Isolation and Screening of Extracellular PGPR from the Rhizosphere of Tomato Plants after Long-Term Reduced Tillage and Cover Crops

Maria Chiara Guerrieri 1, Elisabetta Fanfoni 1, Andrea Fiorini 2, Marco Trevisan 1,* and Edoardo Puglisi 1

1 Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy; mariachiara.guerrieri@unicatt.it (M.C.G.); elisabetta.fanfoni01@icatt.it (E.F.); edoardo.puglisi@unicatt.it (E.P.)

2 Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy; andrea.fiorini@unicatt.it

* Correspondence: marco.trevisan@unicatt.it; Tel.: +39-0523-599152

Received: 8 April 2020; Accepted: 20 May 2020; Published: 25 May 2020

Abstract: Plant growth promoting rhizobacteria provide an innovative solution to address challenges in sustainable agro-ecosystems, improving plant growth as well as acting as agents of biocontrol. In this study autochthonous bacteria were isolated from the rhizosphere of processing tomato plants (Solanum lycopersicum L.) cultivated with conservation agriculture practices (i.e., reduced tillage and cover crops), and evaluated for both growth-promoting activities (PGPAs), and antagonistic potential against the phytopathogenic pest Sclerotinia sclerotiorum. Considering the several activities of PGPR, we decided to structure the screening with a hierarchic approach, starting from testing the capability of fixing nitrogen. The obtained bacteria were processed through the molecular typing technique rep-PCR (Repetitive Extragenic Palindromic) in order to discriminate microbial strains with the same profiles, and identified via 16S rDNA sequencing. Thirty-eight selected isolates were screened in vitro for different activities related to plant nutrition and plant growth regulation as well as for antifungal traits. Isolated bacteria were found to exhibit different efficiencies in indoleacetic acid production and siderophore production, phosphate solubilization and biocontrol activity against the widespread soil-borne plant pathogen S. sclerotiorum. All the 38 bacterial isolates showed at least one property tested. With a view to detect the suitable candidates to be developed as biofertilizers, the selected isolates were ranked by their potential ability to function as PGPR. Thus, consortium of native PGPR bacteria inoculants may represent a suitable solution to address the challenges in sustainable agriculture, to ensure crop yield and quality, lowering the application of chemicals input.

Keywords: conservation agriculture; biostimulants; diazotrophs; phosphate solubilizers; IAA production; siderophores; Sclerotinia sclerotiorum

1. Introduction

Plants can no longer be seen as standalone entities; it has now been ascertained the role of plant microbiota in promoting plants fitness thanks to an intimacy of interactions between the plant and a wide diversity of microorganisms both inside and outside plant tissues, in the endosphere and rhizosphere respectively [1]. This is especially the case for soils where conservation agriculture practices (i.e., reduced tillage, cover crops) are performed [2,3], due to enhanced soil fertility conditions [4]. Bacteria able to colonize plant root systems and promote plant growth are referred to as plant growth promoting rhizobacteria (PGPR) [5,6]. PGPR exert a number of positive effect to plants, ranging from direct influence mechanisms, aimed at the plant nutrition and plant growth regulation, to indirect effects, related to the biocontrol activity [7,8]. Thus, PGPR appear to promote plant growth by
We focused on the identification of potential PGPR isolates by using selective or semi-selective culture media, well known that CCs increase the biomass of a microbial community [37,38], there is still a paucity of information on the effects of long-term RT management on soil microbial structure and diversity, and belonging to genera other than Pseudomonas and Bacillus are under-explored, in comparison to the amount of agricultural land cultivated worldwide [17]. Interest in the bacterial diversity detected in the rhizosphere is represented mainly by species belonging to three dominant phyla such as Proteobacteria, Firmicutes, and Actinobacteria [35], where the most common genera reported include Bacillus, Pseudomonas, Enterobacter, Erwinia, Serratia, Arthrobacter, Rhizobium, Agrobacterium, Burkholderia, Azospirillum, Azotobacter, Mycobacterium, Flavobacterium, and Micrococcus [36]. However, a shortage of studies is referred to the microbial diversity that is potential induced by reduced tillage (RT) and cover crops (CCs) on field. Indeed, while it is now well known that CCs increase the biomass of a microbial community [37,38], there is still a paucity of information on the effect of long-term RT management on soil microbial structure and diversity, rather than a conventional tillage (CT) management based on soil plowing [37,39].

The objective of this study was to isolate and characterize beneficial bacteria that are present in processing tomato (Solanum lycopersicum) rhizosphere after a long-term RT soil management. We focused on the identification of potential PGPR isolates by using selective or semi-selective culture media, by characterizing their specific metabolic features, by screening them with rep-PCR and by determining the identities of selected bacteria using 16S rRNA gene sequencing. Our approach was to assess the plant growth promoting activities (PGPAs) of the isolates of Solanum lycopersicum rhizosphere soil by conducting in vitro qualitative and quantitative assays for traits related to (i) mineral nutrition, including phosphate (P) solubilization, nitrogen (N) fixation and siderophores production, (ii) the production of growth hormones like indole acetic acid (IAA), and (iii) antagonism against fungal pathogens.
pathogen such as *Sclerotinia sclerotiorum*. Finally, isolates were ranked by their potential ability to function as PGPR.

2. Material and Methods

2.1. Field Site and Samples Collection

Soil sampling occurred in July 2019 in a commercial field situated in Gabbioneta-Binanuova (45°12′03.0″ N 10°12′27.8″ E), Cremona, Po Valley (Northern Italy). At the selected field site conservation agriculture practices have been adopted since 2011. Specifically: (i) A mixture of cover crops (CCs), composed by rye (*Secale cereale* L.), hairy vetch (*Vicia villosa* Roth), and radish (*Raphanus sativus* L.), was sown each year after harvesting the previous main crop and terminated right before the next seedbed preparation; (ii) reduced-tillage (RT) operations, which consisted of a ripper passage (25 cm depth) and one/two spring harrowing (10–15 cm depth), were annually performed before planting the main crop. The crop sequence was a 3-year crop rotation including maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and processing tomato (*Solanum lycopersicum* L.).

Soil samples adhered to the roots of tomato plants were manually separated from the surrounding bulk soil and collected. The plants were carefully uprooted from soil, collected in sterile polybags and stored at 4 °C for the isolation of rhizobacteria. The rhizosphere and rhizoplane soil were separated from the bulk soil following the method proposed by Barillot et al. [40]. Briefly, bulk soil was removed shaking plants by hand for 10 min vigorously, paying attention to the roots’ integrity, as long as the roots’ non-adhering soil particles were completely removed. In order to collect rhizosphere and rhizoplane soil, the root system was washed with 500 mL of 0.9% NaCl added with Tween80 (0.01% v/v) and afterwards 150 mL of bacterial suspension were incubated at 25 °C for 90 min with shaking at 180 rpm.

2.2. Isolation of Putative Diazotrophic Bacteria

Considering the many activities of PGPR, it has been decided to start the screening analysis of bacteria that may fix atmospheric nitrogen using two different N-free semi-solid media, NFb (New Fábio Pedrosa) and LGI (Liquid Glucose Ivo) [41] and comparing the potential growth with the bacterial strain *Azospirillum brasilense* Sp7 (DSM 1690), used as positive control [42]. The isolation of the putative diazotrophic bacteria was carried out following the method proposed by Ambrosini and Passaglia [43]. After incubation, each suspension was diluted in a sterile NaCl 0.9% solution until reaching 10−3, in triplicates. One hundred µL of the initial suspension (100) and serial 3-fold dilutions (10−1, 10−2, 10−3) were inoculated in 4 mL of NFb and LGI, in duplicate, and incubated for 4–7 d at 30 °C until the growth of a veil-like pellicle near the surface of the culture medium. The colour change of the medium from green-blueish to blue for NFb and from green to yellow for LGI, was another indicator for the bacterial growth. Pellicles were re-inoculated in new vials under the previous conditions in order to reduce the number of scavenger bacteria. Usually, from each vial only one bacteria isolate will be obtained, thus biofilms were streaked in LB agar (Luria-Bertani) (Oxoid, Basingstoke, UK) to confirm this and bacterial cultures were incubated at 30 °C for 15–24 h under aerobic conditions. After incubation, a morphological visual screening was performed, and a first selection was made to exclude the same isolates.

2.3. Rep-PCR

The genetic diversity among the isolates obtained was assessed by means of rep-PCR genotyping analysis, in order to discriminate same microbial strains profile [44]. DNA extraction from bacterial isolates was performed using the Microlysis Plus kit (Microzone, Haywards Heath, UK). Repetitive extragenic palindromic PCR was performed using GTG5 (5′-GTGGTGTTGGGTGTGGT-3′) as a primer [45]. PCR amplification was carried out in a 25 µL reaction mixture containing 1 µL DNA sample, 0.5 µL of GTG5 primer (100 µM), and 23.5 µL Megamix (Microzone). PCR was performed in a T100™ Thermal
Cycler (Bio-Rad Laboratories, Hercules, CA, USA) with the following steps: Initial denaturation at 95 °C for 7 min, followed by 30 cycles of denaturation at 90 °C for 30 s, annealing at 40 °C for 1 min, extension at 65 °C for 8 min and a final extension at 65 °C for 16 min. The PCR products were electrophoresed in a 2.5% agarose gel in 1x Tris-acetate-EDTA (TAE) buffer solution at 80V for 2 h and the profiles were visualized with the software Image Lab (Bio-Rad). The comparative analysis of the resulting fingerprints was performed using the software Geljv.2.0 [46].

2.4. Taxonomic Identification of Unique Isolates

The taxonomical identification of the bacterial isolates was carried out by amplification and sequencing of the 16S rRNA using the primers P1 (5′-GCGGCGTGCTAATACATGC-3′) and P6 (5′-CTACGGCTACCTTGTTACGA-3′) [47]. PCR amplification was carried out in a 25 µL reaction mixture containing 2 µL DNA sample, 0.5 µL of each primer (50 µM), 22 µL Megamix (Microzone). PCR was performed in a T100™ Thermal Cycler (Bio-Rad) with the following steps: Initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 92 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. PCR products were run on 1% agarose gel in 1x Tris-acetate-EDTA (TAE) buffer solution at 100V for 60 min and the profiles were visualized with the software Image Lab (Bio-Rad). After the DNA purification by NucleoSpin® Gel and PCR clean-up (MACHEREY-NAGEL, Duren, Germany) and quantification by electrophoresis using 1% agarose gel and Marker II (Roche diagnostics GmbH, Mannheim, Germany), the amplified fragments were subjected to Sanger sequence analysis. Sanger sequencing of PCR products was carried out at GATC Biotech (Ebersberg, Germany). The 16S ribosomal DNA sequences were then analysed through the NCBI-BLAST server. The BLAST result revealed the identity of the query sequences based on their percentage query coverages and sequence identities. Bacterial isolates with human and plant pathogenic properties were identified on the basis of literature info and discarded.

