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RESEARCH

Exiguobacterium sp. as a bioinoculant for plant-growth promotion and Selenium biofortification strategies in horticultural plants

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Abstract

Plant growth-promoting rhizobacteria (PGPR) have a positive effect on plant development and being a promising way to enhance crop productivity and as substitution of chemical fertilizers. Selenium (Se) is an important trace element and its intake is usually lower than the daily minimum amount required for humans; hence, there is a demand on the design of Se biofortification strategies. Here, the genetic traits known to be associated with Plant-Growth Promotion (PGP) and Se biotransformation of *Exiguobacterium* sp. S17 were evaluated through genome analysis. Its growth-promoting capacity was tested through plant-growth promotion assays in laboratory and field conditions, using *Brassica juncea* (indian mustard), *Beta vulgaris* (chard), and *Lactuca sativa* (lettuce). Additionally, the Se biotransformation ability of *Exiguobacterium* sp. S17 was evaluated and the obtained selenized bacteria were tested in mustard plants. The sequenced bacteria genome revealed the presence of multiple genes involved in important functions regarding soil and plant colonization, PGP and Se biotransformation. Moreover, it was demonstrated that *Exiguobacterium* sp. S17 enhanced plant growth and could be useful to produce Se accumulation and biofortification in accumulator plants such as mustard. Thereby, *Exiguobacterium* sp. S17 might be used for developing new, sustainable, and environmentally friendly agro-technological strategies.

Keywords Plant-growth promotion · Selenium nanoparticles · Bioinoculant, *Exiguobacterium* spp.

Introduction

Bacteria of the genus *Exiguobacterium* are commonly found in soil, water, and stromatolite and are characterized by their ability to transform or degrade multiple organic and inorganic toxins (Ordoñez et al. 2013, 2015; Akkoyun et al. 2020). *Exiguobacterium* strains could be of biotechnological interest due to their diverse functions and possible applications (Chauhan et al. 2015; Kasana et al. 2018). Several studies have focused on different activities relevant to economic and ecological processes, such as bioremediation, heavy metal fortification in crops, and agricultural applications as plant-growth promoters (Kumar et al. 2006; Okeke 2008; Bharti et al. 2013; Kasana and Pandey 2018). In agronomic systems, it has been suggested that *Exiguobacterium* species may promote plant growth, solubilize phosphate, and produce indole-3-acetic acid (IAA) and exopolysaccharide (EPS) (Dastager et al. 2010; Bharti et al. 2013; Chauhan et al. 2015). In addition to the reported properties, *Exiguobacterium* strains could be used in new biotechnological processes.

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For this reason, the present study explored the possible application of *Exiguobacterium* sp. S17 as a bioinoculant for plant-growth promotion and its utilization in selenium (Se) biofortification strategies in horticultural plants.

In horticultural production and sustainable agriculture, the substitution of chemical fertilizers by plant growth-promoting bacteria (PGPB) is desirable due to the reduction in costs and the low environmental impact. Although the application of fertilizers plays a fundamental role in increasing agricultural production, their excessive use not only causes pollution and a potential risk to humanity but also irreversibly alters the ecology of the soil, further reducing the area available for crop production (Jones et al. 2001; Vejan et al. 2016; Wang et al. 2019). In this sense, the isolation, characterization, and use of plant growth-promoting bacteria as bio-based fertilizers is one of the promising ways to enhance crop productivity and manage plant diseases (Sturz and Nowak 2000; Venkadesaperumal et al. 2014).

Although Se is not essential in plants, it promotes their growth and development (Pilon-Smits and Quinn 2010) and induces the expression of genes related to antioxidant defense and Se-assimilation. Moreover, Se induces the production of polyphenols, which are known to scavenge free radicals and inhibit membrane lipid peroxidation, enhancing plant resistance to oxidative stress (Zahedi et al. 2019). Selenium is also an essential trace element in humans and other vertebrates. It is involved in immune system modulation, cancer prevention, brain development and cognitive aspects, thyroid hormone metabolism, and cardiovascular protection (Beckett and Arthur 2005; Pedrero et al. 2006; Papp et al. 2007; Nicastro and Dunn 2013; Palomo-Siguero and Madrid 2017). In several countries, Se intake is lower than the minimum daily requirement due to the shortage of this element in crop food sources and the low concentration of Se in soils (Combs 2001; Rayman 2008). To overcome this problem, various Se biofortification strategies for different crops have been used, such as exogenous incorporation in ionic forms and, more recently, in the form of nanoparticles (Moreno-Martin et al. 2019). These strategies showed promising results in coping with Se deficiency (Perez et al. 2019). In nature, Se is found as selenide (Se^{2-}), elemental Se (Se^0), and soluble salts selenite (SeO_2^{-3}) and selenate (SeO_2^{-4}), which are the most toxic Se forms (Martínez et al. 2020). Some bacteria can biotransform Se salts into the seleno-amino acids selenomethionine (SeMet) and selenocysteine (SeCys) (Pescuma et al. 2017; Martínez et al. 2020), and into seleno-nanoparticles (SeNPs) containing mainly Se^0 (Palomo-Siguero et al. 2016; Moreno-Martín et al. 2020). Organic Se species (such as SeCys and SeMet) and seleno-nanoparticles (SeNPs) are less toxic and can be absorbed and transported by plants more efficiently than Se salts (Moreno-Martin et al. 2020).

The aims of the present work were to analyze the plant growth-promoting capacity and the Se biotransformation ability of *Exiguobacterium* sp. S17 using genome sequence as a tool to identify the key functions of the bacterial strain and to confirm these activities both in laboratory and field conditions. Additionally, the beneficial effects of the application of selenized *Exiguobacterium* sp. S17, such as plant growth promotion, micronutrient biofortification, and an increase in Phenolic compounds content and antioxidant activity were evaluated in mustard, a Se secondary accumulator model species.

Materials and methods

Bacterial strain

For this study, bacterial strain *Exiguobacterium* sp. S17, isolated from Socompa lake stromatolite (Ordoñez et al. 2013) in the high-altitude Andean Puna, was obtained from the culture collection of LIMLA-PROIMI-CONICET. The strain was stored at −20 °C in distilled H_2O with 20% (v/v) glycerol. Before performing the assays, cells were transferred to fresh Luria–Bertani (LB) broth (Britania) and cultured overnight (16 h) at 30 °C.

Analysis of genes that contribute to plant-growth promotion and Se biotransformation in Exiguobacterium sp. S17 genome

A comprehensive set of 78 genes coding for known Plant Growth-Promoting Rhizobacteria (PGPR) related proteins and Se metabolism were selected from the literature; their protein sequence IDs were retrieved from genomes of species of the genus *Bacillus,* using the NCBI Protein and the KEGG databases (*B. megaterium* QM B1551, *B. cereus* ATCC 14579, and *B. subtilis* QB928). Then, other protein sequence IDs were retrieved from the Gram-negative proteobacteria genomes (*Burkholderia* sp. KJ006, *Pseudomonas fluorescens* F113, and *Pseudomonas aeruginosa* PAO1), using the NCBI Batch Entrez. Homolog genes of these proteins/protein sequence were searched using Blastp in *Exiguobacterium* sp. S17 genome (DDBJ/EMBL/GenBank accession number ASXD01000000, Ordoñez et al. 2013). Only best hits with an E-value cut-off of $1e^{-05}$ (≤ 0.00001) were retained. Moreover, proteins were considered to be homologs only if they shared the same Pfam functional protein domains. Protein domain assignment was performed using InterProScan.

