Research Article

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Increase in phenolic compounds of *Coriandrum sativum* L. after the application of a *Bacillus halotolerans* biofertilizer

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Abstract

BACKGROUND: There is an urgent need for a new sustainable way of satisfying the increasing demand for food worldwide. One of the main challenges is replacing chemical fertilizers with biofertilizers, which include plant root-associated beneficial microorganisms. The present study reports, for the first time, the effects of SCCPVE07 bacterial strain with respect to improving not only plant development, but also the nutritional content and bioactive compounds content of *Coriandrum sativum* L., one of the most economically important crops, even for plant growth under salinity stress.

RESULTS: Innoculated coriander plants (*C. sativum* L.) showed an increase in potassium, carbon, calcium and iron content. A significant improvement in phenolic compounds contents was also observed. The contents of 5-*O*-caffeoylquinic acid, cinnamic acid, 4-methoxy-cinnamic acid hexoside, *K*-3-*O* rutinoside, *Q*-3-*O*-rutinoside, *Q*-3-*O*-glucoside and *Q*-3-*O*-glucuronide were significantly enhanced. Moreover, an efficient bacterial root colonization and a noted growth promotion were demonstrated. Bacterial genome was sequenced and analysed. Gene coding related to Plant growth promotion (PGP) mechanisms and proteins involved in plant defence from salinity or in the metabolism of phenolic compounds, such as quercetin 2,3-dioxygenase and phenolic acid decarboxylase, were identified.

CONCLUSION: The results obtained in the present study show, for the first time, the beneficial effects of the inoculation of a bacterial *Bacillus halotolerans* biofertilizer on coriander crops with respect to increasing the content in bioactive compounds and plant development.

Keywords: PGPR; coriander; bioactive compounds; flavonols; phenolic acids

INTRODUCTION

It is well known that the intake of fruits and vegetables in the human diet is correlated with wellness as aq result of proven health benefits according to the protective effect of the bioactive substances such as phenolic compounds and carotenoids.[1] Among phenolic compounds, flavonoids and phenolic acids have been related to a decreased risk in many chronic diseases.[2]

Flavonols are the most widespread of the flavonoids in plant food. They can be found not only as free forms, but also as glycosylated derivatives, with quercetin, kaempferol and myricetin being the most abundant flavonol structures, which are usually glycosylated mainly at their C-3 position. The presence of the glycosylation has an important impact on both the flavonol biological activity and its bioavailability. Free forms have shown lower bioavailability than the corresponding glycosylated forms and the type of sugar moiety linked to the quercetin molecule plays an important role in its absorption.[3]

Nowadays, vegetables with high antioxidant potential such as coriander are in high demand by consumers, as a result of the beneficial effects for human health. Coriander (*Coriandrum sativum* L.) is currently one of the most economically important aromatic crops.[4] Apart from its uses in folk medicine, pharmacy and food industries, coriander is a herb used throughout the world as a spice.[5] The presence of phenolics compounds in coriander plant are associated with a significant antioxidant activity.[5] In addition to food preservation, the importance of the antioxidant content and its beneficial effect on many chronic diseases have been investigated in many scientific studies according to the

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essential oil content.[6] These compounds vary strongly depending on the cultivar, climatic conditions and geographic position.

On the other hand, climate change is helping to deteriorate overall yield productions as a result of the severity of many non-biological stresses such as high temperatures, droughts or soil salinity. Up until now, farmers worldwide apply to agricultural lands a significant quantity of chemical fertilizers, which are extensively reported not only to be hazardous for human health, but also to affect ecological harmony in the environment to maintain crop productions.[7] The major issue for agriculture over the world is soil salinity; every year about 1–2% in the arid and semi-arid zones turns to useless agricultural land.[8] Salinity limits growth and plant development causing serious biochemical and physiological changes in crops.[9]

The world urgently needs a new sustainable and eco-friendly way of satisfying the current and increasing demand for food. One of the main challenges is replacing chemical fertilizers with biofertilizers, which include plant root-associated beneficial microorganisms (PGPR) with several direct and indirect plant growth promotion mechanisms, such as phosphate solubilization, nitrogen fixation or phytohormones production.[10] Beginning many decades ago, researchers have focused on the use of PGPRs to increase growth and yield plants further mineral content.[11] However, the role of these bacteria in management of abiotic stress and quality food is becoming more and more important.

Many rhizospheric bacteria are described as potential candidates for useful biofertilizers, although food safety practices must be considered, requiring the use of innocuous bacteria, particularly on vegetables that are consumed raw.[12] *Bacillus* is one bacterial genus that has received most extensive attention as a result of *in vitro* mechanisms, as well as specific metabolic and physiological traits, including the formation of stress resistant-endospores, which facilitate the formulation into commercial fertilizers.[13] *Bacillus halotolerans*, is a rhizobacterium with the potential to promote plant growth and increase tolerance to salinity stress. The availability of an efficient genome analysis is currently improving the application of new isolated *Bacillus* strains as biofertilizers.[14]

The present study aimed to analyse the effect on the plant growth development and on the phenolic composition and mineral content of coriander plants after the inoculation of a probiotic bacterial strain from genus *Bacillus* under saline conditions.