2.5. Phosphate Solubilization Ability Assessment

All bacterial isolates were screened for phosphate solubilization on the GY/Tricalcium phosphate medium [43] containing tricalcium phosphate (Ca$_3$PO$_4$)$_2$ as insoluble source of phosphorus. Each bacterial isolate was spot inoculated onto these plates and incubated at 30 °C for seven days. Plates were observed for development of a halo zone around the colony, which is indicative of tricalcium phosphate solubilization. This phosphate solubilization ability was analyzed measuring the halo’s diameter according the method proposed by Ambrosini and Passaglia [43]: isolates without a halo (halo = 0 cm) were considered as non-phosphate solubilizers or level 1 phosphate solubilizers, isolates with a halo bigger than 0 cm up to 1 cm were considered level 2 phosphate solubilizers, and isolates with a halo bigger than 1 cm were considered level 3 phosphate solubilizers.

2.6. Determination of the Indole Acetic Acid Production

IAA production by bacterial strains was estimated using the Salkowski reagent, which consisted of 12 g of FeCl$_3$ per L in 7.9 M H$_2$SO$_4$ [48]. Briefly, 1.5 mL of each bacterial cultures (24 h old) were centrifugated at 5000–8000 rpm for 10 min, supernatant was discarded and the pellet was resuspended in the same amount of sterile distilled water. The concentration of bacterial cultures was adjusted to an O.D$_{600}$ = 0.1. An aliquot of 10 µL from each culture was inoculated both in 5 mL of LB Broth supplemented with 500 µL DL-Tryptophan (0.01%) and as well in LB broth without DL-tryptophan and then incubated at 30 °C for 72 h, with shaking at 180 rpm. After incubation, 1000 µL of bacterial cultures were centrifugated at 6000 rpm for 10 min. Five hundred microliters of Salkowski reagent was added to 1 mL of collected supernatant and after 30 min incubation in dark the reddish color developed which indicated the IAA production. To quantify IAA, absorbance was taken at 540 nm by using UV/visible spectrophotometer. The IAA concentration was estimated with a standard curve of IAA.
2.7. Siderophores Production Assay

Quantitative estimation of siderophores production was done using CAS (Chrome Azurol Sulphonat) reagent [49]. This test was carried out following the protocol proposed by Arora and Verma [50], the modified microplate (96 wells plate) method was performed. Briefly, bacterial cultures (48 h old) were centrifuged at 10,000 rpm for 10 min, cell pellets were discarded, and supernatant was used to estimate siderophores. Supernatant (100 µL) of each bacterial culture was added in separate wells of microplate followed by the addition of 100 µL CAS reagent. After 20 min optical density was taken at 620 nm using microplate reader. Siderophore produced by strains was measured in percent siderophore unit (psu) which was calculated according to the following formula:

\[
\text{Siderophore production} = \frac{(A_r - A_s) \times 100}{A_r} \tag{1}
\]

where \(A_r\) = absorbance of reference (CAS solution and uninoculated broth), and \(A_s\) = absorbance of sample (CAS solution and cell-free supernatant of sample). The chelating agent EDTA (Ethylenediaminetetraacetic acid) was used as positive control.

2.8. In Vitro Assessment of Antifungal Activity

Bacterial isolates were screened for antifungal activities against Sclerotinia sclerotiorum (DSM 1946) using dual culture assay [51] on a potato dextrose agar (PDA) medium. A mycelial agar disc of 5 mm diameter of the pathogen was taken from an actively grown PDA culture of fully grown 7-day-old culture and was placed on one side of the PDA plates about 3 cm from the edge. A loopful of each antagonistic bacterial isolate, from an overnight culture, was streaked 3 cm away from mycelia disc. Only the culture of the fungal pathogen was inoculated in control plate. After 5–7 days of incubation at 25 °C, the antagonistic activity was observed by measuring the size of the growth inhibition zone and the percentage of growth inhibition (PGI) was calculated using the formula:

\[
\text{PGI} (\%) = \frac{KR - R1}{KR} \times 100 \tag{2}
\]

where \(KR\) represents the colony diameter of the pathogen in the control plate, and \(R1\) represents the colony diameter in the treated plate.

2.9. Data Analysis

Results were expressed as means + standard errors (SE). For the antifungal activity analysis differences between means were determined by one-way ANOVA with post-hoc Tukey’s HSD (honestly significant difference) test with the level of significance established at \(p < 0.05\).

In order to select the most performing bacterial isolates, the strains were ranked for their potential to promote plant growth and defense ability. The ranking covers all the data obtained from the in vitro assays. Briefly, the ranking was made assigning to each isolate a value in a range from zero to one to each PGP and antifungal traits. For the biocontrol activity, IAA and siderophore production, the maximum value for each evaluated trait was converted to the 100%, then, all the other values were proportioned to it. For phosphate solubilization, to each of four solubilization level identified, a value was assigned in a ratio scale of 0.25, that is, level 1 = 0.25, level 2 = 0.50, level 2 + = 0.75 and level 3 = 1. While for the nitrogen fixation trait, only two output were obtained from the assay, that is the positive (+) or the negative (−) growth, hence only the two value 0 or 1 were appointed. For every single bacterial strain all the values obtained for each PGPR traits tested were added up. Furthermore, the ranking obtained was compared with the bonitur scale, an arbitrary ranking commonly used to assess the bacteria isolates with high plant growth promotion potential [30,52].
3. Results

3.1. Isolation, Putative Diazotrophic Bacteria Identification, Rep-PCR, and Molecular Characterization of Rhizobacteria

According to the protocol proposed by Ambrosini and Passaglia [43], from each vial only one bacteria isolate is obtained. However, in our study, streaking the veil-like pellicle developed in NFB semi-solid medium on LB agar plates, from some single vials more than one isolate were obtained. Hence, a total of 129 bacterial isolates were discerned from the rhizosphere and rhizoplane soil of tomato plant *Solanum lycopersicum*. Rep-PCR analysis of the all 129 isolates was performed in order to reduce genetic redundancy, and the clustering scheme was constructed by the software Geljv2.0 (Figure 1). In accordance with the dendrogram of genetic similarity using Dice similarity coefficient index only 34 isolates had similar repetitive-element PCR genomic fingerprint to others isolates, indicating the high diversity in the system. Hence, the bacterial isolates were reduced from 129 to 95. Molecular identification of resulting bacterial strains was done by 16S rRNA gene sequencing analysis. The sequences obtained were submitted to NCBI GenBank using BLAST (Basic Local Alignment Tool). Based on scientific literature we discarded bacterial isolates with the highest possibilities to have human and plant pathogenic properties. The most common human pathogens found were *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, and *Klebsiella variicola*. After this literature research the number of bacterial isolates was further decreased from 95 to 38. The BLASTn phylogenetic analyses revealed that these 38 isolates belonged to 9 different genera. Out of these 38 isolates, 10 (26.3%) isolates belonged to genus *Pseudomonas*, 7 (18.4%) were from genus *Stenotrophomonas*, 5 (13.2%) from genus *Klebsiella*, 4 (10.5%) each from genus *Chryseobacterium* and *Enterobacter*, 3 (7.9%) each from genus *Sphingobacterium* and *Kosakonia*, and 1 (2.6%) isolate each belonged to genera *Aeromonas* and *Delftia*. All the bacterial isolates obtained, excluding the pathogenic strains, are reported in Table 1. Since more than one bacterial isolate was obtained from each vial containing the N-free semi solid medium NFB and LGI, the 38 isolates screened were re-inoculated in new vials with NFB and LGI medium under the previous condition in order to confirm their potential ability to fix nitrogen. Among the 38 screened isolates, a total of 29 bacteria were confirmed to be putative diazotrophic bacteria, in which *Pseudomonas* was the most abundant nitrogen fixer in tomato rhizosphere and rhizoplane soil (Table 2). Not wanting to focus only on the potential to fix nitrogen, the whole 38 bacteria isolates were assessed for the others potential PGPAs, such as: Tricalcium phosphate solubilization, IAA production, siderophore production, and antifungal activity.
Figure 1. Dendrogram analysis of rep-PCR (Repetitive Extragenic Palindromic) fingerprints performed using the software Geljv.2.0. Same bacterial strains belong to the same group.