Plant-growth promotion assays

The ability of *Exiguobacterium* sp. S17 to enhance plant growth was first tested in the laboratory under controlled conditions using *Brassica juncea* (Indian mustard). Indian mustard was chosen as a model species because it is a well characterized and fast growing plant, tolerant to Se, and with the ability to accumulate Se (Hanson et al. 2003). Then, plant growth promotion was tested in a field assay using commercial production conditions. For the field assay, two of the most popular crops and with the largest production area among salad crops in the world were used: *Beta vulgaris* L. var Bressane (chard) and *Lactuca sativa* L. var Criolla verde (lettuce)**.**

In the laboratory assay, to evaluate whether *Exiguobacterium* sp. S17 could actually promote mustard growth, two treatments and two control groups were carried out with 10 replicates (10 independent pots) each. In the first treatment, mustard seeds were immersed in an *Exiguobacterium* sp. S17 suspension (1.5×10^8 CFU/mL) for 12 h at room temperature and shaking speed of 120 rpm. Bacterial suspensions used in these treatments were grown in Standard Medium 1 broth (ST1) (meat peptone 15 g/L, yeast extract 3 g/L, NaCl 6 g/L and glucose 1 g/l) at pH 7.6 (selected in previous assays as the optimal media) for 48 h at 30 °C. As control groups, mustard seeds were immersed in sterilized water and ST1 broth without any bacteria. Then, ten mustard seeds were sown in each 2-L plastic pot (10 independent pots per treatment) filled with a mixture of soil and volcanic sand (1:1). After 1 week, plants were thinned by leaving three plants per pot. A second set of ten pots with pre-inoculated seeds was prepared for the re-inoculated treatment. In these treatments, plants obtained from seeds immersed in bacterial suspension (as mentioned above) were re-inoculated after 40 days with 100 mL of the corresponding bacterial suspension (1.5×10^8 CFU/mL), directly on plant substrates. At the same time, control groups were irrigated with 100 mL of ST1 media or 100 mL of sterilized water. Plants were grown in a growth chamber with high-pressure sodium (HPS) lamps with a 17/7 h photoperiod at 21 °C, $50 \pm 10\%$ relative humidity, and regularly irrigated at field capacity. After 8 weeks, ten plants from each treatment were carefully removed from the pots, cleaned, and dried at 60 °C until constant weight in order to determine dry weight.

In the field assay, to evaluate whether *Exiguobacterium* sp. S17 could act as a bioinoculant to induce plant-growth promotion in chard and lettuce, a bacterial suspension was applied to both plant species. The study was carried out in the Experimental Organic Orchard of the CIEFAP (Esquel, Chubut Province, Argentina) from November to December 2021. Seeds were immersed in an *Exiguobacterium* sp. S17 suspension growth in ST1 broth for 12 h at room temperature and shaking speed of 120 rpm (2×10^8 CFU/mL). Control groups were incubated in distilled water or ST1 broth without bacteria for the same period. Seeds were sown directly in soil in continuous rows (60 cm × 12 m) with a density of 15 plants/m^2. A drip irrigation system was installed to provide the required water, and weeds were manually removed. Plants obtained from seeds were re-inoculated after 24 days with 370 mL/m^2 of the bacterial suspension (2×10^8 CFU/mL) directly on plant substrates. Control plants were watered with distilled water or sterile ST1 broth. After 40 days, ten plants were harvested, cleaned and dried as described above. To evaluate plant growth promotion, five variables were determined: leaf length and width, root length, fresh weight, and dry weight.

Then, for laboratory and field assays, Kruskal–Wallis nonparametric tests and Conover-Inman a posteriori tests were used for all pairwise comparisons ($\alpha = 0.016$, including Bonferroni correction, assuming chi-square distribution with 2 df). Statistical analyses were performed with SYSTAT 13 software Version 13.00.005 (Systat Software, Inc.).

Selenium biotransformation by Exiguobacterium sp. S17

Optimal microbial growth in the presence of Se was evaluated. Cultures of *Exiguobacterium* sp. S17, previously grown in LB media broth (pH 7.6), were inoculated at 2% (v/v) in LB broth supplemented with different Na_2SeO_3 (Sigma-Aldrich Chemical Co., MO, USA) concentrations (0, 10, 20, 40, 80, 160, 320, 640 mg/L of Se) in a microplate at 30 °C and 150 rpm for 48 h. The optimal Na_2SeO_3 concentration and incubation period were determined when the deepest red color of the cultures was observed and cell growth inhibition was detected.

Additionally, to evaluate the uptake of Se and the Se biotransformation ability of *Exiguobacterium* sp. S17, the bacterium was grown in LB broth with or without 160 mg/L of Se at 30 °C and at a shaking speed of 150 rpm for 48 h. Concentration of total Se in the cell pellets was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after total digestion of the samples in closed vessels containing 1 mL of concentrated HNO_3 and 0.5 mL of H_2O_2 30% (v/v), using a microwave oven (MSP microwave oven, CEM, Matthews, NC, USA). The resulting solutions were cooled down, diluted to a 25 mL final volume with MilliQ water, and further analyzed for Se concentration with a Perkin Elmer NexION 350X ICP-MS with hydrogen gas as collision gas in Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI)-CONICET, Rosario, Argentina. Finally, the presence of the seleno-nanoparticles (SeNPs) produced by *Exiguobacterium* sp. S17 was observed by Transmission Electron Microscopy (TEM FEI TECNAI F20 G2) at Laboratorio de Microscopía de Física de Materiales, Centro Atómico Bariloche, Comisión Nacional de Energía Atómica.

Samples were prepared by placing a drop of culture onto 300-mesh lacey carbon copper TEM grids. The film on the TEM grids was dried for 5 min at room temperature before analysis. The diameter of SeNPs was measured from the TEM images by using the image-processing software ImageJ 1.52 (Wayne Rasband, National Institutes of Health, USA; website: https://imagej.nih.gov/ij/).

Effect of selenized Exiguobacterium sp. S17 on plant growth, Se and nutrient accumulation, total phenolic compound concentration, and antioxidant activity

To analyze the plant growth-promoting capacity of selenized bacterium, *Exiguobacterium* sp. S17 was grown in LB media or in LB media with Se (160 mg/L), at 30 °C and 150 rpm, for 48 h. Then, cells were washed twice and suspended in distilled water. Mustard plants were inoculated in soil with 50 mL of a *Exiguobacterium* sp. S17 (3.1×10^9 cfu/mL) or selenized *Exiguobacterium* sp. S17 cells (3.1×10^9 cfu/mL containing 1 mg/L of Se as SeNPs or organic Se) in distilled water. A second control group was treated with sterile water. In all treatments, ten replicates were carried out (one pot per replicate, three plants per pot). The plant growth-promoting assay was performed as described above.