MATERIALS AND METHODS

Bacterial isolation and taxonomical identification

Bacillus halotolerans SCCPVE07 was isolated from the nodules of *Phaseolus vulgaris* L. plants, collected in San Cristobal de la Cuesta, Salamanca, España (41°01′41.0″N, 5°37′24.1″W). The protocol of isolation was described by Vincent[15] using plates containing tryptic soy agar (TSA) medium Difco (Franklin Lakes, NJ, USA) (pancreatic digest of casein 15.0 g L^{-1}, papaic digest of soybean 5.0 g L^{-1}, sodium chloride 5.0 g L^{-1} and agar 15.0 g L^{-1}).

DNA for amplifying and sequencing 16S rRNA was extracted using the REDExtract-n-Amp™ (Sigma, St Louis, MO, USA) in accordance with the manufacturer's instructions. First, amplification was performed using 2 µL of bacterial DNA (100 µg mL^{-1}) as the template, 6 µL of miliQ sterile water, 12.5 µL of REDExtract Ready Mix, 2.5 µL of 27F 1 µmol L^{-1} (5′ GAGGGTGGCGGTTCT 3′) and 2.5 µL of 1522R 1 µmol L^{-1} (5′AAGGAGGTGATCCANCCRCA 3′). The polymerase chain reaction (PCR) conditions comprised: preheating at 95 °C for 9 min, 35 cycles of denaturing at 94 °C for 1 min; annealing at 56.5 °C for 1 min and 30 s and extension 72 °C for 2 min, and a final extension at 72 °C for 7 min.

PCR product was purified using QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) in accordance with the manufacturer´s specifications. The sequence reaction was performed on an ABI PRISM sequencer (EEUU; Applied Biosystems, Foster City, CA, USA) using a 'Dye Terminator Cycle Sequencing Ready Reaction Kit' (Sequencing Service, University of Salamanca, Salamanca, Spain). The isolate was identified using Blastn.[16]

Draft genome sequencing and annotation

The genomic DNA for genome sequencing was obtained from pure colonies of SCCPVE07 strain grown on TSA plates and collected after 24 h at 28 °C, using the ZR Fungal/Bacterial DNA MiniPrep (Zymo Research, Irvine, CA, USA).

The draft genome sequence of the isolated was obtained by shotgun sequencing on an MiSeq platform (Illumina, Inc., San Diego, CA, USA) via a paired-end run (2 × 251 bp). The sequence data was assembled using Velvet 1.2.10.[17] Gene calling and annotation was performed using RAST 2.0 (Rapid Annotation using Subsystem Technology) (http://rast.theseed.org). The SEEDviewer 2.0 framework[18] was used to search for genes.

Colonization of coriander roots

Coriander seeds (*C. sativum* L.) were surfaced-sterilized with a solution of NaClO 5% for 10 min. The seeds were then washed six times with sterile water and germinated on water-agar plates. The seedlings were kept in the dark at 28 °C for 4 days, and then transferred to 1.5% agar square plates (12 × 12 cm), with a distribution of five seedlings per plate. To prepare the suspension, the SCCPVE07 strain was grown for 1 day at 28 °C on TSA plates flooded with sterile water to obtain bacterial suspension, which was transferred to a sterile flask. The suspension was adjusted to an optical density (OD) at 600 nm of 0.6, 10^8 colony-forming units (CFU) mL^{-1}. A micropipette was used to inoculate each seedling with 250 µL of the suspension on the roots. The plates were maintained in a growth chamber and observed at 7 and 13 days post inoculation.

After the incubation days the coriander roots were immersed in a solution of 3% milk and Triton 0.03% in 1 × phosphate-buffered saline (PBS) for 1 h at room temperature. Subsequently, the roots were washed three times with PBS for 5 min. Then, uricase antibody (dilution 1: 50) was added and left overnight at 4 °C. Roots were again washed with a solution of PBS three times, after fluorescein isothiocyanate goat antibody (dilution 1:200) was added and the mix was incubated 1 h at room temperature. Three PBS washes were performed again for 5 min each. Next, coriander roots were incubated with DAPI (4′,6-diamino-2-phenylindol, which is a fluorescent label) for 10 min. Finally, three washes with 5-min PBS were made again. Propidium iodide 10 µm was added into the roots 10 min before microscopy observation. Fluorescence microscopy was carried out with a Eclipse 80i fluorescence microscope (Nikon, Tokyo, Japan).

