonads (Dubuis and Haas 2007; Dubuis et al. 2007) as well as for the diffusible signal factor system found in *Stenotrophomonas* and *Xanthomonas* (Ryan et al. 2008). Furthermore, signalling distances between in the rhizosphere exceeded the size of the individual cells up to 30-fold suggesting that induction via quorum sensing requires much lower population thresholds than previously thought (Gantner et al. 2006).

In vitro systems, Suckstorff and Berg (2003) had shown a strong dose-dependency of plant growth promotion by *S. rhizophila* in strawberries, and doses above 10^4 CFU plant^{-1} had deleterious effects; these results could be confirmed in tomato and cotton. This dose dependency may also explain the negative effect of the strain on tomato in vitro, while its effect in non-sterile soil was beneficial. Even with low initial doses of 10^4 CFU plant^{-1}, populations in vitro rose to high densities of 10^8 CFU g^{-1} root fresh weight in gnotobiotic systems, which were four- to fivefold higher than in soil. However, these final population sizes were not deleterious in other crops. The time–course of population build-up appears to be the crucial element here; the non-germinated or germinating seed may represent a particular sensitive stage, which is particularly susceptible to phytotoxic effects of high initial populations. In this context, it is noteworthy that oilseed rape (*Brassica napus*), was particularly resistant against the phytotoxic effects of high initial doses of *S. rhizophila* DSM14405^T but was also not positively affected by the strain (data not shown). This may be the result of long-term adaptation to *S. rhizophila*, as oilseed rape was the source of the strain (Minkwitz and Berg 2001; Wolf et al. 2002)

Our work demonstrated that *S. rhizophila* DSM14405^T, when applied to the seed, does also colonise the above-ground parts of the plants. However, it does appear to need high humidity, as the shoots of sweet pepper plants were densely colonised only in the water-saturated atmosphere of closed microcosms (unpublished results), but not in open top microcosms with ambient humidity. Populations on tomato shoots were significantly higher than on all other plant species at ambient humidity. This may be attributed to their dense coverage with trichomes which may provide for a moister more sheltered micro-environment close to the plant surface, ensuring a better survival of *S. rhizophila*. *Stenotrophomonas* isolates have also been shown to colonise plants endophytically (Berg et al. 2005b; Ryan et al. 2009); therefore, the isolate may settle into the even more sheltered environment in the inside of the trichomes. In this study, we could demonstrate that *S rhizophila* DSM14405^T microcolonies are located in the vicinity of and possibly also inside root hairs. However, our results obtained by serial dilution plating appear to be contrary to that, as almost all of the population of *S. rhizophila* DSM14405^T was recovered in the ectophytic fractions (surface washings) and no cells could be recovered from the inside of plant parts after surface sterilisation. Possibly, the root hairs (and trichomes) break during vortexing and sonication, releasing their inhabitants into the surrounding solution or exposing them to the hypochloride treatment during the later operations for surface sterilisation. Cells of *S. rhizophila* growing endophytically in plant hairs may therefore end up in the surface washing or may be killed during surface sterilisation. Therefore, they may be falsely attributed to the epiphytic population in plating experiments.

Colonisation of above-ground plant parts raises the question whether the strain poses a health risk if it colonises harvestable products, especially as the species is closely related to *S. maltophilia*, an opportunistic human pathogen. However, *S rhizophila* forms a clearly distinguished clade consisting only of non-clinical isolates (Minkwitz and Berg 2001; Ribbeck-Busch et al. 2005) and lacks important traits making the latter a harmful pathogen such as the multidrug efflux pump SmeDEF and the gene for a zonula occludens-like toxin (Ribbeck-Busch et al. 2005; Hagemann et al. 2006). Colonisation of the smooth and waxy surfaces of sweet pepper and tomato fruit (Suslow 2004) may be absent or reduced at lower humidity, as was observed on sweet pepper leaves. However, colonisation of aboveground plant parts by *S. rhizophila* deserves further attention, especially as it also may also have positive implications and highlights a possible potential of the strain for the control of aerial pathogens such as tomato blight (*Phytophthora infestans*).

In conclusion, the plant growth promoting effect of *S. rhizophila* DSM14405^T appears to be largely based on antagonism towards deleterious and pathogenic rhizosphere microorganisms. This is suggested by (1) a greater effect in non-sterile soil than in sterile systems and (2) the profound effect of the isolate on the rhizosphere community composition. This effect should be further investigated in different soil types, and under conditions of increasing salinity, as the *S. rhizophila* DSM14405^T is envisaged to be used under saline conditions (Egamberdieva et al. 2011).

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