To evaluate the increase in micronutrient uptake and accumulation (including Se) by mustard plants, two composite samples per treatment (six plants) were analyzed. Each composite sample was obtained by combining three dried mustard plants from two pots. Samples were homogenized, and 0.2 g was digested, diluted with MilliQ water, and further analyzed by ICP-MS as described above. The micronutrients evaluated were Se, iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), magnesium (Mg), calcium (Ca), and molybdenum (Mo). For macronutrients uptake and accumulation, three composite samples per treatment (nine plants) were analyzed, and nitrogen (N), phosphorus (P) and potassium (K) were measured. Nitrogen was evaluated by material digestion and determination by distillation and manual titration. P and K were calculated using calcination and determination by flame photometry. Techniques for N, P and K were carried out following Sadzawka et al. 2007.

Phenolic compounds were extracted from three dried mustard plants from each pot (3 pots). Plants were homogenized, suspended in methanol/formic acid (97:3 v/v), and incubated for 16 h at room temperature with agitation speed of 60 rpm. A centrifugation step was used to separate the supernatant with two washing steps of the residue (4 h incubation each) with the same solvent. The extract was stored at 4 °C. To determine the total phenolic content of the plant extracts, the modified Folin-Ciocalteu method described by (Crespo et al. 2021) was applied. Briefly, each extract (10 µl) was mixed with Folin-Ciocalteu reagent (50 µl). The mixture was allowed to stand at room temperature for 2 min. Sodium carbonate was added to the mixture (150 µl, 2% w/v), and the total volume was adjusted to 750 µl with distilled water and then mixed gently. After the mixture stood at room temperature for 20 min, the absorbance was measured at 760 nm using a Microplate Reader (Multiskan ™ SkyHigh—Thermo Scientific). The standard calibration curve was plotted using gallic acid (2.5–100 µg/mL). The total phenolic content was expressed as mg gallic acid equivalents (GAE) per g of dried biomass.

The antioxidant capacity of extracts obtained from control plants and from plants inoculated with *Exiguobacterium* sp. S17 or with *Exiguobacterium* sp. S17 with Se (SeNPs) was determined by applying the DPPH, ABTS (TEAC), and FRAP methods. The DPPH radical scavenging capacity was evaluated according to the method of Isas et al. (2020). To perform this technique, mustard plant extracts were diluted 1/100 (v/v). The DPPH radical scavenging activity was calculated using the following equation:

$$\mathrm{RSA\%} = \left[\left(A_b - A_s\right)/A_b\right] \times 100$$

where A_b is the absorbance of the blank reaction (all reagents except mustard extract), and A_s is the absorbance of the sample (subtracting the absorbance of the sample with DPPH from the one without DPPH).

The Trolox Equivalent Antioxidant Capacity (TEAC) method was performed according to Re et al. (1999), based on the capacity of a sample to inhibit the 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical ($ABTS^{\bullet+}$) in comparison with a reference antioxidant standard (Trolox). For performing this technique, the samples were diluted 1/1000 to enter the Trolox calibration curve (50–250 µM). Results were expressed as Trolox equivalent per gram of dried biomass.

Ferric-reducing power was determined according to Kozarski et al. (2011) with some modifications. The samples were diluted 1/10 and mixed with 0.25 mL 0.2 M sodium phosphate buffer (pH 6.6) and 0.25 mL of 1% potassium ferricyanide. The mixture was vortexed and incubated at 50 °C for 20 min. Then, 0.25 mL of 10% trichloroacetic acid (w/v) was added, and 0.5 mL of the mixture was mixed with 0.5 mL of distilled water and 0.1 mL of 0.1% ferric chloride. The absorbance of 0.2 mL was measured at 700 nm against a blank using a UV/Vis spectrophotometer (Multiskan Sky High, Thermo Scientific, Waltham, Massachusetts, USA). The blank was the solution with all reagents but without the mustard extracts. A higher absorbance indicates a higher reducing power. Results were expressed as ascorbic acid equivalents per g of dried biomass.

Results

Analysis of genes that contribute to plant-growth promotion and Se biotransformation in Exiguobacterium sp. S17 genome

To determine whether the *Exiguobacterium* sp. S17 strain displayed one or several of the genetic traits known to be associated with Plant-Growth Promotion (PGP), or with Se biotransformation, its sequenced genome was analyzed. Briefly, with the data identified from the literature and database searches, a detailed list of PGP-related genes and their associated protein sequences was done. Results of this analysis revealed that the genome of *Exiguobacterium* sp. S17 encodes a complete set of proteins involved in PGP activity linked to phosphate solubilization and mineralization, nitrogen assimilation, reduction, and fixation, siderophore synthesis, Fe-uptake, flagellar assembly, bacterial chemotaxis, and synthesis of several compounds (L-tryptophan, indole, auxin, spermidine, acetoin butanediol nitric oxide and exopolysaccharide) (Table 1). Additionally, the genome of *Exiguobacterium* sp. S17 encodes genes involved in the formation of SeNPs and selenoprotein synthesis: glutathione reductase, thioredoxin reductase, methyl-tRNA synthase, selenocysteine lyase, cysteine-S-conjugate beta–lyase, methionyl-tRNA synthase.

Plant-growth promotion assays

The comparison between the dry weight of mustard seedlings subjected to different treatments (simple and double inoculation) and the control groups showed statistical differences (KW = 19.237, p = 0.001). Seedlings with double inoculation of *Exiguobacterium* sp. S17 had significantly greater dry weight than seedlings from the control group and from the groups with single and double inoculations with culture medium ($p < 0.009$) (Fig. 1). Mustard seedlings with reinoculation showed a mean dry weight of 1.4 g, whereas the mean dry weight of the control group (non-inoculated) was 0.71 g. Single-inoculated seeds, without reinoculation, showed a slightly lower growth than the control groups (KW = 2,509 p = 0,016) and much lower growth in comparison with seedlings with double inoculation (KW = 5.696, p = 0,000) (Fig. 1). Culture media had no effect on plant growth ($p > 0.213$).

Similarly, *Exiguobacterium* sp. S17 inoculation significantly promoted plant growth in comparison with the control group under field conditions. The inoculation with the bacterial strain in chard produced an increment in most of the measured variable in leaves and roots. Increases of 39% in leaf length (K = 12.143, p = 0.002), 156% in leaf fresh weight (K = 9.857, p = 0.007), and 211% in leaf dry weight (K = 10.035, p = 0.007) were observed. Roots were 49% longer (K = 10.574, p = 0.005) and their weight (dry and fresh) was 168% and 177.6% greater than in the control group rinsed with water and ST1 medium (K = 8.909, p = 0.012 and K = 9.323, p = 0.009, respectively) (Fig. 2a, b, c, d). Regarding lettuce, the effect of *Exiguobacterium* sp. S17 was observed only in leaves. The leaves were 35.2% longer (K = 9.975, p = 0.007) and 53.2% wider (K = 8.250, p = 0.016) than in control plants, and fresh and dry weights were 196% and 154% greater than in the control group (K = 9.890, p = 0.007 and K = 8.674, p = 0.013) (Fig. 2e, f, g, h).

