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Halotolerant Endophytic Bacterium *Serratia rubidaea* ED1 Enhances Phosphate Solubilization and Promotes Seed Germination

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Abstract: Quinoa is renowned for its nutritional value and ability to withstand harsh environmental conditions such as salinity. In the present work, we isolated 34 phosphate solubilizing endophytic bacteria associated with the roots of quinoa plants. Based on phosphate solubilization efficiency and biochemical characterization, we selected one isolate named ED1. Ribotyping using partial 16S RNA gene analysis revealed that the selected isolate shares 99.7% identity with *Serratia rubidaea*. Plant growth promoting (PGP) studies showed that the ED1 strain solubilized complexed forms of phosphate ($Ca_3(PO_4)_2$). Zinc release from ZnO, $Zn_3(PO_4)_2$, or $ZnCO_3$ revealed the efficient ZnO solubilization by the ED1 strain. Except for proteases, the strain ED1 produced siderophores, cellulase, ammonia and exhibited oligonitrophilic features. Indole acetic acid (IAA) production was detected with and without the L-tryptophan precursor. Next, we demonstrated that the ED1 strain tolerated 1.5 M NaCl final concentration and exhibited intrinsic resistance to seven antibiotics frequently prescribed for medical use. Moreover, we found that ED1 strain withstood 2 mg/L of Cadmium and 1 mg/L of either Nickel or Copper. Furthermore, we observed that *S. rubidaea* ED1 stimulated quinoa seeds germination and seedlings growth under salt stress conditions. Lastly, we discuss the advantages versus disadvantages of applying the *S. rubidaea* ED1 strain as a beneficial agent for salty and/or heavy metals contaminated soils.

Keywords: endophyte; *Chenopodium quinoa* willd.; *Serratia rubidaea*; plant growth promotion; salt stress; antibiotics resistance; heavy metals tolerance

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1. Introduction

Plant associated endophytic microorganisms are considered as the second functional genome of host plants [1]. In 1995, endophytes were defined as symbiotic microorganisms (bacteria, archaea, fungi, and protists) residing within the plant endosphere without causing any detrimental impact to the host plant [2]. Hardoim et al. (2015) stated that any colonizing microbe of plant tissues could be classified as endophyte regardless of the outcome of its association with the host plant [3]. However, colonization by endophytes is crucial for providing benefits to plants [4]. Indeed, the interaction of endophytes with plants is characterized as a symbiotic relationship because of mutual benefits. The plant provides protective niches to the internal microorganisms which, in turn, act directly by promoting plant growth through nitrogen (N) fixation, phytohormone, and metabolites production, and indirectly by stimulating availability and absorption of macro and micronutrients and

water. In addition, endophytic microorganisms induce host tolerance to abiotic stresses such as osmotic stress, exposure to heavy metals and xenobiotic molecules, and assist in the biocontrol of plant pathogens [5,6].

Plant growth promoting microorganisms (PGPM), including endophytes, have an immense potential to boost plants' performance and to reduce the environmental impacts caused by chemical inputs as increasing agricultural productivity is of the upmost priority around the globe [4,7]. Indeed, bacterial endophytes have been reported to prime plants for faster and more intense defense responses upon pathogen attacks at low physiological cost to the plant [8]. Endophytes also stimulate seedlings' emergence and enhance plant growth under adverse conditions [9,10]. Inoculation of plant growth promoting endophytic (PGPE) bacteria in plants has low environmental impact, requires low-cost techniques, and could be of immense economic importance.

Plants are complex micro-ecosystems where the different tissue and surface compartments are exploited by a wide variety of microorganisms [11]. PGPE bacteria have been isolated from several plants such as wheat roots [12], sweet potato roots [13], rice roots [14], sugarcane stems [15], bean leaves [16], and quinoa seeds [17]. Plants interact with microbes primarily at the roots. Despite the crucial role of root bacterial communities in plant health and nutrient assimilation, the current understanding of the complex plant–microbe interactions is still in its infancy. At the soil-root interface, roots provide different microhabitats: rhizospheric soil, rhizoplane, and endorhizosphere [18].

Among phytohormones, auxins (e.g., indole acetic acid (IAA)) are the main plant growth regulators having many physiological actions including elongation of primary roots, differentiation of tissues, formation of pigments, stimulation of nitrogen fixation, and resistance to various stresses. Plants associated microorganisms can synthesize IAA via tryptophan-dependent pathways by converting tryptophan to different intermediates. This pathway of IAA biosynthesis has been demonstrated in the majority of soil bacteria [19]. However, some microorganisms are endowed with the ability to produce it via tryptophan-independent routes [20].

Salinity in one of the most devastating stresses in agriculture, especially in arid and semi-arid environmental areas [21]. It considerably reduces plant growth and productivity because of nutritional imbalance in plants (increase in Na^+ and decrease in K^+ uptake), inhibition of protein synthesis, enzyme inactivation, early senescence, decrease in photosynthesis and respiration, and loss of cellular integrity [22,23]. Nowadays, methods to increase tolerance of plants to salinity involve the use of salt tolerant crops, transgenic plant genetic engineering, and traditional breeding [24]. Unfortunately, these strategies are labor-intensive and highly technical [25]. The alternative strategy to sustain plant growth under salty conditions is the use of PGPB (plant growth promoting bacteria). These latter can be found both at the root surface and in endophytic associations with host plant. These bacteria can either directly or indirectly enhance plant growth in normal and stressful conditions [25–27]. Several bacterial species have been reported to mitigate salinity stress and stimulate plant growth. They include *Klebsiella* sp., *Burkholderia* sp., *Enterobacter* sp. [23], *Microbacterium* sp., *Alcaligenes* sp. [28], *Ochrobactrum* sp. [28], *Bacillus* sp. [23,29], *Arthrobacter* sp. [30], *Pseudomonas* sp. [31], *Bacillus licheniformis* and *Enterobacter asburiae* [32], and finally *Serratia* sp. [33].

Several studies have tackled the emergence and propagation of antibiotic resistance (ARG) genes in different environmental reservoirs through different anthropogenic activities such as the application of manure, slurry, and soil amendments with regard to their different transfer pathways and threats to public health and ecology [34]. However, ARG-harboring PGPB inoculants are mostly unexplored, while most of these PGPB are still recommended as potential effective biofertilizers. Despite the importance of antibiotic resistance in helping biofertilizers-based bacteria to survive and compete the respective native microflora in open microbial habitats [35], large-scale application of ARG-harboring biofertilizers into the soil can rise new resistances and worsen the spread of ARG in other ecosystems.

Quinoa (*Chenopodium quinoa* Willd.), an herbaceous plant species belonging to the amaranthaceae family [36], is one of the most promising crops for tomorrow's food demand

and nutritional security. Originally from South America, quinoa was heavily cultivated by the ancient Andean civilization, but it was not until the 1970s that quinoa began to be introduced all over the world. Quinoa is renowned for its ability to withstand harsh environmental conditions such as drought, salinity, wind, and heat [37,38] and its outstanding nutritional value [39] with high content of vitamins, and good quality of proteins and saponins [40]. Quinoa crop has recently gained attention and its cultivation is spreading worldwide. In the developing countries of Africa and Asia, quinoa represents a miracle solution to provide highly nutritious food [41] due to its growth adaptability to various environmental conditions. In Morocco, it has started to be cultivated for its income-generating potential, its adaptability to different soil and climatic conditions, and its potential to improve cropping systems [42]. More importantly, it grows up to an altitude of 4000 m above sea level, resists a wide daily temperature ranges, needs only 300 mm of precipitation per year, and is produced in a short period of time [40]. Given the ability of halophyte quinoa plants to cope with abiotic stresses and extreme environments, we hypothesized that quinoa-associated endophytic bacteria may be involved in the acquisition of such tolerance as well as in promoting plant growth. Herein, we identified a phosphate solubilizing endophytic bacterium inhabiting the roots of quinoa plants cultivated in Morocco and characterize its PGP properties in vitro. We also assessed its ability to withstand extreme conditions such salt and heavy metals stresses.

2. Materials and Methods

2.1. Plant Material and Sampling

Root samples were collected from *Chenopodium quinoa* plants growing in different locations at the experimental farm (32.219731 E, −7.892268 N) of Mohammed VI Polytechnic University, Ben Guerir, Morocco. Sampling was carried out in June 2018, peak growing season for quinoa. Six plants were randomly selected for root sampling, and roots without visible damage were collected under aseptic conditions and transported to the laboratory for further analysis.

2.2. Isolation of Root-Borne Bacterial Endophytes

Primary and secondary roots were washed under running tap water to remove soil debris, then surface-sterilized with ethanol (70%) for 3 min and sodium hypochlorite (3%) for 1 min, followed by three rinses in sterile distilled water for 3 min each [43,44]. To ensure the success of surface sterilization treatments, the last water wash solutions as well as sterilized roots were respectively deposited on TSA (Trypticase Soy Agar, BIOKAR Diagnostics, BEAUVAIS, France) plates and cultivated overnight [44]. The sterilized root pieces were ground in 0.85% aqueous NaCl using a mortar and pestle. Next, serial dilutions (10^{-1} to 10^{-5}) were generated, and each dilution was spread on a TSA medium using a glass spreader [16]. Plates were then incubated at 30 °C for 24 h. Emerging colonies were subcultured to obtain pure isolates.

