Effect of Microbial Inoculants Endowed with Multifarious Plant Growth-Promoting Traits on Grape Growth and Fruit Quality under Organic Fertilization Scenarios

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Abstract: Plant growth-promoting bacteria (PGPB) have growth-promoting and disease-resisting effects and can be used as new types of plant growth promoters. This study was conducted to determine the plant growth-promoting traits of five strains and follow a 2-year field trial to evaluate their effects on grape growth and fruit quality. The five PGPB were combined with two organic fertilizers (cow dung fertilizer and distillers' grain fertilizer) for application on grape fields; the control group only received the corresponding organic fertilizer. The five strains showed different growth promoting abilities, as indicated by their differing production of indole acetic acid (IAA) and siderophores and ability to dissolve phosphorus and potassium, fix nitrogen, and resist saline and alkali. During the field trial, vine growth and fruit quality were significantly better in the distillers' grain fertilizer (high nutrient content) alone treatment than in the cow dung fertilizer (low nutrient content) alone treatment. However, after the two fertilizers were inoculated with the five different PGPB, only the five treatments with cow dung fertilizer inoculated with PGPB showed significant improvement. The five treatments of cow dung fertilizer inoculated with PGPB exhibited varied impacts on plant growth and fruit quality. And the promotion effects persisted significantly after two consecutive years. Among the PGPB, Bacillus velezensis 18, B. velezensis 20, and Rahnella aquatilis 5 emerged as consistently effective performers over the two-year period, demonstrating stable and commendable outcomes. These strains are recommended for prolonged application in grape cultivation to optimize growth and yield. This study provides a theoretical reference and an experimental basis for organic fertilizer inoculated with PGPB to improve grape production.

Keywords: microbial inoculant; organic fertilizer; PGP trait; plant growth promotion; grape

1. Introduction

Grape belongs to the family Vitaceae, Vitis, and woody vine. Its fruit can be used for fresh food, wine making, juice making, and drying, and it is greatly loved by the public because of its diverse flavors and delicious taste [1]. Since the 21st century, China’s grape planting area, output, grape consumption, and import of grape products have ranked among the highest in the world [2]. However, one factor contributing to China’s low grape exports may be the heavy use of chemical fertilizers and pesticides by some orchards to maximize yields, which leads to soil hardening and degradation, fertility decline, and grape quality deterioration [3,4]. In addition, due to land constraints and regional developmental needs, orchards are operated under continuous crop systems. Long-term irrational cultivation may lead to soil deterioration, the accumulation of self-toxic substances, and an imbalance in microbial communities, resulting in a nutrient imbalance in the soil, which affects crop yield and quality [5]. In recent years, to obtain high-quality organic grapes, large amounts of organic fertilizers have been applied. However, due to the uneven quality of the organic fertilizers used and the improper application methods, the amount applied often far exceeds the amount actually required for grape growth,
resulting in poor plant growth [6, 7]. Studies have shown that the soil nutrient content of vineyards is generally at very high or extremely high levels at present [8], and the fertilization methods should be adjusted as soon as possible for large-scale and high-quality vineyards to achieve sustainable and efficient utilization of nutrients and improve the economic and environmental benefits of vineyards. How to improve fertilizer utilization efficiency, enhance soil conditions, and strengthen the effective absorption and utilization of nutrients by grapevines, thereby promoting grape growth and fruit quality, is a critical issue that needs to be addressed in contemporary vineyard production.

Plant growth-promoting bacteria (PGPB) [9] colonize the plant rhizosphere, which can improve plant growth, increase yield, enhance soil fertility, and reduce pathogens as well as biotic or abiotic stressors [10]. PGPB help plants by producing plant growth phytohormones such as indole acetic acid (IAA), cytokinins, and gibberellins [11], fixing nitrogen [12], solubilizing inorganic phosphate [13], and producing siderophores, antibiotics, and fungicidal compounds with antagonistic effects against phytopathogenic microorganisms [14]. Liu et al. [15] showed that *Priestia aryabhattai* JY17 and *P. aryabhattai* JY22B possessed a variety of PGP traits and can be used as biofertilizers to increase soil soluble phosphorus content. Rolli et al. [16] indicated that beneficial bacteria rapidly and intimately colonize the rhizoplane and the root system of grapevine and can be successfully used to promote the growth of grapevines in the field. The use of PGPB in the form of biofertilizers or inoculants to reduce the application of fertilizers and pesticides, thereby improving the soil environment, and increase plant yield is currently a significant research area in the fields of agriculture, microbiology, and biotechnology [17]. However, few studies have combined organic fertilizers with PGPB to improve plant growth.