Effect of selenized Exiguobacterium sp. S17 on plant growth, Se and nutrient accumulation, total phenolic compounds concentration, and antioxidant activity

Exiguobacterium sp. S17 was able to grow in LB broth at different Se concentrations, and the deeppest reddish color was obtained at a concentration of 160 mg/L; however, growth after 48 h incubation was 1 U log lower than in the control LB media. The Se concentration in the cell pellets in the presence of Se was 19.4 mg/L, and in the control cells 0.002 mg/L. *Exiguobacterium* sp. S17 produced irregular and spherical SeNPs with a diameter of 0.146 μm ± 0.052 (Fig. 3a, b, c), and the three emission peaks of Se [Lα (1.4 keV), Kα (11.22 keV), and Kβ (12.49 keV)] were confirmed by XEDS (Fig. 3c).

Selenized cells of *Exiguobacterium* sp. S17 inoculation had a positive effect on fresh weight of mustard plants (K = 10.640, p = 0.005). A significant increase of 28% in the fresh weight with respect to the control group (p = 0.015) and 46% in comparison with plants pre-inoculated with *Exiguobacterium* sp. S17 (p = 0.007) was observed. However, differences in dry weight were not significant between plants inoculated with the selenized cells (SeNPs) and control plants (p = 0.079) or between plants inoculated with control *Exiguobacterium* cells compared with the control group (p = 0.136). Plants inoculated with non-selenized bacteria showed significantly lower growth than plants treated with the selenized *Exiguobacterium* sp S17 cells (p = 0.002) (Fig. 4a). Moreover, an increase in root growth was detected in the SeNPs samples compared to the control group (Fig. 4b). Besides the plant growth-promoting effect, Se concentration in the SeNPs inoculated plants was at least 7.8 fold higher than in the control plants (Table 2), while an increase in the concentration of Fe and Mn, and an subtle reduction of Cu were also observed (Table 2).

Total phenolic content (TPC), DPPH radical scavenging activity (RSA), total Trolox equivalent antioxidant capacity (TEAC) and Ferric-reducing antioxidant power

Table 1 Genes involved in plant-growth promotion and selenium absorption, biotransformation and SeNPs synthesis present in *Exiguobacterium* sp. S17 genome

Gene funcion	Gene	Protein name	Protein domains_query (InterproScan)	N° of protein from NCBI
Phosphate solubilization and mineralization	*pqqC*	Thiaminase-2/PQQC	PF03070	EPE62114.1
	pqqF	Pyrroloquinoline quinone biosynthesis protein F	PF00675 PF05193	EPE63096.1 EPE61974.1
	pqqG	Coenzyme PQQ synthesis protein G	PF00326	EPE61541.1 EPE62325.1 EPE60501.1
	phoP	Two-component response regulator PhoP	PF00072 PF00486	EPE62878.1
	phoR	Two-component sensor histidine kinase PhoR	PF00512	EPE62893.1
	PhoU	Phosphate transport system regulatory protein PhoU"	PF01895	EPE60460.1
	Ppx/GppA	Exopolyphosphatase Ppx	PF02541	EPE63236.1
	Ppa	Inorganic pyrophosphatase	PF00719	EPE63415.1
Nitrogen assimilation, fixation and reduction	*Nark*	Nitrite extrusion protein	PF07690	EPE63399.1 EPE61627.1 EPE63221.1
	nasC	Nitrate reductase, catalytic subunit	PF00384,PF04879,PF01568	EPE63354.1
	nasE, nirD	Rieske [2Fe-2S] domain protein	PF00355	EPE63438.1
	nifU	Nitrogen fixation protein NifU	PF01106	EPE62189.1 EPE63469.1 EPE60784.1
Nitric oxide synthesis	*Nos*	Nitric oxide synthase oxygenase	PF02898	EPE61167.1
	nirC	Formate/nitrite transporter family protein	PF01226	EPE63345.1 EPE61861.1
Siderophore synthesis/Fe-uptake	*yfmC/yfiY*	*Putative siderophore-binding lipoprotein*	*PF01497*	*EPE61426.1* *EPE61771.1* *EPE60445.1*
	yfmD	ABC transporter permease proteins	PF01032	EPE61745.1
	yfmF / YusV	Putative siderophore transport system ATP-binding protein YusV	PF00005	EPE61432.1
	yfmR	ABC transporter C-terminal domain	PF16326	EPE62925.1
	Fiu	TonB-dependent siderophore receptor family protein	PF00593	EPE61707.1
L-tryptophan, indole synthesis	*trpA*	Tryptophan synthase alpha subunit	PF00290	EPE63062.1
	trpB	Tryptophan synthase beta subunit	PF00291	EPE63032.1
	trpC	Indole-3-glycerol phosphate synthase family protein	PF00218	EPE63036.1
	trpD	Anthranilate phosphoribosyltransferase	PF02885,PF00591	EPE63041.1
	trpE	Anthranilate synthase component I	PF04715	EPE61760.1
	trpF	N-(5'phosphoribosyl)anthranilate (PRA) isomerase family protein	PF00697	EPE63069.1

Table 1 (continued)

Gene funcion	Gene	Protein name	Protein domains_query (InterproScan)	N° of protein from NCBI
Auxin (Indole-3-Acetic acid) synthesis	*iaaM*	Flavin containing amine oxidoreductase	PF01593	EPE60951.1
	gatA, iaaH	Glutamyl-tRNA(Gln) amidotransferase subunit A	PF01425	EPE61486.1
	gatB	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B	PF02934	EPE61473.1
	gatC	Glutamyl-tRNA(Gln) and/or aspartyl-tRNA(Asn) amidotransferase, C subunit	PF02686	EPE61492.1
ACC (1-Aminocyclopropane-1-Carboxylate)-deamination	*Accd*	Cysteine synthase A, corresponds to aminocyclopropane-1-carboxylate deaminase	PF00291	EPE61720.1
Spermidine synthesis	*speA*	Arginine decarboxylase	PF03711,PF01276	EPE61834.1
	speB	Agmatinase	PF00491	EPE62789.1
	speH, speD, AMD1	S-adenosylmethionine decarboxylase	PF02675	EPE63280.1
	speE, SRM	Sermidine synthase	PF17284,PF01564	EPE62734.1
		Gltathionylspermidine synthase family protein		EPE63007.1
Acetoine/2,3-butanediol synthesis	*AcuC*	Actoin utilization protein	PF00850	EPE62868.1 EPE62901.1
	bdhA	NADH-dependent butanol dehydrogenase A	PF 00465	EPE61553.1
Flagellar assembly	*flgB*	Flaellar basal-body rod protein FlgB	PF00460	EPE63077.1
	flgC	Flaellar basal-body rod protein FlgC	PF00460, PF06429	EPE63120.1
	flgD	Flaellar hook capping family protein	PF03963	EPE63114.1
	flgE	Flaellar hook-basal body s family protein	PF00460,PF06429	EPE63112.1
	FlgK	Flaellar hook-associated protein FlgK	PF06429,PF00460	EPE62042.1
	FlgL	Flaellar hook-associated protein 3	PF00669,PF00700	EPE62066.1
	FlgM	Ati-sigma-28 factor, FlgM family protein	PF04316	EPE62069.1
	FlgN	flgN family protein	PF05130	EPE62037.1
	flhA	Flagllar biosynthesis protein FlhA	PF00771	EPE63104.1
	flhB	Fagellar biosynthetic protein FlhB	PF01312	EPE63102.1
		flhB protein		EPE62715.1
	hag, fliC	Flellin	PF00669,PF00700	EPE62687.1 EPE62719.1
	FliD	Flagellar hook-associated 2 domain protein	PF02465,PF07195	EPE62686.1