2.3. Plate Assay for Phosphate Solubilizing Activity

The purified bacterial isolates were subjected to phosphate solubilization activity screening. Using nitrocellulose membranes, a total of 34 endophytic bacterial colonies grown on TSA plates were transferred to NBRIP (National Botanical Research Institute's phosphate) agar medium plate [45] consisting of (g/L) dextrose 10; hydroxyapatite 5 (purum p.a., ≥90% (as $Ca^3(PO4)_2$,KT); ammonium sulphate 0.5; potassium chloride 0.2; sodium chloride 0.2; magnesium sulphate 0.1; ferrous sulphate trace; manganese sulphate trace; agar 15; and the pH was adjusted to 6.75 ± 0.25 before autoclaving [46]. Plates were incubated at 30 °C and checked daily for 7 days for the appearance of transparent halos indicating P-solubilizing ability. Based on the growth on selective medium and discrete halo zones appearance, one selected isolate (named ED1) was subcultured several times, purified on the same medium, then stored at −80 °C in cryotubes using 10% dimethyl sulfoxide (DMSO) as a cryoprotective agent.

2.4. Ribotyping Identification of Isolated Strains

The polymerase chain reaction (PCR) was performed on bacterial 16S rRNA gene using primers: forward pA (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse 926R (5′-CCGYCAATTYMTTTRAGTTT-3′) [47] generating an amplicon of 910 bp. The PCR reaction mixture contained 23 µL DNAase free water, 1 µL of forward and reverse primers at 20 µM final concentration, 25 µL PCR SuperMix (Invitrogen, Carlsbad, CA, USA), and 1 µL of fresh bacterial cultures as a DNA matrix. The amplification process was launched according to the following program: initial denaturation step for 5 min at 94 °C, denaturation for 1 min at 94 °C, hybridization for 1 min at 52 °C, elongation step for 1 min 30 s at 72 °C with 35 cycles, and finally an incubation for 10 min at 72 °C. Then, 5 µL of each PCR sample was checked on agarose gel. The amplicons were sequenced and generated nucleotide sequences were aligned using the ExPASy Bioinformatics Resource Portal (https://www.expasy.org/ (accessed on 20 November 2020)), and compared to available homologous sequences using the BLAST search (Basic Local Alignment Search Tools, NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi (accessed on 20 November 2020))) and the High-Quality Ribosomal RNA database (SILVA (https://www.arb-silva.de/ (accessed on 20 November 2020)) [48]. The phylogenetic dendrogram was generated by the Neighbor-Joining method using Unipro UGENE software, version 1.32.0 [49].

2.5. In Vitro Assessment of PGP Attributes and Extreme Growth Properties

2.5.1. Heat, Salt, and pH Tolerance

To assess for heat tolerance, bacteria were streaked on TSA plates and further incubated for 24 h at various temperatures: 20, 25, 30, 37, 42, 45, and 50 °C [50]. Salt tolerance was firstly evaluated on a TSA medium by supplementing plates with various concentrations of NaCl (0 to 14%) (*w/v*) and incubated at 30 °C for 48 h [51]. The salt and heat tolerance of the isolate was confirmed by observing its growth on salt-supplemented and nutrient agar media. *B. licheniformis* QA1 [32] and *Esherichia coli* DH5α were used as positive (C+) and negative (C−) controls, respectively. To determine the optimum salinity tolerance of the selected bacterium, we used 48-well microtiter microplates; each well was filled with 500 µL of TSB broth supplemented with various NaCl concentrations (0, 2, 4, 6, 8, 10, 12, 14, and 16%) (*w/v*). In addition, pH tolerance was assessed by adjusting the pH of the media at different levels (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12). Five microliters of an overnight bacterial culture (OD_{600nm} = 0.8) was inoculated in each well and incubated at 30 °C under shaking at 150 rpm. Growth was measured after 24 h by colorimetry at OD_{600nm} using the VICTOR NivoTM Multimode Plate Reader (Perkin Elmer, Casablanca, Morocco) [52].

2.5.2. Phosphate Solubilization Assay under Salt Stress Conditions

Inorganic phosphate solubilization by bacteria was studied in a liquid medium. Bacterial suspension of OD_{600nm} = 0.8 was inoculated into 50 mL of NBRIP broth containing 0%, 4%, and 8% NaCl concentrations and incubated in an incubator shaker at 30 °C/150 rpm [51]. Solubilization of hydroxyapatite (purum p.a., ≥90% (as $Ca_3(PO_4)_2$, KT) was measured after 5- and 10-days post incubation (DAI; day after incubation). The bacterial broth was centrifuged at 5000 rpm for 10 min, filtered using 0.22 µm sterile syringe filters, and 5 mL of each supernatant was transferred into 50 mL centrifuge tubes. *E. coli* DH5α was used as a negative control (C−) [46]. Soluble P concentrations (orthophosphates (PO_4)) in each sample were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES) at the Agricultural Innovation and Technology Transfer Center (AITTC) of the Mohammed VI Polytechnic University (UM6P).

2.5.3. Oligonitrophilic Activity

To monitor the oligonitrophilic property of the selected bacterium, we used the nitrogen free Jensen's medium [53] containing the following composition (in 1 L): Sucrose 20 g, Dipotassium phosphate 1 g, Magnesium sulphate 0.5 g, Sodium chloride 0.5 g, Ferrous sulphate 0.1 g, Sodium molybdate 0.005 g, Calcium carbonate 2 g, and Agar 15 g. Plates

were streaked with fresh bacterial culture of isolated bacterium and checked for growth following incubation at 30 °C for 24 h. *B. licheniformis* QA1 [32] and *E. coli* DH5α were used as positive (C+) and negative (C−) controls, respectively.

2.5.4. Monitoring Indole Acetic Acid (IAA) Production

To quantify the amounts of IAA produced by the P-solubilizing endophyte, a colorimetric method of *Van Urk Salkowski* reagent was performed [54]. The isolate was inoculated in LB medium supplemented with 0, 0.2, and 0.5% of L-tryptophan and incubated at 28 ± 2 °C for 11 days under shaking of 150 rpm [55]. The determination of produced IAA was assessed after 3, 7, and 11 days of incubation (DAI). The bacterial cultures were centrifuged at 13,000 rpm for 5 min at 4 °C. Next, two mL of Van Urk Salkowski reagent (1 mL of 0.5 M $FeCl_3$ and 50 mL of 35% $HClO_4$) were mixed with 1 mL of each supernatant. Mixtures were incubated in the dark for 30 min [52]. IAA quantification was determined spectrophotometrically at OD_{533nm} using a standard curve of pure IAA (Sigma Aldrich, Overijse, Belgium) for concentrations in the 0–100 µg/mL range.

2.5.5. Solubilization of Insoluble Zinc Compounds

The ability of the selected endophytes to solubilize insoluble Zn compounds was evaluated on Tris-mineral agar medium [56] containing (in 1 L): D-glucose 10 g, $(NH_4)_2SO_4$ 1 g, KCl 0.2 g, K_2HPO_4 0.1 g, $MgSO_4$ 2 g, pH = 6.75 ± 0.25. Prepared media were separately amended with three sources of insoluble Zn namely zinc oxide (ZnO, 15.23 mM), zinc phosphate ($Zn_3(PO_4)_2$, 5.0 mM), and zinc carbonate (CO_3Zn, 5.2 mM) at 0.1% Zn final concentration [57]. The selected isolate was spot inoculated on each medium and plates were incubated at 30 °C for 10 days. The appearance of a clear zone around colonies indicate positive Zn solubilization. *B. licheniformis* QA1 [32] and *E. coli* DH5α were used as positive (C+) and negative (C−) controls, respectively.

2.5.6. Siderophores Production Assay

The production of siderophores by bacterial isolates was monitored according to the method described by *Schwyn* and *Neilands* (1987) [58] using Chromium Azurol S medium (CAS) which contains the ternary complex CAS-Fe^{+3}-Hexadecyltrimethyl-Ammonium bromide as an indicator. The isolate was spot inoculated on CAS plates and incubated at 28 ± 2 °C for 5 days. The efficiency to produce siderophores was checked by monitoring the size of the halo zone and the intensity of the color change (blue to yellow or orange). The halo diameter was taken as an indicator of siderophores production intensity. *B. licheniformis* QA1 [32] and *E. coli* DH5α were used as positive (C+) and negative (C−) controls, respectively.

2.5.7. Ammonia Production Assay

ED1 isolate was tested for ammonia production by adopting the method described by Cappuccino and Sherman (1992) [59]. Fresh bacterial cultures of selected isolate were inoculated into 10 mL of peptone water and incubated at 28 ± 2 °C with shaking at 150 rpm. Post 48 h incubation, 0.5 mL of Nessler reagent was added to each bacterial culture. A faint yellow color indicated a small amount of ammonia and a deep yellow to brownish color indicated high production of ammonia. The absorbance was measured at OD_{450nm} and the concentrations of ammonia were estimated using a standard curve of ammonium sulphate ($(NH_4)_2SO_4$) for concentrations in 0–0.3 µmol/mL range [60]. *B. licheniformis* QA1 [32] and *E. coli* DH5α were used as positive (C+) and negative (C−) controls, respectively.