Figure 1. Cont.
Figure 1. Dendrogram analysis of rep-PCR (Repetitive Extragenic Palindromic) fingerprints performed using the software Geljv 2.0. Same bacterial strains belong to the same group.
| Code     | Identity                                      | Query Length | Bit-Score | Query Cover (%) | E-Value | Ident (%) | Accession No. | a Code for the selected strains with best PGP traits. | b GeneBank sequence accession numbers of selected strains. |
|----------|-----------------------------------------------|--------------|-----------|----------------|---------|-----------|--------------|------------------------------------------------------|-------------------------------------------------------------|
| UC4080   | *Sphingobacterium* detergens                  | 816          | 1485      | 100            | 0.0     | 99.51     | MT435019     |                                                      |                                                             |
| UC4081   | *Chryseobacterium* oraninense                 | 1073         | 1940      | 99             | 0.0     | 99.35     | MT435020     |                                                      |                                                             |
| UC4082   | *Pseudomonas* pseudocaligenes                 | 1041         | 1855      | 99             | 0.0     | 99.13     | MT435021     |                                                      |                                                             |
| UC4083   | *Stenotrophomonas* acidaminiphila             | 1182         | 2132      | 100            | 0.0     | 99.24     | MT435022     |                                                      |                                                             |
| UC4084   | *Kosakonia* radicincintans                   | 1123         | 2061      | 99             | 0.0     | 99.82     | MT435023     |                                                      |                                                             |
| UC4086   | *Klebsiella* oxytoca                         | 1155         | 2093      | 99             | 0.0     | 99.56     | MT435024     |                                                      |                                                             |
| UC4087   | *Pseudomonas* indoloxydans                   | 1204         | 2176      | 99             | 0.0     | 99.33     | MT435025     |                                                      |                                                             |
| UC4088   | *Pseudomonas* indoloxydans                   | 1243         | 2268      | 100            | 0.0     | 99.60     | MT435026     |                                                      |                                                             |
| UC4089   | *Stenotrophomonas* pictorum                  | 1136         | 2076      | 100            | 0.0     | 99.65     | MT435027     |                                                      |                                                             |
| UC4090   | *Aeromonas* caviae                           | 800          | 1458      | 99             | 0.0     | 99.87     | MT435028     |                                                      |                                                             |
| UC4091   | *Pseudomonas* pseudocaligenes                | 1178         | 2109      | 100            | 0.0     | 98.98     | MT435029     |                                                      |                                                             |
| UC4092   | *Kosakonia* radicincitans                    | 1068         | 1954      | 100            | 0.0     | 99.72     | MT435030     |                                                      |                                                             |
| UC4093   | *Stenotrophomonas* pictorum                  | 1184         | 2145      | 99             | 0.0     | 99.58     | MT435031     |                                                      |                                                             |
| UC4094   | *Enterobacter* tabaci                        | 1102         | 2021      | 99             | 0.0     | 99.82     | MT435032     |                                                      |                                                             |
| UC4096   | *Stenotrophomonas* patani                   | 996          | 1808      | 100            | 0.0     | 99.40     | MT435033     |                                                      |                                                             |
| UC4098   | *Stenotrophomonas* rhizophila               | 990          | 1810      | 99             | 0.0     | 99.70     | MT435034     |                                                      |                                                             |
| UC4099   | *Enterobacter* tabaci                        | 1075         | 1965      | 99             | 0.0     | 99.81     | MT435035     |                                                      |                                                             |
| UC4101   | *Klebsiella* grimmontii                   | 1056         | 1914      | 99             | 0.0     | 99.82     | MT435036     |                                                      |                                                             |
| UC4102   | *Chryseobacterium* urethriticum              | 1143         | 2025      | 99             | 0.0     | 98.77     | MT435037     |                                                      |                                                             |
| UC4103   | *[Pseudomonas]* hibiscicola                  | 1159         | 2080      | 99             | 0.0     | 99.30     | MT435038     |                                                      |                                                             |
| UC4104   | *Stenotrophomonas* rhizophila               | 1023         | 1873      | 100            | 0.0     | 99.71     | MT435039     |                                                      |                                                             |
| UC4105   | *Stenotrophomonas* pictorum                  | 1188         | 2128      | 99             | 0.0     | 99.32     | MT435040     |                                                      |                                                             |
| UC4106   | *Enterobacter* ludwigii                     | 1155         | 2115      | 99             | 0.0     | 99.91     | MT435041     |                                                      |                                                             |
| UC4107   | *Sphingobacterium* canadense                | 1152         | 2080      | 99             | 0.0     | 99.13     | MT435042     |                                                      |                                                             |
| UC4108   | *Chryseobacterium* rhizosphaerae            | 1162         | 2117      | 99             | 0.0     | 99.83     | MT435043     |                                                      |                                                             |
| UC4109   | *Enterobacter* tabaci                        | 1164         | 2102      | 99             | 0.0     | 99.48     | MT435044     |                                                      |                                                             |
| UC4110   | *Kosakonia* oryzensphoptyca                 | 1150         | 2045      | 98             | 0.0     | 99.12     | MT435045     |                                                      |                                                             |
| UC4112   | *Pseudomonas* taiwanensis                   | 1036         | 1890      | 99             | 0.0     | 99.81     | MT435046     |                                                      |                                                             |
| UC4113   | *[Pseudomonas]* hibiscicola                  | 1251         | 2244      | 99             | 0.0     | 99.04     | MT435047     |                                                      |                                                             |
| UC4117   | *Pseudomonas* taiwanensis                   | 1066         | 1949      | 99             | 0.0     | 99.81     | MT435048     |                                                      |                                                             |
| UC4118   | *Klebsiella* oxytoca                        | 1185         | 2158      | 99             | 0.0     | 99.66     | MT435049     |                                                      |                                                             |
| UC4120   | *Chryseobacterium* rhizosphaerae            | 1212         | 2215      | 99             | 0.0     | 99.75     | MT435050     |                                                      |                                                             |
| UC4121   | *Sphingobacterium* siyangense               | 985          | 1725      | 99             | 0.0     | 98.28     | MT435051     |                                                      |                                                             |
| UC4122   | *Pseudomonas* taiwanensis                   | 1162         | 2102      | 99             | 0.0     | 99.31     | MT435052     |                                                      |                                                             |
| UC4123   | *Klebsiella* oxytoca                        | 1150         | 2084      | 99             | 0.0     | 99.56     | MT435053     |                                                      |                                                             |
| UC4125   | *Delftia* tsuruhutensis                     | 1022         | 1869      | 99             | 0.0     | 99.90     | MT435054     |                                                      |                                                             |
| UC4126   | *Pseudomonas* japonica                      | 1151         | 2087      | 99             | 0.0     | 99.31     | MT435055     |                                                      |                                                             |
| UC4127   | *Klebsiella* oxytoca                        | 1187         | 2165      | 99             | 0.0     | 99.83     | MT435056     |                                                      |                                                             |
3.2. Qualitative Estimation of Phosphate Solubilization Ability

Among the 38 selected isolates, 18 bacterial strains are able to solubilize phosphate by producing clear zone around the colonies after 7 days of incubation. Two different strains of *Pseudomonas taiwanensis* (isolates UC4117 and UC4122) showed highest phosphate solubilization level (level 2 +). Both isolates showed a significant clear zone around the colonies, wider than the other level 2 bacteria, and close to 1 cm in diameter, hence their ability of phosphate solubilization was indicated as level 2 +. During this assay no level 3 phosphorus solubilizers were obtained, while 11 strains were not able to grow on GY/Tricalcium phosphate medium. Results for the phosphate solubilization assay are shown in Table 2.

3.3. Determination of the Indole Acetic Acid Production

To screen for indole acetic acid production the Salkowski reagent was used, which gave different degrees of red to the solution according to the different levels of IAA produced. Qualitative and quantitative analysis of culture supernatant of selected strains isolated revealed production of variable amount of IAA both in the absence and presence of tryptophan (0.01%). The concentration of IAA produced by the rhizobacteria showed variations between 0 and 32.99 µg/mL in presence of L-tryptophan, *Enterobacter tabaci* isolate UC4109, produced maximum IAA (32.99 µg/mL), followed by the isolates UC4086, *Klebsiella oxytoca*, (17.85 µg/mL) and the isolate UC4094, *Enterobacter tabaci*, (15.33 µg/mL). An important variation in the concentration of IAA produced was observed between 0.05 and 33.07 µg/mL even without the IAA precursor tryptophan, in which the isolates UC4098 (*Stenotrophomonas rhizophila*) produced the highest level of IAA (33.07 µg/mL). Quantitative analysis for the indole acetic acid production are shown in Table 2.
| Code     | Identity                          | N Fixation | P Solubilization | IAA Production (µg/mL) |
|----------|-----------------------------------|------------|------------------|------------------------|
|          |                                   | Growth on Nfb | w/Try s.e.m | Tukey s.e.m | w/o Try Tukey |
| UC4080   | Sphingobacterium detersens        | –          | –                | 1.42 ± 1.11           | ab 0.39 ± 0.14     | b          |
| UC4081   | Chrysobacterium aurantii          | –          | –                | 1.34 ± 0.01           | ab 0.86 ± 0.02     | b          |
| UC4082   | Pseudomonas pseudoalcaligenes     | +          | Level 1          | 0.50 ± 0.05           | b 0.84 ± 0.09      | b          |
| UC4083   | Stenotrophomonas acidaminiphila   | –          | –                | 1.61 ± 1.35           | ab 1.45 ± 1.11     | b          |
| UC4084   | Kosakonia radicincitans          | +          | Level 2          | 1.20 ± 0.91           | ab 0.50 ± 0.25     | b          |
| UC4086   | Klebsiella oxytoca                | –          | –                | 17.84 ± 0.94          | ab 10.30 ± 0.55    | ab         |
| UC4087   | Pseudomonas indoloxylans         | +          | –                | 1.62 ± 0.97           | ab 2.05 ± 0.26     | b          |
| UC4088   | Pseudomonas indoloxylans         | +          | –                | 1.88 ± 0.71           | ab 1.99 ± 0.20     | b          |
| UC4089   | Stenotrophomonas pittorum        | +          | –                | 5.82 ± 5.01           | ab 0.66 ± 0.43     | b          |
| UC4090   | Aeromonas caviae                  | +          | Level 2          | 5.82 ± 2.37           | ab 3.99 ± 0.26     | ab         |
| UC4091   | Pseudomonas pseudoalcaligenes    | +          | –                | 7.32 ± 5.71           | ab 1.61 ± 0.46     | b          |
| UC4092   | Kosakonia radicincitans          | +          | Level 2          | 4.20 ± 0.51           | ab 4.17 ± 0.91     | ab         |
| UC4093   | Stenotrophomonas pittorum        | +          | Level 1          | 0.50 ± 0.12           | b 0.38 ± 0.25      | b          |
| UC4094   | Enterobacter tabaci               | +          | Level 2          | 15.33 ± 11.40         | ab 13.88 ± 11.54   | ab         |
| UC4096   | Stenotrophomonas putrinii        | +          | Level 1          | 1.42 ± 1.04           | ab 0.90 ± 0.34     | b          |
| UC4098   | Stenotrophomonas rhizophila      | +          | Level 2          | 5.20 ± 1.72           | ab 33.07 ± 29.25   | a          |
| UC4099   | Enterobacter tabaci               | +          | Level 2          | 11.84 ± 7.00          | ab 3.05 ± 0.03     | ab         |
| UC4101   | Klebsiella grimonii              | +          | Level 2          | 8.14 ± 0.18           | ab 8.20 ± 0.35     | ab         |
| UC4102   | Chrysobacterium urelyticum       | –          | Level 1          | 1.23 ± 1.23           | ab 1.13 ± 0.17     | b          |
| UC4103   | [Pseudomonas] hibiscicola        | +          | Level 1          | 1.24 ± 1.23           | ab 1.16 ± 0.51     | b          |
| UC4104   | Stenotrophomonas rhizophila      | +          | Level 1          | 1.25 ± 1.15           | ab 0.81 ± 0.37     | b          |
| UC4105   | Stenotrophomonas pittorum        | +          | Level 1          | 5.65 ± 5.51           | ab 0.93 ± 0.15     | b          |
| UC4106   | Enterobacter ludwigi             | +          | Level 2          | 11.95 ± 3.33          | ab 8.51 ± 0.91     | ab         |
| UC4107   | Sphingobacterium canadense       | –          | –                | 1.90 ± 1.27           | ab 0.67 ± 0.26     | b          |
| UC4108   | Chrysobacterium rhiziholae       | –          | –                | 1.22 ± 0.90           | ab 0.90 ± 0.18     | b          |
| UC4109   | Enterobacter tabaci              | +          | Level 2          | 33.00 ± 27.14         | a 3.71 ± 1.12      | ab         |
| UC4110   | Kosakonia oryzendophylica        | +          | Level 2          | 2.05 ± 1.12           | ab 1.27 ± 0.83     | b          |
| UC4112   | Pseudomonas taiwanensis          | +          | Level 2          | 0.84 ± 0.06           | b 1.02 ± 0.12      | b          |
| UC4113   | [Pseudomonas] hibiscicola        | +          | Level 1          | 1.41 ± 1.37           | ab 1.05 ± 0.61     | b          |
| UC4117   | Pseudomonas taiwanensis         | +          | Level 2 (+)      | 2.21 ± 1.18           | ab 2.81 ± 0.37     | ab         |
| UC4118   | Klebsiella oxytoca               | +          | Level 2          | 8.55 ± 1.11           | ab 7.37 ± 0.75     | ab         |
| UC4120   | Chrysobacterium rhizoholae       | –          | –                | 5.80 ± 5.70           | ab 0.72 ± 0.10     | b          |
| UC4121   | Sphingobacterium stiengense      | –          | –                | 0.03 ± 0.03           | b 0.05 ± 0.05      | b          |
| UC4122   | Pseudomonas taiwanensis         | +          | Level 2 (+)      | 0.00 ± 0.00           | b 1.00 ± 0.00      | b          |
| UC4123   | Klebsiella oxytoca               | +          | Level 2          | 8.35 ± 1.71           | ab 7.11 ± 1.04     | ab         |
| UC4125   | Delphi tsuruhatersis            | +          | –                | 0.17 ± 0.10           | b 0.49 ± 0.20      | b          |
| UC4126   | Pseudomonas japonica            | +          | Level 2          | 5.94 ± 5.38           | ab 4.30 ± 3.78     | ab         |
| UC4127   | Klebsiella oxytoca               | +          | Level 2          | 8.38 ± 1.21           | ab 7.12 ± 0.72     | ab         |