In vitro growth promotion of seedlings

Surfaced-sterilized coriander seedlings were transferred to pots (diameter 20 cm^3) containing sterilized vermiculite 'SEED PRO 6040' (PROJAR, Valencia, Spain) as substrate. The coriander plants were inoculated with 2 mL of bacterial suspension, 10^8 CFU mL^{-1} (OD at 600 nm of 0.6). Sixty pots were used as a negative control and 60 pots received the addition of bacterial suspension. In each treatment, half of the plants were irrigated with water from a

bottom reservoir every 48 h and the other half with a saline condition (NaCl 100 mmol L^{-1}). Pots were maintained in a growth chamber. Fifteen days post inoculation data of fresh weight per plant, dry weight per plant and leaf size were recorded.

Coriander growth promotion and plant samplings

The ability to promote plant growth was investigated on coriander plants using a mix of non-sterilized soil and vermiculite (3:1 v/v) as substrate. Volumes of 2.4 L of substrate were placed in plastic pots with a capacity of 2.5 L.

The seeds were previously surface-sterilized and pregerminated as explained above. They were then transferred into the substrate and inoculated with 5 mL of the strain suspensions with a final concentration of 10^8 CFU mL^{-1} (OD at 600 nm of 0.6). To obtain these suspensions, the cells of the strain SCCPVE07 cultivated on TSA plates overnight at 28 °C were suspended in sterile water. Uninoculated coriander plants were included as negative controls under the same conditions. The plants were irrigated with water from a bottom reservoir every 48 h and one solution of NaCl 100 mmol L^{-1} (to obtain saline conditions).

The plants were maintained in a greenhouse illuminated with natural light in summer (night temperature ranging from 15 to 20 °C, and day temperatures ranging from 25 to 35 °C), with humidity controlled for 40 days. In total, 18 plants divided into nine pots were used in each treatment. Stem length, shoot fresh weight and shoot dry weight per plant were recorded. Chlorophyll relative content was obtained by the successive measuring of leaves with a chlorophyll meter SPAD-502PLUS (Konica Minolta, Osaka, Japan).

The dry plants were used for the analysis of C, Fe, N, P, K, Ca and Mg, which was performed by the Ionomics Service at CEBAS-CSIC (Murcia, Spain), using Elemental Analyst model TruSpec CN628 equipment (Leco, St Joseph, MI, USA) for the N analysis, and ICP THERMO ICAP 6500DUO equipment (Thermo Fisher, Waltham, MA, USA) for the analysis of the remaining elements.

Phenolic compound analysis

Freeze-dried plant samples (5 mg) were extracted for 30 min using MeOH:H2O 80:20 (8 mL) in an bath of ultrasound. Extraction was repeated three times and the supernatants were gathered, cleaned-up by liquid–liquid extraction with hexan for chlorophyll separation, and concentrated under reduced pressure until a final volume of 2 mL. The phenolic composition of the extracts was determined by high-performance liquid chromatography (HPLC)-diode ray detection-mass spectroscopy using a Hewlett-Packard 1200 series liquid chromatograph (Agilent Technologies, Waldbronn, Germany) coupled to an API 3200 Qtrap (Applied Biosystems, Darmstadt, Germany) equipped with an electrospray ionization source and a triple quadrupole-ion trap mass analyser. A Spherisorb® S3 ODS-2 C18 reversed phase, 3 µm, 150 × 4.6 mm column (Waters Corporation, Milford, MA, USA) was used with thermostat at 35°C. The HPLC conditions were optimized for the analysis of theses samples, using an aqueous solution (0,1%) of formic acid (A) and acetonitrile (B) as solvents. The gradient used for the phytochemical characterization of the extracts was: from 0% to 15% B in 5 min, isocratic 15% B for 5 min, from 15 to 20% B in 5 min, from 20 to 25% B in 10 min and from 25 to 35% B in 10 min. Detection was carried out at 280, 330 and 370 nm as the preferred wavelengths and spectra were recorded from 220 to 600 nm. Mass spectrometry analysis was performed using the previously developed methodology for non-anthocyanin compounds.[19] Briefly, zero grade air was used as the nebulizer gas (30 psi) and turbo gas (40 psi, 400 °C and nitrogen served as the curtain (20 psi) and collision gas (medium). The ion spray voltage was set at −4500 V in the negative mode and spectra were recorded in negative ion mode between *m/z* 100 and 1000. The MS detector was programmed to perform an enhanced MS to show full scan spectra followed by an enhanced product ion to show the fragmentation pattern of the main detected compound.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) followed by Fisher's protected least-significant difference test. Statistical analysis was performed using StatView, version 5.0 (SAS Institute Inc., Cary, NC, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Bacterial strain isolation and genome mining

The SCCPVE07 strain was isolated from surface-sterilized bean root nodules. Based on the SCCPVE07 16S rRNA gene sequence obtained, the highest sequence showed a 100% similarity with *B. halotolerans* ATCC 25096^T. The draft genome obtained contains 33 contings with an average genome length of ∼4 Mbp, a G + C content of 43.9% and 4188 predicted coding sequences. The draft genome sequence was deposited in GenBank under accession number VMOB00000000.

