This is a preprint of an article published in Plant and Soil. The final authenticated version is available online at: https://doi.org/10.1007/s11104-022-05304-9

REGULAR ARTICLE

Stenotrophomonas sp. SRS1 promotes growth of Arabidopsis and tomato plants under salt stress conditions

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Electronic supplementary material The online version of this article contains supplementary material, which is available to authorized users.

ABSTRACT

Aims Plant Growth-Promoting Rhizobacteria (PGPR) support plant growth by alleviating plant stresses, among which those triggered by saline soils. We isolated *Stenotrophomonas* sp. SRS1 from salt-resistant *Carex distans* (distant sedge) roots to understand how this growth promotion was enabled and whether an active contribution of the bacteria and/or plant was required.

Methods Various growth assays were used to analyze the effect of bacterial inoculation on *Arabidopsis thaliana* and *Solanum lycopersicum* (cherry tomato MicroTom) growth. Furthermore, droplet microfluidics, bacterial genome mining, and bacterial and plant gene expression analysis combined with plant mutant analysis were used for in-depth analysis.

Results SRS1 application enhanced plant growth in both saline and nonsaline environments. The fresh weight of SRS1-inoculated plants was higher than that of noninoculated plants, whereas the fresh weight ratio between SRS1-inoculated and noninoculated plants differed whether the plants were grown on agar plates, white sand or in soil. We demonstrated that the strain grew well in high salt-containing media and that, besides plant-growth-promotion-related genes, the bacterium contained various active stress genes. Interestingly, inoculation with the strain increased the induction of plant genes related to abscisic acid and auxin signaling pathways under saline conditions.

Conclusions SRS1 inoculation promoted the growth of Arabidopsis and MicroTom tomato under saline and nonsaline conditions, also when the plants were grown in white sand and potting soil. Overall, genetic traits related to stress alleviation, derived from both the bacteria and the plants, play a crucial role in the impact of this novel PGPR strain on plant performance.

Keywords: Arabidopsis · Auxin · Genomics · Mutant · Plant growth-promoting bacteria · PGPR · Root endosphere · Tomato · qRT-PCR · Salt tolerance · *Stenotrophomonas*

Abbreviations

½MS	half-strength Murashige and Skoog
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
ANOVA	analysis of variance
AUX1	auxin transporter protein 1 gene
CFU	colony-forming units
Col-0	Columbia-0 accession
DAT	days after treatment
DMRT	Duncan Multiple Range Test
EPS	exopolysaccharides
ggpS	*glucosylglycerol-phosphate synthase* gene
IAA	indole-3-acetic acid (IAA
IAA12	auxin-responsive protein 12 gene
IAR4	IAA-alanine resistant 4 gene
katG	*catalase peroxidase* gene
mdoB	*phosphoglycerol transferase* I gene
MES	2-morpholinoethanesulfonic acid
ompA	*outer membrane protein A* gene
PBS	phosphate-buffered saline
Pga	polysaccharide adhesin required for biofilm formation
PGPR	plant growth-promoting rhizobacteria
R2A	Reasoner's 2A
ROS	reactive oxygen species
SOD	superoxide dismutase
ycaD	*glucosylglycerol transporter* gene

Introduction

Salinization caused by climate change is one of the prevalent environmental problems of the last century (FAO 2002; Hall 2001; Shrivastava and Kumar 2015). What is more, by 2050, approximately 50% of the cultivated land has been predicted to suffer from salinity stress (Martinez-Beltran and Manzur 2005; Vaishnav et al. 2017). Soil salinity strongly affects plant growth and hence affects agricultural yield by changing various biochemical and physiological processes (Jamil et al. 2011; Patil 2013; Paul and Lade 2014).

Tomato (*Solanum lycopersicum* L.), one of the most important cultivated cash crops worldwide, is highly susceptible to high soil salinity, with major yield losses of up to 50% as a result (Shrivastava and Kumar 2015). Increasing the tolerance of tomato to salt is being attempted by understanding how plants cope with this type of stress (Maksimovic and Ilin 2012; Philippot et al. 2013; Saleem and Moe 2014; Vos et al. 2014; Raza et al. 2016). Additionally, one of the suggested methods is through the application of plant growth-promoting rhizobacteria (PGPR). Soils are well known to be one of the richest sources of microorganisms, of which several have been shown to promote plant growth under different abiotic stress conditions, such as drought, salt, heat, and chilling stress (Tank and Saraf 2010; Tkacz and Poole 2015; Wang et al. 2016; Yuan et al. 2016; Beirinckx et al. 2020).

Numerous PGPR, from a diverse array of bacterial genera, such as *Stenotrophomonas*, *Bacillus*, and *Pseudomonas*, have been demonstrated to alleviate salt stress in different cereal and legume crops (Gayathri et al. 2010; Singh and Jha 2017; Natarajan et al. 2016; Wang et al. 2016; Yuan et al. 2016; Sarkar et al. 2018; Yasin et al. 2018; Jatan et al. 2019). Also in tomato, different PGPR strains have been described that enhance plant growth under saline conditions, such as *Streptomyces*, *Achromobacter*, and *Enterobacter* (Mayak et al. 2004; Tank and Saraf 2010; Palaniyandi et al. 2014; Kim et al. 2014). Albeit these many examples, little is known about the molecular mechanisms underlying the observed induction of salt stress resilience, but different scenarios could be envisioned. For instance, the PGPR themselves could be tolerant to salt stress and as such, prevent salt to enter the roots by colonizing the plant roots (Bhat et al. 2020). Alternatively, and not exclusively, PGPR could promote growth through the production of phytohormones, such as auxins that enhance root growth (Gupta et al. 2015) or cytokinins and gibberellins that are involved in the abiotic stress alleviation on plants (Gupta et al. 2015; Kumar et al. 2015). Besides the production of phytohormones, some PGPR influence the plant hormone balances indirectly, thereby, inducing plant resistance, for instance through

the expression of 1-aminocyclopropane-1-carboxylate (ACC) deaminases that reduce ethylene production, a well-known plant stress hormone (Mayak et al. 2004; Glick 2014).

The genus *Stenotrophomonas* has often been described as a genus with great potential in agriculture (Berg et al. 2010; Ryan et al. 2009). For instance, *Stenotrophomonas maltophilia* SBP-9 has been reported to improve growth and the ionic balance of wheat (*Triticum* sp.) when grown under saline conditions (Singh and Jha 2017). Although the underlying mechanisms are still unknown, the strain has been shown to contain genes involved in the transport of osmoprotectants (Singh and Jha 2017). In addition, *Stenotrophomonas rhizophila* strain DSM14405^{T}, isolated from a saline soil, could increase growth of cucumber (*Cucumis sativa*) up to 180% (Egamberdieva et al. 2011). Genome analysis revealed that this strain contains various salt stress resistance genes, such as glucosylglycerol-phosphate synthase (*ggpS*) and glucosylglycerol transporter (*YcaD*) (Alavi et al. 2014). However, whether *Stenotrophomonas* sp. can activate the abiotic stress pathways in plants is not known yet.

We have isolated *Stenotrophomonas* SRS1 from the roots of the salt-resistant plant *Carex distans* (distant sedge) that grows in a naturally saline-rich environment in Belgium. We show that the strain is able to promote the growth of *Arabidopsis thaliana* and tomato (var. MicroTom) under both saline and nonsaline conditions, but the effect depends on the growth assay used. We additionally investigated the mechanisms by which the growth promotion occurs both from a bacterial and a plant point of view. This study demonstrates that a bacterial strain isolated from the roots of a plant adapted to grow under high saline conditions can contribute to the growth promotion of tomato under salt stress and opens possibilities to use this strain for the alleviation of salt stress on crops grown in salt-affected regions.

