Article

Characterization of *Streptomyces* spp. from Rice Fields as a Potential Biocontrol Agent against *Burkholderia glumae* and Rice Plant Growth Promoter

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Abstract: The usage of plant growth-promoting (PGP) *Streptomyces* to improve plant growth is an alternative to existing strategies of chemical fertilizers that commonly caused environmental pollution. The aim of this study was to isolate and characterize PGP *Streptomyces* from Malaysian rice fields with antagonistic activity against Bacterial Panicle Blight disease in rice caused by *Burkholderia glumae*. A total of 50 bacterial isolates were recovered from the soil, rhizosphere, and endosphere; 22% showed antagonistic activity against *B. glumae*. Molecular identification using 16S rRNA amplification and phylogenetic tree analyses revealed that the antagonistic isolates belonged to the genus *Streptomyces*. Among the antagonistic isolates, *Streptomyces* sp. TBS5 (26.35 ± 0.14 mm) demonstrated the most antagonistic activity ($p < 0.05$) against *B. glumae*. Through API[®] ZYM analysis, the antagonistic isolates were revealed to have phosphoric hydrolase, ester hydrolase, peptide hydrolase, and glycosidase activities that play a crucial role in plant growth promotion. The antagonistic isolates demonstrated the highest (80%) PGP traits including able to fix nitrogen and solubilize phosphate, as well as produce siderophore and indole-3-acetic acid. Plant growth promotion assay under laboratory and greenhouse conditions were analyzed with the treatment of rice, *Oryza sativa*, seeds with the antagonistic isolates. Seeds treated with *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 showed improvement in vigor index by more than 100% compared to water-treated control plants under both conditions. Augmentation of root length showed an overall increment of more than 101.5% under laboratory condition and 151.9% under greenhouse condition using *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 treatments. Results suggest that *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 are a good candidate to be developed as both biocontrol agent against *B. glumae* and PGP agent in rice.

Keywords: *Oryza sativa*; *Streptomyces* spp.; biocontrol agents; plant growth-promoting; Bacterial Panicle Blight; *Burkholderia glumae*

1. Introduction

Bacterial Panicle Blight (BPB) is a severe seed-borne bacterial disease of rice. It was first reported in the 1950s in Japan. This disease is primarily caused by *Burkholderia glumae*,

a rod-shaped, Gram-negative, and polar flagellated bacterium [1,2]. BPB has been widely spotted in rice-growing countries, including Asia [3,4], South and Central America [5–7], and Africa [8]. The occurrence of BPB is correlated by climatic change and is frequently found in tropical countries [9,10]. Rice plants infected by *B. glumae* show several disease symptoms which include seedling rot, panicle blighting, and leaf-sheath browning [11]. It is noteworthy that *Burkholderia gladioli* infection also produced similar symptoms as *B. glumae*; suggesting that BPB also can be caused by *B. gladioli* [12].

The reduction of rice yield caused by BPB has reached up to 75% worldwide due to the reduction in milling quality, floret sterility, grain weight, and seed germination [13]. The current approach to control BPB includes: (i) development of BPB-resistant rice cultivars [14]; (ii) genomic analysis of pathogen virulence genes [15,16]; (iii) transcriptomic analysis of *B. glumae* along with rice development [17,18]; and (iv) diagnostic tools improvement in the rice fields by farmers for early detection of *B. glumae* symptoms to minimize the frequency of *B. glumae* infection [19,20]. Unfortunately, the effectiveness of these approaches is somehow inefficient to chemical resistance and polymorphisms within the pathogenic strains [21,22]. Interestingly, biocontrol strategy offers a possible alternative in managing plant diseases by the usage of disease-suppressive organisms to improve plant health and control pathogens [23].

The implementation of biocontrol strategy to inhibit the growth of pathogenic organisms has become widely widespread due to environmental concerns [24,25]. Biocontrol strategies to control plant diseases have received great attention because it is potentially safe and environmentally friendly, long-lasting, and inexpensive [26]. Plenty of studies have successfully proven the applicability of biocontrol agent to control rice disease caused by bacteria in an attempt to replace existing strategies of pesticides and chemical fertilizers that frequently lead to environmental pollution [27–29]. As a tool to control rice disease caused by bacteria, biocontrol agents displayed themselves as a remarkable alternative in enhancing rice plants' health and at the same time controlling pathogens in vitro and in vivo [30]. Among bacterial groups, *Streptomyces* spp. were broadly examined as biocontrol agents due to their plant growth-promoting (PGP) and biocontrol characteristics [31–33]. *Streptomyces* spp. also received wide attention based on their ability to secrete varieties of extracellular enzymes [34] and secondary metabolites such as antibiotics [35].

The usage of plant growth-promoting (PGP) *Streptomyces* as biocontrol agents are known as an alternative method in improving rice plant growth [24]. Thus, this study aimed to isolate and characterize PGP *Streptomyces* from rice fields with antagonistic activity against the causal agent of BPB disease, *B. glumae*, in rice. Here, the soil, rhizosphere, and endosphere samples collected from rice fields were subjected for the *Streptomyces* spp. isolation. The obtained isolates were evaluated for their antibacterial activity against *B. glumae* and were identified molecularly. The PGP trait of *Streptomyces* isolates were determined using API[®] ZYM and biochemical assays including nitrogen fixation, phosphate solubilization, as well as siderophore, indole-3-acetic acid, and hydrogen cyanide productions. The effect of *Streptomyces*-treatment upon seed germination and vigor index of rice were also determined. Studies on the determination of potential PGP *Streptomyces* with the antagonistic activity against *B. glumae* are necessary to illustrate their contributions for growth and yield improvement in rice crop and this can be manipulated for the bioformulations development.

2. Materials and Methods

2.1. Sampling and Isolation of *Streptomyces* spp.

Isolation of *Streptomyces* spp. was conducted according on the method as described by Mohd Hata et al., 2015 [32]. Soil, rhizosphere, and endosphere of rice plants were obtained from different rice fields [Kg. Padang Teluk, Mukim Jeram, Kedah (6°28' N, 100°34' E) and Kg. Imam, Mukim Tunjang, Kedah (6°33' N, 100°36' E)] in Malaysia. Briefly, 10 g of soil, rhizosphere, and root samples were mixed with sterile distilled water (90 mL) respectively. To harvest *Streptomyces* from rice plant endospheres, root samples were

surface sterilized and macerated using sterile pestle and mortar before the addition of sterile distilled water. Next, the sample suspensions in the 250 mL conical flask were homogenized and sonicated for 15 min before being subjected to heat treatment at 50 °C for 15 min. The samples were further serially diluted (10^{-1} to 10^{-10}) and 100 µL of each diluted samples were spread onto actinomycetes isolation agar supplemented with nalidixic acid (10 µg/mL) and cycloheximide (40 µg/mL). The plates were then incubated at 30 °C for 7 days and the direct count method for total plate count (TPC) was determined. Pure isolates were obtained by sub-culturing and isolates were preserved for long-term preservation in glycerol at −80 °C.