Five PGPB, *Bacillus velezensis* YH-18, *B. velezensis* YH-20, *B. megaterium* ZS-3, *P. aryabhattai* SK1-7, and *Rahnella aquatilis* JZ-GX1, were screened in the forest pathology laboratory of Nanjing Forestry University, and they showed good growth promotion, yield increases, insect resistance, and disease resistance in pot experiments [18–20]. These microbial strains have substantial application potential. Nonetheless, studies have shown that many bacterial strains are able to exert beneficial effects in laboratory cultures, but fewer strains are successful in promoting disease resistance in laboratory greenhouses, and far fewer bacterial strains function under practical conditions, i.e., in commercial greenhouses or in fields, which is closely related to the complex soil environment of fields and weather [21]. For example, high soil alkalinity and salinity, heavy metal contamination, poor nutrient availability, water scarcity, high ultraviolet radiation, and extreme temperature fluctuations can limit the growth of many PGPB. It is very important for the market expansion of microbial inoculants to apply successful strains from the laboratory to field environments and verify their effectiveness. Introducing PGPB to organic fertilizers for combined application may not only enhance the survival rate of microbial inoculants in the field but also improve the efficiency of organic fertilizer utilization.

The main objectives of this study included the following: 1. to explore the PGP characteristics of the five PGPB and their possible growth-promoting mechanisms; 2. to investigate the effects of the five PGPB on the nutritional growth and fruit quality of grapes in combination with different organic fertilizers under field conditions and to screen for suitable microbial fertilizer types and successful application protocols for high-quality organic grape production; and 3. to investigate the stability of the PGPB in field trials by inoculating them for two consecutive years. The results of this study provide a theoretical and practical basis for the field application of five PGPB on grapes.

### 2. Materials and Methods

#### 2.1. Bacterial Strains

The tested strains *B. velezensis* YH-18 and *B. velezensis* YH-20 were isolated from healthy *Cerasus yedoensis* tissue in Shanghai. *B. megaterium* ZS-3 was isolated from healthy *Cinnamomum camphora* tissue in Shanghai, and *P. aryabhattai* SK1-7 was isolated from the rhizosphere of *Populus alba* L. *R. aquatilis* JZ-GX1 was isolated from the rhizosphere soil of
a 28-year-old individual of *Pinus massoniana* in Nanning, Guangxi. All five strains were preserved at the Laboratory of Forest Pathology, Nanjing Forestry University.

2.2. **PGP Trait Determination**

2.2.1. Determination of Salt Tolerance of Strains

The five strains were streaked on Luria–Bertani (LB) plates with different salt concentrations, and their growth was observed after 24–48 h [19].

2.2.2. Determination of Alkali Resistance of Strains

The pH value of the LB medium was adjusted to 7, 8, 9, 10, 11, and 12 by varying concentrations of NaOH and HCl; the five strains were streaked on LB plates with different pH values, and their growth was observed after 24–48 h.

2.2.3. Quantitative Measurement of Phosphate Solubilization

The strains were inoculated on Luria-Bertani (LB) medium for activation, and then single colonies were selected and transferred to shake flasks containing LB liquid medium; the flasks were then incubated at 28 °C and 200 rpm. The bacterial liquid was diluted to $1 \times 10^8$ CFU/mL with phosphate buffer at pH 7.8. Flasks containing 20 mL NBRIP broth were inoculated with 1% diluted culture and an uninoculated medium as a control [22]. The flasks were incubated at 28 °C for 7 days on a rotary shaker at 200 rpm.

On the 1st, 3rd, 5th, and 7th days, 1 mL of culture was removed, the number of viable bacteria in this culture was determined by the colony counting method, 2 mL of culture was removed, and the supernatant was collected by centrifugation at 5000 rpm for 10 min. A vanadomolybdophosphoric acid colorimetric method was used to quantitatively estimate phosphate solubilization in the broth using a spectrophotometer (UV-1800 Spectrophotometer; Shimadzu corporation, Kyoto, Japan). The amount of solubilized phosphate was quantified from the standard curve and expressed as micrograms per milliliter.