Materials and methods

Isolation of *Stenotrophomonas* SRS1

Plant roots of *Carex distans* L. (distant sedge, Cyperaceae family), known to survive under high salt conditions, were sampled in July 2017 from the natural reserve “Het Zwin”, located between the Belgian North Sea and a high dike, and consists of sandy dunes, salt flats, and marshes (https://knokkeheist.com/en/zwin.php). With an electrical conductivity of 7.45, the soil was moderately saline according to the soil classification of Wicke et al. (2011), confirming its higher salt content due to regular flooding of the region. The endophytic bacteria from *Carex distans* were collected as described by Beirinckx et al. (2020). The resulting bacterial

suspension was subsequently applied to the roots of 2-week-old MicroTom tomato seedlings grown on half-strength Murashige and Skoog (½MS) media (for 1 L ½MS, 2.3 g MS powder, 0.5 g 2-morpholinoethanesulfonic acid [MES], and 8 g agar) plates containing 150 mM NaCl. Next, the roots of the best-growing tomato plants were sampled as described by Beirinckx et al. (2020). Possible root endophytic bacteria were isolated from the resulting bacterial suspension through both the dilution-to-extinction method and classical plating. For the dilution-to-extinction, the suspension was diluted 1,000 fold with Reasoner's 2A (R2A) medium (Difco Laboratories) supplemented with 4% (w/v) NaCl (Reasoner and Geldreich 1985), after which the diluted suspension was plated on R2A agar plates. The colonies were picked and streaked until purity of the cultures. The isolated strains were identified by amplification and sequencing of the 16S rRNA gene (Eurofins) as described by Beirinckx et al. (2020). Taxonomy was assigned with the Nucleotide BLAST in the National Center for Biotechnology Information (NCBI). Finally, after repetitive streaking, a total of six pure strains were isolated (data not shown). These strains were screened *in vitro* for growth promotion in Arabidopsis and MicroTom tomato plants under salt stress conditions (75 mM and 150 mM NaCl for Arabidopsis and tomato, respectively). The best performing strain, *Stenotrophomonas* SRS1, was selected for further experiments.

Plant material

Seeds of three tomato cultivars (MicroTom [Pieterpikzonenb.v., Luinjeberd, The Netherlands], Tolstoi F1 [Sluis Garden b.v., Enkhuizen, The Netherlands], and Money Maker [Sluis Garden]) were used. For *Arabidopsis thaliana* (L.) Heynh. plant assays, the accession Columbia-0 (Col-0) and different mutant lines (in Col-0 background) were used, namely *aba1* (SALK_027326C), *abi4-102* (CS3837), *abi5-1* (SALK_013163) for abscisic acid (ABA) biosynthesis and signaling, *axr1-30* (Gray et al. 2001), *35S:iaaL* (Jensen et al. 1998), and *tir1afb2/3* (Dharmasiri et al. 2005) for auxin biosynthesis and signaling. All seeds were gas-sterilized.

In vitro Arabidopsis assay

To test the effect of *Stenotrophomonas* SRS1 on Arabidopsis, gas-sterilized Arabidopsis Col-0 seeds and the mutant lines were shaken for 3 h in the bacterial suspension at OD_{600} 0.001 or with a phosphate-buffered saline (PBS) solution only. In total, five inoculated seeds were sown on one ½MS plate (12×12 cm) with or without addition of 75 mM NaCl, after which they were

placed at 4ºC for 2 days to synchronize germination. The plants were grown for 14 days at 21ºC under long-day conditions (18 h light/6 h dark regime). The roots, shoots, and total fresh weight were measured at 18 days after treatment (DAT). Each experiment was done in three repeats with four plates (20 plants) per repeat for every treatment.

Tomato plant assays

Firstly, an *in vitro* assay was performed for the analysis of growth promotion in which sterilized tomato seeds (MicroTom) were sown on ½ MS media plates for vernalization at 4ºC for 2 days, after which they were placed at 24ºC for 1 day in the dark to synchronize germination. Next, seeds were placed at 24ºC under a 16 h light/8 h dark photoperiod for germination. Four days after germination, eight uniformly germinated seedlings were transferred to one ½MS plate (24×24 cm) supplemented with or without 150 mM NaCl. After transfer, each seedling was inoculated with 20 µL *Stenotrophomonas* SRS1 resuspended in a PBS solution (OD_{600} 0.001; approximately $8.73\pm0.2\times10^6$ colony-forming units [CFU]/mL) or with 20 µL mock solution (PBS only) and returned to growth chamber conditions (24ºC, 16 h/8h light/dark). At 18 DAT, the roots, shoots, and total fresh weights were measured. The experiment was done in triplicate with 16 tomato plants per treatment.

In a second assay, the growth promotion was evaluated on tomato (MicroTom) grown in sterilized white sand (Nature Gravel, HS Aqua, The Netherlands). To this end, synchronized 4-day-old germinated seedlings (see above) were transferred from ½MS plates to pots (7.5×7.5 cm) filled with white sand. For each *Stenotrophomonas* SRS1 treatment, 12 plants (one plant/pot) were grown. The plants were kept in the greenhouse at 24ºC. Three days after transfer to white sand, 25 mL of a bacterial suspension (OD_{600} 0.001) or the mock solution (only PBS) was added to each pot. Fifteen and 18 days after bacterial inoculation, the salt treatment was done by adding 25 mL of 150 mM NaCl or H_2O (mock) solution to each pot. Every three days, each pot was watered with 25 mL of tap water. Fifteen days after the last salt treatment, the roots, shoots, and total fresh weights were measured. The experiment was done in triplicate with 12 plants per repeat.

Lastly, the growth promotion of *Stenotrophomonas* SRS1 was tested in a greenhouse assay on different tomato cultivars (MicroTom, Money Maker, and Tolstoi F1) grown in potting soil (assay 3). Before sowing, the seeds were inoculated by shaking for 3 h in a bacterial suspension (OD_{600} 0.001) or in a mock (PBS) solution. After inoculation, the seeds were pregerminated on germination paper at 24ºC for 4 days. The seedlings were transferred to

square pots (8×8×8.5 cm) filled with potting soil on trays containing 16 pots and grown at 24ºC in the greenhouse. The salt treatments were performed 15 and 18 days after bacterial inoculation by adding 2 L of 150 mM of NaCl solution to each tray. Every 7 days, two liters of tap water was added to each plant tray. The shoot fresh weight and leaf area were measured 15 days after the first salt treatment. The weight experiments were done in triplicate with 16 plants and 4 plants were used for the leaf area experiment.

Analysis of tomato fruit number and developmental stage

To analyze the fruit yield of tomato plants, the previously established greenhouse assay in potting soil was modified, i.e., 30 days after transfer to soil, just before the flowering stage, the salt treatment was done by adding 2 L of 150 mM NaCl to each tray (as described above). The fruits were harvested 60 days after the salt treatment, their fresh weight was analyzed, and their numbers were counted per color group (green, orange, and red), representing the ripening stage of the fruits. The experiment was done in triplicate with 16 plants for each treatment.

Analysis of root colonization by *Stenotrophomonas* SRS1

To observe the root colonization in Arabidopsis and tomato (MicroTom) plants grown at different salt concentrations, we used the previously established *in vitro* assay for tomato. The seedlings were inoculated with 1 µL and 5 µL of the bacterial suspension (OD_{600} 0.001) and transferred to ½MS plate (12×12 cm) supplemented with 0, 37, 75, and 115 mM NaCl and with 0, 75, and 150 mM NaCl for Arabidopsis and tomato, respectively. At 18 DAT, the roots were removed, weighted, and surface sterilized as described above. The last washing step of the surface sterilization was plated on R2A medium as a control for the sterilization (data not shown). Next, plant roots were crushed with sterile pestles and mortars and the disrupted tissue was resuspended in 1 mL PBS. Colony forming units per fresh weight (CFU/g FW) was determined by serial dilutions of the extracts on R2A plates after 72 h at 28°C.