Pure isolates were inoculated onto yeast malt (ISP-2) agar [0.004% (*w/v*) yeast extract, 0.01% (*w/v*) malt extract, 0.004% (*w/v*) D-glucose, and 1.5% (*w/v*) bacteriological agar] using the four-way streak-plate technique and incubated at 30 °C. After seven days of incubation, macroscopic analysis was determined according to Shirling and Gottlieb, 1966 [36] based on the colony size, aerial mycelium, substrate mycelium, pigmentation, and margin.

2.2. Antibacterial Activity of Isolates against *Burkholderia glumae*

The pathogen, *B. glumae* (GenBank accession No: MW020265), was provided by the Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, Malaysia. The pathogen was inoculated into nutrient broth (10 mL) at 30 °C for 24 h with shaking at 120 × *g*. For long-term preservation, the bacterial pathogen was preserved in glycerol at −80 °C.

Antibacterial activity of isolates against *B. glumae* were tested [32]. Bacterial isolates were inoculated vertically at the center of ISP-2 agar from a 5 mm inoculant and incubated at 30 °C. After 7 days of incubation, the growing isolates were inactivated over chloroform for 3 min [37]. The plates were then flooded with *B. glumae* suspension (2 mL) adjusted to approximately 10^8 CFU/mL using a spectrophotometer at a 600 nm. The suspension was left to dry, and the plate was incubated at 30 °C. After two days of incubation, antibacterial activity was evaluated by the formation of inhibition zone surrounding the isolate growth colony. The inhibition zone was measured in mm.

2.3. Molecular Identification of Antagonistic Isolates

All of the antagonistic isolates were identified based on the 16S ribosomal RNA (rRNA) gene sequence analysis. Universal primer sets [27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGTTACCTTGTACGACTT-3')] and KOD FX Neo (TOYOBO, Osaka, Japan) were used in polymerase chain reaction (PCR). Briefly, each PCR mixture (50 µL) contained 2.0 mM dNTPs, 0.5 µM 27F primer, 0.5 µM 1492R primer, 1.0 µL of KOD FX Neo (1.0 U/µL), 25 µL of 2 × PCR buffer, and 2.0 µL of DNA template (100 ng/µL). Thermal cycling condition was performed at 95 °C for 2 min for initial denaturation followed by 30 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 2 min. One cycle of final extension step at 72 °C for 10 min was also included. The 1.0% (*w/v*) agarose gel electrophoresis was used to verify the amplified PCR products before being subjected for sequencing. The sequencing results were analyzed using Chromas Lite software and compared against the sequences in the NCBI database by using the nucleotide BLAST (blastn) program.

MEGA X software was used for phylogenetic tree analyses [38]. The 16S rRNA gene sequences of the isolates were compared to the sequences of closely related species available in the GenBank. Clustal Omega program (<https://www.ebi.ac.uk/Tools/msa/clustalo/>; accessed on 27 March 2021) and MEGA X software were used for the multiple sequence alignments. The aligned sequence was trimmed at the same position manually. To construct a phylogenetic tree, neighbor-joining [39] and *p*-distance methods [40] with 1000 bootstrapping [41] were used. As for the outgroup, *Kitasatospora paracochleata* NBRC 14769^T was used.

2.4. Evaluation of Enzymatic Fingerprinting of Antagonistic Isolates

The API[®] ZYM kit (BioMérieux, Marcy-l'Etoile, France) was used for the evaluation of enzymatic activities of antagonistic isolates. Bacterial isolates grown in ISP-2 agar at 30 °C for 7 days were suspended in API suspension medium to approximately to 5.0 McFarland standard. The bacterial isolate suspension (65 µL) was inoculated into API ZYM test strips. The strips were incubated at 30 °C for 4 h. For the colorimetric analysis, the strips were then incubated for 5 min after the addition ZYM A and B reagents. Enzyme activity classification was determined from 0 (no activity) to 5 (maximum activity) based on the API ZYM color reaction chart.

2.5. Bioassays for Plant Growth-Promoting (PGP) Traits of Antagonistic Isolates

2.5.1. Nitrogen Fixation

The ability of isolates to fix nitrogen was tested by culturing the isolates on nitrogen-free (NF) agar [32]. Bacterial isolates grown in ISP-2 broth at 30 °C for seven days were further inoculated on NF agar and incubated at 30 °C. After seven days of incubation, the ability of isolates to fix nitrogen was observed by the blue coloration zone around the colony and the diameter of the zone was measured.

2.5.2. Siderophore Production

The production of siderophore was determined on chrome azurol S (CAS) agar [42]. Bacterial isolates grown in ISP-2 broth at 30 °C for seven days were further inoculated on CAS agar and incubated at 30 °C. After seven days of incubation, the diameter of yellow halo zone around the colony was measured and indicated as a positive CAS reaction.

2.5.3. Phosphate Solubilization

Phosphate solubilization activity was determined on pikovskaya's agar (HiMedia, Mumbai, India). Bacterial isolates grown in ISP-2 broth at 30 °C for seven days were further inoculated on pikovskaya's agar and incubated at 30 °C. After seven days of incubation, the formation of halo zone around the colony indicated as a positive phosphate solubilization activity. The diameter of the zone was measured.

2.5.4. Hydrogen Cyanide (HCN) Production

Bacterial isolates were streaked on the entire surface of ISP-2 agar supplemented with 4.4 g/L glycine. Filter paper (Whatman No. 1) was saturated with picric acid solution [5.0% (v/v) picric acid and 2.0% (w/v) Na₂CO₃] and placed aseptically on the lid of the Petri plates. Inoculated plates were carefully sealed and further incubated at 30 °C. After seven days of incubation, the HCN production was indicated by the filter paper color changes from yellow to orange-brown [43].

2.5.5. Indole-3-Acetic Acid (IAA)

Production of IAA was analyzed [44]. Bacterial isolates grown in ISP-2 broth at 30 °C for seven days were further inoculated in ISP-2 broth supplemented with 0.2% (w/v) L-tryptophan. The broth culture was incubated at 30 °C with shaking at 120× g. The broth culture was centrifuged at 10,000× g for 10 min after seven days of incubation. The supernatant was collected and mixed with Salkowski reagent [1.0 mL of 0.5 M FeCl₃ in 50 mL of 35% (v/v) HClO₄] at a ratio of 1:2. The mixture was incubated at 30 °C for 25 min. The IAA production was determined using a spectrophotometer at 535 nm. Un-inoculated broth served as the negative control. The value of IAA concentration was obtained by a standard curve of IAA solution (0 to 100 µg/mL). The IAA amount produced was determined as µg IAA secreted per unit of optical density.