SCCPVE07 genome encoded genes related to the biosynthesis of indol-3-acetic acid. Indole-3-glycerol phosphate synthase (EC 4.1.1.48), tryptophan synthase beta chain (EC 4.2.1.20) and tryptophan synthase alpha chain (EC 4.2.1.20) were found.

Related to bacterial plant root attachment, the SCCPVE07 genome encoded genes involved in extracellular polysaccharide biosynthesis and proteins that form part of the biofilm matrix (tasA) important to bacterial adhesion or genes involved in cellulose production [beta-1,4-glucanase (cellulase) (EC 3.2.1.4)], as also reported for its function in bacterial root colonization.[20]

Moreover, the SCCVPVE07 genome encodes genes involved in the acquisition, transport or biosynthesis of essential elements for bacterial and plant development. It was observed to encode genes involved in siderophore biosynthesis, such as bacillibactina (*dhbA*, *dhbB*, *dhbC*, *dhbE* and *dhbF*) or anthrachelin (*AsbA*, *AsbB*). In this sense, the SCCPVE07 genome also encoded genes related to enhanced element content, such as those involved in nitrogen fixation and assimilation (nitrogen regulatory protein P-II) or magnesium transport (magnesium transport protein CorA). Thus, SCCPVE07 genome encodes enzymes involved in making insoluble phosphorous compounds available, such as lysophospholipase (EC 3.1.1.5), pyrophosphatase (EC 3.6.1.1), manganese-dependent inorganic pyrophosphatase (EC 3.6.1.1) and alkaline phosphatase (EC 3.1.3.1), which are involved in transforming inorganic substrates into more easily assimilable chemical forms.

The accumulation of solutes such as choline, proline and glycine betaine is especially important in terms of comprising a natural defence system against salinity.[21] The SCCPVE07 genome encodes a wide range of genes important in the osmotic response, as related to the biosynthesis, transport and the utilization of these solutes, such as alcohol dehydrogenase GbsB (type III), essential for the utilization of choline (EC 1.1.1.1) and the choline ABC transport system, as well as permease protein OpuBA, OpuBB, OpuBC and OpuBD. Proline dehydrogenase (EC 1.5.5.2), proline/sodium symporter PutP (TC 2.A.21.2.1) and proline dipeptidase (EC 3.4.13.9) were also found.

The SCCPVE07 genome encodes a wide variety of genes important in the metabolism of flavonoid and phenolic acids, such as quercetin 2,3-dioxygenase QdoI (EC 1.13.11.24) involved in the catabolism of quercetin and rutin,[22] phenolic acid decarboxylase related to the food-processing industry and the catalysation and the synthesis of phenols,[23] or cinnamyl alcohol dehydrogenase/reductase (EC 1.1.1.195) involved in cinnamic acid biosynthesis.[24]

Analysis of coriander root colonization

Bacterial colonization was studied via a root immunolocalization experiment and analysed by fluorescence microscopy. It was observed that the SCCPVE07 strain attached to the root surfaces of innoculated coriander seedlings increased gradually during the observations. Figure 1 reveals that the strain colonizes the root surfaces, occupying intercellular spaces in cortical cells, as well as the base of emerging lateral roots, and forms typical biofilm initiation microcolonies.

Effects of *Bacillus* strain on plant growth both *in vitro* and in greenhouse

The results of the plant growth promotion *in vitro* experiments (Table 1) showed that the strain SCCPVE07 promotes the growth of coriander plants in several parameters under non-stress conditions and under salt stress. In both cases, higher growth values were always shown in coriander plants induced by the SCCPVE07 strain. The stem length was significantly increased by 22.6% and 19.4% in the case of SCCPVE07 strain application compared to the respective control in both treatments (Table 1). In the same way, for the SCCPVE07 strain, coriander plants showed higher fresh and dry shoot weights. The largest increases (47.8% and 32.3%) were shown for the shoot dry weight of coriander plants inoculated with SCCPVE07 under non-saline conditions and under saline stress, respectively.