The symbol + represents the presence of growth, while symbol – represents the absence of growth. S.e.m is indicative for Standard Error of the Mean. Try is indicative for DL-Tryptophan. Values are the Mean ± SE. Data for N fixation and P solubilization are the mean values of three replicates for each isolate. Data for IAA production are the mean values of two replicates for each isolate. Tukey-test: Values with different combinations of letters are significantly different from each other (p < 0.05).
3.4. Siderophores Production Assay

The quantitative microplate method for siderophore estimation revealed that the concentration of siderophore produced by bacterial isolates varied from 0 to 52.28 psu. *Sphingobacterium canadense*, (isolate UC4107), produced maximum amount of siderophore (52.28 psu) followed by *Stenotrophomonas pictorum*, (isolate UC4089) > *Chryseobacterium urelyticum*, (isolate UC4102), > *Chryseobacterium rhizosphaerae*, (isolate UC4120) > *Stenotrophomonas pictorum*, (isolate UC4093) > *Chryseobacterium oranimense*, (isolate UC4081). The concentration of siderophore produced by these bacterial strains varied from 51.36 to 47.66 psu. Quantitative analysis results for the siderophore production are shown in Table 3.

![Table 3. Biocontrol traits of the rhizobacteria.](image)

| Code   | Identity                          | Antifungal Activity vs. *Sclerotinia sclerotiorum* | Siderophore |
|--------|-----------------------------------|--------------------------------------------------|-------------|
|        |                                   | Tukey s.e.m                                      | Tukey       |
| UC4080 | *Sphingobacterium detersens*      | 21.40 ± 10.02 ab                                 | 41.50 ± 4.04 abcdedefghijklm |
| UC4081 | *Chryseobacterium oranimense*     | 31.77 ± 10.45 ab                                 | 47.66 ± 4.94 abcdedefghijklm |
| UC4082 | *Pseudomonas pseudoalcaligenes*   | 43.16 ± 2.45 ab                                  | 31.40 ± 3.11 abcdedefghijklm |
| UC4083 | *Stenotrophomonas acidaminiphila* | 37.00 ± 7.74 ab                                  | 42.91 ± 1.48 abcdedefghijklm |
| UC4084 | *Kosakonia radiicincintans*       | 31.03 ± 2.83 ab                                  | 30.38 ± 4.42 abcdedefghijklm |
| UC4086 | *Klebsiella oxytoca*              | 20.00 ± 11.55 ab                                 | 10.08 ± 3.55 abcdedefghijklm |
| UC4087 | *Pseudomonas indoloxydans*        | 39.20 ± 4.39 ab                                  | 28.80 ± 1.25 abcdedefghijklm |
| UC4088 | *Stenotrophomonas pictorum*       | 41.08 ± 2.81 ab                                  | 27.58 ± 0.78 abcdedefghijklm |
| UC4089 | *Stenotrophomonas pictorum*       | 39.08 ± 5.44 ab                                  | 51.36 ± 3.76 abcdedefghijklm |
| UC4090 | *Aeromonas caviae*                | 35.31 ± 13.80 ab                                 | 15.91 ± 7.60 abcdedefghijklm |
| UC4091 | *Pseudomonas pseudoalcaligenes*   | 44.19 ± 2.48 ab                                  | 29.50 ± 2.57 abcdedefghijklm |
| UC4092 | *Kosakonia radiicincintans*       | 31.79 ± 6.41 ab                                  | 3.21 ± 2.00 mn          |
| UC4093 | *Stenotrophomonas pictorum*       | 28.92 ± 13.80 ab                                 | 47.73 ± 2.16 abcdedefghijklm |
| UC4094 | *Enterobacter tabaci*             | 24.53 ± 4.55 ab                                  | 26.86 ± 1.27 abcdedefghijklm |
| UC4096 | *Stenotrophomonas parani*         | 36.49 ± 8.46 ab                                  | 40.17 ± 1.08 abcdedefghijklm |
| UC4098 | *Stenotrophomonas rhizophila*     | 27.94 ± 3.11 ab                                  | 0.00 ± 0.00 n             |
| UC4099 | *Enterobacter tabaci*             | 21.38 ± 6.48 ab                                  | 26.51 ± 2.84 abcdedefghijklm |
| UC4101 | *Klebsiella gerroni*              | 29.19 ± 9.22 ab                                  | 0.56 ± 0.56 n             |
| UC4102 | *Chryseobacterium urelyticum*     | 35.46 ± 6.22 ab                                  | 51.20 ± 3.11 abcdedefghijklm |
| UC4103 | *Chryseobacterium urelyticum*     | 45.10 ± 9.59 ab                                  | 35.41 ± 0.83 abcdedefghijklm |
| UC4104 | *Stenotrophomonas rhizophila*     | 26.96 ± 9.46 ab                                  | 30.51 ± 2.80 abcdedefghijklm |
| UC4105 | *Stenotrophomonas pictorum*       | 35.98 ± 8.49 ab                                  | 40.00 ± 2.22 abcdedefghijklm |
| UC4106 | *Enterobacter ludwigii*           | 29.03 ± 5.49 ab                                  | 3.95 ± 2.56 mn          |
| UC4107 | *Sphingobacterium canadense*      | 22.12 ± 8.13 ab                                  | 52.28 ± 2.65 a           |
| UC4108 | *Chryseobacterium rhizosphaerae*  | 31.40 ± 0.36 ab                                  | 42.59 ± 8.01 abcdedefghijklm |
| UC4109 | *Enterobacter tabaci*             | 18.07 ± 6.69 ab                                  | 8.79 ± 3.65 lmn          |
| UC4110 | *Kosakonia orzendlaphylta*        | 29.02 ± 9.36 ab                                  | 9.64 ± 4.47 lmn          |
| UC4112 | *Pseudomonas taiwanensis*         | 8.09 ± 0.26 ab                                   | 12.25 ± 1.14 lmn          |
| UC4113 | *Pseudomonas taiwanensis*         | 40.85 ± 7.09 ab                                  | 35.53 ± 1.69 abcdedefghijklm |
| UC4117 | *Pseudomonas taiwanensis*         | 28.17 ± 5.93 ab                                  | 20.29 ± 8.28 abcdedefghijklm |
| UC4118 | *Klebsiella oxytoca*              | 20.59 ± 6.29 ab                                  | 5.86 ± 4.45 mn          |
| UC4120 | *Chryseobacterium rhizosphaerae*  | 39.36 ± 8.38 ab                                  | 50.69 ± 1.16 abc          |
| UC4121 | *Sphingobacterium siyangsese*     | 19.02 ± 6.57 ab                                  | 45.71 ± 5.14 abcdedefghijklm |
| UC4122 | *Pseudomonas taiwanensis*         | 9.73 ± 3.34 ab                                   | 11.30 ± 2.24 lmn          |
| UC4123 | *Klebsiella oxytoca*              | 44.56 ± 5.46 ab                                  | 1.24 ± 1.24 n             |
| UC4125 | *Delftia tsuruhatensis*           | 42.31 ± 3.95 ab                                  | 14.39 ± 1.44 lmn          |
| UC4126 | *Pseudomonas japonica*            | 5.23 ± 2.62 ab                                   | 21.77 ± 5.78 fgghijklm     |
| UC4127 | *Klebsiella oxytoca*              | 48.01 ± 1.97 ab                                  | 4.93 ± 3.18 mn          |

s.e.m indicates Standard Error of the Mean.