2.6. Effect of Antagonistic Isolates on the Growth of Rice Seedlings

2.6.1. Bacterial Inocula Preparation for Rice Seed Treatment

All of the antagonistic isolates were cultured in 25 mL ISP-2 broth and further incubated at 30 °C with 120× g while being shaken for seven days. Broth culture was centrifuged at 5000× g for 10 min and bacterial pellets were collected. The bacterial pellets were washed with sterile distilled water three times aseptically. Next, the bacterial pellets were suspended in sterile distilled water (100 mL) adjusted to approximately 10⁸ CFU/mL using a spectrophotometer at a 600 nm. Rice seeds were surface sterilized in 70% (v/v) ethanol for 1 min and further washed with sterile distilled water three times. Dry seeds (10 g) were immersed in the bacterial suspension at 30 °C shaken at 120× g overnight. The treated seeds were dried overnight at 40 °C.

2.6.2. Effect of Bacterial Seed Treatment on Germination and Vigor Index in Rice

The effects of bacterial seed treatment on germination and vigor index in rice were determined under laboratory and greenhouse conditions [33,45]. In laboratory conditions, 25 seeds inoculated with each antagonistic isolate were incubated in a sterile plastic container (30 cm in diameter) impregnated with sterile moistened tissue paper (45 cm × 28 cm). To maintain sufficient moisture, 5 mL sterile distilled water was added into the container daily to promote germination. The seeds were incubated at 28 ± 2 °C in a light incubator. An in vivo experiment was conducted in greenhouse using rice husk ash as growing media. The rice husk ash was sterilized in an autoclave at 121 °C for 10 min three times. Approximately, 1.0 kg sterile rice husk ash was added to sterile plastic container (30 cm in diameter) containing 25 seeds. To promote germination, 10 mL sterile distilled water was added into the container daily to maintain sufficient moisture. Rice seeds treated with sterile distilled water was used as control. The germination percentage, root, and shoot length were determined after 10 days of growth on each condition. The laboratory experiment was conducted as a completely randomized design (CRD) meanwhile greenhouse experiment was conducted as a randomized complete block design (RCBD) with three replications for each antagonistic isolate treatments. The germination rate and vigor index were calculated using the following formulae:

$$\text{Germination rate (\%)} = [(\text{number of seeds germinated}) / (\text{total number of seeds})] \times 100 \quad (1)$$

$$\text{Vigor index} = \text{germination rate} \times \text{total plant length} \quad (2)$$

2.7. Statistical Analysis

NCSS software version 2020 (NCSS LLC, Kaysville, UT, USA) was used for statistical analysis. One-way ANOVA and Tukey's tests were used to analyzed data. Statistical significance was attributed to $p < 0.05$. Data were expressed as mean values generated from three different experiments.

3. Results

3.1. Isolation of *Streptomyces* spp.

The TPC was obtained after seven days of plate incubation. The TPC recovered from soil, rhizosphere, and endosphere samples showed significant differences (Table 1). The TPC of soil ranged from 151.30×10^4 to 230.17×10^4 CFU/g, meanwhile rhizosphere ranged from 130.08×10^4 to 185.52×10^4 CFU/g. The TPC of endosphere ranged from 1.06×10^4 to 2.82×10^4 CFU/g. Among the three samples, soil showed the highest TPC value (230.17×10^4 CFU/g) followed by rhizosphere (185.52×10^4 CFU/g) and endosphere (2.82×10^4 CFU/g) samples.

Overall, a total of 50 isolates (24 isolates from the soil, 14 isolates from rhizospheres, and 12 isolates from endospheres) were obtained from this study (Table S1). Streptomycetes were screened by morphological characterization based on colony size, aerial mycelium, substrate mycelium, pigmentation, and margin.

Table 1. Total plate count in the soil, rhizosphere, and endosphere of rice plants. Results were the average of data in triplicates $\pm$ SD (standard deviation). Means with different superscript letters in the same column indicated significant difference at $p < 0.05$ (One-way ANOVA).

Location	Plot	Growth Phase (Day)	Rice Variety	Total Plate Count (10^4 CFU/g)		
				Soil	Rhizosphere	Endosphere
Kg. Padang Teluk	A	Ripening (100 days)	MR297	151.30 ^b $\pm$ 11.49	185.52 ^a $\pm$ 19.81	2.82 ^a $\pm$ 0.07
	B	Ripening (100 days)	UPutra	125.37 ^c $\pm$ 5.92	130.08 ^d $\pm$ 9.05	1.06 ^c $\pm$ 0.00
Kg. Imam	A	Ripening (110 days)	MR297	230.17 ^a $\pm$ 8.23	162.55 ^b $\pm$ 10.88	1.75 ^b $\pm$ 0.03
	B	Ripening (110 days)	MR297	219.7 ^a $\pm$ 3.67	138.92 ^c $\pm$ 1.71	1.76 ^b $\pm$ 0.00

3.2. Antibacterial Activity against *Burkholderia glumae*

The antagonistic activity of all 50 isolates were determined by the production of inhibition zone on the media inoculated with *B. glumae*. Overall, 22.0% (11 out of 50 isolates) demonstrated antagonistic activities against *B. glumae* (Figure 1). Most of the antagonistic isolates were isolated from the soil (81.8%) and rhizosphere (18.2%). No antimicrobial activity of the isolates against *B. glumae* from the endosphere was detected using this method. The inhibition zone that represented as a positive result for antagonistic activity ranged from 1.13 to 26.35 mm. Isolate TBS5 (26.35 ± 0.14 mm) from the soil demonstrated the highest antagonistic activity ($p < 0.05$) against *B. glumae* followed by TKR8 (16.35 ± 0.14 mm), JAS2 (11.00 ± 0.16 mm), and R2-7 (9.65 ± 0.14 mm) compared to other active antagonistic isolates (Figure 1).

Figure 1. Antimicrobial activity against *Burkholderia glumae*. (a) Inhibition zone of isolates against *B. glumae* grown on (b) ISP-2 agar. Plates inoculated only with the pathogen served as the negative control (NC). The isolates' name/abbreviation is provided at the bottom of the photo. Results were the average of data in triplicates $\pm$ SD (standard deviation). Means with different superscript letters (a–j) differ significantly at $p < 0.05$ (One-way ANOVA) significance level.

3.3. Molecular Identification of Antagonistic Isolates Using 16S Ribosomal RNA

The partial fragment of 16S rRNA gene sequences at approximately 1.5-Kbp was successfully amplified using PCR and the sequences were compared to the sequences available in the GenBank database (Figure 2). The sequences showed maximum similarity ($\geq 98\%$) to the closest known species in the database. The 16S rRNA gene sequences were used for the phylogenetic analysis. All of the antagonistic isolates were found belonged to the genus *Streptomyces*. All the 16S rRNA gene sequences have been deposited in the NCBI database.

Figure 2. Analysis of 16S rRNA gene sequence. (a) The comparison of 16S rRNA gene sequences of *Streptomyces* isolates with the 16S rRNA gene sequence in GenBank. (b) Phylogenetic tree analysis of 16S rRNA sequence of *Streptomyces* isolates. *Streptomyces* isolates retrieved in this study are in bold, accession numbers are indicated in parenthesis. *Kitasatospora paracochleata* NBRC 14769^T was chosen as an outgroup.