Table 1 (continued)

Gene funcion	Gene	Protein name	Protein domains_query (InterproScan)	N° of protein from NCBI
	FliE	Flagellar hook-basal body complex protein FliE	PF02049	EPE63097.1
	FliF	Flagellar M-ring protein FliF	PF08345,PF01514	EPE63072.1
	FliG	Flagellar motor switch protein FliG	PF14842, PF01706, PF14841	EPE63121.1
	FliW	Flagellar assembly factor FliW	PF02108	EPE62048.1
	FliL	Flagellar basal body-associated FliL family protein	PF00006, PF18269, PF02874	EPE63122.1
	FliJ	Hypothetical protein L479_00499 FliJ	PF02050	EPE63105.1
	FliK	Flagellar hook-length control family protein	PF02120	EPE63088.1
	FliM	Flagellar motor switch protein FliM	PF02154,PF01052	EPE63094.1
	FliN, FliY	cheC, inhibitor of MCP methylation/FliN fusion protein	PF01052,PF04509	EPE63099.1
	FliP	Flagellar biosynthetic protein FliP	PF00813	EPE63076.1
	FliQ	Flagellar biosynthetic protein FliQ	PF01313	EPE63092.1
	FliR	Flagellar biosynthetic protein FliR	PF01311	EPE63128.1
	FliS	Flagellar protein FliS	PF02561	EPE62721.1
		Flagellar protein FliS		EPE62710.1
	motA	MotA/TolQ/ExbB proton channel family protein	PF01618	EPE61447.1
	motB	OmpA family protein	PF13677,PF00691	EPE61467.1
Bacterial chemotaxis	*cheA*	Hpt domain protein	PF02895,PF02518,PF07194,PF01627,PF01584	EPE63124.1
	cheB	Response regulator	PF01339,PF00072	EPE61480.1
	cheD	Chemoreceptor glutamine deamidase CheD	PF03975	EPE63070.1
	cheR	cheR methyltransferase, all-alpha domain protein	PF01739,PF03705	EPE60660.1
	cheY	Response regulator receiver protein	PF00072	EPE63087.1
	cheW	cheW-like domain protein	PF01584	EPE63139.1
	cheV	cheW-like domain protein	PF01584	EPE62002.1
	cheC	cheC-like family protein	PF04509	EPE63115.1
Extracellular polymeric substances (EPS)	*eps*	Exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase family protein	PF02397	EPE62571.1
	eps	Capsular exopolysaccharide family domain protein	PF02706	EPE61872.1

Table 1 (continued)

Gene funcion	Gene	Protein name	Protein domains_query (InterproScan)	N° of protein from NCBI
Selenocompounts metabolism	*nfrA1*	NADPH-dependent nitro/flavin reductase	PF00881	EPE63147.1
	trx	Thioredoxin reductase	PF00085	EPE62732.1 EPE62695.1
	metG	Methionyl-tRNA synthetase	PF09334	EPE60650.1
	Bsu PatB	Cystathionine beta-lyase	PF00155	EPE62634.1
	Csd	Cysteine desulfurase	PF01053	EPE60782.1

(FRAP) were significantly different among treatments ($p \leq 0.016$, Table 3). The total phenolic compound concentration in the mustard plants with SeNPs was greater than in the plants of the control group and those inoculated with *Exiguobacterium* sp. S17 alone. In comparison with the control group, selenized plants showed a 27% increase in phenolic concentration, and plants inoculated with non-selenized bacteria exhibited an 18% increase (Table 3). The antioxidant capacity determined by the DPPH was slightly higher in the plants inoculated with SeNPs. However, the FRAP values in SeNPs-treated plants were significantly higher than those obtained from the control and *Exiguobacterium* sp. S17 inoculated plants (Table 3). Furthermore, the antioxidant activity measured by ABTS (TEAC) in plants inoculated with *Exiguobacterium* sp. S17 and SeNPs was also higher than the TEAC in the control plants.

Discussion

In the present study, *Exiguobacterium* sp. S17 was evaluated in its ability to promote plant growth in different species and to produce Se biotransformation and Se accumulation and biofortification in mustard plants. Assays carried out in this study revealed that this bacterial strain possesses all these capabilities and, then, it could be used as a PGPR and in biofortification strategies.

In order to confirm the plant growth-promoting capacities of *Exiguobacterium* sp. S17, plants under field conditions were inoculated and the results revealed that *Exiguobacterium* sp. S17 displays evident plant growth-promoting activities, mainly in leafy greens such as chard and lettuce. The increase in leaf and root biomass and size is in agreement with previous studies which demonstrated that other strains in the genus *Exiguobacterium* also play an important role in plant-growth promotion (Dastager et al. 2010; Selvakumar et al. 2010; Bharti et al. 2013).

Analysis of the whole genome of *Exiguobacterium* sp. S17 supports the positive effect found on plant growth. It is well known that PGPR promote plant growth by providing nutrients to the host plant or by preventing infections by pathogenic organisms (Castro-Sowinski et al. 2007; Glick 2012). Examples of the supply of extra nutrients include nitrogen fixation, which provides reduced nitrogen; the production of phytohormones, which increases root surface area for absorption of nutrients; siderophores, which capture and solubilize iron; and organic acids, which solubilize phosphates (Lifshitz et al. 1987; Vessey 2003; Santoyo et al. 2016; Liu et al. 2017). In this sense, genes involved in the supply of nutrients have been found, such as those linked to phosphate solubilization and mineralization, nitrogen assimilation and reduction, nitrogen fixation, siderophore synthesis, and Fe-uptake. Biological nitrogen fixation and phosphorus uptake, together with microbial production of phytohormones, were the major factors responsible for plant-growth improvement by PGPR, which contributes to the development of an efficient root system with enhanced water and nutrient uptake (Lifshitz 1987; Ahmed and Hasnain 2014). The genome sequence suggests that *Exiguobacterium* sp. S17 has the capability to synthesize phytohormones like auxin (Indole-3-Acetic acid), spermidine, cetoin, utanediol, and nitric oxide (NO). Auxins, as well as NO as an intermediary in auxin-regulated signaling cascade, control most of plant processes and, for this reason, they are considered responsible for most of the developmental patterns in plants (Tanimoto 2005; Molina-Favero 2007; Wu et al. 2011). PGPR produces auxins and NO regulates root development, stimulating root formation and lateral root initiation, along with the growth of new leaves at the apical meristem (Molina-Favero 2007; Ahmed and Hasnain 2014). Polyamines, including putrescine, spermine, spermidine, and cadaverine, are natural small-molecular-weight polycationic compounds that are present in mammals, fungi, bacteria, and plants. Polyamines play important physiological roles in organisms and are involved in various processes, including cell division and differentiation, nucleic acid replication, transcription, translation, protein synthesis, and membrane stability (Kusano et al. 2008). Moreover, spermidine has attracted widespread interest because it is essential for eukaryotic cell viability, and its presence is correlated with lateral root development (Xie et al. 2014).