Analysis of the proline content

The effect of bacterial inoculation on the proline content of Arabidopsis and tomato (MicroTom) was measured with the *in vitro* assay for Arabidopsis and the potting soil assay for tomato. The proline content was measured (Bates et al. 1973) at 4, 8, 12 DAT and the proline

levels were determined by absorbance at a wavelength of 520 nm. Proline solutions of 0, 20, 40, 100, 200, 400 µg/mL were used to generate a standard curve. The proline concentrations were measured and calculated per gram fresh weight (µmol/g fresh weight). The experiment was done in triplicate.

Analysis of the *Stenotrophomonas* SRS1 genome, phylogeny, and phylogenomics

Pure DNA of *Stenotrophomonas* SRS1 was extracted with an automated Maxwell DNA preparation instrument (Promega) as described by Tahon et al. (2018) and its full genome was sequenced with an Illumina HiSeq 4000 platform PE150 by the Oxford Genomics Center (University of Oxford, UK). The read quality, the genome assembly, the assembly quality, and the genome annotation were checked as described by Luo et al. (2019).

A maximum likelihood tree was constructed based on the 16S rDNA genes, including all the type strains of the *Stenotrophomonas* genus. The sequence alignment and the tree were created by means of the MEGA v10.0.5 software (Kumar et al. 2008) and visualized with the interactive Tree of Life v4.2.3 (Letunic and Bork 2016). To further analyze the phylogeny, 45 available genomes previously classified as *Stenotrophomonas* were downloaded from the NCBI database (https://www.ncbi.nlm.nih.gov/). By comparison of 107 essential core genes, a phylogenomic tree was constructed (Luo et al. 2019). Three strains belonging to the *Thermomonas* genus were used as an outgroup.

Bacterial gene expression analysis

For the analysis of the influence of a salt shock on the bacterial gene expression, *Stenotrophomonas* SRS1 was grown overnight at 28°C in R2A medium until OD_{600} 0.75, after which NaCl was added to the bacterial suspension to a final concentration of 4% (w/v) (approximately 684 mM NaCl). The salt shock experiment was done for 2 h at 28°C under shaking conditions. Bacterial pellets were obtained by centrifuging at 4,400*g* for 10 min and the resulting pellet was used for RNA extraction.

Additionally, the bacterial gene expression was monitored during plant inoculation. To this end, an overnight bacterial suspension culture was diluted to OD_{600} 0.001 and inoculated on plants grown in agar plates as indicated above with and without the addition of 75 mM NaCl and 150 mM NaCl for Arabidopsis and tomato, respectively. Seven days later, the roots were sampled and stored at -20°C until RNA extraction.

The total RNA of the bacterial suspensions and inoculated plant material was extracted with the TriZol method (Chomczynskiand Mackey 1995). cDNA was obtained with the qScript cDNA Synthesis Kit (Quantabio, Beverly, MA, USA) with the reaction components qScript cDNA superMix (5×) and RNA template (1 µg-10 pg). Quantitative real-time PCR (LightCycler 480II, Roche Diagnostics, Belgium) was run on three independent repeats and started with a preincubation at 95ºC for 5 min, followed by 45 cycles at 95ºC for 10 s, 60ºC for 10 s, and then at 72ºC for 10 s. The genes analyzed by qRT-PCR are described in Supplementary Table S1 and the primers were designed based on the *Stenotrophomonas* SRS1 whole-genome sequence with the Primer3 software (Supplementary Table S1).

Plant gene expression analysis

The expression of a selection of plant stress-responsive genes (Supplementary Table S2) was analyzed by qRT-PCR. Arabidopsis plants were grown in the *in vitro* plant assay as described above with some modifications. The Arabidopsis seeds were inoculated with a bacterial suspension (OD_{600} 0.001) or mock solution (PBS) and grown on ½MS with a nylon mesh (Sefar AG, Heiden, Switzerland). After 12 days of growth, the nylon mesh with the seedlings was transferred to a new ½MS plate supplemented with or without 75 mM NaCl. The root samples were collected at 3 h and 6 h after salt treatment. The RNA was extracted with the TriZol method as described above.

Measurement of bacterial salt and H_2O_2 tolerance and indole-3-acetic acid (IAA) production

To assess the salt and H_2O_2 tolerance of *Stenotrophomonas* SRS1, the strain was grown overnight in liquid R2A at 28ºC. The bacterial cultures were centrifuged and the pellets were resuspended in PBS buffer and the OD_{600} was adjusted to 0.001. PBS was used as mock treatment. Next, 1 µL of the bacterial suspension was added to each well of a 96-well plate filled with 200 µL R2A medium per well. In total, seven different concentrations of NaCl (from 0 to 7% or from 0 to 1.2 M) and H_2O_2 (from 0 to 7 mM) were added to the wells. Each treatment was tested in 12 wells (one row). The plates were incubated at 28ºC for one week. Three independent repeats were performed.

Additionally, the automated MilliDrop (Paris, France) system was used to identify the effect of 0, 2, 4, 6% of NaCl or 0, 0.17, 0.35, 0.5 mM of H_2O_2 on the growth curve of bacteria grown in R2A medium. The MilliDrop experiments were done according to the MilliDrop

protocol. In brief, the bacterial growth was measured in droplets containing the bacterial suspension (OD_{600} 0.01) by a spectrophotometer at different timepoints. The growth curves were finalized after 40 h and 20 h for the NaCl and H_2O_2 experiments, respectively, to prevent coalescence of the droplets. The bacterial droplets were grown at 28ºC.

The indole-3-acetic acid (IAA) produced by *Stenotrophomonas* SRS1 was determined via the Salkowski staining according to the protocol of Gordon and Weber (1951) with some modifications. The bacteria were grown in R2A medium supplemented with tryptophan (0.1%) and at salt concentrations ranging from 0 to 5% of NaCl for 2 days at 28ºC. Hereafter, the supernatant was obtained by centrifuging the bacterial suspension at 13,000*g* for 5 min. The IAA production was determined by adding the Salkowski reagent (81 mL Salkowski reagent: 1 mL of $FeCl_3$ 0.5 M, 30 mL H_2SO_4, 50 mL distilled water) to the supernatant solution. After incubation for 25 min at room temperature, the OD_{530} of the cultures was measured. The IAA concentration was quantified via a standard curve based on different IAA concentrations: 0, 5, 10, 20, 50, 100 µg/mL.

Statistical data analysis

The experiments were done in completely randomized designs and the data were expressed as means ± standard errors of three independent replicates**.** All data were tested for significance between control and bacterial treatments under saline and nonsaline conditions with the analysis of variance (ANOVA), followed by *post hoc* testing with the Duncan Multiple Range Test (DMRT). All analyses were done with the SPSS software version 25.0. The significance level is shown by different letters (a, b, c, and d) indicating significant differences ($\alpha = 0.05$).

Results

Stenotrophomonas SRS1 positively affects growth of Arabidopsis and tomato in different growth conditions

To test whether *Stenotrophomonas* SRS1, isolated from the roots of *Carex distans* increases growth when applied to salt-sensitive plants, we inoculated the bacteria on Arabidopsis grown on a vertical agar-based medium in the presence or absence of 75 mM of NaCl. Fresh shoot and root weights were analyzed at 18 DAT. Both under saline and nonsaline conditions, an increase in shoot, root and total fresh weights was observed (Fig. 1A). Detailed analysis further indicated

that inoculation with SRS1 increased the leaf area under both conditions (Fig. 1B). Furthermore, the root length of plants treated with SRS1 was longer than that of the control, but an increase in lateral root density was detected only when SRS1-inoculated plants were grown in the presence of 75 mM NaCl (Figs. 1C and 1D).