2.5.8. Cellulase and Protease Production

To check the production of extracellular cellulase by selected bacterium, we used the mineral–salt agar plates containing 0.4% $(NH_4)_2SO_4$, 0.6% NaCl, 0.1% K_2HPO_4, 0.01% $MgSO_4$, 0.01% $CaCl_2$ with 0.5% carboxymethyl cellulose, and 2% agar, pH = 6.75 ± 0.25. The plates were then spot inoculated and incubated at 30 °C. After 2 days of incubation, a Congo red solution (1%) was added to the surface of each plate. 20 min later, the plates

were flooded with a 1M NaCl solution and then left to stand for 30 min. The appearance of a clear halo around colonies indicates the degradation of the CMC and reflects the presence of extracellular cellulase [61].

The protease activity was performed according to the method of Kavitha et al. (2013) [62] using the medium having the following composition (g L^{-1}): Pancreatic casein (5); Yeast extract (2.5); Glucose (1); and Agar (15). The pH of medium was adjusted to 6.75 ± 0.25 and autoclaved. After cooling, 100 mL of a 10% skimmed milk solution was aseptically prepared and added to the medium, then seeded by the spot inoculation method. Bacteria with protease activity show a transparent halo around colonies over 48 h of incubation [63]. *B. licheniformis* QA1 [32] and *E. coli* DH5α were used as positive (C+) and negative (C−) controls, respectively.

2.6. Antibiotic Sensitivity Assay

Bacterial isolate was tested for its potential resistance to antibiotics on Mueller-Hinton (MH) media (BIOKAR Diagnostics, BEAUVAIS, France) [64] using agar dilution assay, disc diffusion method, and Etest [65]. Freshly prepared MH plates were amended with various concentrations of antibiotics frequently used in the treatment of infectious diseases namely ampicillin (100 µg/mL), kanamycin (50 µg/mL), tetracycline (10 µg/mL), streptomycin (100 µg/mL), chloramphenicol (20 µg/mL), and spectinomycin (60 µg/mL). As for disc diffusion and Etest, 200 µL of 18 h old bacterial culture (OD_{600nm} = 1) was spread over the entire MH agar surfaces using a swab stick and left to dry for five minutes before placing the antibiotic-impregnated discs and/or strips. Antibiotic discs of ceftazidime (CAZ; 30 µg/disc), ciprofloxacin (CIP; 5 µg/disc), meropenem (MRP; 30 µg/disc), ertapenem (ETP; 10 µg/disc), and vancomycin (VA; 30 µg/disc) were placed in triplicate. Etest was adopted using the *Liofilchem*® Minimum Inhibitory Concentration (MIC) Test Strips with a predefined concentration gradient of imipenem (IMI; 0.002–32 µg/mL), gentamycin (CN; 0.06–1024 µg/mL), and cefotaxime (CTX; 0.002–32 µg/mL). As a negative control, we used the *E. coli* DH5α strain as it is sensitive to all tested antibiotics [66]. In addition, we used a PGPR (Plant Growth Promoting Rhizobacterium) *Bacillus atrophaeus* strain S8 (MW295957) from our lab collection as an additional control. Resistance patterns were determined following incubation at 30 °C for 24 h. Positive resistances were considered in antibiotic MH amended plates by observing the growth of the bacterium on each medium. In disk diffusion plates, the zone of inhibition surrounding discs was measured in millimeters (mm) using calipers near the agar surface [67]. However, the minimum inhibitory concentration (MIC) was read directly from the scale in terms of µg/mL, at the intersection point of the edge of the inhibition ellipse and the MIC Test Strip. Susceptibilities and resistances were analyzed using Zone Size Interpretative Chart (ZSIC) [68,69], *Liofilchem*®—Antibiotic Disc Interpretative and CLSI Standards for Antimicrobial Susceptibility Testing of Clinical and Laboratory Standards Institute (https://clsi.org/ (accessed on 18 October 2020)).

2.7. Monitoring Trace Elements Tolerance

The ED1 isolate was tested for its growth under various concentrations ranging from 0 to 2000 µg/mL of trace metals: $CdSO_4$, $CuO_4S.5H_2O$, and N_2NiO_8. Stock solutions were prepared in distilled water, sterilized through 0.45 µm using sterile syringe filters, and stored at 4 °C. Agar dilution method was followed [70]. Freshly prepared agar plates were amended with increasing soluble heavy metal salts concentrations [71]. The ED1 isolate was streaked in both metal amended and control plates (free metal media). Trace metal tolerance was determined after 48 h of incubation at 30 °C. *B. licheniformis* QA1 [32] and *E. coli* DH5α were used as positive (C+) and negative (C−) controls, respectively.

2.8. Quinoa Seed Germination Assay

Quinoa seeds of *Titicaca* variety were used for in vitro seed bacterization to evaluate the early plant growth promoting potential of selected bacterium. Firstly, seeds were sorted and those with damageable aspects were excluded [32]. Seeds were surface sterilized using

3% of sodium hypochlorite for 1 min, soaked in 70% ethanol for 1 min, rinsed 5 times by sterilized distilled water, and air-dried under a laminar flow hood. The bacterial pellets (OD_{600nm} = 0.8) were obtained from an overnight bacterial culture by centrifugation at 5000 rpm for 5 min. The pellets were then resuspended in 10 mL of phosphate-buffered saline (PBS) and used as seed drench [72]. Bacterial suspension was applied as seed drenches for 1 h with gentle shaking [73]. Afterwards, seeds were air-dried and transferred into 9 cm sterile Petri dishes on filter paper with a ratio of 30 seeds per plate. Each treatment was performed in triplicate, and seeds treated with PBS were used as negative control [74]. Each filter paper was aseptically wetted with 3 mL of sterilized distilled saline water solution at 0, 200 (1.16%), or 400 mM (2.32%) NaCl [74]. To prevent evaporation and contamination, the plates were closed with parafilm. The plates were labeled and maintained at 25 °C for 48 h in the dark [73] and germination rates were monitored 24 h and 48 h post-incubation. At the third day, the plates were maintained at room temperature in a day/night cycle (~12/12 h) for an additional 72 h to compute their morphological traits mainly total length, fresh and dry weight. The germination percentage and vigor index were calculated using the following equations [74,75].

$$\text{Germination percentage (\%)} = (n/N) \times 100 \quad (1)$$

n: number of germinated seed and N: total number of seeds.

$$\text{Vigor index} = \text{Germination percentage (\%)} \times \text{Total seedling length (cm)} \quad (2)$$

2.9. Statistical Analysis

All results obtained were subjected to statistical analysis using IBM SPSS Statistics 20 software. Comparison between means were performed using one-way analysis of variance (ANOVA) followed by the post-hoc analysis with Tukey test. Significant differences were set at $p \leq 0.05$. The results were expressed as the mean of 3 replicates ± SD. The experiments performed in this study were done in triplicates using a complete randomized design (CRD).

3. Results

3.1. In Vitro PGP Properties of Selected Quinoa Endophytic Bacteria

3.1.1. P and Zn Solubilization, and Oligonitrophilic Activity

A total of 34 isolated endophytic bacteria were screened for mineral phosphate solubilization on NBRIP agar plates. One isolate named ED1 was further selected based on its remarkable capacity to solubilize inorganic P on plates as evidenced by the halos surrounding colonies (Figure 1). We also checked its ability to solubilize insoluble Zn from three minerals: ZnO, $Zn_3(PO4)_2$, and CO_3Zn. The ED1 isolate was able to solubilize ZnO at a greater extend compared to the two other forms, while *B. licheniformis* QA1 and *E. coli* DH5α only solubilized ZnO (Table 1).

In contrast to *B. licheniformis* QA1 and *E. coli* DH5α used as positive and negative controls, respectively, the ED1 isolate was able to grow on Jensen's medium which is very poor in nitrogen, indicating its oligonitrophilic activity (Table 1).

Table 1. Summary of relevant plant growth promoting (PGP) traits of the ED1 isolate, the PGPR strain *Bacillus licheniformis* QA1, and *E. coli* DH5α (C−).

Strain/Isolate	Oligonitrophilic Activity	Siderophore	Zinc Solubilization			Ammonia (µmol/mL)	Extracellular Enzymes	
			ZnO	$Zn_3(PO)_2$	CO_3Zn		Cellulase	Protease
E. coli DH5 α (C−)	−	−	+	−	−	0.065 ± 0.048	−	−
B. licheniformis QA1 (C+)	−	+++	++	−	−	0.48 ± 0.11	++	+
ED1 isolate	+	+	+++	+	+	0.68 ± 0.04	+	−

The values represent means of replicates (n = 3) ± SD. The '+' and '−' signs indicate efficiencies as follow: −, negative result; +, weakly positive; ++, moderately positive; +++, highly positive. Previously published *B. licheniformis* QA1 [32] was used as the PGPR reference strain (C+; positive control) and *E. coli* DH5α (C−; negative control).