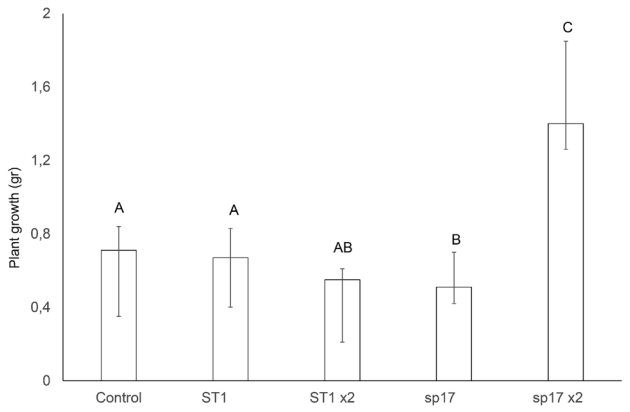

Fig. 1 Plant-growth promotion of mustard seedlings (measure as dry biomass) after simple and double inoculation with *Exiguobacterium* sp. S17. Control: seedlings watered with distilled water. St1: seedlings watered with culture medium. St1×2: seedlings with a double inoculation of culture medium. sp17: seedlings pre-inoculated with *Exiguobacterium* sp. S17. sp17×2: seedlings with a re-inoculation of *Exiguobacterium* sp. S17. Different letters indicate significant statistical differences

Previous studies showed that spermidine was the pivotal plant growth-promoting substance produced by *B. subtilis* OKB105, by inducing expansin expression and decreasing root-inhibiting factors like ethylene in plant cells (Xie et al. 2014). *Exiguobacterium* sp. S17 genome showed the presence of genes involved in the synthesis of air-borne chemicals that are released from specific bacterial strains able to trigger growth promotion and induce systemic resistance (ISR), such as acetoin (which was found to trigger ISR and protect plants against pathogens) and butanediol synthesis (Ryu et al. 2003; Rudrappa et al. 2010). Lastly, the capacity of rhizobacteria to migrate towards the roots and colonize them in response to root exudates also requires a set of genes, including some involved in flagellar biosynthesis/regulation that were also found in the *Exiguobacterium* sp. S17 genome (Cole et al. 2017).

*S*elenium is a metalloid considered a vital micronutrient in the human diet. Selenium replaces sulfur in cysteine and it is incorporated as selenocysteine (SeCys) in selenoproteins (Mounicou et al. 2006). The main selenoenzymes are glutathione peroxidase, iodothyronine deiodinase, and thioredoxin reductase, which are involved in antioxidant defense, detoxification, and thyroid functions (Palomo-Siguero and Madrid 2017). Selenium deficiency in humans is associated with hypothyroidism, cardiovascular disease, and immune system weakness (Pedrero et al. 2006). However, there is a fine line between Se essentiality and toxicity. Bacteria have a specific scavenging, uptake, and removal system for many elements including Se. Selenium is found within the biosphere in all its oxidation states but mainly as Se VI and Se IV, which are highly toxic. Elemental Se is less toxic and water-insoluble and many bacteria can bio-transform Se salts into less toxic Se^0 containing nanoparticles as a detoxifying mechanism that will affect its Se resistance capacity. *Exiguobacterium* sp. S17 was able to incorporate and transform Se into irregular and spherical seleno-nanoparticles (SeNPs). Different mechanisms have been proposed to be involved in selenite reduction by microorganisms. Thiol-mediated Se reduction is the most widely recognized mechanism and involves glutathione and a NADPH-dependent reductase. However, others include the thioredoxin reductase system and siderophore-mediated reduction. Reduced thioredoxin can react with selenodiglutathione and form oxidized thioredoxin, reduced glutathione and selenopersulfide anion from which Se^0 is released. In addition, an iron siderophore produced by *Pseudomonas stutzeri* KC was proposed to be responsible for selenite reduction to Se^0. Although the mechanism by which *Exiguobacterium* sp: S17 produces SeNPs has not been elucidated, this mechanism could be responsible for Se^0 formation and SeNPs production. Moreover, the presence of the gene coding for a selenocysteine lyase could also be involved in Se^0 production from selenocysteine. Besides, the enzymes cysteine S-conjugate beta lyase and methionyl tRNA synthase in selenoprotein synthesis were also found in *Exiguobacterium* sp. S17 genome. The SeNPs produced by the analyzed bacteria showed a moderate effect on the growth of mustard roots and leaves. Mustard plants inoculated with SeNPs showed a moderate increase in fresh weight of 46% whereas differences in dry weight were not observed. In addition to the plant growth promotion effect, Se concentration in the SeNPs inoculated plants was far greater than in the control plants, suggesting that biofortification using selenized bacteria is feasible. Additionally, an increase in the concentration of Fe and Mn was also observed in SeNPs-inoculated plants suggesting that SeNPs improve the essential element uptake by mustard plants. Similar results were observed for wheat when Se was added to their leaves (Nawaz et al. 2015) and also when SeNPs were foliar applied to pomegranate plants (Zahedi et al. 2019).

Finally, SeNPs inoculation increased the antioxidant capacity of plants as measured by DPPH and FRAP, and the total phenolic was also higher in the SeNPs inoculated mustard plants. Similar results were observed by Zahedi et al. (2019), who found significant differences in anthocyanin concentration and antioxidant activity by foliar applying SeNPs to pomegranate plants. Consistently, Badawy et al. (2017) found that Se fertilizer significantly increased the total polyphenol content in several plant species.