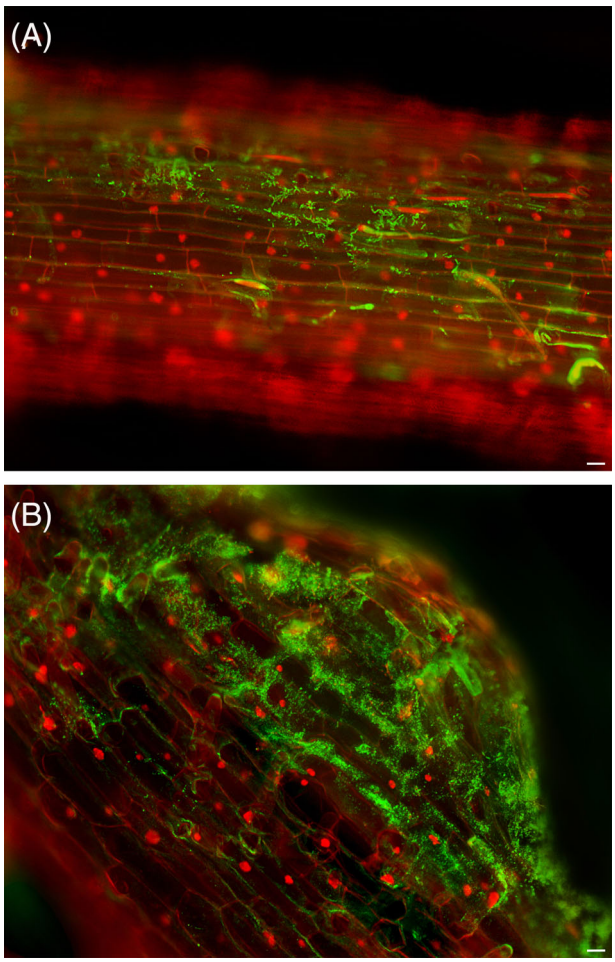

Figure 1. Bacterial root colonization. Fluorescence optical micrographs of coriander seedlings roots obtained at (A) 7 days and (B) 14 days after inoculation. The micrographs show the ability of SCCPVE07 strain (green colour) to colonize the root surfaces, base of emerging roots and the initiation of microcolonies (scale bar = 150 μm).

The results described above were also checked in greenhouse experiments (Table 1), which showed that the SCCPVE07 strain promotes the growth of coriander plants because innoculation with this strain led to an increase in several plant growth parameters. Bacterial inoculation enhanced the stem length by up to 5.9% and 17.6%, respectively, compared to controls. The shoot dry weights were significantly increased by the SCCPVE07 strain not only under non-saline conditions (+15.8%), but also under salt stress (+23.5%). Bacterial innoculation also improved the chlorophyll content under both conditions. However, the largest increase was shown under saline conditions; coriander plants inoculated with the SCCPVE07 strain increased by up to 34.1% compared to the respective un-inoculated plants.

According to the significant enhancement of growth parameters, the nutrient content of coriander plants in both treatments was also analysed (Table 2). The results showed important improvements in nutrient plant content compared to un-innoculated plants. Most values are statistically higher under bacterial innoculation. However, the phosphorus content showed the greatest growth, by up to 38.2% under non-saline conditions and 111.9% under saline stress, compared to the respective controls. The plant content of elements necessary for crops, such as nitrogen, were also increased under bacterial innoculation, by up to 11.9% and 13.1%, respectively.

Effects of *Bacillus* strain on plant phenolic composition

The phenolic composition of the coriander plants is reported in Table 3. The identity of the phenolic compounds was determined on the basis of the UV-visible spectra and the retention time in the chromatographic analysis and the molecular ion $[M\text{-}H]^-$ and the fragmentation pattern in the mass spectrometry analysis. Different phenolic acids and flavanol structures were determined. Within phenolic acids, vanillic, cinnamic and caffeoylquinic acid derivatives were the most abundant. On the other hand, quercetin derivatives (rutinoside, glucuronide and glucoside) were the main flavonols determined in the samples.

Table 4 reports the effect of the *B. halotolerans* SCCPVE07 inoculation on the concentration of phenolic acids in coriander plants. Under non-saline conditions, the innoculation leads to a small but significant increase in the total phenolic acid content. The content of vanillic acid was much higher in the inoculated plants, whereas the levels of cinnamic acid derivatives decreased as a result of the inoculation. By contrast, under saline conditions (100 mmol L^{-1} NaCl) a significant increase was observed in the inoculated coriander plants compared to the non-inoculated ones not only for the total phenolic acid concentration, but also for the concentration of all the phenolic acid derivatives, except vanillic acid. Likewise, the concentration of the 5-*O*-caffeoylquinic acid in the inoculated plants was almost twice the concentration of this compound compared to the corresponding control plants.

Table 1. Results from *in vitro* growth promotion experiments and effects of *Bacillus halotolerans* SCCPVE07 inoculation on the growth and chlorophyll content of coriander plants under greenhouse conditions

	Non-saline conditions			100 mM NaCl		
	Control	SCCPVE07		Control	SCCPVE07	
	Mean ± SE	Mean ± SE	%	Mean ± SE	Mean ± SE	%
Results from *in vitro* growth promotion experiments						
Stem length (cm)	10.81 ± 1.56	13.25 ± 2.32*	+22.58	11.37 ± 2.02	13.57 ± 2.51*	+19.35
SFW (g)	0.212 ± 0.01	0.272 ± 0.01*	+28.31	0.282 ± 0.01	0.310 ± 0.01*	+9.93
SDW (g)	0.023 ± 0.01	0.034 ± 0.01*	+47.83	0.031 ± 0.01	0.041 ± 0.01*	+32.26
Greenhouse conditions						
Stem length (cm)	32.30 ± 0.62	34.20 ± 0.54	+5.89	27.90 ± 0.28	32.81 ± 0.58*	+17.60
SFW (g)	13.71 ± 0.19	15.63 ± 0.33*	+14.00	12.10 ± 0.14	14.84 ± 0.25*	+22.64
SDW (g)	1.52 ± 0.02	1.76 ± 0.04*	+15.79	1.36 ± 0.02	1.68 ± 0.03*	+23.53
Chlorophyll (SPAD Units)	31.16 ± 0.61	31.26 ± 0.56	+0.32	24.39 ± 0.40	32.70 ± 0.61*	+34.07