3.4. Enzymatic Fingerprinting of *Streptomyces* spp.

All the *Streptomyces* spp. that actively against *B. glumae* were further characterized by their enzymatic activities. Nineteen types of enzyme activities of *Streptomyces* spp. were verified using API[®] ZYM (Figures 3 and S1). Enzymes tested in this study grouped into four different types; phosphoric hydrolases, ester hydrolases, peptide hydrolases, and glycosidases. Isolate *Streptomyces* sp. TBS5 produced the highest number of enzymes tested (17 out of 19), followed by *Streptomyces* sp. R2-7 and JBS6, with 15 enzymes respectively.

Figure 3. Heatmap of enzymatic activities of *Streptomyces* isolates. All activities were determined using the API[®] ZYM kit. The experiment was repeated in triplicate. The *Streptomyces* isolates' name/abbreviation is provided at the top of the photo.

Most of *Streptomyces* spp. in this study was able to produce all phosphoric hydrolyses enzymes (alkaline phosphatase, acid phosphatase, and naphthol phosphohydrolase) except for *Streptomyces* sp. TBS2 and TAS10. For ester hydrolases enzymes group, all *Streptomyces* spp. tested were found able to produce esterase (C4) and esterase/lipase (C8), while only 3 isolates namely *Streptomyces* sp. TBS5, R2-7, and JAS2 produced lipase which able to catalyze the hydrolysis of long chain fatty acid, C14. In addition, most *Streptomyces* spp. tested in this study are peptide hydrolases enzymes producers, where leucine arylamidase showed an intense enzyme activity, followed by cystine arylamidase and valine arylamidase. As for glycosidases enzymes group, *N*-acetyl- β -glucosaminidase was actively produced by all *Streptomyces* isolates, except *Streptomyces* sp. NA9 and St-1.

3.5. Determination of Plant Growth-Promoting (PGP) Activities

All the antagonistic *Streptomyces* isolates against *B. glumae* were further characterized of their PGP activities based on their ability to fix nitrogen, produce siderophore, solubilize phosphate, produce HCN, and produce IAA (Figures 4 and S2). Overall, all the antagonistic isolates demonstrated single or multiple PGP activities (either two, three, or four PGP activities), except for *Streptomyces* sp. TAS10, where none of the PGP activities were detected.

Overall, *Streptomyces* sp. JAS2 was positive on 4 (80.0%) of PGP traits including nitrogen fixation, siderophore production, phosphate solubilization, and IAA production. *Streptomyces* sp. TKR2, TKR8, NA9, and St-1 were all positive on 3 (60.0%) of PGP traits including fixed nitrogen, solubilize phosphate, and produce IAA. Meanwhile, *Streptomyces* sp. TBS5 and R2-7 were both positive on 3 (60.0%) of PGP traits including fixed nitrogen,

produce siderophore, and solubilize phosphate. *Streptomyces* sp. TKS4 was found positive on fixed nitrogen and solubilized phosphate, whereas *Streptomyces* sp. TBS2 was positive on produced siderophore and IAA. *Streptomyces* sp. JBS6 was shown only able to solubilize phosphate. No HCN production was found from all the tested isolates.

Figure 4. Evaluation of different plant growth-promoting (PGP) traits of *Streptomyces* isolates. (a) Nitrogen fixation, (b) siderophore production, (c) phosphate solubilization, and (d) IAA production. Results were average of data in triplicates $\pm$ SD (standard deviation). Means with different superscripts (a–g) differ significantly at $p < 0.05$ (One-way ANOVA) significance level.

Among the 11 *Streptomyces* isolates, 8 (72.7%) were able to fix nitrogen and were identified as potential nitrogen-fixing bacteria based on the blue coloration zone around the colony on NF agar. Nitrogen-fixing activity varied from 1.0 to 2.4 cm among the isolates. The highest nitrogen fixation activity was detected in *Streptomyces* sp. TBS5 (2.4 ± 0.1 cm) followed by *Streptomyces* sp. NA9 (2.0 ± 0.1 cm), *Streptomyces* sp. St-1 (2.0 ± 0.1 cm), and *Streptomyces* sp. TKR8 (1.4 ± 0.1 cm).

Siderophore production was found in 4 (36.4%) isolates. The four isolates produced siderophore at levels ranging from 1.1 to 2.1 cm. *Streptomyces* sp. TBS5 (2.1 ± 0.1 cm) produced the most siderophore followed by *Streptomyces* sp. R2-7 (1.8 ± 0.1 cm), *Streptomyces* sp. JAS2 (1.2 ± 0.1 cm), and *Streptomyces* sp. TBS2 (1.1 ± 0.1 cm).

Out of 11 *Streptomyces* isolates, 9 (81.8%) were able to solubilize phosphate. The efficiency of phosphate solubilization varied from 0.8 to 2.7 cm among the isolates. *Streptomyces* sp. R2-7 (2.7 ± 0.1 cm) showed the highest activity followed by *Streptomyces* sp. TBS5 (2.5 ± 0.1 cm), *Streptomyces* sp. JBS6 (1.9 ± 0.1 cm), and *Streptomyces* sp. TKR2 (1.7 ± 0.1 cm).

Among the 11 *Streptomyces* isolates, 6 (54.5%) were detected to produce IAA. The quantitative range of IAA production was determined from 0.038 to 6.674 $\mu\text{g/mL}$. *Streptomyces* sp. JAS2 (6.674 ± 0.005 $\mu\text{g/mL}$) produced the most IAA followed by *Streptomyces* sp. NA9 (3.490 ± 0.001 $\mu\text{g/mL}$), *Streptomyces* sp. TBS2 (3.475 ± 0.03 $\mu\text{g/mL}$), and *Streptomyces* sp. St-1 (2.186 ± 0.007 $\mu\text{g/mL}$).

3.6. The Effect in Rice Germination and Vigor Index Using *Streptomyces*-Treatments

The effect in rice germination and vigor index using *Streptomyces*-treatments varied among the different isolates used. A significant effect on the vigor index (VI), germination rate (GR), root length (RL), and shoot length (SL) compared to the control were detected from all different treatments (Figure 5). Numerical data are shown in Supplementary Materials Table S2.

Figure 5. Heatmap of the effect of *Streptomyces*-treated rice seedlings on germination and vigor index in rice. The vigor index (VI), germination rate (GR), root length (RL), and shoot length (SL) were obtained after 10 days of growth under laboratory and greenhouse conditions. Data was presented as a percentage of *Streptomyces*-treated rice seedling's relative to non-treated control seedlings. The experiment was repeated in triplicates. The *Streptomyces* isolates' name/abbreviation is provided at the top of the photo.

Overall, the VI increased up to 139.0% under laboratory conditions while in greenhouse conditions the VI increased up to 118.1% compared to the control. *Streptomyces*-treatments improved the GR of rice seeds up to 119.4% under laboratory conditions while under greenhouse conditions the GR improved up to 110.7% compared to the control.