2.2.4. Determination of IAA Production

The bacteria were grown in flasks containing 50 mL of LB broth supplemented with 0.1% L-tryptophan to quantify IAA. The inoculated culture flasks were incubated on an orbital shaker at 30 °C for 5 days at 200 rpm. On the 1st, 3rd, and 5th days, the supernatant was collected by centrifugation at 5000 × g for 10 min. One milliliter of the supernatant was added to 1 mL of Salkowski’s reagent (2 mL of 0.5 M iron(III) chloride and 98 mL of 35% perchloric acid). After 30 min of incubation in a dark environment, the intensity of the red color was quantified by a spectrophotometer (UV-1800 Spectrophotometer; Shimadzu) at 530 nm [23]. A calibration curve for calculating IAA concentration was established using pure IAA and expressed as micrograms per milliliter.

2.2.5. Quantitative Measurement of Dissolved Potassium

The potassium-solubilizing fermentation medium was composed of 10.0 g of sucrose, 1 g of Na$_2$HPO$_4$, 1 g of MgSO$_4\cdot7$H$_2$O, 0.0005 g of FeCl$_3$, 0.5 g (NH$_4$)$_2$SO$_4$, 0.2 g of yeast extract, 12 g of potassium feldspar powder, and 1000 mL of deionized water at pH 7.2.

The seed culture was inoculated into 20 mL of the potassium fermentation medium at a 5% inoculant rate, with 3 replicates performed for each group. The same volume of LB broth was used as a blank control, and the culture was incubated at 30 °C for 7 days with shaking at 200 rpm. Fermentation broth samples were collected at 1, 3, 5, and 7 days, centrifuged at 8000 rpm for 10 min, and then 5 mL of the supernatant was reserved [18]. The soluble potassium content was determined by flame spectrophotometry (FP6450, Shanghai, China).

2.2.6. Qualitative Siderophore Production

Quantitative estimation of siderophore was conducted by the method described in the CAS assay [24]. The bacterial culture was inoculated into an iron-free modified sucrose-asparagine medium (MSA) and incubated at 28 °C in a rotary shaker at 200 rpm. Every
6 h, 2 mL of culture was collected and centrifuged at 5000 rpm for 10 min. Thereafter, 1 mL of supernatant was mixed in 1 mL CAS solution (1:1 v:v). At the same time, uninoculated MSA medium and CAS solutions (1:1 v:v) were used to prepare reference samples. The mixtures were incubated for 30–45 min at room temperature; after that, the absorbance was measured at 630 nm. Siderophore production by bacterial strains was evaluated as the percentage of siderophore units. Siderophore (%) = (Ar − As)/Ar × 100, where Ar = the absorbance of the reference solution and As = the absorbance of the sample at 630 nm.

2.2.7. Nitrogen Fixation Assay

The tested strains were streaked on Ashby’s medium, and plates were incubated at 28 °C for 7 days. The growth on Ashby’s medium showed the ability of isolates to fix nitrogen [25].

2.3. Field Trial

2.3.1. Overview of the Test Site

The test site was located in the village of Baiyang in the town of LvXiang, Jinshan District, Shanghai, China (121° E, 30° N, and 10 m above sea level). Jinshan District is located south of the Yangtze River and has a subtropical monsoon climate. The region had an annual average temperature of 17.4 °C and an annual average precipitation of 1404.4 mm in 2019; an annual average temperature of 17.8 °C and an annual average precipitation of 1555 mm in 2020; and an annual average temperature of 18.1 °C and an annual average precipitation of 1386.2 mm in 2021. The initial basic soil properties of the field trial site were as follows: pH 6.21, total nitrogen 101.22 mg/kg, total phosphorus 253 mg/kg, total potassium 325.8 mg/kg, and organic matter (OM) 25.37 g kg⁻¹.