3.5. In Vitro Assessment of Antifungal Activity

Antifungal activity of the bacterial isolates was assayed against *Sclerotinia sclerotiorum* (DSM 1946) using PDA (Potato dextrose agar) media. Results of dual culture assay showed that all strains have different efficiencies in the inhibition of the mycelial growth (Table 3). On the basis on the in vitro dual culture experiment, the antifungal activity of all bacterial isolates checked varied with percent of growth inhibition (PGI), from 5.23% to 48.02%. Isolate UC4127 (*Klebsiella oxytoca*) showed the strongest antagonisms against the pathogen with the highest PGI value (48.02%), followed by the isolate UC4103...
(Pseudomonas hibiscicola) with a PGI of 45.10% and the isolate UC4123 (Klebsiella oxytoca) with a PGI of 44.57%.

3.6. Ranking of Different Plant Growth Promoting Traits

With a view to organize the selected bacterial isolated into a hierarchy, a nonarbitrary ranking approach was elaborated. Each PGPR isolate was ranked on the basis of its in vitro PGP (Plant Growth Promoting) and antifungal assay, considering a range from 0 to 1 for each assayed property. According to the ranking scale the first three positions were obtained by isolate UC4094 (Enterobacter tabaci), isolate UC4098 (Stenotrophomonas rhizophila), and the isolate UC4109 (Enterobacter tabaci). A complete list of ranked strains is shown in Table 4. Furthermore, the collection of bacterial strains was also ranked with the arbitrary bonitur scale approach (data not shown). Same results were obtained for the bacteria with the highest score.
Table 4. Ranking of the rhizobacteria based on their in vitro PGP (plant growth promoting) and antifungal assay.

| Code    | Identity               | Fixation | Solubilization | IAA Production | Antifungal Activity vs. S. sclerotiorum | Siderophore | Rank  |
|---------|------------------------|----------|----------------|----------------|-----------------------------------------|-------------|-------|
| w/Try  | w/o Try                |          |                |                |                                         |             |       |
| UC4094  | Enterobacter tabaci    | 1        | 0.5            | 0.46           | 0.42                                    | 0.51        | 3.41  |
| UC4098  | Stenotrophomonas rhizophila | 1    | 0.5            | 0.16           | 1.00                                    | 0.57        | 3.22  |
| UC4109  | Enterobacter tabaci    | 1        | 0.5            | 1.00           | 0.11                                    | 0.38        | 3.16  |
| UC4127  | Klebsiella oxytoca     | 1        | 0.5            | 0.25           | 0.22                                    | 1.00        | 0.09  | 3.06  |
| UC4089  | Stenotrophomonas pictorum | 1    | 0.5            | 0.25           | 0.22                                    | 1.00        | 0.09  | 2.99  |
| UC4105  | Stenotrophomonas pictorum | 1    | 0.25           | 0.17           | 0.03                                    | 0.75        | 0.78  | 2.98  |
| UC4103  | [Pseudomonas] hibiscicola | 1      | 0.25           | 0.04           | 0.04                                    | 0.94        | 0.68  | 2.94  |
| UC4123  | Klebsiella oxytoca     | 1        | 0.5            | 0.25           | 0.21                                    | 0.93        | 0.02  | 2.92  |
| UC4099  | Enterobacter tabaci    | 1        | 0.5            | 0.36           | 0.09                                    | 0.45        | 0.51  | 2.90  |
| UC4117  | Pseudomonas taiwanensis | 1     | 0.75           | 0.07           | 0.09                                    | 0.59        | 0.39  | 2.88  |
| UC4113  | [Pseudomonas] hibiscicola | 1      | 0.25           | 0.04           | 0.03                                    | 0.85        | 0.68  | 2.86  |
| UC4096  | Stenotrophomonas paucini | 1      | 0.25           | 0.04           | 0.03                                    | 0.76        | 0.77  | 2.85  |
| UC4090  | Aeromonas cavae        | 1        | 0.5            | 0.18           | 0.12                                    | 0.74        | 0.30  | 2.84  |
| UC4106  | Enterobacter ludwigi   | 1        | 0.5            | 0.36           | 0.26                                    | 0.60        | 0.08  | 2.80  |
| UC4093  | Stenotrophomonas pictorum | 1     | 0.25           | 0.02           | 0.01                                    | 0.60        | 0.91  | 2.79  |
| UC4082  | Pseudomonas pseudoalcaligenes | 1   | 0.25           | 0.01           | 0.03                                    | 0.90        | 0.60  | 2.79  |
| UC4084  | Kosakonia radicicinctans | 1      | 0.5            | 0.04           | 0.02                                    | 0.65        | 0.58  | 2.78  |
| UC4091  | Pseudomonas pseudoalcaligenes | 1   | 0              | 0.22           | 0.05                                    | 0.92        | 0.57  | 2.76  |
| UC4101  | Klebsiella grimonii    | 1        | 0.5            | 0.25           | 0.25                                    | 0.61        | 0.01  | 2.61  |
| UC4118  | Klebsiella oxytoca     | 1        | 0.5            | 0.26           | 0.22                                    | 0.43        | 0.11  | 2.52  |
| UC4088  | Pseudomonas indoloxydans | 1      | 0              | 0.06           | 0.06                                    | 0.86        | 0.53  | 2.50  |
| UC4087  | Pseudomonas indoloxydans | 1      | 0              | 0.05           | 0.06                                    | 0.82        | 0.55  | 2.48  |
| UC4092  | Kosakonia radicicinctans | 1      | 0.5            | 0.13           | 0.13                                    | 0.66        | 0.06  | 2.48  |
| UC4104  | Stenotrophomonas rhizophila | 1     | 0.25           | 0.04           | 0.02                                    | 0.56        | 0.58  | 2.46  |
| UC4110  | Kosakonia oryzendophytic | 1      | 0.5            | 0.06           | 0.04                                    | 0.60        | 0.18  | 2.39  |
| UC4126  | Pseudomonas japonica   | 1        | 0.5            | 0.18           | 0.13                                    | 0.11        | 0.42  | 2.33  |
| UC4122  | Pseudomonas taiwanensis | 1      | 0.75           | 0.00           | 0.03                                    | 0.20        | 0.22  | 2.20  |
| UC4125  | Delphi tsurahatanesis  | 1        | 0              | 0.01           | 0.01                                    | 0.75        | 0.28  | 2.05  |
| UC4102  | Chryseobacterium urelyticum | 0     | 0.25           | 0.04           | 0.03                                    | 0.74        | 0.98  | 2.04  |
| UC4120  | Chryseobacterium rhizophilae | 0      | 0              | 0.18           | 0.02                                    | 0.82        | 0.97  | 1.99  |
| UC4086  | Klebsiella oxytoca     | 0        | 0.5            | 0.54           | 0.31                                    | 0.42        | 0.19  | 1.96  |
| UC4112  | Pseudomonas taiwanensis | 1      | 0.5            | 0.01           | 0.03                                    | 0.17        | 0.23  | 1.95  |
| UC4081  | Chryseobacterium oraminense | 0     | 0.25           | 0.04           | 0.03                                    | 0.66        | 0.91  | 1.89  |
| UC4083  | Stenotrophomonas acidamphila | 0   | 0              | 0.05           | 0.04                                    | 0.77        | 0.75  | 1.61  |
| UC4107  | Sphingobacterium canadense | 0     | 0              | 0.06           | 0.02                                    | 0.46        | 1.00  | 1.54  |
| UC4108  | Chryseobacterium rhizophilae | 0      | 0              | 0.04           | 0.03                                    | 0.65        | 0.81  | 1.53  |
| UC4080  | Sphingobacterium detergens | 0      | 0              | 0.04           | 0.01                                    | 0.45        | 0.79  | 1.29  |
| UC4121  | Sphingobacterium siyangense | 0   | 0              | 0.00           | 0.00                                    | 0.40        | 0.87  | 1.27  |

w/Try and w/o Try stands for with or without DL-Tryptophan.
4. Discussion

In the current study, 38 promising rhizobacterial isolates were selected out of 129 isolates on the basis of their genetic diversity and in vitro PGP and antifungal assay. These potential PGPR were isolated from rhizosphere soil of tomato plants (*Solanum lycopersicum* L.) after a long-term RT plus CCs soil management, and they were screened in vitro for the different PGP traits, such as nitrogen fixation, phosphate solubilization, IAA and siderophore production, and also for antagonistic potential against the phytopathogenic pest *S. sclerotiorum*.

Isolation of putative PGPR from rhizosphere environment usually results in a large number of isolates [53,54]. Hence, we established a strategy to sort out culturable bacteria strains and restrict them to those highly suspected to have PGP and antifungal traits. We decided to follow a hierarchic approach, starting from testing the capability of fixing nitrogen. Nitrogen is an essential element in plant growth. Hence, the biological nitrogen fixation (BNF) can be considered as one of the major mechanisms by which plants can benefit from microorganisms [55]. Moreover, according to Islam et al. [56] inoculation to tomato plants, under gnotobiotic conditions, with some nitrogen-fixing bacterial strains belonging to phyla Proteobacteria and Firmicutes, had significantly impacted on a variety of growth parameters, such as root and shoot length, seedling vigor and dry biomass. Among the 38 screened isolates, after a re-inoculation in \( N \)-free semi solid medium, a total of 29 bacteria were confirmed to be diazotrophic bacteria. These data confirmed the occurrence of effective nitrogen-scavenging bacteria in the rhizosphere of tomato plant, and the close association with diazotrophic strains [43,57]. In our study the higher isolation frequency of putative diazotrophic bacteria was for the genus *Pseudomonas*.

Isolates were screened at strains level by using rep-PCR genotyping analysis and then the phylogenetic affiliations were determined. It was necessary to carry out a literature research in order to discard bacterial isolates which have characterizing human and plant pathogenic properties. The BLASTn similarity searches revealed that the most common human pathogens found were *Stenotrophomonas maltophilia* and *Klebsiella pneumoniae*, both global opportunistic pathogen increasingly resistant to multiple antimicrobial agents and responsible for the emergent incidence of nosocomial infections, mainly in debilitated and immunosuppressed individuals [58,59], and *Klebsiella variicola*, a versatile bacterium capable of colonizing different hosts such as plants, humans, insects and animals but currently recognized as a cause of several human infections [60]. The phylogenetic analyses, of the non-pathogenic strains, revealed that 38 isolates belonged to 9 different genera, among which the genus *Pseudomonas* was the most abundant; however, strains belonging to the *Bacillus* genus were not identified, possibly even because the phylum Firmicutes, as a whole, tend to be less abundant in the rhizosphere than other phyla [61]. At phylum level our strains can be divided into only two different phyla, such as Proteobacteria and Bacteroidetes, with the full dominance of Proteobacteria.