To evaluate the growth-promoting effect of SRS1 on tomato, we first used an assay similar to the Arabidopsis *in vitro* assay. SRS1 inoculation of tomato increased the shoot and total fresh weights significantly under both saline and nonsaline conditions, but the root fresh weight increased only in the absence of NaCl (Supplementary Fig. S1). For both Arabidopsis and tomato, we assessed whether addition of salt would affect colonization in the *in vitro* set-up. In both Arabidopsis and tomato roots, the colonization decreased with increasing salt concentrations (Supplementary Fig. S2).

After identification of the growth-promoting effect of SRS1 on tomato in the *in vitro* set-up, we carried out two additional experiments, one in white sand (Supplementary Fig. S3) and one in potting soil (Fig. 2) to analyze whether the positive impact was also observed in other substrates. When grown in white sand, the shoot fresh weight increased significantly after bacterial inoculation, with and without addition of 150 mM NaCl with a 1.16- and 1.41-fold change, respectively (Supplementary Figs. S3 A-C). Accordingly, the leaf area of the SRS1-inoculated plants was higher than those of the mock-inoculated plants under both saline and nonsaline conditions (1.38- and 1.23-fold change, respectively), but SRS1 had no significant effect on leaf numbers (Supplementary Figs. S3D and S3E). A similar effect was observed when the tomato plants were grown in potting soil. The shoot fresh weight was 1.37-fold higher after SRS1 inoculation than that of uninoculated plants under mock conditions and 1.42-fold higher under saline conditions (Figs. 2A and 2C). In agreement, the leaf area and leaf number increased under nonsaline and saline conditions upon SRS1 inoculation (1.31- and 1.41-fold change for the leaf area and 1.04- and 1.10-fold for the leaf number, respectively) (Figs. 2B, 2D, and 2E).

After analysis of the growth promotion by SRS1 on MicroTom in the different experimental set-ups, we examined the effect on the cultivars Money Maker and Tolstoi F1, grown in potting soil under saline and nonsaline conditions (Supplementary Fig. S4), but the SRS1 inoculation had no impact under both conditions. Thus, SRS1 inoculation promoted the growth of Arabidopsis and MicroTom tomato in saline and nonsaline conditions, also when the plants were grown in white sand and potting soil, although the effects varied between the different set-ups and cultivars used.

359 Impact of *Stenotrophomonas* SRS1 inoculation on tomato fruit development
360
361 In a next step, we wanted to evaluate whether inoculation with SRS1 could modify the tomato
362 fruit development. We analyzed the effect of SRS1 inoculation on fruit number, fruit weight,
363 and the fruit developmental stage of MicroTom tomato grown under saline and nonsaline
364 conditions. For the developmental stage, the number of green, orange, and red fruits was
365 counted, corresponding to immature, partially mature, and mature fruits, respectively. In
366 contrast to the previous analysis, the bacterial inoculation was done just before flowering (see
367 Materials and Methods). After SRS1 inoculation, the total number of fruits increased 1.23-fold
368 under nonsaline conditions (Figs. 3A and 3B). This result was obtained by enhancing the
369 number of green fruits (1.49-fold change) (Fig. 3B). Moreover, the total fruit weight increased
370 by a 1.17- and 1.20-fold change after bacterial treatment, without and with 150 mM NaCl,
371 respectively (Fig. 3C). Hence, inoculation with SRS1, not only promoted plant growth, it also
372 increased the fruit weights under both nonsaline and saline conditions, but the effect on the
373 developmental stage of the fruits was rather small and depended on the environmental
374 condition.
375
376 Whole-genome, phylogenomics and phylogenetic analyses of *S. rhizosphila* SRS1
377
378 The genome of SRS1 was sequenced and analyzed to identify potential genes involved in the
379 observed growth promotion activity. Additionally, to assess whether strain SRS1 belongs to an
380 already described *Stenotrophomonas* species, a maximum likelihood tree was constructed with
381 the full 16S rRNA of all the type strains that belonged to this genus (Supplementary Fig. S5).
382 The closest neighbor of strain SRS1 was *S. rhizophila* DSM14405^{T} (*S. rhizophila* e-p10),
383 followed by *S. bentonitica* BII-R7. For a deeper insight into the phylogeny of this strain and to
384 determine whether it belongs to the species *S. rhizophila*, a phylogenomic analysis was
385 performed. A phylogenomic tree was constructed with bcgTree (Ankenbrand and Keller 2016)
386 that integrates all the available strains of *S. rhizophila*, as well as some outliers belonging to the
387 different *Stenotrophomonas* species (Ankenbrandand Keller 2016). The tree topology indicated
388 that the SRS1 strain clustered together with some *S. rhizophila* strains (Fig. 4). *S. rhizophila*
389 BIGb0145, *S. rhizophila* IS26, and *S. rhizophila* USBAGBX843 were the most closely related
390 strains, with ANI values of 98.57, 93.48, and 93.45 %, respectively. However, strain SRS1 only
391 shared 85.95% identity with the type strain *S. rhizophila* DSM14405^{T}, well below the species
392 boundary of 95% ANI, suggesting that it does not belong to this species. Surprisingly, only *S.*

rhizophila CFBP 13529 and *S. rhizophila* CFBP 13503 shared an ANI value above a 95% with the type strain, implying that the species delimitation within the *S. rhizophila* clade might not be well established (Jain et al. 2018).

Next, we looked for bacterial genes related to salt tolerance (Supplementary Table S3). The *outer membrane protein A precursor* (*ompA*) and the osmoregulators *glucosylglycerol-phosphate synthase* (*ggpS*) and *glucosylglycerol transporter* (*ycaD*) that are involved in the synthesis and transport of the osmolyte glucosyl glycerol and that play an important role in the bacterial salt stress responses, were present in the genome (Alavi et al. 2013, 2014). Seven genes related to the spermidine biosynthesis pathway (*speE*) and its transport (*mdtI* and *mdtJ*) were also found. Spermidine can enhance plant growth under high and low temperatures, salinity, hyperosmosis, hypoxia, and atmospheric pollutants (García-Jiménez et al. 2007; Liu et al. 2007). Furthermore, we detected genes encoding the polysaccharide adhesin required for biofilm formation (*PgaA*, *PgaB*, *PgaC*, and *PgaD*) that can improve plant salt tolerance (Wang et al. 2004).

Additionally, the genome comprised several genes related to reactive oxygen species (ROS) scavenging (Supplementary Table S3), including catalases, peroxidases, alkyl hydroperoxide reductases, and superoxide dismutases, all involved in abiotic stress responses (Lázaro et al. 2013), and genes coding for trehalose biosynthesis (*ost*AB), which is linked to drought tolerance (Suárez et al. 2008). Regarding root colonization, genes that encode flagella biosynthesis and chemotaxis clustered together in the SRS1 genome (Supplementary Table S3). Moreover, additional genes involved in abiotic stress responses, such as heat and cold shock proteins, were found as well (Supplementary Table S3).

Hence, the genome of *S. rhizophila* SRS1 carries many genes that are related to salt stress tolerance as well as to root colonization. However, its phylogenetic position is not so clear, implying that the species delimitation within the *S. rhizophila* clade needs to be further ameliorated.