In both conditions (laboratory and greenhouse), the maximum increase of VI [laboratory (139.0%) and greenhouse (119.0%)] and GR [laboratory (119.4%) and greenhouse (110.7%)] were obtained with the *Streptomyces* sp. TBS5 treatment. This was followed by the treatment using *Streptomyces* sp. JAS2, R2-7, and TKR8. In particular, the VI of *Streptomyces* sp. JAS2, R2-7 and TKR8 increased more than 108.6% compared to the control under both conditions.

The improvement of RL using the *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 treatments showed an overall increment of more than 101.5% (4.6 cm) under laboratory conditions and 151.9% (0.8 cm) under greenhouse conditions (Figure 6 and Table S3). Meanwhile, SL improvement using the *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 treatments showed an overall increment of more than 109.0% (4.6 cm) under laboratory conditions and 94.6% (3.7 cm) under greenhouse conditions. These results suggested that treatment with *Streptomyces* could improve the germination and vigor of rice seeds.

Figure 6. Effect of *Streptomyces*-treated rice seedlings on germination in rice after 10 days of growth under laboratory and greenhouse conditions. Seeds treated with sterile distilled water were used as control. The *Streptomyces* isolates' name/abbreviation is provided at the bottom of the photo.

4. Discussion

In this study, the highest bacterial population was recorded in soil compared to the rhizosphere and endosphere samples. Soil microbes have been suggested as a major component of soil fertility [46]. As reported by Yu et al. 2003 [47], the population of microbes in the soil is closely correlated with its healthy status and the soil's fertilization. It is not clear whether this impact is caused by the texture of the soil or other environmental conditions. Moreover, plant varieties and growth stages are also important to determine the development of microbial population in the soil [48]. In 2015, the isolation of rice soil actinobacteria were reported from the Tiruchirappalli district, Tamil Nadu, India [49]. Based on the isolation of actinobacteria from 12 different rice fields, 31.1% of the isolates were recognized as bacteria from the genus *Streptomyces*. This suggested that soil fertility correlated with the diversity of soil actinobacteria that indicates a positive relationship with the physicochemical properties of soil. It is worth mentioning that *Streptomyces* spp. are essential groups of soil bacteria. Various of *Streptomyces* spp. have been recognized as a producer of antibiotics and extracellular enzymes [50]. To date, detailed information on the characteristics of *Streptomyces* spp. isolated from Malaysian rice fields is scarce. Thus, *Streptomyces* spp. characterization as a biocontrol agent against *B. glumae* and as rice plant growth promoters is needed.

In 2019, the PGP abilities and biocontrol properties of *Streptomyces* strain A20 to control *B. glumae* were reported [31]. The laboratory tests have showed the ability of *Streptomyces* strain A20 to produce siderophores, solubilize phosphate, produce IAA, and produce extracellular enzymes. Noteworthy, *Streptomyces* strain A20 was suggested can colonize rice plants as well as promote rice plant growth under greenhouse experiments. Also, antimicrobial compounds from *Streptomyces* strain A20 that are antagonistic against *B. glumae* were found as streptotricins D, E, and F. Overall, these results suggested the potential application of *Streptomyces* strain A20 as a biocontrol agent against *B. glumae* infection in rice. Here, antibacterial activity of *Streptomyces* spp. against *B. glumae* was

observed. Analysis of various properties considered to be favorable for the promotion of rice plant growth clearly indicated that *Streptomyces* isolates (as identified by 16S rRNA gene sequencing) are potential PGP agents and can be applied to suppress *B. glumae*.

Taxonomical analyses of 16S rRNA gene have proven that all the antagonistic isolates (TBS5, TKR2, TKS4, TBS2, TAS10, R2-7, JAS2, TKR8, JBS6, NA9, and St-1) were from the genus *Streptomyces* with maximum similarity calculations ($\geq 98\%$). Phylogenetic tree analysis indicated that isolate TBS5 was related to clade 97, having 99.65% similarity to *S. misionensis* [51]. Both isolates TKR2 and TKS4 have 98.30% and 98.17% similarity to *S. griseoluteus*, respectively. Isolate TBS2 is 99.01% similar to *S. geysiriensis*, meanwhile TAS10 has a similarity value of 98.94% to *S. kebangsaanensis*. Isolate R2-7 is similar to *S. panaciradicis* (99.57%) and *S. sasae* (99.50%). A similar analysis located isolates JAS2 and TKR8 within clade 2 with 99.15% and 99.36% similarity to *S. cylabdanicus* and *S. corchorusii* respectively [51]. Isolate JBS6 has 99.58% similarity to *S. bungoensis*. Isolates NA9 and St-1 are closely related to *S. cavourensis* with 99.79% similarity which placing both isolates within the clade 31 [51].

The results of the enzyme profile using the API[®] ZYM kit have depicted the *Streptomyces* isolate specificity and difference in enzyme profiles (Figure 4). To note, *Streptomyces* spp. have received wide attention due to their ability to secrete many types of extracellular enzymes with various applications in agriculture and industrial practices [52]. In this study, most *Streptomyces* isolates exhibited phosphoric hydrolase activities. It is noteworthy that the phosphoric hydrolase activities including phosphohydrolase and phosphatase by the rhizosphere microbiota are crucial for the organic phosphorus sources conversion in the soil into easily assimilated forms for plants [53,54]. Moreover, it has been found that in all *Streptomyces* isolates investigated, ester hydrolase, peptide hydrolase, and glycosidase activities were detected too. Microbial enzymes including ester hydrolases, peptide hydrolases, and glycosidases play an important role in preserving soil quality as well as promoting plant growth [55]. It has been reported that the secretion of hydrolytic enzymes such as esterase and lipase by the rhizospheric microbiota improved the mechanisms of plant to control soil-borne phytopathogens infection [56]. In another related study, peptide hydrolases enzymes such as leucine arylamidase has been suggested involved in N mineralization in soils [57]. Interestingly in this study, *N*-acetyl- β -glucosaminidase, an enzyme that required for the degradation of chitin was detected in the most of *Streptomyces* isolates. *N*-acetyl- β -glucosaminidase was suggested to have antifungal activity and was used in agriculture to control phytopathogens [58,59]. Results suggested that the *Streptomyces* isolates were able to produce varieties of enzymes that play an essential role in plant growth and biocontrol activities.

Data from this study showed that all the antagonistic *Streptomyces* spp. displayed positive characteristics as PGP agents except for *Streptomyces* sp. TAS10, where none of the PGP activities were detected. The assessments of PGP activities are crucial for the best selection of biocontrol agent candidates [60]. Here, all of the antagonistic *Streptomyces* spp. were analyzed in the laboratory for characteristics that are known to be crucial for PGP activities including fix nitrogen, produce siderophores, solubilize phosphate, produce IAA, and produce HCN [61]. It has been suggested that nitrogen-fixing as well as phosphate-solubilizing bacteria play important roles in plant nutrition by regulating the elemental cycle in soil [62]. From this study, among the 11 antagonistic *Streptomyces* isolates, 8 (72.7%) were found able to fix nitrogen and 9 (81.8%) able to solubilize phosphate. Siderophores production was detected only from *Streptomyces* sp. TBS5, TBS2, R2-7, and JAS2. Among 11 antagonistic *Streptomyces* isolates, 6 (54.5%) were found able to produce the phytohormone IAA. Overall, all the antagonistic *Streptomyces* isolates demonstrated PGP traits, which is related to plant growth promotion.