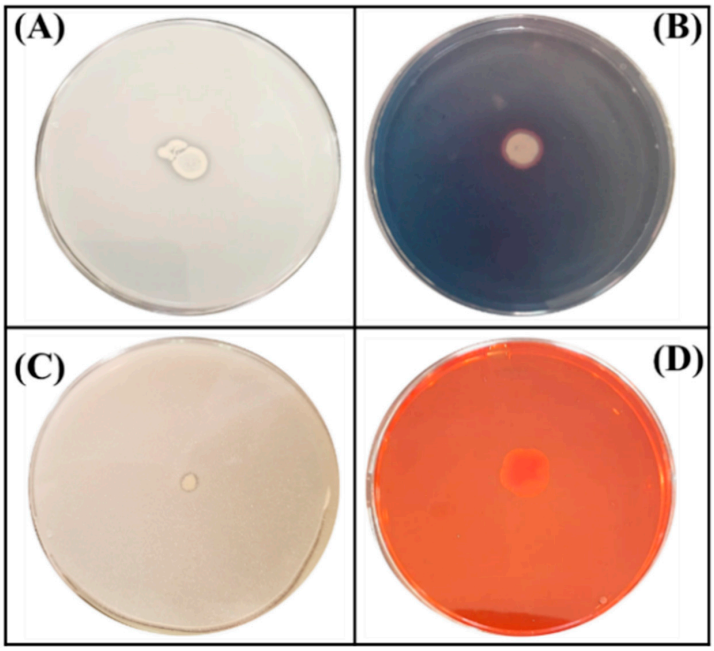

Figure 1. Schematic illustration of four tests performed on plates for the assessment of plant growth promoting traits of the ED1 isolate. (**A**) Phosphate solubilization, (**B**) Siderophores production, (**C**) Zinc oxide solubilization, (**D**) Cellulase production.

3.1.2. Siderophores, Ammonia and Extracellular Enzymes Production by the ED1 Isolate

We next tested the capacity of the ED1 isolate to produce molecules and metabolites of agricultural interest. We showed that it liberated siderophores and produced ammonia and cellulase enzyme (Figure 1). In contrast, no protease activity was detected. In addition, the ED1 isolate was able to grow in peptone water, indicating its capacity to produce ammonia which was 10-fold higher than in *E. coli* DH5α. Comparatively with the PGPR reference strain *B. licheniformis* QA1, the selected isolate produced less amounts of siderophores and cellulase but higher concentrations of ammonia (Table 1).

3.2. The ED1 Isolate Is Related to the Genus of Serratia Rubidaea

Using the genotyping analysis based on 16S rDNA sequencing and sequences comparison to the NCBI and SILVA databases, we found that the ED1 isolate is 99.7% identical to the genus of *Serratia rubidaea*. Next, the amplified 16S rDNA sequence was submitted to GenBank and the accession number MW173145 was provided. The phylogenetic tree analysis reflected the same identity with *Serratia rubidaea* (Figure 2).

3.3. The Strain S. rubidaea ED1 Tolerates Growth under Stressful Conditions

We first checker the tolerance of *S. rubidaea* ED1 to salt. Bacteria were plated on TSA containing various NaCl concentrations. We found that the ED1 strain grew up to 9% of NaCl, while the PGPR reference strain, *B. licheniformis* QA1, tolerated up to 11%. However, the growth arrest happened in *E. coli* DH5α at only 3%. In addition, ED1 is a mesophilic bacterium as its optimal growth ranged from 20 to 42 °C (Table 2). When grown on liquid TSB media with increasing NaCl concentrations, the maximum growth of the *S. rubidaea* ED1 strain was detected at 4% NaCl. However, although at reduced growth level, it tolerated up to 10% NaCl. Total growth inhibition occurred at 12% NaCl. In contrast to the ED1 strain, the growth of *E. coli* DH5α proportionally decreased as the concentration of NaCl increased (Figure 3A). Next, we monitored bacterial survival at different pH and found that the optimum growth of both *S. rubidaea* ED1 strain and *E. coli* DH5α occurred at pH 7. However, *S. rubidaea* ED1 could still grow in a range of pH from 4 to 9 (Figure 3B).

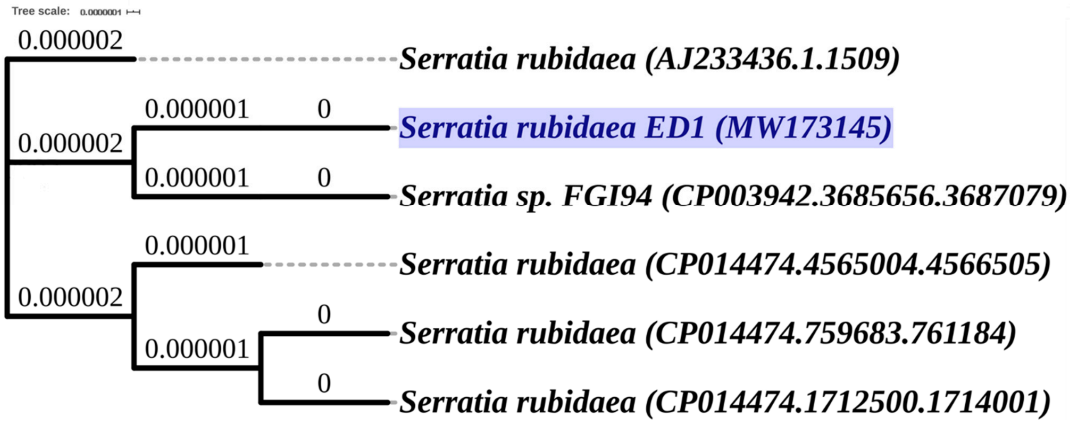

Figure 2. Phylogenetic tree of selected bacterium *Serratia rubidaea* ED1 (MW173145) based on the PHYLIP (PHYLogeny Inference Package) Neighbor-Joining method of the 16S rRNA gene sequences using (Unipro UGENE software, version 1.32.0. Branch lengths are displayed as age. The 16S rRNA gene sequences and tree of related species were downloaded from the High-Quality Ribosomal RNA database (SILVA).

The ability of the *S. rubidaea* ED1 strain to grow on plates containing various trace metals concentrations was also evaluated. Because of their great interest, cadmium, copper, and nickel were chosen. We found that *S. rubidaea* ED1 supported $CdSO_4$ at 2000 µg/mL, $CuO_4S.5H_2O$ at 1000 µg/mL, and finally, N_2NiO_8 at 1000 µg/mL. For both the positive and negative control strains, maximum tolerances were seen at 300 µg/mL, 1000 µg/mL and 1000 µg/mL, of $CdSO_4$, N_2NiO_8, and CuO_4S, respectively (Table 2).

Table 2. Summary of relevant extreme properties of isolated endophytic bacterium *Serratia rubidaea* ED1, the PGPR reference strain *B. licheniformis* QA1, and *E. coli* DH5α (C−).

Species	Maximum Tolerable Temperature	NaCl Tolerance Range (%)	Heavy Metals Tolerance		
			Max Concentration (µg/mL)		
			$CdSO_4$	N_2NiO_8	CuO_4S
E. coli DH5α (C−)	37 °C	0–3	300	500	1000
B. licheniformis QA1 (C+)	55 °C	0–11	200	1000	500
S. rubidaea ED1	42 °C	0–9	2000	1000	1000

Previously published *B. licheniformis* QA1 [32] was used as the PGPR reference strain (C+; Positive control) and *E. coli* DH5α (C−; negative control).

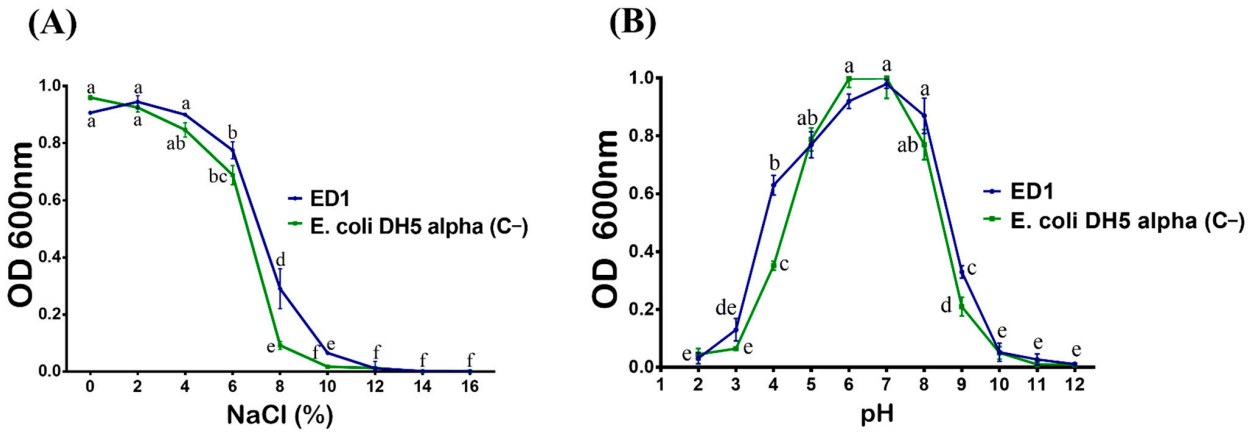

Figure 3. Effect of NaCl (**A**) and pH (**B**) on the growth of the *S. rubidaea* ED1 strain. Media inoculated with *Esherichia coli* DH5α were used as a negative control (C−). The values represent means of three replicates (n = 3) ± SD. The different letters in superscript (a–f) indicate the statistically significant difference at 95% between treatments.