*Significant difference between values, $P = 0.05$ according to Fisher's protected least significant difference.
SFW, shoot fresh weight; SDW, shoot dry weight.

Table 2. Effects of *Bacillus halotolerans* SCCPVE07 inoculation on nutrient contents of coriander plants

	Non-saline conditions			100 mM NaCl		
	Control	SCCPVE07		Control	SCCPVE07	
	Mean ± SE	Mean ± SE	%	Mean ± SE	Mean ± SE	%
N (g 100 g^{-1})	2.44 ± 0.09	2.73 ± 0.02	+ 11.89	3.06 ± 0.02	3.46 ± 0.11	+ 13.07
C (g 100 g^{-1})	40.56 ± 0.07	41.16 ± 0.08	+ 1.48	37.39 ± 0.06	41.36 ± 0.08*	+ 10.62
P (g 100 g^{-1})	0.68 ± 0.04	0.94 ± 0.05*	+ 38.24	0.67 ± 0.01	1.42 ± 0.03*	+ 111.94
Mg (g 100 g^{-1})	0.91 ± 0.06	0.94 ± 0.07	+ 3.30	0.81 ± 0.01	0.81 ± 0.01	=
Ca (g 100 g^{-1})	1.62 ± 0.08	1.64 ± 0.07	+ 1.23	1.60 ± 0.01	2.24 ± 0.05*	+ 40.00
Fe (mg kg^{-1})	123.51 ± 6.31	344.80 ± 59.2*	+ 179	277.29 ± 14.82	254.38 ± 6.55	-

*Significant difference between values, $P = 0.05$ according to Fisher's protected least significant difference.

Table 3. Phenolic composition of coriander plants

Compound	t_R (min)	UV λ_{max} (nm)	$[M-H]^-$ (*m/z*)	Fragment ions (*m/z*)	Identification
1	10.4	284	167	152	Vanillic acid
2	10.7	328	369	207 189	Dimethoxy-cinnamic acid hexoside
3	11.4	328	369	207 189	Dimethoxy-cinnamic acid hexoside
4	13.2	314	353	189 127	Cinnamic acid derivative
5	13.7	324	353	191	5-*O*-caffeoylquinic acid
6	14.3	322	339	177	4-methoxy-cinnamic acid hexoside
7	16.2	322	355	193	Ferulic acid glucoside
8	26.4	354	609	301	Quercetin-3-*O*-rutinoside
9	26.9	355	477	301	Quercetin-3-*O*-glucuronide
10	27.9	356	463	301	Quercetin-3-*O*-glucoside
11	30.5	354	593	285	Kaempherol-3-*O*-rutinoside

Under both saline and non-saline conditions, the inoculated plants showed higher total concentration of flavonols (Table 5), with the difference for the control samples being more important in the case of saline conditions. Under non-saline conditions, the inoculated plants showed the highest total flavonol content but the lowest levels of quercetin 3-*O*-rutinoside. Moreover, in both cases (saline and non-saline conditions), quercetin 3-*O*-glucuronide is the flavonol derivative for which levels showed the most important increase.

Table 4. Effect of *Bacillus halotolerans* SCCPVE07 inoculation on the concentration of phenolic acids of coriander plants

	Non-saline conditions		100 mmol L^{-1} NaCl	
	Control	SCCPVE07	Control	SCCPVE07
Vanillic acid	14.31 ± 0.47	18.80 ± 0.54*	14.55 ± 0.47	12.90 ± 0.18*
Dimethoxy-cinnamic acid hexoside	0.61 ± 0.07	0.70 ± 0.05	0.61 ± 0.04	0.73 ± 0.04*
Dimethoxy-cinnamic acid hexoside	13.79 ± 0.24*	11.30 ± 0.41	12.72 ± 0.23	14.47 ± 0.21*
Cinnamic acid derivative	0.61 ± 0.03*	0.48 ± 0.02	0.75 ± 0.01	0.91 ± 0.01*
5-*O*-caffeoylquinic acid	3.00 ± 0.05	3.68 ± 0.02*	5.52 ± 0.03	10.23 ± 0.03*
4-methoxy-cinnamic acid hexoside	0.98 ± 0.03*	0.83 ± 0.02	1.55 ± 0.04	1.69 ± 0.04*
Ferulic acid hexoside	0.86 ± 0.05*	0.62 ± 0.03	0.73 ± 0.05	0.77 ± 0.04
Total phenolic acids	34.14 ± 0.42	36.43 ± 0.61*	36.42 ± 0.41	41.69 ± 0.27*

*An asterisk (*) within the same row and within each type of condition (non-saline and saline) indicates a significant difference according to the least significant difference test ($P < 0.05$).