Stenotrophomonas SRS1 is resistant to high salt concentrations through the activation of salt stress-related genes

The genome analysis indicated that SRS1 might increase plant growth because it is adapted to grow in high salt concentrations and, hence, can trigger plant growth-promoting activities under these conditions. We tested whether the strain could indeed tolerate increasing salt concentrations during growth. Additionally, in high salt environments, plant roots increase the

production of H_2O_2 (Yen et al. 2013), which is toxic for bacteria (Clifford and Repine 1982). As several ROS-scavenging genes were detected within the SRS1 genome, we therefore examined whether SRS1 was tolerant to high H_2O_2 concentrations.

Bacterial growth was evaluated at different H_2O_2 and salt concentrations in a 96-well plate assay (Figs. 5A and 5B). SRS1 could thrive well up to 4% (w/v, approximately 684 mM) and much less in 5% (w/v, approximately 855 mM) NaCl, whereas growth was not detected anymore at higher concentrations. The salt concentration that SRS1 could stand was much higher than that of other soil-isolated bacterial strains, such as *Burkholderia phytofirmans* PSJN (Nowak et al. 1998) and *Caulobacter* RHG1 (Luo et al. 2019) (Supplementary Fig. S6A). The concentration of H_2O_2 in the medium also impacted the growth of SRS1, because this strain only survived up to the addition of 2 mM H_2O_2 (Supplementary Fig. S6B).

These results were further analyzed by a microfluidics-based technology (MilliDrop, Paris, France) that allowed us to see the influence of salt on the entire growth curve (Fig. 5C) (Jiang et al. 2016). Millidroplets (1 µL) were generated containing bacteria and growth medium at different salt concentrations. The start of the exponential growth phase was delayed already by the addition of 2% (approximately 342 mM) NaCl. This effect was even more pronounced at 4% and no growth was detected anymore at 6% (approximately 1.02 M) NaCl, in accordance with the results obtained in the 96-well plates.

Next, we assessed whether the expression of several salt-tolerant and stress-related bacterial genes changed when the bacteria were exposed to 4% NaCl for 2 h (see Materials and Methods). The genes analyzed are described in Supplementary Table S1. The mRNA levels of the salt stress-related *ycaD* and the osmoregulator-related genes *aquaporin* (*aqpZ*) and *ompA* were strongly and significantly induced after treatment with 4% NaCl. Additionally, *phosphoglycerol transferase* I (*mdoB*), a negative regulator of osmolarity (Miller et al. 1986), was significantly reduced upon the salt shock. Concerning the ROS-scavenging genes, only *catalase-peroxidase* (*katG*) and *superoxide dismutase* (Cu-Zn) (*SOD* [*Cu/Zn*]), were significantly upregulated, whereas the expression of the other analyzed ROS-scavenging genes did not vary upon salt treatment. Interestingly, the expression of the tested heat and cold shock-responsive genes showed a more than 2-fold significant increase upon salt treatment (Table 1). Together, this analysis reveals that SRS1 has the genetic capability to survive under high salt conditions and can capture salt through the osmoregulators *ggpS* and *ycaD* that decrease the intracellular osmotic potential necessary for osmotic water uptake and an increase in turgor and growth (Hagemann et al. 2008).

Understanding the growth-promoting mechanisms in Arabidopsis and tomato after SRS1 inoculation: contribution of the bacteria

To assess the underlying growth-promoting mechanisms, we analyzed the expression of bacterial genes related to plant growth promotion and stress tolerance during the interaction with Arabidopsis and tomato by qRT-PCR (Table 2). Inoculation of Arabidopsis under normal conditions had no effect on the expression of the bacterial genes related to plant growth and salt tolerance, whereas under salt stress conditions, *ycaD* and *ggpS* expression was upregulated compared to the expression found in the inoculum before application to the plant. By contrast, bacterial inoculation of tomato resulted in a downregulation of *speE, MdtI*, and *MdtJ* under both saline and nonsaline conditions, but in an upregulation of *ycaD, ggpS*, and *IroN* compared to the expression found in the inoculum before application. Hence, the expression of the genes of SRS1 related to plant growth promotion and salt stress tolerance were modulated in the presence of the plant, albeit in a different manner in the two plants analyzed.

Understanding the growth-promoting mechanisms in Arabidopsis and tomato after SRS1 inoculation: contribution of the plant

Next, we investigated whether SRS1 inoculation affected the mechanisms that Arabidopsis and tomato might use to enable the observed responses. First, we assessed whether SRS1 inoculation influenced the levels of proline, a well-known plant indicator of abiotic stress, in the plant leaves of both Arabidopsis and tomato plants. In both Arabidopsis and tomato, the presence of salt increased the proline levels, but treatment with SRS1 decreased the proline levels at 4 and 8 DAT and at 4, 8, and 12 DAT in salt-grown Arabidopsis and tomato, respectively (Fig. 6A).

As ABA is an essential phytohormone for plant growth that plays an important role in abiotic stress tolerance (Shinozaki and Yamaguchi-Shinozaki 2007; Sah et al. 2016), we analyzed by qRT-PCR whether SRS1 inoculation could modulate the expression of the ABA-dependent salt stress-related dehydration-responsive *Arabidopsis* gene *RD29A* and *RD29B* 3 h or 6 h after salt treatment (Fig. 6B; Supplementary Table S4). The expression of both genes was enhanced upon salt treatment and the enhancement was even more pronounced after inoculation with SRS1. The effect of SRS1 inoculation on the ABA pathway was further confirmed with the *ABI4::GUS*-expressing Arabidopsis line (Shkolnik-Inbar and Bar-Zvi 2011) (Fig. 6C). Under control conditions, GUS staining was observed in the shoot apex, vascular

system of the hypocotyl, and in the root tip. This expression pattern was unaffected by the SRS1 inoculation, but a more pronounced blue staining was visible in salt-grown plants. Interestingly, in salt-grown Arabidopsis plants, the staining in the region between the hypocotyl and the root was even more intense in SRS1-treated than that in untreated plants (red arrow, Figs. 6C, k and e).

Because of these changes, we tested the growth-promoting effect of SRS1 in the Arabidopsis ABA mutant lines *aba1*, deficient in the ABA biosynthesis process (Assmann et al. 2011) and *abi4-102* and *abi5-1*, insensitive to ABA signaling (Reeves et al. 2011). The growth promotion ratio of SRS1 inoculation compared to mock conditions (fold change) in Col-0 and different mutant lines was examined under both saline and nonsaline conditions (Supplementary Fig. S7A). Under normal growth conditions, the effect of SRS1 inoculation was similar for Col-0 and the different mutant lines, whereas under saline conditions, the SRS1 growth promotion was lost in the *aba1* and *abi4-102* mutant lines and did not differ in the *abi5-1* line. These results showed that SRS1 can alleviate salt stress on the plant through the ABA biosynthesis (ABA1) and the ABA signaling pathway (ABI4, *RD29A*, and *RD29B*).

Even though the common IAA production pathways in bacteria (indole-3-pyruvic acid and indole-3-acetamide) did not seem to occur in the SRS1 genome, the Salkowski reagent was used to corroborate the presence or absence of pathways for the biosynthesis of these auxins in SRS1. The capability of this strain to produce auxins was measured under different salt concentrations. When grown in 0-3% salt, SRS1 produced between 1.2 to 1.6 µg/mL IAA. When the salt concentrations exceeded 4%, the production of indolic compounds strongly decreased to become insignificant (Supplementary Fig. S6C). The expression of the auxin-responsive genes *IAR4* (IAA-alanine resistant 4), *AUX1* (auxin transporter protein 1), and *IAA12* (auxin-responsive protein 12) was analyzed by qRT-PCR in SRS1-treated Arabidopsis under both saline and nonsaline conditions (Fig. 7A; Supplementary Table S4). Salt treatment decreased the expression of all auxin-responsive genes in at least one timepoint. At 6 h after transfer, inoculation with SRS1 dampened this effect.