3.4. Effect of NaCl on P solubilization by Strain S. rubidaea ED1

Salinity is considered as one of the main factors influencing P solubilization by bacteria [76]. Here, we assessed the ability of ED1 strain to solubilize hydroxyapatite at increasing NaCl final concentrations: 0%, 4%, and 8%. P solubilization by the *S. rubidaea* ED1 strain decreased following the increase of NaCl concentration (R^2 = 0.87–0.99). In addition, P solubilization by ED1 was significantly increased over the incubation time from 5 to 10 DAI (Figure 4A, Table 3). Soluble PO_4 levels significantly deceased following the increase of NaCl concentration (Figure 4A) with correlation coefficients of up to −0.99 (Table 3). The mineral P was solubilized at its maximum at 0% NaCl at 10DAI where it reached 1085.22 ± 84.34 mg/L by ED1 strain and 307.81 ± 20 mg/L by *E. coli* DH5α (C−). It turned out that compared to the *S. rubidaea* ED1 strain, 8% NaCl has a greater reducing effect on *E. coli* DH5α solubilizing capacity at 5DAI. Interestingly, the level of solubilized P under 8% NaCl is the same as the one obtained at 0% NaCl using ED1 strain. More precisely, soluble P reached 350.63 ± 82.40 mg/L in 8% NaCl medium using the *S. rubidaea* ED1 strain and 307.81 ± 20 mL/L in 0% NaCl medium using *E. coli* DH5α. In salty media, solubilized P by ED1 increased from 5 to 10DAI, while no statistically significant increase was noticed in *E. coli* DH5α (C−) (Figure 4A).

The solubilization of P by the endophyte strain was accompanied by a significant drop in pH of the culture supernatants in both saline and non-saline NBRIP media (Figure 4B). Highly negative correlations were noticed between soluble *p* values and final pH of the supernatants (r = 0.9–0.99, R^2 = 0.82–0.98) (Table 3). The maximum pH decline, 3.04 ± 0.11, was observed using ED1 in the non-saline medium at 10DAI and it proportionally increased with NaCl concentrations over the incubation period. For instance, at 8% NaCl, the pH at 10DAI was 5.86 ± 0.29 in *E. coli* DH5α, and 3.76 ± 0.92 in *S. rubidaea* ED1 strain (Figure 4B).

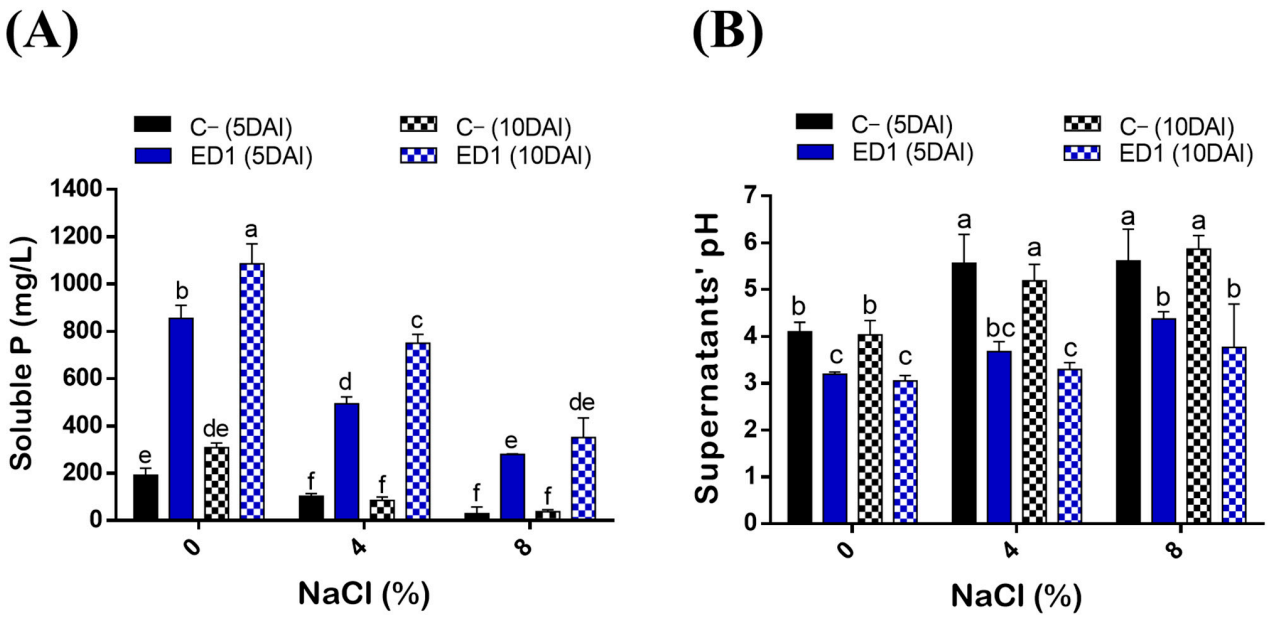

Figure 4. Quantitative determination of solubilized P (PO_4) in the National Botanical Research Institute's phosphate (NBRIP) broth under salt stress conditions. (**A**) Solubilized P concentrations by the *S. rubidaea* ED1 strain and *E. coli* DH5α (C−) at 0%, 4%, and 8% NaCl final concentrations. (**B**) pH variation of final culture supernatants. DAI means day after incubation. The values represent means of replicates (n = 3) ± SD. The different letters in superscript (a–f) indicate the statistically significant difference at 95% between treatments.

Table 3. Analysis of the variation of soluble P concentrations released by *S. rubidaea* ED1 and *E. coli* DH5α strain as a function of NaCl levels and pH values.

Soluble P vs. NaCl (%)				
	C− (5DAI)	ED1 (5DAI)	C− (10DAI)	ED1 (10DAI)
Coefficient of determination (R^2)	0.996	0.978	0.877	0.997
Correlation coefficient (r)	−0.998	−0.989	−0.936	−0.998
Soluble P vs. pH				
	C− (5DAI)	ED1 (5DAI)	C− (10DAI)	ED1 (10DAI)
Coefficient of determination (R^2)	0.820	0.940	0.957	0.983
Correlation coefficient (r)	−0.905	−0.969	−0.978	−0.991

DAI: Day after incubation. C−: *E. coli* DH5α strain used as negative control.

3.5. Indole Acetic Acid Production by the Serratia Rubidaea ED1 Strain Is Partly L-Tryptophan Dependent

The production of IAA by the *S. Rubidaea* ED1 strain correlated proportionally to added L-tryptophan concentrations (r = 0.88, 0.99, 0.98, R^2 = 0.77, 0.99 and 0.96) and to period of incubation (r = 0.95, 0.99, 0.99, R^2 = 0.91, 0.98 and 0.99) (Figure 5). IAA production was significantly higher using 0.5% L-tryptophan at day 11 (Figure 5C) compared to 0.2% L-tryptophan at day 7. In the negative control media, IAA did not exceed 0.17 ± 0.09 µg/mL over the three tested incubation periods (Figure 5A–C). Unexpectedly, IAA was produced even in the absence of L-tryptophan as the measured quantities were: 25.23 ± 1.81 µg/mL at day 3 (Figure 5A), 66.79 ± 18.88 µg/mL at day 7 (Figure 5B), and 76.47 ± 17.7 µg/mL at day 11 (Figure 5C).