2.3.2. Test Material

The experiment was set up in a field where grape vines (variety Vitis vinifera cv. Zui Jinxiang) were planted 8 years ago. The grape variety was a tetraploid table grape bred by crossing Shenyang Rose (7601) as the female parent and Jufeng grape as the male parent. Vines were spaced 2 × 3 m and vertically trellised on a double cordon system. Three years before the experiment was conducted, in order to establish an organic grape brand, organic viticulture, i.e., excluding mineral fertilization and chemical pesticide use, was adopted for the plot. During the experiment, unified cultivation and management were carried out in the experimental field, such as pest management, trellising, and pruning.

The experiment involved two organic fertilizers: the cow dung organic fertilizer, a widely used traditional fertilizer, was an organic fertilizer with organic matter content ≥ 30% obtained from ordinary composting and fermentation methods using cow manure as raw material. The distillers’ grain fertilizer, a new, environmentally friendly, and efficient fertilizer, was an organic fertilizer with an organic matter content ≥ 60%, humic acid content ≥ 20%, trace element content ≥ 2%, and organic NPK content ≥ 5% obtained by adding a variety of microbial inoculants to accelerate the composting process of distillers’ grains as the raw material.

2.3.3. Preparation of Microbial Inoculants

Five single colonies were selected and inoculated into 20 mL of nutrient broth (NB) medium each, followed by cultivation at 30 °C and 200 rpm for 18–24 h to generate primary seed cultures. These primary seed cultures were then inoculated at a 1% concentration into 1200 mL of NB medium and cultivated at 30 °C and 200 rpm for 18–24 h to produce the seed culture required for the 100 L fermenter. The five strains were cultured in a 100 L fermenter with NB medium for 24 h under the following conditions: 60% liquid loading, 2% seed liquid inoculation, 200 rpm rotation speed, pH 7.0–7.2, aeration rate 100 L/h, and temperature 30 °C. The fermentations were concluded upon reaching a broth culture with viable bacterial counts of approximately 10⁹ CFU mL⁻¹, and the resulting fermentation products were utilized as microbial inoculants (Figure 1). The viable bacterial counts of the microbial inoculants from the four batches are presented in Table 1.
The process of microbial inoculant preparation and application.

Table 1. Number of viable bacteria in four batches of microbial inoculants.

<table>
<thead>
<tr>
<th></th>
<th>2019 Spring</th>
<th>2019 Autumn</th>
<th>2020 Spring</th>
<th>2020 Autumn</th>
</tr>
</thead>
<tbody>
<tr>
<td>YH-18</td>
<td>$1.8 \times 10^8$ CFU/mL</td>
<td>$1.6 \times 10^9$ CFU/mL</td>
<td>$1.4 \times 10^9$ CFU/mL</td>
<td>$1.9 \times 10^9$ CFU/mL</td>
</tr>
<tr>
<td>YH-20</td>
<td>$1.5 \times 10^8$ CFU/mL</td>
<td>$1.4 \times 10^9$ CFU/mL</td>
<td>$1.9 \times 10^9$ CFU/mL</td>
<td>$1.5 \times 10^9$ CFU/mL</td>
</tr>
<tr>
<td>ZS-3</td>
<td>$1.3 \times 10^8$ CFU/mL</td>
<td>$1.3 \times 10^9$ CFU/mL</td>
<td>$1.7 \times 10^9$ CFU/mL</td>
<td>$1.6 \times 10^9$ CFU/mL</td>
</tr>
<tr>
<td>SK1-7</td>
<td>$1.3 \times 10^8$ CFU/mL</td>
<td>$1.6 \times 10^9$ CFU/mL</td>
<td>$1.2 \times 10^9$ CFU/mL</td>
<td>$1.7 \times 10^9$ CFU/mL</td>
</tr>
<tr>
<td>JZ-GX1</td>
<td>$1.6 \times 10^8$ CFU/mL</td>
<td>$1.8 \times 10^9$ CFU/mL</td>
<td>$1.3 \times 10^9$ CFU/mL</td>
<td>$1.5 \times 10^9$ CFU/mL</td>
</tr>
</tbody>
</table>