A metagenomic approach should be performed in order to be able to provide conclusions and results about microbial diversity of our selected environment; however, previous metagenomic investigation about biodiversity in reduce tillage (RT) with CCs compared with in conventional tillage (CT) practice added value to our results. Legrand et al. [62] discovered that phyla Proteobacteria and Bacteroidetes were more abundant in soil under RT, while the presence of Firmicutes strains was higher under CT. Other studies showed that the highest bacterial richness of prokaryotes is typically found in the top soil layer under RT [39], while at deeper layer the richness of phyla under RT is usually lower than under CT [63]. Moreover, a meta-analysis research carried out on the impact of crop rotation by Venter et al. [64] highlighted how longer study trials produced larger increases in microbial richness, although the opposite was true for microbial diversity; nevertheless the addition of legumes to rotation had no consistent effects on microbial diversity or richness. In addition, Buyer et al. [65] pointed out how vetch CC may increase the amount of Gram-negative bacteria in the rhizosphere of tomato plants.

Hariprasad and Niranjana [66] screened the rhizosphere of tomato plants in order to specifically detect phosphate solubilizing rhizobacteria. Phosphate solubilizing microorganisms (PSM), including bacteria, have an important role in plant growth, making the unavailable insoluble sources of \( P \) available to the actively growing plants. [67]. Generally, strains belonging to the genera *Pseudomonas*...
are among the most powerful phosphate solubilizers [68]. In this study 18 isolates out of 38 showed phosphate solubilization, where two different strains of \textit{P. taiwanensis} showed the highest level (Table 2). \textit{Pseudomonas taiwanensis} is a novel species bacterium isolated in 2010 [69], Volmer et al. [70] utilized this bacterium as a biocatalyst by considering its capacity as an organic solvent tolerant, showing its potential for use in contaminated sites. According to the literature, only a few studies report its ability to solubilize phosphate [71,72].

Bacterial IAA producers (BIPs), by input of IAA into the plant’s auxin pool, can have a positive effect on root system elongation and development, thereby helping water and nutrient uptake [73]. Our isolates, with a range between 0 and 32.99 \(\mu\)g/mL of IAA production in presence of the precursor L-tryptophan, produce lower IAA concentration as compared to previous reports [74,75]. \textit{Enterobacter tabaci}, produce maximum IAA (32.99 \(\mu\)g/mL) level. In previous investigation, a strain of \textit{E. tabaci} was isolated from the fruits of tomato plant for its ability to produce the polygalacturonase enzyme, an enzyme that aids in microbial spoilage of fruits and vegetables, with the aim to produce important enzymes in food, drinks, and pharmaceutical industries at affordable prices [76]. So, this property should be taken into account and evaluated before its use as an inoculum in agriculture. In the absent of L-tryptophan, generally a reduction or even a lack of production of IAA was observed [77,78]. However, in the current study, a concentration of IAA with the value of 33.07 \(\mu\)g/mL was achieved for \textit{S. rhizophila} (Table 2). A previous study confirmed the capability of \textit{S. rhizophila} to product high level of IAA also without the precursor L-tryptophan [75].

The siderophores production ability can be classifies either as a direct mechanism or an indirect mechanism of plant growth promoting rhizobacteria. They are organic compound with low molecular masses, produced by microorganisms in order to provide plants with Fe nutrition to enhance their growth under low iron conditions. At the same time, siderophore produced by the PGPR bind the iron in order to reduce the Fe availability and efficiently prevent the propagation of fungal pathogens [79]. In the present study (Table 3) the isolates selected produce higher siderophores concentration, within a range of 0 to 52.28 psu, compare to other reports [52,80]. \textit{Sphingobacterium canadense}, showed the higher level of siderophores production. This is in line with another investigation which indicates some strains of \textit{Sphingobacterium} spp. as greater siderophore-producing bacteria [81]. However, in particular for \textit{S. canadense}, according to the literature, there is no information regarding this microorganism, except scientific proof of its isolation from corn roots [82].

Beside stimulating plant growth by direct mechanisms, PGPR isolated were also screened for their suppressive effects against \textit{S. sclerotiorum}, a widespread soilborne plant pathogen affecting yield and product quality of more than 400 plant species, among which is tomato [83]. In the current study \textit{Klebsiella oxytoca} showed higher antagonistic activity against the pathogen, with a percent growth inhibition value of 48.01\%. \textit{Klebsiella} is a borderline genus, known for its pathogenic properties as well as for their potential in agriculture, indeed \textit{K. pneumoniae} and \textit{K. oxytoca} are able to fix atmospheric nitrogen [84]. The PGP properties of \textit{K. oxytoca} were highlighted in several report [85,86], as well for its capacity to induced systemic resistance (ISR) against soft-rot disease pathogen in tobacco [87]. In general, according to the literature there is a lack of information concerning the antagonistic potential of \textit{K. oxytoca} against the phytopathogenic pest \textit{S. sclerotiorum}.

In order to evaluate the potential PGPR isolates to be possibly commercialized as biofertilizers, biopesticide or biostimulant, our first decision-making approach was to rank the selected isolated bacteria. The PGPR were ranked on the basis of their in vitro PGP and antifungal assayed property. According to the ranking scale the first three positions were obtained by isolates UC4094, UC4098, and UC4109 (Table 4). It is noteworthy to consider that ranking approach is based on the sum of all the assayed property: This is means that every rhizobacteria isolate is evaluated on its capability to express more than one PGP or antifungal property. In the current study, all the 38 bacterial isolates showed at least one property tested. Observing the results achieved by the ranking, with the exception of the isolate UC4109, the other two isolated at the top of the scale, are able to exert all the properties tested but these are expressed at lower levels compared to others that express maybe only one property but at a
higher level. So, the use of this index should only help in the thinking of the decision-making approach, and its output should not be considered in the strongest term. Moreover, the use of microbial consortia in the form of bio-product for reduction in the application of chemical fertilizers, pesticides, and related agrochemicals, without compromising the plant yield is currently a significant research area in the field of agriculture [88]. From the perspective of developing a bacterial consortium we can observe that in the current study 38 bacterial isolates with at least one plant growth promoting property were isolated from the rhizosphere and rhizoplane of *Solanum lycopersicum* of a local farm; efforts aimed to discovering indigenous microorganisms that can improve crop development and growth are therefore promising. Consortium of native PGPR bacteria inoculants can indeed have the potential to alleviate challenges of local chemical fertilizers production. Further research will be necessary in order to evaluate the effective PGPR behavior of selected isolates, indeed greenhouse and field trials will be performed. Before the in vivo assay, genome analyses will be needed in order to confirm the absence of pathogenic genes and the presence of potential genes involved in PGPR activities.

**Author Contributions:** E.P. and M.T. conceived and supervised the study; M.C.G. wrote the manuscript and carried out microbiological analyses; E.F. carried out microbiological analyses; A.F. provided agronomical support and soil analyses. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Regione Lombardia (Italy) project PROBIOPOM (Biostimulant microorganisms for the biological protection of tomatoes from insects, fungi and mycotoxins), grant number 32 dds 5 March 2020—n. 2995.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Vandenkoornhuyse, P.; Quaiser, A.; Duhamel, M.; Le Van, A.; Dufresne, A. The importance of the microbiome of the plant holobiont. *New Phytol.* **2015**, *206*, 1196–1206. [CrossRef] [PubMed]
2. Virk, H.K.; Singh, G.; Sharma, P. Effect of Tillage, Crop Residues of Preceding Wheat Crop and Nitrogen Levels on Biological and Chemical Properties of Soil in the Soybean–Wheat Cropping System. *Commun. Soil Sci. Plant. Anal.* **2017**, *48*, 1764–1771. [CrossRef]
3. Mishra, P.; Singh, P.P.; Singh, S.K.; Verma, H. Sustainable agriculture and benefits of organic farming to special emphasis on PGPR. In *Role of Plant Growth Promoting Microorganisms in Sustainable Agriculture and Nanotechnology*; Elsevier Inc.: Amsterdam, The Netherlands, 2019.
4. Fiorini, A.; Boselli, R.; Maris, S.C.; Santelli, S.; Ardenti, F.; Capra, F.; Tabaglio, V. May conservation tillage enhance soil C and N accumulation without decreasing yield in intensive irrigated croplands? Results from an eight-year maize monoculture. *Agric. Ecosyst. Environ.* **2020**, *296*, 106926. [CrossRef]
5. Klopper, J.W.; Leong, J.; Teintze, M.; Schroth, M.N. Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* **1980**, *286*, 885–886. [CrossRef]
6. Klopper, J.W. Plant Growth-Promoting Rhizobacteria and Plant Growth Under Gnotobiotic Conditions. *Phytopathology* **1981**, *81*, 97–168. [CrossRef]
7. Boyetchko, S. Plant Growth Promoting Rhizobacteria. *Encycl. Pest. Manag.* **2002**, *2011*, 1–30.
8. Glick, B.R. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* **1995**, *41*, 109–117. [CrossRef]
9. Bhattacharyya, P.N.; Jha, D.K. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1327–1350. [CrossRef]
10. Saia, S.; Rappa, V.; Ruisi, P.; Abenavoli, M.R.; Sunseri, F.; Giambalvo, D.; Fresa, A.S.; Martinelli, F. Soil inoculation with symbiotic microorganisms promotes plant growth and nutrient transporter genes expression in durum wheat. *Front. Plant. Sci.* **2015**, *6*, 1–10. [CrossRef]
11. Ali, S.; Hameed, S.; Shahid, M.; Iqbal, M.; Lazarovits, G.; Imran, A. Functional characterization of potential PGPR exhibiting broad-spectrum antifungal activity. *Microbiol. Res.* **2020**, *232*, 126389. [CrossRef]
12. Kudoyarova, G.; Arkhipova, T.; Korshunova, T.; Bakaeva, M.; Loginov, O.; Dodd, I.C. Phytohormone Mediation of Interactions Between Plants and Non-Symbiotic Growth Promoting Bacteria Under Edaphic Stresses. *Front. Plant. Sci.* **2019**, *10*, 1368. [CrossRef] [PubMed]
13. Park, Y.G.; Mun, B.G.; Kang, S.M.; Hussain, A.; Shahzad, R.; Seo, C.W.; Kim, A.Y.; Lee, S.U.; Oh, K.Y.; Lee, D.Y.; et al. Bacillus aryabhattaii SRB02 tolerates oxidative and nitrosative stress and promotes the growth of soybean by modulating the production of phytohormones. PLoS ONE 2017, 12, e0173203. [CrossRef] [PubMed]