Table 5. Effects of *Bacillus halotolerans* SCCPVE07 inoculation on the flavonol composition of coriander plants

	Non-saline conditions		100 mmol L^{-1} NaCl	
	Control	SCCPVE07	Control	SCCPVE07
Q-3-*O*-rutinoside	7.14 ± 0.096*	5.89 ± 0.02	6.15 ± 0.03	8.46 ± 0.046*
Q-3-*O*-glucuronide	2.71 ± 0.01	5.17 ± 0.086*	2.64 ± 0.06	5.42 ± 0.066*
Q-3-*O*-glucoside	0.31 ± 0.02	0.27 ± 0.01	0.30 ± 0.01	0.41 ± 0.026*
K-3-*O*-rutinoside	0.83 ± 0.036*	0.48 ± 0.03	0.69 ± 0.03	1.01 ± 0.016*
Total flavonols	10.98 ± 0.07	11.81 ± 0.076*	9.78 ± 0.08	15.30 ± 0.076*

An asterisk (*) within the same row and within each type of condition (non-saline and saline) indicates a significant difference according to the least significant difference test ($P < 0.05$).
Q, quercetin; K, kaempherol.

DISCUSSION

Subsequent to the emergence of genome mining technology, it has been considered as a useful tool for identifying functionally important genes and, as more and more genomic information becomes available, the development of genomic technologies is able to provide further insights into plant–microorganism mutualistic interactions.[25]

Based on *in silico* analyses, strain SCCPVE07 appears to exhibit tremendous potential as a plant growth-promoting bacterium. The SCCPVE07 genome encodes important genes related to plant–microbe interaction. They include some that are related to the biosynthesis of phytohormones, which are reported to be one of the mechanisms through which bacteria interact with crops in plant development and many physiological processes.[26] The SCCPVE07 genome encodes several genes related to the biosynthesis of indol-3-acetic acid, which is reported to be one of the most important phytohormones for stimulating plant growth promotion.[27]

Moreover, one of the most important factors in microbial plant growth promotion is coping with other microorganisms in the plant rhizosphere. In this sense, as *B. halotolerans* SCCPVE07 colonizes the plant root, it produces siderophores, which are described as an efficient mechanism for inhibiting the growth of bacteria or fungi by depriving them of iron or protecting from the action of antibiotic secreted by other competing microorganisms.[28]

Soil microorganisms have been described having as an important role in natural phosphorus cycling, as a result of solubilizing and precipitating phosphorus processes. In general, these processes depend on pH and soil type.[29] According to several enzymes involved in making insoluble phosphorous compounds available for plant cellular growth, biological solubilization is currently a focus of great special attention. The results of the present study are in agreement with the studies by Banerjee *et al.*[30] and Maheswar and Sathiyavani,[31] which also report several *Bacillus* strains with higher phosphate solubilizing activity.

The most effective mechanism in plants for coping with stress conditions is the biosynthesis of secondary metabolites such as phenolic compounds[32] or osmolytes.[33] According to the soil-salinity problem and its negative effects, a biofertilizer capable of reducing this problem in plants is very promising. In this sense, we suggest that biofertilization with SCCPVE07 can diminish the negative impact as a result of the potential production and biosynthesis of several phenolics acids and flavonols.

Related to *Bacillus* genus, many studies have described genes (as also found in the present study) involved in plant growth promotion. Whole transcriptomic analysis of the strain *Bacillus amyloliquefacines* SQR9 colonising maize plant roots revealed that some of the most upregulated genes were related to biofilm formation.[34] The genome of other reported PGPR *Bacillus* strains also included genes linked to mineral phosphate solubilization, siderophore and exopolysaccharide production, and root surface attachment.[29]

It is commonly accepted that efficient PGPR strains colonize roots under a wide range of conditions, predominantly by forming biofilms.[35] Bacterial plant root attachment is essential for successful colonization. This process relies on a variety of cell, such as

pili, flagella and extracellular polysaccharides, which play a major role in the initial surface adhesion.[36] The prevalence of biofilm formation is well-recognized and enhances the role of PGP traits. Root colonization is an essential step for plant growth-promoting bacteria,[37] and bacterial adhesion is essential for the production of several metabolites involved in plant growth, such as indole acetic acid and siderophores, or for the ability to mobilize plant nutrients, which are relevant characteristics in PGPR.

The results of the present study revealed that SCCPVE07 presented a colonization dynamic as reported in other *Bacillus* strains in different horticultural crops roots.[34] However, the present study is the first to report *B. halotolerans* as an efficient colonizer of coriander roots.