2.3.4. Field Experimental Design

The twelve treatments of grape vines were (1) cow dung organic fertilizer control (CK1) with no inoculant; (2) cow dung organic fertilizer with YH-18 inoculant (T1); (3) cow dung organic fertilizer with YH-20 inoculant (T2); (4) cow dung organic fertilizer with ZS-3 inoculant (T3); (5) cow dung organic fertilizer with SK1-7 inoculant (T4); (6) cow dung organic fertilizer with JZ-GX1 inoculant (T5); (7) distillers’ grain organic fertilizer control (CK2) with no inoculant; (8) distillers’ grain organic fertilizer with YH-18 inoculant (D1); (9) distillers’ grain organic fertilizer with YH-18 inoculant (D2); (10) distillers’ grain organic fertilizer with YH-18 inoculant (D3); (11) distillers’ grain organic fertilizer with YH-18 inoculant (D4); and (12) distillers’ grain organic fertilizer with YH-18 inoculant (D5). In different treatments, these organic fertilizers or mixtures of organic fertilizer and inoculant (diluted 10 times to approximately $10^8$ CFU/mL) were distributed evenly around the roots of grape vines in autumn and then mixed with the surface soil layer of approximately 15 cm. In spring, the corresponding inoculant (diluted 10 times to approximately $10^8$ CFU/mL) was introduced via irrigation to the roots. The process of microbial inoculant preparation and application is shown in Figure 1. Each inoculation experiment was conducted in sunny weather at 15–25 °C. Each treatment had 30 replicates, covering an area of 167 hm$^2$. The CK1 and T1-T5 treatments were conducted in the same continuous rain-sheltered greenhouse, while the CK2 and D1-D5 treatments were in another one. The two rain-sheltered greenhouses were closely linked (Figure 2). The specific application ratios are shown in Table 2. Images of plants, fields, and microbial inoculant are presented in Supplementary Figure S1.
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The specific application ratios are shown in Table 2. Images of plants, fields, and microbial inoculant are presented in Supplementary Figure S1.

![Field experimental design diagram](image)

### Figure 2. Filed experimental design diagram.

### Table 2. Specific proportion and experimental design of different treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Microbial Inoculant Type</th>
<th>Fertilization in Autumn of 2019/hm$^2$</th>
<th>Inoculation in Spring of 2020/hm$^2$</th>
<th>Fertilization in Autumn of 2020/hm$^2$</th>
<th>Inoculation in Spring of 2021/hm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cow Dung Organic Fertilizer/ton</td>
<td>Distillers’ Grain Organic Fertilizer</td>
<td>Microbial Inoculation/L</td>
<td>Microbial Inoculation/L</td>
<td>Cow Dung Organic Fertilizer/ton</td>
</tr>
<tr>
<td>CK1</td>
<td>/</td>
<td>/</td>
<td>0</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>T1</td>
<td>YH-18</td>
<td>/</td>
<td>15</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>T2</td>
<td>YH-20</td>
<td>/</td>
<td>15</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>T3</td>
<td>ZS-3</td>
<td>15</td>
<td>/</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>T4</td>
<td>SK1-7</td>
<td>15</td>
<td>/</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>T5</td>
<td>JZ-GX1</td>
<td>15</td>
<td>/</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>CK2</td>
<td>/</td>
<td>/</td>
<td>15</td>
<td>0</td>
<td>/</td>
</tr>
<tr>
<td>D1</td>
<td>YH-18</td>
<td>/</td>
<td>15</td>
<td>150</td>
<td>/</td>
</tr>
<tr>
<td>D2</td>
<td>YH-20</td>
<td>/</td>
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<td>150</td>
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<tr>
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<td>D4</td>
<td>SK1-7</td>
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<td>15</td>
<td>150</td>
<td>/</td>
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<tr>
<td>D5</td>
<td>JZ-GX1</td>
<td>/</td>
<td>15</td>
<td>150</td>
<td>/</td>
</tr>
</tbody>
</table>

2.4. Field Measurements

2.4.1. Determination of Grape Leaf Parameters

One month after inoculation, 15 seedlings of vines were randomly selected from each replicate, and three branches were selected on each vine to determine the leaf index. The relative chlorophyll concentrations, leaf area, and leaf thicknesses of leaves taken at the fourth node of each primary shoot without mechanical damage were measured with a SPAD chlorophyll meter (SPAD-502, Konica, Minolta Sensing, Inc., Sakai, Osaka, Japan), a leaf area meter (LA 211, Systronics., New Delhi, India), and Vernier calipers (0.01 mm accuracy), respectively [26].
2.4.2. Determination of Grape Shoot Growth Parameters

After defoliation, 15 vines were randomly selected for each treatment, and three primary shoots were randomly selected from each tree to measure the fourth internode length of the primary shoot, stem–pith ratio, roundness of primary shoots, and shoot internode thickness with a Vernier caliper [27].