We further investigated the expression of the auxin-responsive reporter *DR5* by means of the *DR5::GUS* Arabidopsis line (Fig. 7B). Interestingly, the blue staining was intensified in the leaves, adventitious root primordia in the region between hypocotyl and root, and in the root after SRS1 inoculation under both conditions (red arrow, Fig. 7B). The Arabidopsis auxin mutants and transgenic lines available in the laboratory were analyzed for a growth-promoting response upon SRS1 inoculation under both saline and nonsaline conditions. These mutants include *axr1-30* (auxin signaling mutant of Auxin resistance 1) (Martinez-Garcia et al. 2020),

529 *35S:iaaL* (overexpressing the bacterial IAA lysine synthase that inactivates IAA), and
530 *tir1afb2/3* (auxin signaling mutant deficient in the auxin receptors). The growth-promotion ratio
531 of the SRS1 inoculation was subsequently compared to mock conditions (fold change)
532 (Supplementary Fig. S7B, Supplementary Table S4). The effect of SRS1 inoculation did not
533 differ between Col-0 and the different mutant lines under nonsaline conditions, except for
534 *tir1afb2/3*, of which the growth promotion was enhanced. Under saline conditions, the SRS1
535 growth promotion was lower in the *axr1-30* and *35S:iaaL* mutant lines and remained the same
536 in the *tir1afb2/3* line.
537 These results showed that changes in the auxin signaling in Arabidopsis can modulate the
538 growth-promoting effect of SRS1. Together, from these data we can see that also plant
539 responses are involved in the observed growth-promoting effects.
540
541 **Discussion**
542
543 Salt stress is one of the current threats in agriculture (Hall 2001; Martinez-Beltran and Manzur
544 2005; Shrivastava and Kumar 2015; Vaishnav et al. 2017; Shultana et al. 2020a). Among the
545 many different solutions to this problem, an attractive approach is the use of PGPR that reside
546 in and around plant roots. These PGPR can help plants to cope with abiotic stresses via direct
547 mechanisms, such as phosphate solubilization, nitrogen fixation, and IAA synthesis, or indirect
548 (secondary) mechanisms, such as antioxidant defense, VOC, exopolysaccharides (EPS),
549 biofilm formation, osmotic balance, and phytohormone signaling pathway (Goswami et al.,
550 2016). Indeed, *Stenotrophomonas* strains, such as *Stenotrophomonas maltophilia* SBP-9,
551 *Stenotrophomonas rhizophila* DSM14405^{T}, and *Stenotrophomonas maltophilia* BJ01 are
552 known PGPR that can alleviate salt stress of several crops, such as wheat, cucumber, and peanut
553 (*Arachishypogaea*) (Mayak et al. 2004; Egamberdieva et al. 2011; Alexander et al. 2020).
554 Although genes possibly responsible for the observed plant effects have been identified,
555 detailed underlying mechanisms for this growth promotion are still unknown (Singh et al.
556 2017). Here, to unravel these processes, we combined both physiological and molecular
557 methods and analyzed the interaction both from the bacterial and the plant point of view.
558 The strain used in this study had been isolated from salt-resistant *Carex distans* roots that
559 grew in sandy saline soils. Although 16S rRNA gene analysis revealed that the strain was
560 closely related to *Stenotrophomonas rhizophila*, further genome analysis indicated that it did
561 not belong to the same species, because it only shared an 85.95% ANI when compared to the
562 type strain. Similar outcomes were obtained for other *S. rhizophila*, with only *S. rhizophila*

563 CFBP 13529 and *S. rhizophila* CFBP 13503 sharing an ANI value above a 95% with the type
564 strain. These results suggest that strains currently designated *S. rhizophila* are rather
565 heterogeneous and that not all of them should be classified within this species. In particular,
566 strains SRS1 and BIGb0145 belong to the same *Stenotrophomonas* species (ANI value higher
567 than 95%), but are distinct from the group of the type strains representing the real *S. rhizophila*.
568 We thus confirm that there is an taxonomic inconsistency inside the genus *Stenotrophomonas*
569 (Ochoa-Sánchez and Vinuesa 2017).

570 Application of *Stenotrophomonas* SRS1 to MicroTom tomato cultivated *in vitro* on agar
571 plates and grown on white sand and soil, increased the total fresh weight of the plant shoots
572 whether salt had been added to the medium or not. On the agar medium, SRS1 inoculation
573 affected shoots and roots distinctly and enhanced the root fresh weight only in the absence of
574 salt. Additionally, further detailed analysis indicated an enlarged total leaf area. *S. rhizophila*
575 DSM14405^{T} had been reported to have a negative or no effect on root and shoot lengths,
576 respectively, whereas it positively affected the number of secondary leaves of tomato (cv.
577 Avicenne) when grown *in vitro* (Schmidt et al. 2012). An increase in leaf area could result from
578 the development of additional leaves (only observed on SRS1-inoculated soil-grown tomato)
579 or from an impact on foliar cell division or cell elongation (Luo et al. 2019; Martínez-Viveros
580 et al. 2010). Hence, it would be interesting to investigate on which of these pathways the
581 bacteria impinge to promote growth.

582 Although SRS1 inoculation could trigger growth of MicroTom, it could not of other
583 tomato varieties (Money Maker and Tolstoi F1), in the same soil set-up. Currently, this
584 observation cannot be explained, but one hypothesis is that, because the lifetime of these two
585 varieties is longer than that of MicroTom, the effect was studied too early in development
586 (Cordero et al 2018; Tank and Saraf 2010).

587 Salinity stress during the flowering and fruiting stages has been reported to reduce the
588 tomato yield, due to a decrease in the fruit numbers rather than due to an effect on the fruit size
589 (Zhang et al. 2017). Application of PGPR have been shown to increase the numbers and weights
590 of tomato fruit (Mena-Violante and Olalde-Portugal 2007; Berger et al. 2017; Widnyana 2019).
591 Similarly, SRS1 inoculation to tomato plants augmented the total fruit numbers under nonsaline
592 conditions, enhanced the fruit weights under both normal and salt stress conditions, and
593 increased the numbers of mature (red) fruits. The increase in the numbers of leaves and fruits
594 in SRS1-inoculated plants under salt stress condition may be caused by a rise in auxin levels
595 after inoculation, resulting in an expansion in fruit number and weight (Davies 2004; Cheng et
596 al. 2013; Arikan and Pirlak 2016) or by an improved utilization of the NPK (nitogen,

phosphorus, and potassium) fertilizer in the inoculated plants (Pirlak and Köse 2009; Abdel-Mawgoud et al. 2007). Our observation that auxin markers increased upon SRS1 inoculation in Arabidopsis supports the first hypothesis.

The ultimate goals of this study were to understand the molecular basis of the growth promotion and to investigate whether the growth promotion and salt tolerance were caused by bacterial and/or plant factors. The SRS1 whole-genome analysis revealed the presence of different stress-responsive genes, such as genes encoding osmoregulators, antioxidants, ROS scavengers, and heat and cold shock proteins. The expression of many of these genes was upregulated when the bacteria were exposed to 4% NaCl, indicating that the strain has active mechanisms to cope with high salt conditions. For instance, the genes *ggpS* and *ycaD*, involved in glucosyl glycerol synthesis and transport of osmoprotectants, respectively, have been reported previously to protect bacterial cells against high salt conditions (Ferjani et al. 2003; Hincha and Hagemann,2004). This was confirmed by the analysis of the bacterial growth and the ability of SRS1 to withstand high salt concentrations (approximately 5%) as well as to survive at 2 mM H_2O_2, which is known to accumulate in stress-exposed cells (Hossain et al. 2015).