In our present study, four antagonistic *Streptomyces* spp. (*Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8) were found effective in suppressing *B. glumae* by more than 9 mm inhibition (Figure 1). Plant growth promotion assay by seed treatment with *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 showed improvement in VI, GR, RL, and SL by more than 100%

compared to water-treated control plants under laboratory and greenhouse conditions. Seed treatments using *Streptomyces* sp. TBS5 showed the most promising improvement of VI and GR under both conditions (Figure 5). Augmentation of RL showed an overall increment of more than 101.5% under laboratory condition and 151.9% under greenhouse condition using *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 treatments. The increment in RL will support the plant growth by exploring a more prominent volume of the soil thereby increasing nutrient accessibility and water absorption, especially during drought stress [63,64].

Overall, antimicrobial activities against *B. glumae*, enzymatic activities, PGP traits, and plant growth experiments shown by *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 suggested that these isolates could be used for biocontrol against *B. glumae* and enhancement of rice plant growth, as they were initially recovered from rice fields. Therefore, these results fulfill the premise that microbes used for inoculation need to be selected from their original niches before being reinoculated into the similar environments to establish the specified benefits [31]. Thus, the use of *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 in optimized bioformulations will give us hope towards a more sustainable rice industry in the future. Further studies are necessary to verify the effects of the application of *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 on rice plant growth under natural growth conditions.

5. Conclusions

This study described in the laboratory activities of a potential PGP *Streptomyces* from Malaysian rice fields as a potential biocontrol agent against *B. glumae*. These analyses facilitated the best selection of PGP *Streptomyces* candidates. Plant growth promotion assays also demonstrated the growth effects of seeds treated with *Streptomyces* spp. The improvement in the early stages of rice plant growth from the *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 treatments were supported by their enzymatic activities and PGP traits. Results have indicated the potential of *Streptomyces* sp. TBS5, JAS2, R2-7, and TKR8 as a biocontrol agent against *B. glumae* and plant growth promoter in rice. Overall, this study offers the applicability of *Streptomyces* spp. in improving rice production.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy11091850/s1>. Figure S1: Enzymatic activities of *Streptomyces* isolates as analyzed using the API® ZYM kit; Figure S2: Evaluation of plant growth-promoting (PGP) traits of *Streptomyces* isolates; Table S1: Bacterial isolates obtained from rice fields; Table S2: The effect of *Streptomyces*-treated rice seedlings on germination and vigor index of rice; Table S3: The effect in rice seedlings using *Streptomyces* spp. treatments.

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References

- Goto, K.; Ohata, K. New bacterial disease of rice (brown stripe and grain rot). *Ann. Phytopathol. Soc. Jpn.* **1956**, *21*, 46.
- Tanii, A.; Miyajima, K.; Akita, T. The sheath brown rot disease of rice plant and its causal bacterium, *Pseudomonas fuscovaginae*. *Ann. Phytopathol. Soc. Jpn.* **1976**, *42*, 540–548. [\[CrossRef\]](#)
- Weny; Safni, I.; Lisnawita; Lubis, K. Screening for disease resistance in rice varieties against bacterial panicle blight disease (*Burkholderia glumae*) in Northern Sumatra of Indonesia. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *260*, 012118. [\[CrossRef\]](#)
- Mondal, K.K.; Mani, C.; Verma, G. Emergence of Bacterial Panicle Blight caused by *Burkholderia glumae* in North India. *Plant Dis.* **2015**, *99*, 1268. [\[CrossRef\]](#)
- Mulaw, T.; Wamish, Y.; Jia, Y. Characterization and in plant detection of bacteria that cause Bacterial Panicle Blight of rice. *Am. J. Plant Sci.* **2018**, *9*, 667–684. [\[CrossRef\]](#)
- Quesada-González, A.; García-Santamaría, F. *Burkholderia glumae* in the rice crop in Costa Rica. *Agron. Mesoam.* **2014**, *25*, 371–381. [\[CrossRef\]](#)
- Riera-Ruiz, C.; Vargas, J.; Cevallos-Cevallos, J.M.; Ratti, M.; Peralta, E.L. First report of *Burkholderia glumae* causing Bacterial Panicle Blight on rice in Ecuador. *Plant Dis.* **2014**, *98*, 988. [\[CrossRef\]](#)
- Zhou, X.G. First report of Bacterial Panicle Blight of rice caused by *Burkholderia glumae* in South Africa. *Plant Dis.* **2014**, *98*, 566. [\[CrossRef\]](#)
- Shew, A.M.; Durand-Morat, A.; Nalley, L.L.; Zhou, X.G.; Rojas, C.; Thoma, G. Warming increases Bacterial Panicle Blight (*Burkholderia glumae*) occurrences and impacts on USA rice production. *PLoS ONE* **2019**, *14*, e0219199. [\[CrossRef\]](#) [\[PubMed\]](#)
- Li, L.; Wang, L.; Liu, L.M.; Hou, Y.X.; Li, Q.Q.; Huang, S.W. Infection process of *Burkholderia glumae* before booting stage of rice. *J. Phytopathol.* **2016**, *164*, 825–832. [\[CrossRef\]](#)
- Cui, Z.; Zhu, B.; Xie, G.; Li, B.; Huang, S. Research Status and Prospect of *Burkholderia glumae*, the Pathogen Causing Bacterial Panicle Blight. *Rice Sci.* **2016**, *23*, 111–118. [\[CrossRef\]](#)
- Nandakumar, R.; Shahjahan, A.K.M.; Yuan, X.L.; Dickstein, E.R.; Groth, D.E.; Clark, C.A.; Cartwright, R.D.; Rush, M.C. *Burkholderia glumae* and *B. gladioli* cause Bacterial Panicle Blight in rice in the Southern United States. *Plant Dis.* **2009**, *93*, 896–905. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhou, X. Sustainable strategies for managing Bacterial Panicle Blight in rice. In *Protecting Rice Grains in the Post-Genomic Era*; IntechOpen: London, UK, 2019.
- Zhou, X.G.; McClung, A.M.; Way, M.O.; Jo, Y.K.; Tabien, R.E.; Wilson, L.T. Severe outbreaks of Bacterial Panicle Blight across Texas Rice belt in 2010. *Phytopathology* **2011**, *101*, S205.
- Fory, P.A.; Triplett, L.; Ballen, C.; Abello, J.F.; Duitama, J.; Aricapa, M.G.; Prado, G.A.; Correa, F.; Hamilton, J.; Leach, J.E.; et al. Comparative analysis of two emerging rice seed bacterial pathogens. *Phytopathology* **2014**, *104*, 436–444. [\[CrossRef\]](#) [\[PubMed\]](#)
- Francis, F.; Kim, J.; Ramaraj, T.; Farmer, A.; Rush, M.C.; Ham, J.H. Comparative genomic analysis of two *Burkholderia glumae* strains from different geographic origins reveals a high degree of plasticity in genome structure associated with genomic islands. *Mol. Genet. Genom.* **2013**, *288*, 195–203. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kim, S.; Park, J.; Lee, J.; Shin, D.; Park, D.S.; Lim, J.S.; Choi, I.Y.; Seo, Y.S. Understanding pathogenic *Burkholderia glumae* metabolic and signaling pathways within rice tissues through in vivo transcriptome analyses. *Gene* **2014**, *547*, 77–85. [\[CrossRef\]](#) [\[PubMed\]](#)
- Magbanua, Z.V.; Arick, M.; Buza, T.; Hsu, C.Y.; Showmaker, K.C.; Chouvarine, P.; Deng, P.; Peterson, D.G.; Lu, S. Transcriptomic dissection of the rice—*Burkholderia glumae* interaction. *BMC Genom.* **2014**, *15*, 755. [\[CrossRef\]](#)
- Kawanishi, T.; Shiraishi, T.; Okano, Y.; Sugawara, K.; Hashimoto, M.; Maejima, K.; Komatsu, K.; Kakizawa, S.; Yamaji, Y.; Hamamoto, H.; et al. New detection systems of bacteria using highly selective media designed by SMART: Selective medium-design algorithm restricted by two constraints. *PLoS ONE* **2011**, *6*, e16512. [\[CrossRef\]](#)
- Sayler, R.J.; Cartwright, R.D.; Yang, Y. Genetic characterization and realtime PCR detection of *Burkholderia glumae*, a newly emerging bacterial pathogen of rice in the United States. *Plant Dis.* **2006**, *90*, 603–610. [\[CrossRef\]](#)
- Karki, H.S.; Shrestha, B.K.; Han, J.W.; Groth, D.E.; Barphagha, I.K.; Rush, M.C.; Melanson, R.A.; Kim, B.S.; Ham, J.H. Diversities in Virulence, Antifungal Activity, Pigmentation and DNA Fingerprint among Strains of *Burkholderia glumae*. *PLoS ONE* **2012**, *7*, e45376. [\[CrossRef\]](#)
- Hikichi, Y.; Tsujiguchi, K.; Maeda, Y.; Okuno, T. Development of Increased Oxolinic Acid-resistance in *Burkholderia glumae*. *J. Gen. Plant Pathol.* **2001**, *67*, 58–62. [\[CrossRef\]](#)
- Handelsman, J.; Stabb, E.V. Biocontrol of soilborne plant pathogens. *Plant Cell* **1996**, *8*, 1855–1869. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ngalimat, M.S.; Hata, E.M.; Zulperi, D.; Ismail, S.I.; Ismail, M.R.; Zainudin, N.A.I.M.; Saidi, N.B.; Yusof, M.T. Plant growth-promoting bacteria as an emerging tool to manage bacterial rice pathogens. *Microorganisms* **2021**, *9*, 682. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ngalimat, M.S.; Yahaya, R.S.R.; Baharudin, M.M.A.; Yaminudin, S.M.; Karim, M.; Ahmad, S.A.; Sabri, S. A review on the biotechnological applications of the operational group *Bacillus amyloliquefaciens*. *Microorganisms* **2021**, *9*, 614. [\[CrossRef\]](#)