2.4.3. Determination of Grape Fruit Quality Parameters

Cluster and berry biomass were recorded by weighing 20 clusters randomly chosen from each plot and two berries randomly picked from each of those clusters for a total of forty berries per plot. During harvest, 100 berries were randomly taken from each treatment replicate to measure total soluble solids (TSS), reducing sugar content (RS), and titratable acidity (TA). TSS were determined by a handheld refractometer, RS was determined by the 3,5-dinitrosalicylic acid method [28], and TA was determined by titration [29].

2.5. Data Analysis

Data were statistically analyzed using SPSS, version 19.0, using analysis of variance with subsequent Duncan’s multiple range test to compare the treatments with the control (uninoculated plants). Differences at the 95% confidence level were considered significant.

3. Results

3.1. Plant Growth-Promoting Characteristics of the Five Strains

The growth of bacteria in media in the presence of high salt concentrations is an important manifestation of their salt tolerance. YH-18, YH-20, and ZS-3 can grow on solid plates with NaCl contents in the range of 0 to 9%, but when the NaCl concentration is 9%, the growth is slow, and the number of colonies is small. SK1-7 and JZ-GX1 can grow on solid plates with NaCl contents in the range of 0 to 6%, but when the NaCl concentration is 6%, the growth is slow, and the number of colonies is small (Table 3).

<table>
<thead>
<tr>
<th>Salt Tolerance</th>
<th>Alkali Resistance</th>
<th>Nitrogen-Fixing Ability</th>
</tr>
</thead>
<tbody>
<tr>
<td>YH-18</td>
<td>9%</td>
<td>10</td>
</tr>
<tr>
<td>YH-20</td>
<td>9%</td>
<td>9</td>
</tr>
<tr>
<td>ZS-3</td>
<td>9%</td>
<td>11</td>
</tr>
<tr>
<td>SK1-7</td>
<td>6%</td>
<td>12</td>
</tr>
<tr>
<td>JZ-GX1</td>
<td>6%</td>
<td>11</td>
</tr>
</tbody>
</table>

The symbol + represents the positive reaction/presence of growth.

The growth of bacteria in a strongly alkaline medium is an important index that demonstrates their alkaline resistance. YH-20 can grow on a solid plate at a pH of 7–9. YH-18 grows on a solid plate at a pH of 7–10. ZS-3 and JZ-GX1 can grow on a plate at a pH of 7–11, but the strain grows slowly when the pH of the plate is 11. SK1-7 can grow on a plate at pH 7–12, and growth is still robust on a plate at pH 12 (Table 3).

The five strains of bacteria were inoculated on a nitrogen-free medium, and strain growth was observed to preliminarily determine if they had nitrogen-fixing capacities. The results showed that all five strains could grow on a nitrogen-free medium, and all of them had certain nitrogen-fixing abilities (Table 3).

The IAA synthesis of the five strains was quantified by the Salkowski colorimetric method, and the results are shown in Figure 3A. The IAA production of all five strains stabilized at approximately 3 days. At 5 days, the IAA production capacity of the five strains was SK1-7 > JZ-GX1 > YH-18 > ZS-3 > YH-20, and the amount of IAA produced at 5 days was 65.50, 34.45, 33.59, 26.63, and 17.40 µg/mL, respectively. In general studies, PGPB producing an IAA content of more than 30 µg/mL are considered to be high IAA-producing strains.
was significantly higher than that of CK2. In addition, the five treatments of distillers’ grain YH-20 was significantly higher than that of the other three strains.

Potassium released was 7.13 and 7.60 \( \mu \text{g/mL} \), respectively. After 7 days of culturing, the phosphorus solubilization amounts of YH-18, YH-20, ZS-3, SK1-7, and JZ-GX1 were 143.07, 157.75, 154.54, 157.12, and 181.11 \( \mu \text{g/mL} \), respectively. Figure 3C indicates that the potassium-dissolving capacity of strains YH-18, YH-20, and ZS-3 increased rapidly on the first day of culture and then gradually stabilized, reaching 14.63, 12.50, and 13.6 \( \mu \text{g/mL} \) on the seventh day, respectively. The concentration of potassium ions in the SK1-7 and JZ-GX1 culture media increased rapidly after 1–5 days of culturing and then gradually stabilized after 5 days. By the seventh day, the amount of potassium released was 7.13 and 7.60 \( \mu \text{g/mL} \), respectively.