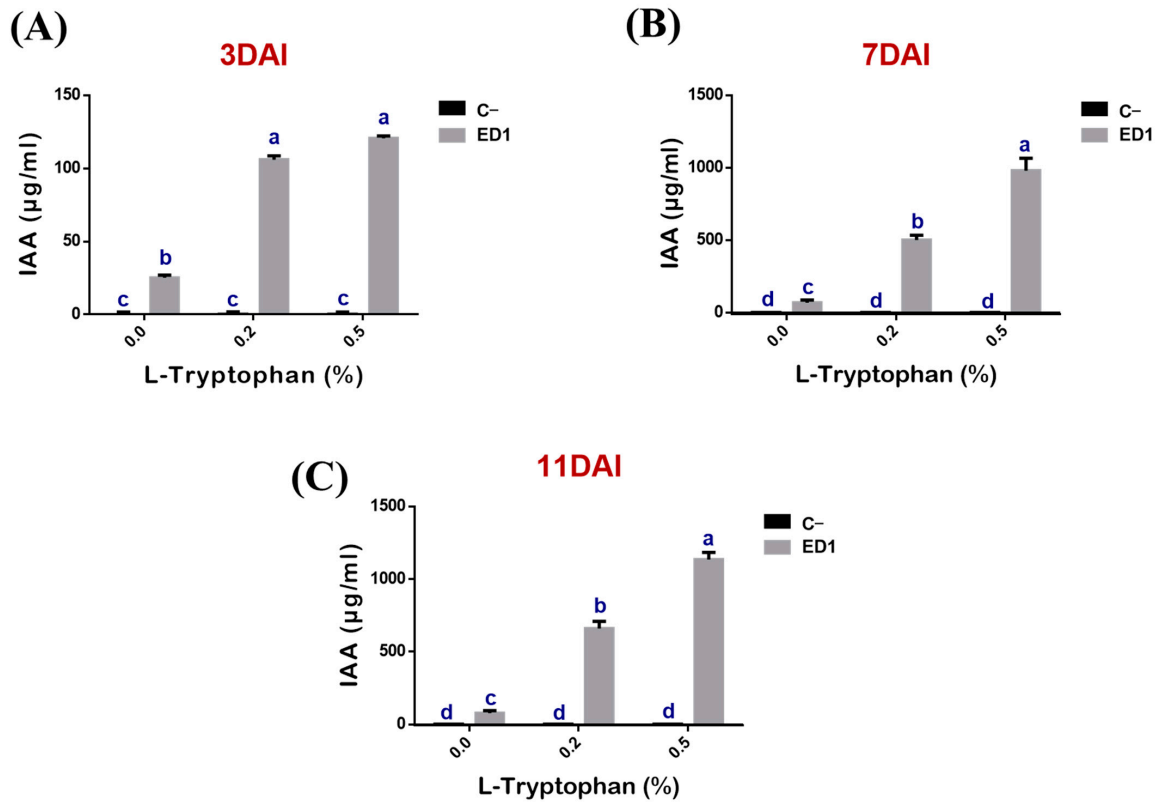

Figure 5. Kinetic of indole acetic acid (IAA) production by *S. rubidaea* ED1 with or without L-tryptophan addition. IAA determination was performed after 3 (**A**), 7 (**B**), and 11 (**C**) days of incubation. Uninoculated media were used as a negative control (C−). The values represent means of three replicates (n = 3) ± SD. The different letters in superscript (a–d) indicate the statistically significant difference at 95% between treatments.

3.6. Strain Serratia rubidaea ED1 Exhibited Intrinsic Antibiotic Resistance

Next, we evaluated the intrinsic resistance phenotype to antibiotics by choosing those that are frequently used in humans to tackle infectious diseases. Based on the Zone Size Interpretative Chart (ZSIC) and MIC strips (Figure 6), it turned out that the *S. rubidaea* ED1 strain is resistant to several antibiotics such as ciprofloxacin, ertapenem, meropenem, ampicillin, chloramphenicol, tetracycline, and spectinomycin, while *Bacillus atrophaeus* S8 strain resists only ceftazidim and ertapenem (Table 4, Figure 6).

3.7. Strain Serratia rubidaea ED1 Enhanced Germination Rate and Seedlings Growth under Salty Amendment

The quinoa seed germination rate of both inoculated and control seeds was gradually reduced along with an increasing of NaCl concentration, especially 24 h post incubation. The *S. rubidaea* ED1 strain significantly enhanced seed germination rate under non-saline (0 mM NaCl) and saline (400 mM or 2.32% NaCl) conditions by 36.35% (after 24 h incubation) and 390.39% (after 48 h incubation), respectively (Figure 7). Compared to non-saline treatment, 400 mM NaCl decreased seed germination by 58.87% in controls seeds and by 45.8% using *S. rubidaea* ED1 strain (Figure 8A,B, Table 5). At a moderate NaCl concentration (200 mM or 1.16% NaCl), the percentage of germination increased in a comparable manner to the control (Figure 8A,B). Inoculation with *Serratia rubidaea* ED1 increased the total length of seedlings under both non-saline and saline treatments. ED1 strain enhanced seedling length by 57.76, 52.97, and 376.19% under 0, 200, and 400 mM NaCl, respectively (Figure 8C, Table 5). Likewise, quinoa seedlings showed 34.78 and 253.39% increase in dry weights under 0 and 400 mM NaCl treatments, respectively. Finally, the vigor index was significantly affected using the ED1 strain, especially under 0 and 400 mM NaCl treatment ($p < 0.05$) (Figure 8F).

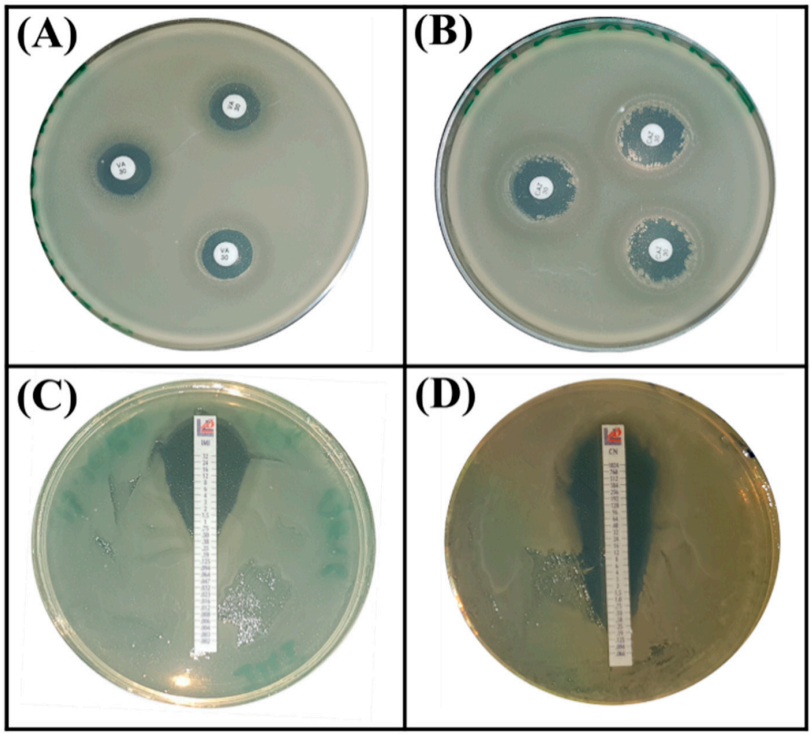

Figure 6. Antibiotic resistance assays using *Serratia rubidaea* ED1 strain on MHA (Mueller Hinton Agar, BIOKAR Diagnostics, BEAUVAIS, France) plates supplemented by antibiotic disc method (**A**,**B**) and Etest (**C**,**D**). (A) vancomycin, (**B**) ceftazidime, (**C**) imipenem, (**D**) gentamycin.

Table 4. Antibiotic resistance profile of the *S. rubidaea* ED1 strain using disc diffusion, Etest, and antibiotic amended agar methods.

Strain Etest (MIC in μg/mL)				**Disc Diffusion Test (Zone of Inhibition in mm)**				
	IMI	CTX	CN	CAZ	CIP	MRP	ETP	VA
				(30 μg)	(10 μg)	(10 μg)	(10 μg)	(30 μg)
S. rubidaea ED1	2.5	1	0.125	18.66 ± 0.57	20 ± 2.64 *	19.93 ± 0.77 *	24.6 ± 1.32 *	13.66 ± 0.57
B. atrophaeus S8	0.38	1.5	0.75	17.66 ± 1.52 *	30 ± 3	29.33 ± 2.08	21 ± 1 *	NT
E. coli DH5α (C−)	−	−	−	−	−	−	−	−
Antibiotic amended agar method (μg/mL)								
	AM	CHL	STR	SPC	K	TET		
	100	20	100	60	50	10		
S. rubidaea ED1	+	+	−	+	−	+		
B. atrophaeus S8	−	−	−	−	−	−		
E. coli DH5α (C−)	−	−	−	−	−	−		

The values represent means of three replicates (n = 3) ± SD. MIC (Minimum Inhibitory Concentration), CAZ (ceftazidim), IMI (imipenem), CIP (ciprofloxacin), CTX (cefotaxim), CN (gentamycin), MRP (meropenem), ETP (ertapenem), VA (vancomycin), AM (ampicillin), CHL (chloramphenicol), STR (streptomycin), SPC (spectinomycin), K (kanamycin), TET (tetracycline), NT (Not tested). Asterisk symbols (*) and the '+' sign indicate resistance while the '−' sign indicates susceptibility. Strains *Bacillus atrophaeus* S8, and *E. coli* DH5α (C−) were used as controls.

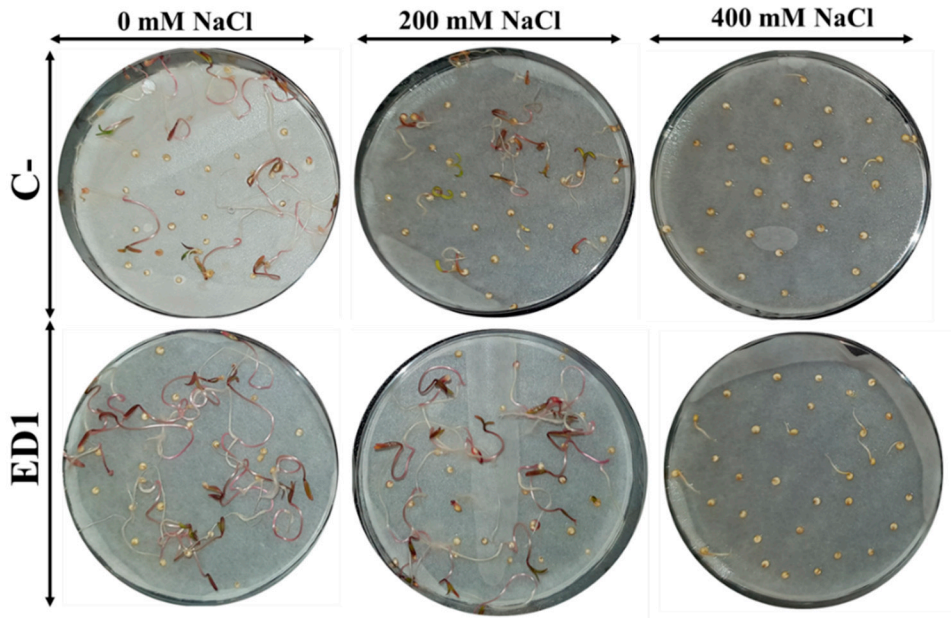

Figure 7. Effect of the *Serratia rubidaea* ED1 strain on quinoa seed germination and early growth seedlings under saline treatments. Seeds inoculated with phosphate-buffered saline (PBS) were used as a negative control (C−).