26. Aghighi, S.; Shadidi Bonjar, G.H.; Rawashdeh, R.; Batayneh, S.; Saadoun, I. First report of antifungal spectra of activity of Iranian actinomycetes strains against *Alternaria solani*, *Alternaria alternata*, *Fusarium solani*, *Phytophthora megasperma*, *Verticillium dahliae*, and *Saccharomyces cerevisiae*. *Asian J. Plant Sci.* **2004**, *3*, 463–471. [\[CrossRef\]](#)
27. Betancur, L.A.; Naranjo-Gaybor, S.J.; Vinchira-Villarraga, D.M.; Moreno-Sarmiento, N.C.; Maldonado, L.A.; Suarez-Moreno, Z.R.; Acosta-González, A.; Padilla-Gonzalez, G.F.; Puyana, M.; Castellanos, L.; et al. Marine Actinobacteria as a source of compounds for phytopathogen control: An integrative metabolic-profiling/bioactivity and taxonomical approach. *PLoS ONE* **2017**, *12*, e0170148. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Ham, Y.; Kim, T.J. Anthranilamide from *Streptomyces* spp. inhibited *Xanthomonas oryzae* biofilm formation without affecting cell growth. *Appl. Biol. Chem.* **2018**, *61*, 673–680. [\[CrossRef\]](#)
29. Shang, N.N.; Zhang, Z.; Huang, J.P.; Wang, L.; Luo, J.; Yang, J.; Peng, T.; Yan, Y.; Ma, Y.T.; Huang, S.X. Glycosylated piericidins from an endophytic streptomyces with cytotoxicity and antimicrobial activity. *J. Antibiot.* **2018**, *71*, 672–676. [\[CrossRef\]](#)
30. Jin, P.; Wang, Y.; Tan, Z.; Liu, W.; Miao, W. Antibacterial activity and rice-induced resistance, mediated by C15surfactin A, in controlling rice disease caused by *Xanthomonas oryzae* pv. *oryzae*. *Pestic. Biochem. Physiol.* **2020**, *169*, 104669. [\[CrossRef\]](#)
31. Suárez-Moreno, Z.R.; Vinchira-Villarraga, D.M.; Vergara-Morales, D.I.; Castellanos, L.; Ramos, F.A.; Guarnaccia, C.; Degraasi, G.; Venturi, V.; Moreno-Sarmiento, N. Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. *Front. Microbiol.* **2019**, *10*, 290. [\[CrossRef\]](#)
32. Mohd Hata, E.; Sijam, K.; Ahmad, Z.A.M.; Yusof, M.T.; Azman, N.A. In vitro antimicrobial assay of actinomycetes in rice against *Xanthomonas oryzae* pv. *oryzicola* and as potential plant growth promoter. *Braz. Arch. Biol. Technol.* **2015**, *58*, 821–832. [\[CrossRef\]](#)
33. Hata, E.M.; Yusof, M.T.; Zulperi, D. Induction of Systemic Resistance against Bacterial Leaf Streak Disease and Growth Promotion in Rice Plant by *Streptomyces shenzhenensis* TKSC3 and *Streptomyces* sp. SS8. *Plant Pathol. J.* **2021**, *37*, 173–181. [\[CrossRef\]](#) [\[PubMed\]](#)
34. El-Tarabily, K.A.; Sivasithamparan, K. Non-streptomycete actinomycetes as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. *Soil Biol. Biochem.* **2006**, *38*, 1505–1520. [\[CrossRef\]](#)
35. Arasu, M.V.; Duraipandian, V.; Agastian, P.; Ignacimuthu, S. In Vitro antimicrobial activity of *Streptomyces* spp. ERI-3 isolated from Western Ghats rock soil (India). *J. Mycol. Med.* **2009**, *19*, 22–28. [\[CrossRef\]](#)
36. Shirling, E.B.; Gottlieb, D. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Bacteriol.* **1966**, *16*, 313–340. [\[CrossRef\]](#)
37. Lemessa, F.; Zeller, W. Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia. *Biol. Control* **2007**, *42*, 336–344. [\[CrossRef\]](#)
38. Kumar, S.; Stecher, G.; Li, M.; Nnyaz, C.; Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [\[CrossRef\]](#)
39. Saitou, N.; Nei, M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **1987**, *4*, 406–425. [\[CrossRef\]](#)
40. Nei, M.; Kumar, S. *Molecular Evolution and Phylogenetics*; Oxford University Press: New York, NY, USA, 2000.
41. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **1985**, *39*, 783–791. [\[CrossRef\]](#)
42. Mahmoud, A.; Abd-Alla, M. Siderophore production by some microorganisms and their effect on *Bradyrhizobium*-Mung Bean symbiosis. *Int. J. Agric. Biol.* **2001**, *3*, 157–162.
43. Reetha, A.K.; Pavani, S.L.; Mohan, S. Hydrogen Cyanide Production Ability by bacterial antagonist and their Antibiotics Inhibition Potential on *Macrophomina phaseolina* (Tassi.) goid. *Int. J. Curr. Microbiol. Appl. Sci.* **2014**, *3*, 172–178.
44. Kaur, T.; Sharma, D.; Kaur, A.; Manhas, R.K. Antagonistic and plant growth promoting activities of endophytic and soil actinomycetes. *Arch. Phytopathol. Plant Prot.* **2013**, *46*, 1756–1768. [\[CrossRef\]](#)
45. Baset Mia, M.; Shamsuddin, Z.H. Enhanced Emergence and Vigor Seedling Production of Rice through Growth Promoting Bacterial Inoculation. *Res. J. Seed Sci.* **2009**, *2*, 96–104.
46. Ruiyu, L.; Hong, R.; Junjian, Z.; Cuiping, Y.; Chenying, Y.; Liangsheng, C.; Wenxiong, L. Impact of allelopathic rice seedlings on rhizospheric microbial populations and their functional diversity. *Acta Ecol. Sin.* **2007**, *27*, 3644–3654. [\[CrossRef\]](#)
47. Yu, S.; He, Z.L.; Huang, C.Y. Advances in their search of soil microorganisms and their mediated processes under heavy metal stress. *Chin. J. Appl. Ecol.* **2003**, *14*, 618–622.
48. Naher, U.A.; Radziah, O.; Shamsuddin, Z.H.; Halimi, M.S.; Razi, M. Isolation of diazotrophs from different soils of Tanjong Karang rice growing area in Malaysia. *Int. J. Agric. Biol.* **2009**, *11*, 547–552.
49. Priyadharsini, P.; Dhanasekaran, D. Diversity of soil Allelopathic Actinobacteria in Tiruchirappalli district, Tamilnadu, India. *J. Saudi Soc. Agric. Sci.* **2015**, *14*, 54–60. [\[CrossRef\]](#)
50. Olanrewaju, O.S.; Babalola, O.O. *Streptomyces*: Implications and interactions in plant growth promotion. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 1179–1188. [\[CrossRef\]](#)
51. Labeda, D.P.; Goodfellow, M.; Brown, R.; Ward, A.C.; Lanoot, B.; Vannanneyt, M.; Swings, J.; Kim, S.B.; Liu, Z.; Chun, J.; et al. Phylogenetic study of the species within the family Streptomycetaceae. *Antonie Van Leeuwenhoek* **2012**, *101*, 73–104. [\[CrossRef\]](#)
52. Kumar, M.; Kumar, P.; Das, P.; Solanki, R.; Kapur, M.K. Potential applications of extracellular enzymes from *Streptomyces* spp. in various industries. *Arch. Microbiol.* **2020**, *202*, 1597–1615. [\[CrossRef\]](#)
53. Calvo, P.; Nelson, L.; Kloepper, J.W. Agricultural uses of plant biostimulants. *Plant Soil* **2014**, *383*, 3–41. [\[CrossRef\]](#)
54. Georgieva, T.; Evstatieva, Y.; Savov, V.; Bratkova, S.; Nikolova, D. Assessment of plant growth promoting activities of five rhizospheric *Pseudomonas* strains. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 285–292. [\[CrossRef\]](#)