Figure 3D shows that the content of siderophore produced by the five strains reached the highest after 42 h of culture, and the content of siderophore produced by ZS-3 and YH-20 was significantly higher than that of the other three strains.

3.2. Effect of Five PGPB in Combination with Two Different Organic Fertilizers on Grape Growth and Fruit Quality in the First Year

As shown in Figure 3B, the amount of dissolved phosphorus in the culture containing the five strains increased rapidly during 1–3 days of culturing, and the amount of dissolved phosphorus increased gradually and steadily after 3 days of culturing. After 7 days of culturing, the phosphorus solubilization amounts of YH-18, YH-20, ZS-3, SK1-7, and JZ-GX1 were 143.07, 157.75, 154.54, 157.12, and 181.11 \( \mu \text{g/mL} \), respectively.

As shown in Table 4, the five treatments of cow dung fertilizer combined with microbial inoculant (T1~T5) showed no significant differences in SPAD values. The SPAD value of CK1 was significantly higher than that of CK2. In addition, the five treatments of distillers’ grain fertilizer combined with microbial inoculant (D1~D5) did not increase the SPAD value.

The results showed that the leaf area of the T1~T5 treatments was significantly higher than that of the CK1 treatment by 12.09–20.10%. The leaf area of CK2 was significantly higher than that of CK1. The D1~D5 treatments had no effect on the leaf area.

There was no significant effect of any of the treatments on leaf thickness.

Different treatments were applied to the grape vines, and the yield parameters of the fruits were determined (Table 5). The T1~T5 treatments significantly promoted clus-
ter biomass. Among them, the T3, T1, and T2 treatments were the most effective and increased fruit biomass by 73.4%, 66.3%, and 63.0%, respectively, relative to CK1. The cluster biomass of CK2 was significantly higher (39.1%) than that of CK1. Nevertheless, the D1~D5 treatments had no noticeable effect on cluster biomass.

The T1~T5 treatments increased the cross and longitudinal diameters of clusters and showed values significantly different from those of CK1; the cross diameters increased by 12.02–26.07%, and the longitudinal diameters increased by 9.28–18.90%. Compared with CK1, the cross and longitudinal diameters of the clusters of CK2 were higher; the cross diameter significantly increased by 8.66%. The cluster cross diameters of the D1~D5 treatments was not significantly different from that of CK2.

The berry biomass of CK2 was significantly heavier than that of CK1. The berry biomass of CK2 was significantly higher (39.1%) than that of CK1. Nevertheless, the D1~D5 treatments had no noticeable effect on cluster biomass.

The T1~T5 treatments showed no significant difference in the TSS content of grapes. There was no significant difference in TSS content between CK1 and CK2. Except for the D3

Table 4. Effects of different treatments on grape leaf parameters in the first year.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SPAD Value</th>
<th>Leaf Area</th>
<th>Leaf Thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK1</td>
<td>37.37 ± 3.31 a</td>
<td>540.31 ± 97.25 a</td>
<td>1.12 ± 0.21 a</td>
</tr>
<tr>
<td>T1</td>
<td>36.23 ± 3.24 a</td>
<td>623.17 ± 98.17 b</td>
<td>1.30 ± 0.22 a</td>
</tr>
<tr>
<td>T2</td>
<td>37.52 ± 5.26 a</td>
<td>648.93 ± 110.62 b</td>
<td>1.14 ± 0.25 a</td>
</tr>
<tr>
<td>T3</td>
<td>37.97 ± 5.31 ab</td>
<td>610.04 ± 82.57 b</td>
<td>1.17 ± 0.15 a</td>
</tr>
<tr>
<td>T4</td>
<td>37.53 ± 4.05 a</td>
<td>627.73 ± 87.57 b</td>
<td>1.31 ± 0.20 a</td>
</tr>
<tr>
<td>T5</td>
<td>38.18 ± 3.3 ab</td>
<td>605.64 ± 98.74 b</td>
<td>1.19 ± 0.19 a</td>
</tr>
<tr>
<td>CK2</td>
<td>40.67 ± 4.85 b B</td>
<td>595.76 ± 77.61 b A</td>
<td>1.11 ± 0.21 a A</td>
</tr>
<tr>
<td>D1</td>
<td>37.78 ± 2.94 A</td>
<td>608.96 ± 77.47 A</td>
<td>1.24 ± 0.27 A</td>
</tr>
<tr>
<td>D2</td>
<td>35.86 ± 3.81 A</td>
<td>615.96 ± 92.97 A</td>
<td>1.21 ± 0.20 A</td>
</tr>
<tr>
<td>D3</td>
<td>38.48 ± 3.79 AB</td>
<td>603.81 ± 76.43 A</td>
<td>1.22 ± 0.20 A</td>
</tr>
<tr>
<td>D4</td>
<td>37.38 ± 3.78 A</td>
<td>615.66 ± 77.99 A</td>
<td>1.24 ± 0.34 A</td>
</tr>
<tr>
<td>D5</td>
<td>38.33 ± 4.04 AB</td>
<td>580.81 ± 76.63 A</td>
<td>1.18 ± 0.16 A</td>
</tr>
</tbody>
</table>