To get a detailed insight into the effect of various salt concentrations on the entire growth curve, we studied growth with the MilliDrop system (Jiang et al. 2016). Increasing salt concentrations delayed the onset of the exponential phase. Hence, SRS1 could promote growth under saline conditions because it possesses the genetic tools to withstand the salinity stress, helping the plants as a consequence. Indeed, during the interaction with the plant, the salt stress-related genes *ycaD*, *ggpS*, *apqZ*, *ompA*, *GSS*, *katG*, *SOD (Cu/Zn)*, and *SoxR*, the heat shock-related genes *DnaK, DnaJ, GroES, GroEL,* and *Hsp90,* and the cold shock-related genes *GrpE, CspA, CspD*, and *CspE* were all upregulated (Alavi et al. 2013; Ferjani et al. 2003; Hincha and Hagemann, 2004). Moreover, genes correlated to plant colonization and growth promotion were also found in the SRS1 genome, for instance, the *PgaA, PgaB, PgaC*, and *PgaD* genes that encode the polysaccharide adhesin required for biofilm formation (Wang et al. 2004). Indeed, biofilm formation is a well-studied mechanism for plant abiotic stress alleviation by PGPR. Strains that produce EPS show potential for growth promotion of plants under salt stress conditions by forming hydrophilic biofilms (Rossi et al., 2012). The formation of such a capsule aids the water-holding capacity, resulting in enhanced nutrient uptake and water potential under salt stress (Upadhyay et al., 2011; Rossi et al., 2012; Shultana et al., 2020a). In future research, it would be interesting to analyze the production of EPS and the formation of a biofilm to confirm whether SRS1 alleviates plant salt stress in such a manner.

To address the question, whether, in addition to bacterial factors, also plant mechanisms might be involved in the observed growth promotion under saline and nonsaline conditions, we not only used tomato, but also the model plant Arabidopsis. SRS1 also promoted growth in Arabidopsis cultured on agar plates. Both with and without addition of salt, the shoot as well as the root weights increased because of an enlargement in the leaf area and mainly a root length elongation, although in the presence of salt, the lateral root density also increased.

One of the described mechanisms used by PGPR to increase salt tolerance in plants is the production of plant osmolytes (Abbas et al. 2019). Plants suffering from abiotic stresses, for example induced by high salt concentrations, accumulate proline to decrease the osmotic stress (Wang and Han 2009; Krasensky and Jonak 2012, Shultana et al. 2019). Previously, it has been reported that an increased proline content could be mediated by both ABA-dependent and ABA-independent signaling pathways (Pál et al. 2018). Our study revealed that SRS1 inoculation decreased the proline content in both Arabidopsis and tomato plants grown under salt stress conditions, which is in agreement with previous studies with *S. maltophilia* SBP-9 on wheat (Singh and Jha 2017) and *Sphingobacterium* BHU-AV3 on tomato (Vaishnav et al. 2020). From this result, it could be speculated that SRS1 colonization might reduce the salt levels sensed by the plant because the bacteria might form a biofilm to protect the plants. However, as already mentioned, more conclusive experiments should be performed to corroborate this hypothesis.

Additionally, PGPR have been described to promote plant growth via the modulation of plant hormonal pathways and the production of phytohormones (Hashem et al. 2016; Shultana et al. 2020b). PGPR have been confirmed to be able to produce various types of phytohormones, such as auxins, gibberellins, cytokinins, but also to utilize these phytohormones to activate stress-tolerant pathways under salt stress (Dodd et al. 2010, Hashem et al. 2016). ABA is a well-known plant hormone that orchestrates plant stress processes. SRS1 inoculation resulted in the upregulation of the salt stress-related gene markers *RD29A* and *RD29B*, and the *ABI4::GUS* expression. Additionally, in the *aba1* and *abi4-102* mutants, but not in the *abi5-1* mutant, the growth promotion was abolished under saline, but not under nonsaline conditions, illustrating that the ABA signaling is definitely an important aspect of the growth promotion under saline conditions.

By contrast, we used several auxin mutants and auxin expression markers to analyze whether auxin is involved in the observed effects. Salt stress reduced the expression of genes encoding *AUX1, IRA4,* and *IAA12* (Liu et al. 2015), whereas SRS1 inoculation dampened this effect and, accordingly, could upregulate the auxin marker *DR5::GUS*. Under saline conditions,

the growth-promoting effects were lost in the *35S:iaaL* and *axr1-30* lines, but did not differ in the triple receptor mutant. These results might indicate that auxin could possibly be only indirectly involved in the salt-induced growth promotion provoked by the SRS1 inoculation, because, for instance, the *axr1-30* mutant is also affected in other plant hormone pathways (Leyser et al. 1993; Timpte et al. 1995; Tiryaki and Staswick 2002). Nevertheless, the marker gene analysis indicated that the SRS1 inoculation seemingly prevented a decrease in auxin levels provoked by the salt treatment. Interestingly, the growth promotion ratio of the *tir1afb2/3* line was higher in nonsaline conditions than that of Col-0, in contrast to the effects seen for the other two mutants under saline conditions, eventually demonstrating that local auxin levels are important, but possibly without being directly involved.

Together and based on previous models about how the ABA and auxin pathways are involved in alleviating abiotic stresses (del Pozo and Estelle 1999; Shinozaki and Yamaguchi-Shinozaki 2007; Sharma et al. 2015), we propose that the SRS1 strain induces ABA biosynthesis, after which signaling via the ABI4-induced *RD29A* and *RD29B* expression activates salt tolerance. Simultaneously, SRS1 inoculation prevents the decrease in auxin biosynthesis and signaling and likewise in growth, resulting in growth promotion and salt stress tolerance (Fig. 8). The latter mechanism could possibly only happen in an indirect manner (Fig. 8).

Conclusion

Together we showed that the root endosphere of salt-tolerant plants is a source of interesting PGPR from which new biologicals can be developed and used in agriculture. Mode-of-action studies revealed that both the bacterial capacity to tolerate high salt conditions and the ability of the SRS1 to activate the ABA pathway and to prevent a decrease in auxin levels are mechanisms by which the growth promotion might happen. We now have a framework that will be used to obtain an in-depth understanding on how the growth is promoted in Arabidopsis and later in the crops themselves.

Acknowledgments

The authors thank Leo Declercq of the nature reserve “Het Zwin” for the collection of plants, Silvie Van den Hoecke (Tech Watch Innovation, VIB) and Jean-Baptiste Abbe (MilliDrop) for

the set-up and processing of the MilliDrop system, Annick De Keyser for technical support, and Martine De Cock for help preparing the manuscript.

Author contribution

H.M.T., M.V. and S.G. designed all the experiments. H.M.T. performed all the experiments analyzed the data statistically. S.G.M. and A.W. analyzed the whole-genome data. H.M.T. wrote the first draft of the manuscript. S.G.M., D.L., S.B., M.V. and S.G. revised it. All authors gave final approval for publication.

Funding

This work was supported by the Research Foundation-Flanders (grant G.0151.18N to A.W. and S.G.). H.M.T. is indebted to the Vietnam International Education Development (VIED), Ministry of Education, Vietnam for a PhD scholarship.

Data availability

The raw sequence data, assembly, and annotation of SRS1 will be deposited to the National Center for Biotechnology Information database.

Declaration of competing interest

The authors declare no conflict of interest.