55. Khare, E.; Yadav, A. The role of microbial enzyme systems in plant growth promotion. *Clim. Chang. Environ. Sustain.* **2017**, *5*, 122–145. [[CrossRef](#)]
56. Verma, P.; Yadav, A.N.; Khannam, K.S.; Kumar, S.; Saxena, A.K.; Suman, A. Molecular diversity and multifarious plant growth promoting attributes of Bacilli associated with wheat (*Triticum aestivum* L.) rhizosphere from six diverse agro-ecological zones of India. *J. Basic Microbiol.* **2016**, *56*, 44–58. [[CrossRef](#)]
57. Tabatabai, M.A.; García-Manzanedo, A.M.; Acosta-Martínez, V. Substrate specificity of arylamidase in soils. *Soil Biol. Biochem.* **2002**, *34*, 103–110. [[CrossRef](#)]
58. Patil, R.S.; Ghormade, V.; Deshpande, M.V. Chitinolytic enzymes: An exploration. *Enzyme Microb. Technol.* **2000**, *26*, 473–483. [[CrossRef](#)]
59. Lorito, M.; Hayes, C.K.; Di Pietro, A.; Woo, S.L.; Harman, G.E. Purification, characterization, and synergistic activity of a glucan 1,3-beta-glucosidase and an N-acetyl-beta-glucosaminidase from *Trichoderma harzianum*. *Phytopathology* **1994**, *84*, 398–405. [[CrossRef](#)]
60. Kumar, A.; Kumar, R.; Kumari, M.; Goldar, S. Enhancement of plant growth by using PGPR for a sustainable agriculture: A review. *Int. J. Curr. Microbiol. Appl. Sci.* **2020**, *9*, 152–165. [[CrossRef](#)]
61. 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*, 1334. [[CrossRef](#)]
62. Tang, A.; Haruna, A.O.; Ab. Majid, N.M.; Jalloh, M.B. Potential PGPR properties of cellulolytic, nitrogen-fixing, and phosphate-solubilizing bacteria of a rehabilitated tropical forest soil. *Microorganisms* **2020**, *8*, 442. [[CrossRef](#)]
63. Sandhya, V.; Ali, S.Z.; Grover, M.; Reddy, G.; Venkateswarlu, B. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul.* **2010**, *62*, 21–30. [[CrossRef](#)]
64. Prasad, J.K.; Gupta, S.K.; Raghuwansh, R. Screening Multifunctional Plant Growth Promoting Rhizobacteria Strains for Enhancing Seed Germination in Wheat (*Triticum aestivum* L.). *Int. J. Agric. Res.* **2017**, *12*, 64–72. [[CrossRef](#)]