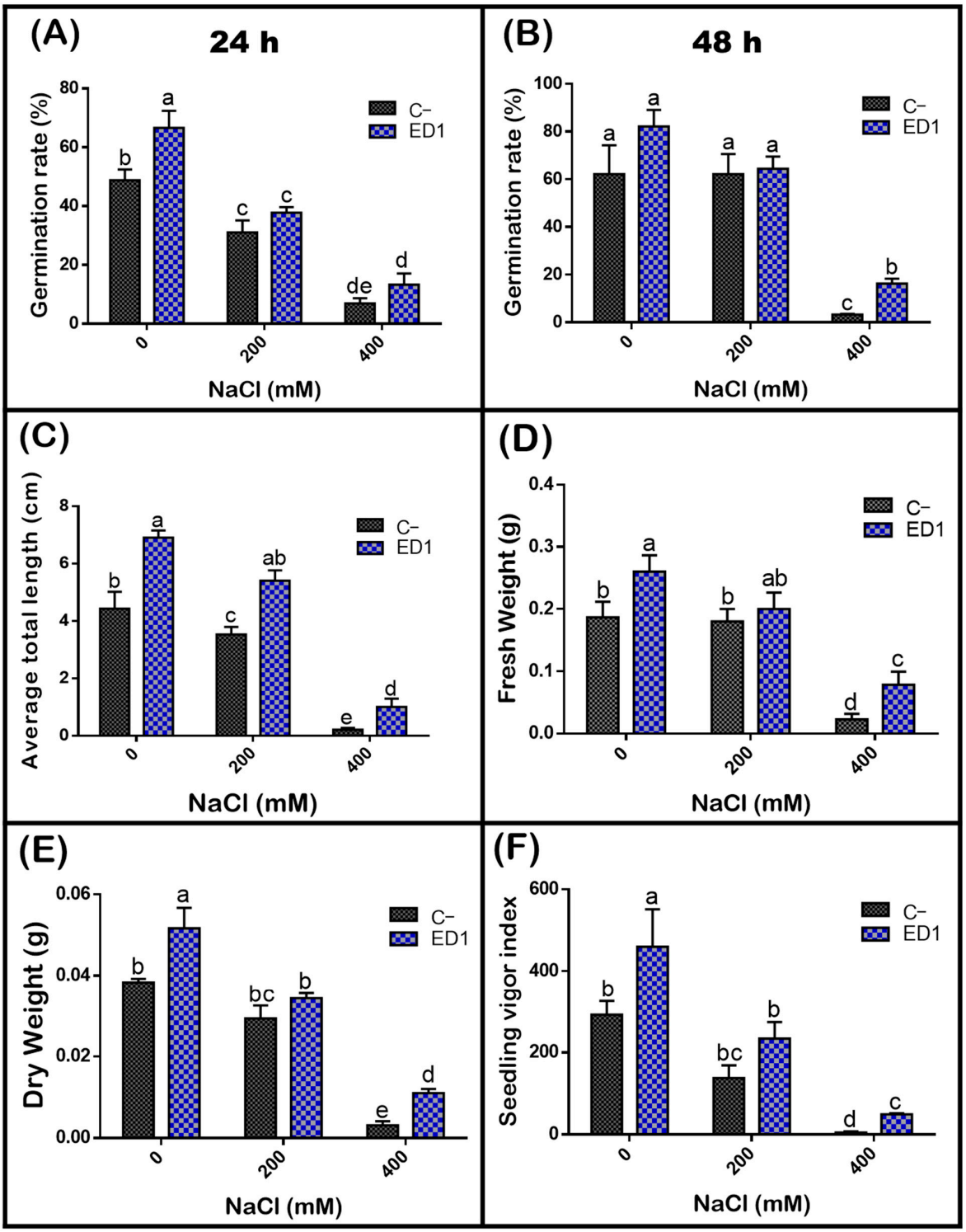

Figure 8. Effect of the *S. rubidaea* ED1 strain on quinoa seed germination parameters under different salinity treatments (0, 200, and 400 mM NaCl) after 48 h of incubation at 25 °C. C− (negative control; Seeds inoculated with phosphate-buffered saline (PBS)), ED1 (Seed treated with the ED1 strain): (**A**) Germination rate after 24 h of incubation, (**B**) germination rate after 48 h of incubation, (**C**) average of total length of seedlings, (**D**) average of fresh weight of seedlings, (**E**) average of dry weight of seedlings, and (**F**) seedling vigor index. The values represent means of three replicates (n = 3) ± SD. The different letters in superscript (a–e) indicate the statistically significant difference at 95% between treatments.

Table 5. Inoculation of quinoa seeds with the *Serratia rubidaea* ED1 strain enhanced the rate of seedling growth parameters.

% Increase						
NaCl (mM)	**Germination Rate (24 h)**	**Germination Rate (48 h)**	**Total Length**	**Fresh Weight**	**Dry Weight**	**Vigor Index**
0	36.36	32.15	55.76	39.29	34.78	57.26
200	21.46	3.59	52.97	11.11	17.05	39.45
400	93.79	390.39	376.19	245.59	253.39	904.50

Seeds inoculated with phosphate-buffered saline (PBS) were used as the growth reference (100%). for each parameter.

4. Discussion

Alongside nitrogen (N) and potassium (K), phosphorus (P) is one of the most limiting nutrients for crop yields, growth, and development. P deficiency in plants is usually translated by inhibited stem and root development, poor flowering, lack of seed and fruit formation etc. In last decades, the use of PGPB-based bio-inoculants as an alternative to chemical fertilizers has emerged [77]. In the present study, we identified an endophytic *S. rubidaea* ED1 bacterium from quinoa roots endosphere, a plant endowed with many phyto-beneficial attributes facing extreme growth stresses. Some PGP results of *Serratia rubidaea* ED1 were expected as they were reported in *Serratia rubidaea* alongside *S. plymuthica*, *S. liquefaciens*, *S. proteamaculans*, and *S. nematodiphila*, isolated from various environments niches [78]. PGP attributes of *Serratia rubidaea* (NR114716) associated with *Origanum* plants was reported for IAA and siderophores production, P solubilization, ACC-deaminase activity, and N fixation [79]. In addition, this strain improved growth performance parameters and biochemicals, and was considered as a potential bio-factor to engineer the essential oil constituents from aromatic plants [79]. The first and foremost activity we tested here was mineral P and Zn solubilization. Although reports on P and Zn solubilization by bacterial endophytes are scarce, we showed that *S. rubidaea* ED1 strain solubilized both mineral compounds of P and Zn. Maximum Zn solubilization was recorded in ZnO compared to $Zn_3(PO_4)_2$, and $ZnCO_3$ (Table 1). The mechanisms underlying this specificity remain unknown and very recently, endophytic *Serratia* sp. ZoB14 has been screened for dual P and ZnO solubilization [80]. Next, we showed that *S. rubidaea* ED1 grew on a N-free solid medium supporting its oligonitrophilic feature (Table 1) as previously reported in *Serratia liquefaciens* and *Serratia marcescens* [81,82]. Indeed, oligonitrophilic bacteria have broad substrate specificities that allow them to maintain their structure in low N environments [83]. However, other environmental factors, such as soil pH, could greatly influence the oligonitrophilic function [84]. This confirms that *Serratia rubidaea* ED1 strain is more prone to oligonitrophilic conditions, e.g., plants' endosphere.

Around the world, 20% of cultivated and 33% of irrigated arable lands are affected by high salinity [85]. Induced salt tolerance in plants has been shown to be associated with various PGPR strains [32,86–88]. We demonstrated here that *Serratia rubidaea* ED1 grew up to 10% NaCl (Figure 3). Taking advantage to this result, we next studied P solubilization under salty conditions. The quantity of orthophosphates (PO_4) released from hydroxyapatite by *S. rubidaea* ED1 decreased with the increase of NaCl concentration. However, substantial amount of soluble P (up to 750 mg/L) was detected at 10DAI using 4% NaCl final concentration. Maximum soluble P level, 1085.22 $\pm$ 84.34 mg/L, was seen in 0% NaCl media at 10DAI (Figure 4). In salty media, the increased P release between days 5 and 10 suggests the adaptation of *Serratia rubidaea* ED1 to the salty medium, as previously reported for *Bacillus megaterium* [51]. Our result is in-line with the reported data on the endophytic *Pseudomonas fluorescens* that solubilized up to 1312 mg/L in non-saline NBRIP medium [46]. As expected, P solubilization was accompanied by a pH decrease which remained almost unchanged under salt treatment. This finding is likely attributed to organic acids production [89].