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Figure legends

Fig. 1 Effects of *Stenotrophomonas* SRS1 inoculation on shoot, root, and total weights (A), leaf area (B), root length (C), and lateral root density (D) of Arabidopsis under nonsaline and saline conditions. Eighteen-day-old Arabidopsis roots and shoots were scanned, leaf area, root length, number of lateral roots were measured, and the fresh weight was sampled in three independent biological repeats. Values represent the mean ± SE. Different letters on each bar indicate significant differences (α= 0.05) after ANOVA and DMRT test

Fig. 2 Effect of *Stenotrophomonas* SRS1 inoculation on total fresh (A, C), leaf area (B, D), and leaf number (E) of soil-grown MicroTom (under nutrient-rich condition) in the greenhouse. Total fresh weight, leaf area, and leaf number of mock or SRS1-inoculated plants with or without 150 mM NaCl were measured after 1 month of growth and at 15 DAT in three independent biological repeats. Values represent the mean ± SE. Different letters on each bar indicate significant differences (α= 0.05) after ANOVA and DMRT test. Scale bar = 5 cm (A) and 2.5 cm (B)

Fig. 3 Effects of *Stenotrophomonas* SRS1 inoculation on fruit number and color (A, B) and weight (C) of six greenhouse-grown MicroTom plants. One-month-old mock and inoculated tomatoes were treated without and with 150 mM of NaCl. Fruit number or weight were measured after 3 months of growth or at 60 DAT in three repeats. Values represent the mean ± SE. Different letters on each bar indicate significant differences (α = 0.05) after ANOVA and DMRT test. Scale bar = 3 cm (A)

Fig. 4 Whole-genome comparison of *Stenotrophomonas* SRS1 with other *Stenotrophomonas* genus. Forty-five available genomes previously classified as *Stenotrophomonas*sp. and three genomes belonging to *Thermomonas* sp. from the NCBI database were used for the classification of the type strains, obtained by comparison of 107 essential core genes.

Fig. 5 Salt and H_2O_2 tolerance of *Stenotrophomonas* SRS1. The 96-well plate assay was used for the determination of the salt (A) and H_2O_2 (B) tolerance and the MilliDrop experiment (C) for the salinity test only. The 96-well plate assay was done after 7 days at 28^{o}C and MilliDrop experiment after 40 h under different salt concentrations with three independent repeats.

Fig. 6 Effects of *Stenotrophomonas* SRS1 inoculation on the proline content and ABA responses. (A) Proline content of 15-day-old Arabidopsis and tomato leaves treated without or with 75 mM and 150 mM NaCl. The leaves were sampled for each condition with four repeats in total. (B) Expression of ABA-responsive genes in 10-day-old Arabidopsis inoculated or mock-treated plantlets without or with 75 mM NaCl and sampled at 3 and 6 h for qRT-PCR. (C) Expression of *ABI4::GUS* in inoculated or mock-treated plants under normal and salt stress conditions. *ABI4::GUS* mock-treated seedlings under normal conditions (a, b, and c) and under saline conditions (d, e, and f); *ABI4::GUS* inoculated seedlings under nonsaline conditions (g, h, and i) and under saline conditions (j, k, and l). The *ABI4::GUS* seeds were treated without and with 75 mM and stained with GUS at 7 DAT. Scale bar = 200 μm. Values represent the mean ± SE. Different letters on each bar indicate significant differences ($\alpha = 0.05$) after ANOVA and DMRT test. The statistics for the proline content (A) and ABA- responsive genes (B) were done separately for each different timepoint

Fig. 7 Effects of *Stenotrophomonas* SRS1 on auxin-responsive genes and auxin-responsive marker lines under normal or saline conditions. (A) Auxin-responsive genes in 10-day-old inoculated or mock-treated Arabidopsis plantlets treated without or with 75 mM NaCl and sampled at 3 and 6 h. (B) Expression of *DR5::GUS* in inoculated or mock-treated seedling under nonsaline and saline conditions. *DR5::GUS* mock-treated seedling under nonsaline (a, b, and c) and on saline conditions; *DR5::GUS* inoculated seedlings under nonsaline (e, f, and g) and saline (h, I, and j) conditions. The *DR5::GUS* seeds were treated without or with 75 mM NaCl and stained with GUS at 7 DAT. Scale bar = 200 μm. Values represent the mean ± SE for all data. Different letters on each bar indicate significant differences ($\alpha = 0.05$) after ANOVA and DMRT test. The statistics on the auxin-responsible genes (A) were done separately for each timepoint

Fig. 8 Mode of action of *Stenotrophomonas* SRS1 promoting plant growth under salt stress conditions. In the presence of salt, SRS1 upregulates salt-responsive genes to cope with the stress and, additionally, might produce IAA that indirectly affects the growth promotion. On the plant side, upon perception of SRS1, two hormonal pathways change, enhancing growth promotion. First, SRS1 inoculation increases the ABA biosynthesis (ABA1) and, possibly, activates the abiotic stress-responsive genes (*RD29A* and *RD29B*) via the transcription factor *ABI4*. At the same time, SRS1 indirectly reduces the negative effects of salt on the auxin pathway, hence, promoting growth

Table 1 Expression of *Stenotrophomonas* SRS1 genes responsive to 4% of NaCl compared to the control. *Stenotrophomonas* SRS1 was grown overnight at 28°C (OD600 0.75) and treated with or without NaCl for 2 h. The experiment was done with three independent replicates. Data were validated based on ANOVA analysis, **P*<0.05, ***P*<0.01, ****P*<0.001; ns, no significant.

Function	**Gene**	**Fold change**
Osmoregulation	*ycaD*	$7.80^{(***)}$
	ggpS	$1.25^{(**)}$
	aqpZ	$9.60^{(**)}$
	mdoD	1.32^{ns}
	mdoB	$0.58^{(***)}$
	ompA	$9.57^{(***)}$
Antioxidation	*GST*	1.11^{ns}
	GSS	$1.42^{(*)}$
	CAT	1.00^{ns}
	GPx	1.26^{ns}
	katG	$2.15^{(***)}$
	SOD (Cu/Zn)	$3.84^{(**)}$
	SOD (Mn)	0.83^{ns}
	SOD (Fe)	0.86^{ns}
	SoxR	$3.32^{(**)}$
	oxyR	1.03^{ns}
	Ccp551	0.91^{ns}
Heat shock	*DnaK*	$4.41^{(***)}$
	DnaJ	$2.31^{(**)}$
	GroES	$2.19^{(**)}$
	GroEL	$2.77^{(*)}$
	Hsp90	$1.64^{(***)}$
Cold shock	*GrpE*	$2.18^{(***)}$
	CspA	$4.10^{(**)}$
	CspD	$2.22^{(***)}$
	CspE	$5.28^{(***)}$

Table 2 Expression of *Stenotrophomonas* SRS1 genes during of Arabidopsis and tomato roots compared to the control (fold change). SRS1-inoculated Arabidopsis and tomato plants were grown without or with NaCl added to the agar medium *in vitro* and sampled at 7 DAT. ANOVA analysis, **P*<0.05, ***P*<0.01, ****P*<0.001; ns, not significant.

No.	**Gene**	**Root**			
		Arabidopsis		MicroTom	
		0 mM	75 mM	0 mM	150 mM
1	*speE*	0.69^{ns}	2.05^{ns}	$0.07^{(***)}$	$0.07^{(***)}$
2	*MdtI*	1.33^{ns}	0.58^{ns}	$0.67^{(**)}$	$0.69^{(*)}$
3	*MdtJ*	0.64^{ns}	1.23^{ns}	$0.35^{(***)}$	$0.34^{(***)}$
	ggpS	2.92^{ns}	$12.21^{(*)}$	$4.12^{(***)}$	$3.96^{(***)}$
5	*ycaD*	3.25^{ns}	$6.57^{(***)}$	$4.95^{(***)}$	$4.60^{(***)}$
6	*IroN*	2.92^{ns}	7.40^{ns}	$2.64^{(***)}$	$2.72^{(***)}$