The results obtained in the present study indicate an increase in *Bacillus* species that promote plant growth, as well as the number of horticultural crops suitable for biofertilization with *Bacillus* species. Both of the plant assays carried out in a growth chamber and in a greenhouse showed that *B. halotolerans* promoted the plant growth of coriander plants under normal conditions and under salt stress. In the initial stages of growth, the coriander seedlings inoculated with SCCPVE07 strain have longer stems than the uninoculated controls, suggesting that this strain exerts a positive effect on plant development, which was confirmed in the greenhouse experiments. In both, SCCPVE07 inoculation promotes the growth of the edible parts of coriander plants. These plants are bigger and have more chlorophyll content, increasing the greenness of the leaves, which is highly appreciated in vegetables that are mostly consumed raw. A significative chlorophyll degradation has been reported under salt stress as a result of chlorophyllase and other enzymes that reduce the production of photosynthetic pigments.[38] However, biofertilization with SCCPVE07 enhances chlorophyll concentration even under saline conditions. Other studies have also reported a significant increased production of photosynthetic pigments in horticultural inoculated plants under salt stress.[39,40]

Collectively, these results suggest that the SCCPVE07 strain increases nutrient plant content via the promotion of plant growth, which significantly enhances the uptake of soil nutrients. This finding is probably related and based on those genes encoded in the SCCPVE07 genome, as previously described to be involved in the biosynthesis of many relevant enzymes.

According to the results reported in Table 4, it appears that, under normal conditions, inoculation has different effects depending on the phenolic acid because an increase in the content of vanillic acid was observed, whereas there was a decrease in the content of cinnamic acid derivatives. By contrast, under saline conditions, inoculation with this strain appears to favour the biosynthesis of phenolic acids, which would lead to higher levels of phenolic acids in coriander plants. Although, in some plants, the antioxidant response to salinity stress stimulates the biosynthesis of phenols,[41] no significant differences were observed between coriander control plants. It is worth highlighting the important increase in the 5-*O*-caffeoylquinic acid concentration in inoculated plants, which demonstrated almost twice the concentration of this compound compared to the corresponding control plants. This compound is the most abundant chlorogenic acid in some foods such as coffee where it has shown remarkable antioxidant activity.[42]

Moreover, the inoculation of coriander plants with *B. halotolerans* SCCPVE07 subjected to both conditions (saline and non-saline conditions) leads to a higher total concentration of flavonols in the inoculated plants, with the difference being more important for the control samples in the case of saline conditions (Table 5). However, considering individual flavonols, the effect is different depending on the developmental conditions. Under non-saline conditions, inoculation with this strain led to a decrease in the rutinoside derivatives and to an increase in the glucuronide derivatives. This could be important because it might indicate that the inoculation could affect the biosynthetic routes of flavonols. Moreover, differences in the glycosylation pattern have been related to differences in the bioavailability of these compounds.[3] The effect of inoculation is different under saline conditions because, in this case, the concentration of flavonols is significantly higher in the inoculated samples compared to the controls. Thus, the inoculated samples under saline conditions can accumulate higher levels of flavonols, which have been related to antioxidant and enzyme inhibitory activities in humans.[19]

According to the genome mining, the results suggest that the SCCPVE07 strain can influence the biosynthesis of phenolics compounds through the production of enzymes involved in their pathways. However, it has been also suggested previously in other PGPR strains that the alteration in plant phenolic compounds in response to bacterial colonization may be the result of a general plant defence response that is subsequently suppressed.[43] Thus, the microbial attenuation of plant phenolic compounds is an important aspect in the establishment of an effective plant–microbe interaction.

CONCLUSIONS

In the present study, we have analysed, for the first time, the effects of inoculation with a *B. halotolerans* strain in coriander plants, showing that it leads to significant increases in plant development, in addition to carbon content, as well as phosphorus and calcium plant contents. The leaves from plants inoculated with the SCCPVE07 strain have significant higher amounts of phenolic acids, such as dimethoxy-cinnamic acid hexoside, cinnamic acid derivate, 5-*O*-caffeoylquinic acid, 4-methoxy-cinnamic acid hexoside and ferulic acid. Moreover, plants inoculated with the SCCPVE07 strain have also in addition significant higher amounts of flavonols (*Q*-3-*O*-rutinoside, *Q*-3-*O*-glucuronide, *Q*-3-*O*-glucoside and *K*-3-*O*-rutinoside). These results showed that inoculation with *Bacillus halotolerans* SCCPVE07 is a good agronomical practice, improving the content of several phenolic compounds of coriander plants, as well as increasing their nutritional and healthy potential. Biofertilization with this bacterium presents advantages over chemical fertilization and further studies on their effects on the phenolic compounds of other food and medicinal plants will be very useful with respect to the design of future bacterial biofertilizers, which could be effectively used also under saline stress conditions.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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