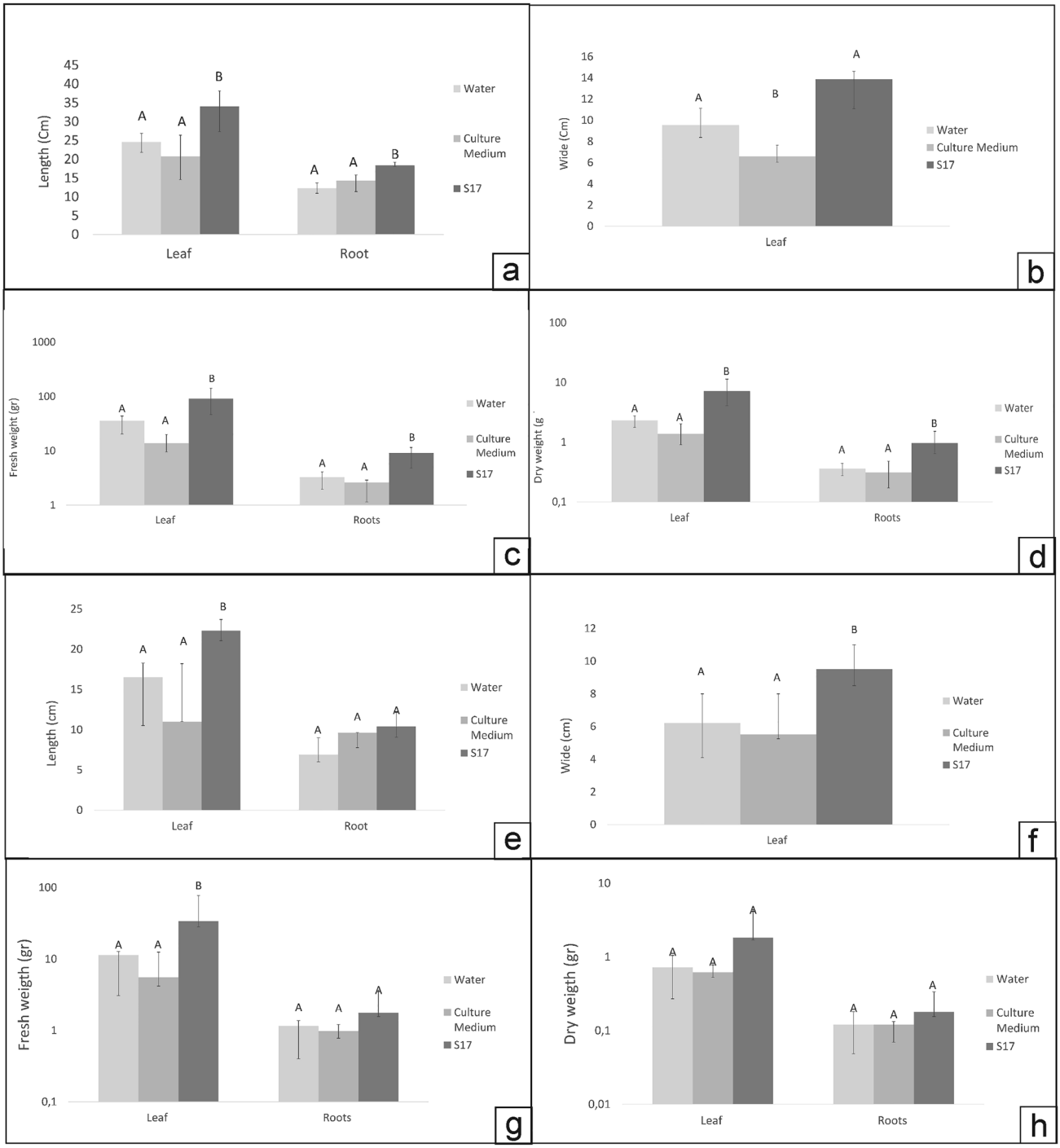

Fig. 2 Plant-growth promotion of chard and lettuce seedlings after simple and double inoculation with *Exiguobacterium* sp. S17. **a**, **b**, **c**, **d**. Chard. **e**, **f**, **g**, **h**. Lettuce. Different letters indicate significant statistical differences

Conclusion

Exiguobacterium sp. S17 showed two capabilities that make this bacterial strain a candidate to be applied in a bioinoculant formulation. On the one hand, the application in soil of *Exiguobacterium* sp. S17 produced high plant-growth promotion in leafy greens such as chard and lettuce. On the other hand, selenized *Exiguobacterium* sp. S17 could be useful to produce Se accumulation and biofortification in accumulator plants to be used as dietary supplement. Finally, this biofortification increase antioxidant capacity and improves the nutritional value of horticultural plants.

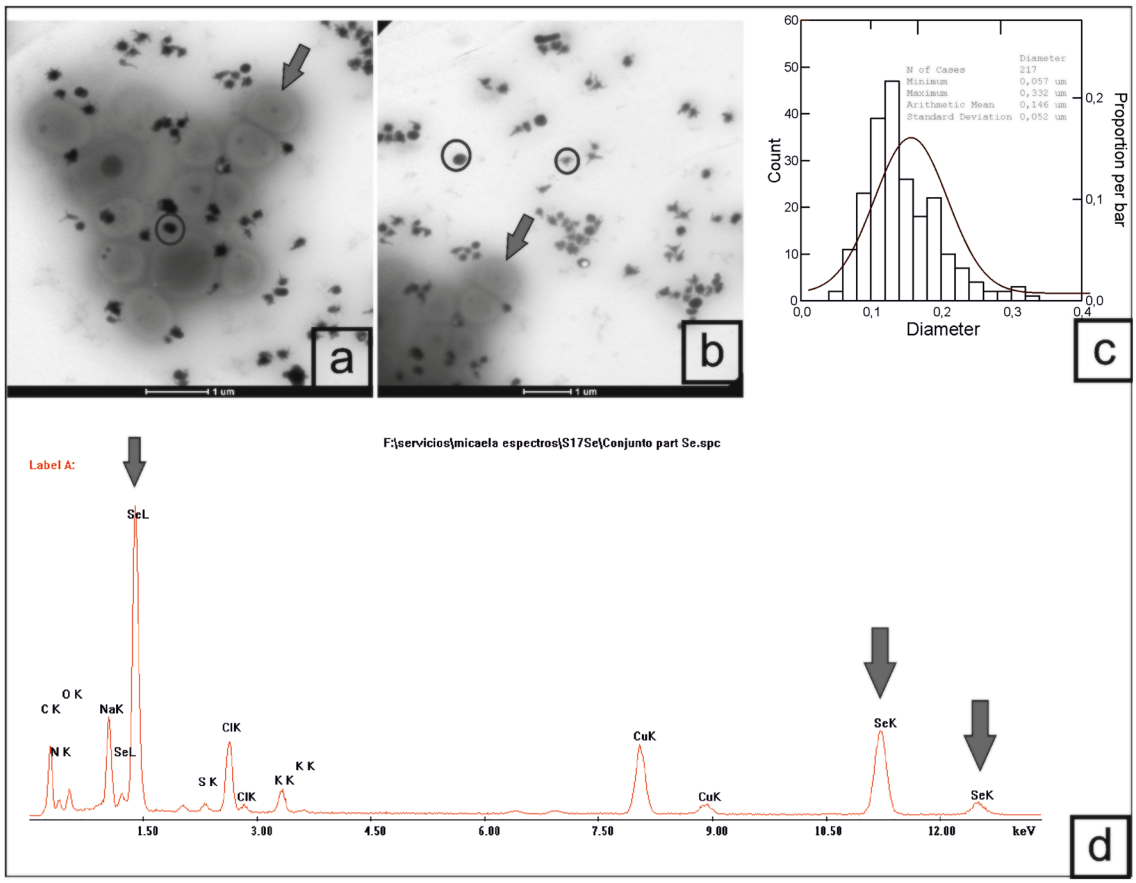

Fig. 3 **a**–**b** TEM images and for the SeNPs produced by Exiguobacterium sp. S17 (some SeNPs are marked with circles and S17 cells are marked with arrows), **c** histogram showing SeNPs size and **d** XEDS analysis graph depicting energy on X-axis and number of counts on Y-axis representing elemental composition derived from SeNPs selected areas. Arrows in XEDS spectra indicate the Se emission peaks consisting of SeLα, SeKα, and SeKβ at 1.4, 11.22, and 12.49 keV, respectively