14. Ilangumaran, G.; Smith, D.L. Plant growth promoting rhizobacteria in amelioration of salinity stress: A systems biology perspective. Front. Plant. Sci. 2017, 8, 1–14. [CrossRef] [PubMed]

15. Gouda, S.; Kerry, R.G.; Das, G.; Paramithiotis, S.; Shin, H.S.; Patra, J.K. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol. Res. 2018, 206, 131–140. [CrossRef] [PubMed]

16. Manoj, S.R.; Karthik, C.; Kadirvelu, K.; Arulselvi, P.I.; Shanmugasundaram, T.; Bruno, B.; Rajkumar, M. Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. J. Environ. Manag. 2020, 254, 109779. [CrossRef]

17. Baez-Rogelio, A.; Morales-García, Y.E.; Quintero-Hernández, V.; Muñoz-Rojas, J. Next generation of microbial inoculants for agriculture and bioremediation. Microb. Biotechnol. 2017, 10, 19–21. [CrossRef]

18. Zhang, L.-N.; Wang, D.-C.; Hu, Q.; Dai, X.-Q.; Xie, Y.-S.; Li, Q.; Liu, H.-M.; Guo, J.-H. Consortium of Plant Growth-Promoting Rhizobacteria Strains Suppresses Sweet Pepper Disease by Altering the Rhizosphere Microbiota. Front. Microbiol. 2019, 10, 1–10. [CrossRef]

19. Kalita, M.; Bharadwaz, M.; Dey, T.; Gogoi, K.; Dowarah, P.; Unni, B.G.; Ozah, D.; Saikia, I. Developing novel bacterial based bioformulation having PGPR properties for enhanced production of agricultural crops. Indian, J. Exp. Biol. 2015, 53, 56–60.

20. Pastor, N.; Carlier, E.; Andrès, J.; Rosas, S.B.; Rovera, M. Characterization of rhizosphere bacteria for control of phytopathogenic fungi of tomato. J. Environ. Manag. 2012, 95, S332–S337. [CrossRef]

21. Title of Site: Il Comparto del Pomodoro da Industria, Dati ISMEA. Available online: https://terraevita.edagricole.it/wp-content/uploads/sites/11/2019/08/Il-comparto-del-pomodoro-da-industria.pdf (accessed on 8 April 2020).

22. Title of Site: I.Stat. Available online: http://dati.istat.it/Index.aspx?DataSetCode=DCSP_COLTIVAZIONI (accessed on 8 April 2020).

23. Vaikuntapu, P.R.; Dutta, S.; Samudrala, R.B.; Rao, V.R.V.N.; Kalam, S.; Podile, A.R. Preferential Promotion of Lycopersicon esculentum (Tomato) Growth by Plant Growth Promoting Bacteria Associated with Tomato. Indian J. Microbiol. 2014, 54, 403–412. [CrossRef]

24. Seleim, M.A.A.; Saeed, F.A.; Abd-El-Moneem, K.M.H.; Abo-Elyour, K.A.M. Biological control of bacterial wilt of tomato by plant growth rhizobacteria. Plant. Pathol. J. 2011, 10, 146–153. [CrossRef]

25. Narendra Babu, A.; Jogaiah, S.; Ito, S.; Kestur Nagaraj, A.; Tran, L.S.P. Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. Plant. Sci. 2015, 231, 62–73. [CrossRef] [PubMed]

26. Agrawal, D.P.K.; Agrawal, S. Original Research Article Characterization of Bacillus sp. strains isolated from rhizosphere of tomato plants, (Lycopersicon esculentum) for their use as potential plant growth promoting rhizobacteria. Int. J. Curr. Microbiol. Appl. Sci. 2013, 2, 406–417.

27. Manfredi, G. Technologies for the Seismic Isolation and Control. Engineering 2009, 1, 271–320.

28. Damodaran, T.; Sah, V.; Rai, R.B.; Sharma, D.K.; Mishra, V.K.; Jha, S.K.; Kannan, R. Isolation of salt tolerant endophytic and rhizospheric bacteria by natural selection and screening for promising plant growth-promoting rhizobacteria (PGPR) and growth vigour in tomato under sodic environment. Afr. J. Microbiol. Res. 2013, 7, 5082–5089.

29. Vacheron, J.; Moeinne-Loccoz, Y.; Dubost, A.; Gonçalves-Martins, M.; Muller, D.; Prigent-Combaret, C. Fluorescent pseudomonas strains with only few plant-beneficial properties are favored in the maize rhizosphere. Front. Plant. Sci. 2016, 7, 1212. [CrossRef] [PubMed]

30. Zuluaga, M.Y.A.; Milani, K.M.L.; Gonçalves, L.S.A.; De Oliveira, A.L.M. Diversity and plant growth-promoting functions of diazotrophic/N-scavenging bacteria isolated from the soils and rhizospheres of two species of Solanum. PLoS ONE 2020, 15, e0227422. [CrossRef]

31. Abdeljalil, N.O.-B.; Vallance, J. Characterization of Tomato-associated Rhizobacteria Recovered from Various Tomato-growing Sites in Tunisia. J. Plant. Pathol. Microbiol. 2016, 7, 12. [CrossRef]
Plants 2020, 9, 668

32. Fernando, W.G.D.; Nakkeeran, S.; Zhang, Y.; Savchuk, S. Biological control of Sclerotinia sclerotiorum (Lib.) de Bary by Pseudomonas and Bacillus species on canola petals. *Crop. Prot.* 2007, 26, 100–107. [CrossRef]

33. Li, H.; Li, H.; Bai, Y.; Wang, J.; Nie, M.; Li, B.; Xiao, M. The use of Pseudomonas fluorescens P13 to control sclerotinia stem rot (Sclerotinia sclerotiorum) of oilseed rape. *J. Microbiol.* 2011, 49, 884–889. [CrossRef]

34. Massawe, V.C.; Hanif, A.; Farzand, A.; Mburu, D.K.; Ochola, S.O.; Wu, L.; Tahir, H.A.S.; Gu, Q.; Wu, H.; Gao, X. Volatile compounds of endophytic Bacillus spp. have biocontrol activity against Sclerotinia sclerotiorum. *Phytopathology* 2018, 108, 1373–1385. [CrossRef] [PubMed]

35. Mendes, R.; Garbeva, P.; Raaijmakers, J.M. The rhizosphere microbiome: Significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiol. Rev.* 2013, 37, 634–663. [CrossRef] [PubMed]

36. Gray, E.J.; Smith, D.L. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biol. Biochem.* 2005, 37, 395–412. [CrossRef]

37. Mbothia, L.W.; Acosta-Martínez, V.; DeBryun, J.; Schaeffer, S.; Tyler, D.; Odoi, E.; Mphesha, M.; Walker, F.; Eash, N. Long term tillage, cover crop, and fertilization effects on microbial community structure, activity: Implications for soil quality. *Soil Biol. Biochem.* 2015, 89, 24–34. [CrossRef]

38. Finney, D.M.; Buyer, J.S.; Kaye, J.P. Living cover crops have immediate impacts on soil microbial community structure and function. *J. Soil Water Conserv.* 2017, 72, 361–373. [CrossRef]

39. Degrune, F.; Theodorakopoulos, N.; Dufrène, M.; Colinet, G.; Bodson, B.; Hiel, M.P.; Taminiau, B.; Nezer, C.; Daube, G.; Vandenbol, M. No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium). *Agric. Ecosyst. Environ.* 2016, 224, 12–21. [CrossRef]

40. Barillot, C.D.C.; Sarde, C.O.; Bert, V.; Tarrnaud, E.; Cochet, N. A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. *Ann. Microbiol.* 2013, 63, 471–476. [CrossRef]

41. Baldani, J.I.; Reis, V.M.; Videira, S.S.; Boddey, L.H.; Baldani, V.L.D. The art of isolating nitrogen-fixing bacteria from non-leguminous plants using N-free semi-solid media: A practical guide for microbiologists. *Plant. Soil* 2014, 384, 413–431. [CrossRef]

42. Kloos, K.; Mergel, A.; Rösch, C.; Bothe, H. Denitrification within the genus Azospirillum and other associative bacteria. *Funct. Plant Biol.* 2001, 28, 991–998. [CrossRef]

43. Ambrosini, A.; Passaglia, L.M.P. Plant Growth-Promoting Bacteria (PGPB): Isolation and Screening of PGP Activities. *Curr. Protoc. Plant Biol.* 2017, 2, 190–209. [CrossRef]

44. Ishii, S.; Sadowsky, M.J. Applications of the rep-PCR DNA fingerprinting technique to study microbial diversity, ecology and evolution: minireview. *Environ. Microbiol.* 2009, 11, 733–740. [CrossRef] [PubMed]

45. Healy, M.; Huong, J.; Bittner, T.; Lising, M.; Frye, S.; Raza, S.; Schrock, R.; Manry, J.; Renwick, A.; Nieto, R.; et al. Microbial DNA typing by automated repetitive-sequence-based PCR. *J. Clin. Microbiol.* 2005, 43, 199–207. [CrossRef] [PubMed]

46. Heras, J.; Domínguez, C.; Mata, E.; Pascual, V.; Lozano, C.; Torres, C.; Zarazaga, M. GelJ—a tool for analyzing DNA fingerprint gel images. *BMCL Bioinform.* 2015, 16, 1–8. [CrossRef] [PubMed]

47. Di Cello, F.; Fani, R. A molecular strategy for the study of natural bacterial communities by PCR-based techniques. *Minerva Biotecnol.* 1996, 8, 126–134.