Bacteria from any environment are exposed to abiotic stress, exemplified by salinity, pH, temperature, trace elements, nutrient deficiency, and biotic stress such as toxic metabolites, pathogens, competing microorganisms [90]. To cope with these stresses, bacteria use several adaptative strategies to modify their signalization pathways [51]. Salt level is one of

the main parameters affecting the growth rate and metabolism of microorganisms and several reports indicated the positive impact of halotolerant microorganisms on plant growth. At present, PGPB are suggested to enhance the productivity of plants facing salinity and can be exploited to sustain saline agroecosystems [20]. In this study, salt tolerance of *S. rubidaea* ED1 to NaCl was about 10%, while total growth arrest happened at 12% under hyper osmotic conditions (Figure 3). Salt-tolerant bacteria activate osmoadaptation pathways mainly by synthesizing compatible solutes and/or accumulation of K^+ to overcome the Na^+ ions associated toxicity [91]. The excess of sodium ions affects the bacterial enzymes activity [92]. In addition, bacterial exopolysaccharides (EPS), antioxidant enzymes, and ACC deaminase activity are among the strongest salt stress-adapting mechanisms [21].

The capacity of selected endophytic bacterium to solubilize unavailable nutrients such as P and Zn and grow in low nitrogen medium is supportive to further investigate its plant growth potential by boosting plant nutrition. To further confirm the halotolerance property of *S. rubidaea* ED1 strain, we performed a seed germination assay and revealed that inoculation of quinoa seeds enhanced seeds germination and seedlings growth under 400 mM (2.32%) NaCl. Noticeably, germination rate in both inoculated and non-inoculated seeds were inversely proportional to the increase of NaCl (Figures 7 and 8), as previously reported [93]. Indeed, inoculation of quinoa seeds showed a prominent effect on vigor index (904.50% increase) (Table 5), pointing out the mitigation of salt stress in both assays. To our knowledge, this finding represents the first report stating that *Serratia rubidaea* promoted seeds germination and seedlings growth under salty treatments. Comparatively, a study on *Ligustrum sinense* seeds was conducted by increasing NaCl concentrations (0–500 mM) using strains: *Isoptericola dokdonensis*, *Arthrobacter soli*, *Streptomyces pactum*, and *Bacillus flexus.* The latter induced seeds germination by reaching 100% under 300–500 mM NaCl treatment [93]. Sorghum seeds inoculated with endorhizospheric *Serratia* sp. promoted seedlings growth [94]. The increase in seeds germination is likely attributed to the production of phytohormones such IAA [95]. Other studies reported the promoting role of *Bacillus* and *Pseudomonas* in seed germination, root development, and plant growth [96] of various crops including wheat, rice, and sugarcane [97]. Indeed, stimulation of seeds germination in the presence of IAA producing bacteria has been described [75]. Here, IAA production by *Serratia rubidaea* ED1 was seen even in the absence of L-Tryptophan supplementation (Figure 5). However, in L-tryptophan-supplemented broth, production of IAA increased significantly (Figure 5), corroborating previous finding on *Serratia* species including *S. rubidaea* highlighting that IAA signaling pathways are either auxin-dependent, auxin-independent, or tryptophan-independent [78,98]. Besides, other halotolerant bacteria species isolated from halophytic plant in coastal soils in Korea, enhanced plant growth under saline stress. In this case, plant growth was linked to the activity of the ACC deaminase that reduced ethylene production [99]. Additionally, it was reported that the halophyte *Salicornia brachiate* induced plant growth by withstanding a high level of salt [100]. More mechanisms by which microorganisms improve physiological response of plants under salt stress were described [101,102]. These include osmolytes accumulation which regulate water homeostasis, hormonal root–shoot signaling inducing salt tolerance in plants, modulation of the source-sink relationships for plant energetics, nutrients and toxic ions uptake by root by modifying host plant physiology, physical barriers around the roots, or by reducing foliar accumulation of toxic ions [101,102].

In another part of this study, we checked the ability of *S. rubidaea* ED1 to resist pH and to withstand trace elements. It was found to grow from pH 4 to 9 but at a significantly low rate compared to optimal pH 7 (Figure 3). Compared to other PGP strains such as *Bacillus megaterium, Staphylococcus haemolyticus,* and *Bacillus licheniformis* [52], ED1 strain tolerates slightly higher acidity, suggesting its ability to grow in acidic soils [103]. Accumulation of trace elements in the environment represent a major concern to public health and agriculture [104]. We found that ED1 strain survives at 2 mg/L of $CdSO_4$, and 1 mg/L of either $CuO_4S.5H_2O$ or N_2NiO_8 (Table 2). In the same range, *Planomicrobium chinense* and *Bacillus cereus* tolerate Cd (1.5 mg/L) and Ni (1 mg/L) by accumulating

heavy metals upon chelating agents release that affect their mobility and availability which consequently enhance phytoremediation and nutrient transformation [105]. For example, *Serratia marcescens* was shown to assist phytoremediation of metals via the reduction of the toxic chromium (VI) to the less soluble and less toxic chromium (III) [106].

To be an effective biofertilizer, PGPR must survive, resist, and colonize root surface and/or interior to establish itself at population densities required to induce beneficial effects on the host plant. We assessed the intrinsic antibiotic resistance of selected strain on MH agar plates. Surprisingly, this topic is very often neglected in agriculture. Here, we report that *S. rubidaea* ED1 is resistant to seven antibiotics, frequently used to treat infection diseases in humans such as ciprofloxacin, ertapenem, meropenem, ampicillin, chloramphenicol, tetracycline, and spectinomycin (Table 4). Our finding supports previous findings for another *Serratia rubidaea* strain conferring resistance to ampicillin, tetracycline, gentamycin, penicillin, vancomycin, and streptomycin [107–109]. The novelty here is that the list of antibiotics can be extended to five additional antibiotics, namely, ciprofloxacin, ertapenem, meropenem, chloramphenicol, and spectinomycin. It is well established that soil, food, and water are the habitats of the *Serratia rubidaea* strain. Interestingly, it was also found among clinical specimens as a nonpathogenic bacterium [110]. However, infection by *Serratia rubidaea* was reported in immunocompromised patients [111,112]. In 2016, the first complete sequence of the genome of *Serratia rubidaea* (CP014474) was isolated from a patient in China [113]. Sequence analysis revealed the presence of several antibiotic resistance genes (ARG) encoding metallo-beta-lactamase, chloramphenicol, and aminoglycoside phosphotransferase. Other *Serratia* strains such as *S. marcescens* FGI94, *S. fonticola* DSM 4576, and *S. ficaria* showed also multi-resistances to antibiotics [114–116]. Thus, the emerging question is how to consider the two-blade weapons of PGP bacterial strains exhibiting intrinsic resistance to several antibiotics. On the one hand, they can be used either as markers to assess bacteria survival either in vitro or in vivo [117,118], and to help bacteria surviving and competing in native and open microbial environmental niches [35]. On the other hand, their use may represent potential risks by disseminating ARG to neighboring bacteria, plant, animals, and later, to humans [119]. Having said that, the alterable functional and adaptive behavior of resistant strains originating from different environments can differ in genome expression profiles, accessory genome sequences, antibiotic resistance pattern, and virulence activities. In addition, polyphasic approaches, mainly multi-locus sequence typing, discriminatory genotyping methods such as *recN* sequencing, and genome hybridization, showed that a same strain (e.g., *Pseudomonas aeruginosa*) could be a singleton among a large group of closely related strains, clustering distantly from the typical clinical isolates [90,120,121]. The occurrence of *S. rubidaea* in human specimens is rare, and there are no data suggesting that it is of a clinical significance [122]. Recently, rhamnolipid biosurfactant production by *Serratia rubidaea* SNAU02 was shown to act as an anti-Fusarium wilt of eggplant [123]. Production of prodigiosin and pyrrolnitrin by *Serratia rubidaea* C27 represents the main antifungal activity mechanisms [124]. In addition, *S. rubidaea* species were isolated from infected pepper fruits as a causal agent of splotches. This phyto-pathogenicity was attributed to the liberation of extracellular enzymes playing a role during the host infection including protease, lipase, polygalacturonase, and alkaline phosphatase that degrade plant cell wall and membrane constituents. However, their production is inducible and usually dependent on several environmental factors [125].

5. Conclusions

The present study corroborates previous findings and bring new insights on the role of endophytic bacteria in mitigating stresses in agriculture. Our results shed light on the role of the newly isolated *S. rubidaea* ED1 strain to mitigate salt stress. Further investigations on plant growth promotion under large-scale experiments would be paramount to uncover various mechanisms and potential applications of *Serratia rubidaea* ED1 in agriculture. Besides, there is a growing need to remediate the heavy metals from contaminated soils using various microbial accumulators capable of growing in heavy metals contaminated

soils [126]. Hence, to follow-up the present work, more studies should address the potential application of *S. rubidaea* ED1 in sustainable rehabilitation of heavy metals contaminated arable lands and to spotlight the underpinning mechanisms. Lastly, our work raises questions on a neglected issue related to the risk in using PGPR bacteria conferring resistance to many antibiotics for crop improvement.

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