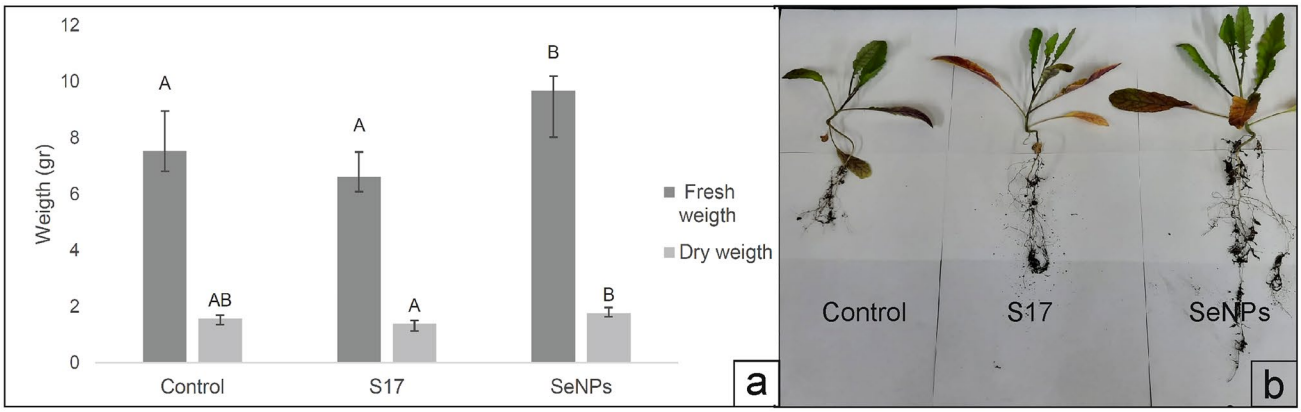

Fig. 4 **a** Plant-growth promotion of mustard seedlings (measure as fresh and dry biomass) after pre-inoculation with non-selenized bacteria (S17) or inoculation with seleno-nanoparticles (SeNPs). **b** Root growth of mustard seedlings

Table 2 Total Se accumulation and essential elements uptake by mustard plants (control) and by plants inoculated with *Exiguobacterium* sp. S17 (S17) or with the selenized bacterial cells (SeNPs)

Micronutriens								
	Se (ug/g)	Fe (ug/g)	Zn (ug/g)	Mn (ug/g)	Cu (ug/g)	Mg (ug/g)	Ca (ug/g)	Mo (ug/g)
Control	0.094 ± 0.00^{a}	237.2 ± 7.5^{a}	33.1 ± 11.7^{a}	16.7 ± 0.7^{a}	4.9 ± 02^{a}	3960.9 ± 716.8^{a}	7517.4 ± 1178.0^{a}	2.92 ± 0.51^{a}
S17	0.057 ± 0.01^{b}	228.4 ± 31.3^{a}	23.9 ± 2.1^{a}	14.9 ± 3.2^{a}	3.9 ± 0.3^{b}	3094.2 ± 153.5^{a}	5125.91 ± 1040.8^{a}	1.80 ± 0.27^{a}
SeNPs	0.742 ± 0.06^{c}	656.6 ± 97.9^{b}	27.4 ± 2.2^{a}	29.9 ± 10.3^{b}	4.7 ± 0.2^{c}	4130.4 ± 658.7^{a}	7018.88 ± 2291.4^{a}	1.87 ± 0.29^{a}
Macronutrients								
	N (mg/g)	P (mg/g)	K (mg/g)					
Control	11.35 ± 0.21^{a}	1.9 ± 0.014^{a}	26.65 ± 5.44^{a}					
S17	10.25 ± 0.07^{a}	1.65 ± 0.49^{a}	24.8 ± 0.84^{a}					
SeNPs	11.5 ± 0.28^{a}	0.8 ± 0.14^{a}	27.1 ± 7.21^{a}					

Data presented in mean ± S.D. Means superscripted by the same letter are not significantly different from each other using non-parametric One-way Analysis of Variance and Conover-Inman Test for All Pairwise Comparisons. Different letters showed significant differences at $p \leq 0.016$

Table 3 Total phenolic compounds content and antioxidant activity of the mustard plant extracts

	TPC (mg GAE/g)	RSA (%)	TEAC (µmol TE/g)	FRAP (mg AAE/g)
Control	848.04 ± 93.29^{a}	89.12 ± 0.06^{a}	1639.40 ± 68.91^{a}	8.48 ± 0.22^{a}
S17	1002.16 ± 19.74^{b}	93.65 ± 0.05^{b}	1899.23 ± 2.47^{b}	9.99 ± 1.07^{a}
SeNPs	1083.57 ± 20.06^{c}	95.92 ± 0.89^{c}	2006.14 ± 13.14^{b}	11.36 ± 0.80^{b}
K	8.346	9.846	9.846	10.881

Control (non-treated), S17 inoculated with *Exiguobacterium* sp. S17 or with the selenized S17 cells (SeNPs). Different letters indicate significant statistical differences ($p \leq 0.016$), K = Kruskall Wallis Test Statistic

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Author contributions Conceptualization: MJA, GAL, FME, VML, PM and OOF. Methodology: MJA (Bacterial production, Plant-growth promotion assays, Effect of selenized Exiguobacterium sp. S17 on plant growth), GAL (Plant-growth promotion assays, total phenolic compound concentration, and antioxidant activity), PM (Selenium biotransformation, Se and micronutrient accumulation, total phenolic compound concentration, and antioxidant activity) and OOF (Analysis of genes in Exiguobacterium sp. S17 Genome, Selenium biotransformation by Exiguobacterium sp. S17, Effect of selenized Exiguobacterium sp. S17 on plant growth). Statistical analysis and Data curation: MJA, GAL, VML. Writing-original draft preparation: MJA, VML. Writing—review and editing: All authors. Funding acquisition: MJA, VML, PM and OOF. Resources: FME, VML, OOF. Supervision: VML.

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Data availability The datasets generated during and analysed during the current study are available in DDBJ/EMBL/GenBank under accession number ASXD01000000 or from the corresponding author on reasonable request.

Declarations

Competing interest The authors declare no competing interest.

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