48. Glickmann, E.; Dessaux, Y. A critical examination of the specificity of the Salkowski reagent for indolic compounds produced by phytopathogenic bacteria. *Appl. Environ. Microbiol.* 1995, 61, 793–796. [CrossRef] [PubMed]

49. Schwyn, B.; Neilands, J.B. Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* 1987, 160, 47–56. [CrossRef]

50. Arora, N.K.; Verma, M. Modified microplate method for rapid and efficient estimation of siderophore produced by bacteria. *3 Biotech.* 2017, 7, 1–9. [CrossRef]

51. Dikin, A.; Sijam, K.; Kadir, J.; Semanz, I.A. Antagonistic bacteria against Schizophyllum Commune FR. In Peninsular Malaysia. *Biotropia* 2006, 13, 111–121.

52. El-Sayed, W.S.; Akhkha, A.; El-Naggar, M.Y.; Elbadry, M. In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. *Front. Microbiol.* 2014, 5, 1–11. [CrossRef]
53. Deka, H.; Deka-Baruah, C.K. Plant Growth Promoting Rhizobacteria for Value Addition: Mechanism of Action. In Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants; Springer International Publishing: Gwerbestrasse, Switzerland, 2015; pp. 305–321.

54. Kennedy, A.C.; de Luna, L.Z. Rhizosphere. In Encyclopedia of Soils in the Environment; Elsevier: Amsterdam, The Netherlands, 2004; ISBN 9780080547954.

55. Santi, C.; Bogusz, D.; Franche, C. Biological nitrogen fixation in non-legume plants. Ann. Bot. 2013, 111, 743–767. [CrossRef]

56. Islam, M.R.; Sultana, T.; Joe, M.M.; Yim, W.; Cho, J.C.; Sa, T. Nitrogen-fixing bacteria with multiple plant growth-promoting activities enhance growth of tomato and red pepper. J. Basic Microbiol. 2013, 53, 1004–1015. [CrossRef] [PubMed]

57. Hurek, T.; Reinhold, B.; Grimm, B.; Fendrik, I.; Niemann, E.G. Occurrence of effective nitrogen-scavenging bacteria in the rhizosphere of kallar grass. Plant. Soil 1988, 110, 339–348. [CrossRef]

58. Brooke, J.S. Stenotrophomonas maltophilia: An emerging global opportunistic pathogen. Clin. Microbiol. Rev. 2012, 25, 2–41. [CrossRef] [PubMed]

59. Diancourt, L.; Passet, V.; Verhoef, J.; Grimont, P.A.D.; Brisse, S. Multilocus sequence typing of Klebsiella pneumonae nosocomial isolates. J. Clin. Microbiol. 2005, 43, 4178–4182. [CrossRef] [PubMed]

60. Rodriguez-Medina, N.; Barrios-Camacho, H.; Duran-Bedolla, J.; Garza-Ramos, U. Klebsiella variicola: An emerging pathogen in humans. Emerg. Microbes Infect. 2019, 8, 973–988. [CrossRef] [PubMed]

61. Maul, J.E.; Buyer, J.S.; Lehman, R.M.; Culman, S.; Blackwood, C.B.; Roberts, D.P.; Zasada, I.A.; Teasdale, J.R. Factors affecting soil microbial community structure and abundance in the rhizosphere and bulk soil of a tomato cropping system that includes cover crops. Appl. Soil Ecol. 2014, 77, 42–50. [CrossRef]

62. Legrand, F.; Picot, A.; Cobo-Díaz, J.F.; Carof, M.; Chen, W.; Le Floch, G. Effect of tillage and static abiotic soil properties on microbial diversity. Appl. Soil Ecol. 2018, 132, 135–145. [CrossRef]

63. Piazza, G.; Ercoli, L.; Nuti, M.; Pellegrino, E. Interaction Between Conservation Tillage and Nitrogen Fertilization Shapes Prokaryotic and Fungal Diversity at Different Soil Depths: Evidence From a 23-Year Field Experiment in the Mediterranean Area. Front. Microbiol. 2019, 10, 1–20. [CrossRef]

64. Venter, Z.S.; Jacobs, K.; Hawkins, H.J. The impact of crop rotation on soil microbial diversity: A meta-analysis. Pedobiologia 2016, 59, 215–223. [CrossRef]

65. Buyer, J.S.; Teasdale, J.R.; Roberts, D.P.; Zasada, I.A.; Maul, J.E. Factors affecting soil microbial community structure in tomato cropping systems. Soil Biol. Biochem. 2010, 42, 831–841. [CrossRef]

66. Hariprasad, P.; Niranjana, S.R. Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant. Soil 2009, 316, 13–24. [CrossRef]

67. Zaidi, A.; Khan, M.S.; Ahemad, M.; Oves, M. Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol. Immunol. Hung. 2009, 56, 263–284. [CrossRef] [PubMed]

68. Rodriguez, H.; Fraga, R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 1999, 17, 319–339. [CrossRef]

69. Wang, L.T.; Tai, C.J.; Wu, Y.C.; Chen, Y.B.; Lee, F.L.; Wang, S.L. Pseudomonas taiwanensis sp. nov., isolated from soil. Int. J. Syst. Evol. Microbiol. 2010, 60, 2094–2098. [CrossRef] [PubMed]

70. Volmer, J.; Neumann, C.; Bühler, B.; Schmid, A. Engineering of Pseudomonas taiwanensis VLB120 for constitutive solvent tolerance and increased specific styrene epoxidation activity. Appl. Environ. Microbiol. 2014, 80, 6539–6548. [CrossRef] [PubMed]

71. Toro, M.; Ramírez-Bahena, M.H.; José Cuesta, M.; Velázquez, E.; Peix, A. Pseudomonas guariconensis sp. nov., isolated from rhizospheric soil. Int. J. Syst. Evol. Microbiol. 2013, 63, 4413–4420. [CrossRef]

72. Dicit, S.; Kuttan, K.K.A.; Shivastava, R. Isolation and characterization of phosphorus solubilizing bacteria from manganese mining area of Balaghat and Chhindwara. Curr. Sci. 2017, 113, 500–504. [CrossRef]

73. Shahab, S.; Ahmed, N.; Khan, N.S. PSB_tested in plant growth. Afr. J. Agric. Res. 2009, 4, 1312–1316.

74. Kumari, P.; Meena, M.; Upadhyay, R.S. Characterization of plant growth promoting rhizobacteria from Vigna radiata (mung bean). Biocatal. Agric. Biotechnol. 2018, 16, 155–162. [CrossRef]

75. Majeed, A.; Kaleem Abbasi, M.; Hameed, S.; Imran, A.; Rahim, N. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Front. Microbiol. 2015, 6, 1–10. [CrossRef]
76. Obafemi, Y.D.; Ajayi, A.A.; Taiwo, O.S.; Olorunsola, S.J.; Isibor, P.O. Isolation of polygalacturonase-producing bacterial strain from tomatoes (Lycopersicon esculentum Mill.). Int. J. Microbiol. 2019, 2019, 7505606. [CrossRef] [PubMed]
77. Ahmad, F.; Ahmad, I.; Khan, M.S. Indole Acetic Acid Production by the Indigenous Isolates of Azotobacter and Fluorescent Pseudomonas in the Presence and Absence of Tryptophan. Turk. J. Biol. 2005, 29, 29–34.
78. Anwar, S.; Ali, B.; Sajid, I. Screening of rhizospheric actinomycetes for various in-vitro and in-vivo plant growth promoting (PGP) traits and for agroactive compounds. Front. Microbiol. 2016, 7, 1–11. [CrossRef] [PubMed]
79. Ahmed, E.; Holmström, S.J.M. Siderophores in environmental research: Roles and applications. Microb. Biotechnol. 2014, 7, 196–208. [CrossRef] [PubMed]
80. Dutta, J.; Thakur, D. Evaluation of multifarious plant growth promoting traits, antagonistic potential and phylogenetic affiliation of rhizobacteria associated with commercial tea plants grown in Darjeeling, India. PLoS ONE 2017, 12, e0182302. [CrossRef]
81. Tian, F.; Ding, Y.; Zhu, H.; Yao, L.; Du, B. Genetic diversity of siderophore-producing bacteria of tobacco rhizosphere. Braz. J. Microbiol. 2009, 40, 276–284. [CrossRef]
82. Mehnaz, S.; Weselowski, B.; Lazarovits, G. Sphingobacterium canadense sp. nov., an isolate from corn roots. Syst. Appl. Microbiol. 2007, 30, 519–524. [CrossRef]
83. Ruiz-Ruiz, J.C.; Peraza-Echeverría, L.; Soto-Hernández, R.M.; San Miguel-Chávez, R.; Pérez-Brito, D.; Tapia-Tussell, R.; Ortiz-Vázquez, E.; Rodríguez-García, C.M. Diospyros cuneata Inhibition of Fusarium oxysporum: Aqueous Extract and its Encapsulation by Ionic Gelation. Plant Pathol. Microbiol. 2016, 7, 1–11.
84. Kim, Y.M.; Ahn, K.J.; Beppu, T.; Uozumi, T. Nucleotide sequence of the nifLA operon of Klebsiella oxytoca NG13 and characterization of the gene products. MGG Mol. Gen. Genet. 1986, 205, 253–259. [CrossRef]
85. Sajjad Mirza, M.; Ahmad, W.; Latif, F.; Haurat, J.; Bally, R.; Normand, P.; Malik, K.A. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. Plant. Soil 2001, 237, 47–54. [CrossRef]
86. Pavlova, A.S.; Leontieva, M.R.; Smirnova, T.A.; Kolomeitseva, G.L.; Netrusov, A.I.; Tsavkelova, E.A. Colonization strategy of the endophytic plant growth-promoting strains of Pseudomonas fluorescens and Klebsiella oxytoca on the seeds, seedlings and roots of the epiphytic orchid, Dendrobium nobile Lindl. J. Appl. Microbiol. 2017, 123, 217–232. [CrossRef] [PubMed]
87. Park, M.R.; Kim, Y.C.; Lee, S.; Kim, I.S. Identification of an ISR-related metabolite produced by rhizobacterium Klebsiella oxytoca C1036 active against soft-rot disease pathogen in tobacco. Pest. Manag. Sci. 2009, 65, 1114–1117. [CrossRef] [PubMed]
88. Woo, S.L.; Pepe, O. Microbial consortia: Promising probiotics as plant biostimulants for sustainable agriculture. Front. Plant. Sci. 2018, 9, 1801. [CrossRef] [PubMed]

© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).