Values within each column followed by different lowercase letters are significant at p < 0.05, and different capital letters are significant at p < 0.05 according to Duncan’s multiple range test.

Table 5. Effects of different treatments on grape quality parameters in the first year.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cluster Biomass/g</th>
<th>The Cross Diameter of Grape Cluster/cm</th>
<th>The Longitudinal Diameter of Grape Cluster/cm</th>
<th>Berry Biomass/g</th>
<th>TSS/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK1</td>
<td>439.18 ± 66.19 a</td>
<td>9.82 ± 0.96 a</td>
<td>17.67 ± 0.87 a</td>
<td>9.64 ± 1.62 a</td>
<td>15.62 ± 1.48 ab</td>
</tr>
<tr>
<td>T1</td>
<td>730.30 ± 81.39 c</td>
<td>11.50 ± 0.29 bc</td>
<td>19.31 ± 1.05 b</td>
<td>12.51 ± 1.97 de</td>
<td>16.00 ± 1.08 b</td>
</tr>
<tr>
<td>T2</td>
<td>715.90 ± 86.47 c</td>
<td>11.88 ± 1.91 bc</td>
<td>21.01 ± 1.64 c</td>
<td>12.89 ± 0.99 e</td>
<td>15.39 ± 1.25 ab</td>
</tr>
<tr>
<td>T3</td>
<td>762.66 ± 18.11 c</td>
<td>11.70 ± 1.11 bc</td>
<td>20.61 ± 0.96 bc</td>
<td>11.43 ± 2.00 bc</td>
<td>15.19 ± 0.97 a</td>
</tr>
<tr>
<td>T4</td>
<td>587.20 ± 67.70 b</td>
<td>12.38 ± 1.07 c</td>
<td>19.75 ± 1.84 bc</td>
<td>11.85 ± 1.84 cd</td>
<td>15.42 ± 0.99 ab</td>
</tr>
<tr>
<td>T5</td>
<td>665.10 ± 120.47 bc</td>
<td>11.17 ± 0.87 bc</td>
<td>20.00 ± 1.63 bc</td>
<td>10.56 ± 1.34 b</td>
<td>14.98 ± 0.79 a</td>
</tr>
<tr>
<td>CK2</td>
<td>611.40 ± 112.90 b A</td>
<td>10.20 ± 0.75 ab A</td>
<td>19.20 ± 1.28 b AB</td>
<td>11.64 ± 1.90 cd AB</td>
<td>15.48 ± 1.13 ab AB</td>
</tr>
<tr>
<td>D1</td>
<td>618.50 ± 93.90 A</td>
<td>11.65 ± 1.06 B</td>
<td>20.76 ± 1.55 AB</td>
<td>12.09 ± 1.43 AB</td>
<td>15.90 ± 1.40 BC</td>
</tr>
<tr>
<td>D2</td>
<td>630.22 ± 81.14 A</td>
<td>12.20 ± 0.59 B</td>
<td>20.95 ± 1.92 B</td>
<td>11.72 ± 1.41 AB</td>
<td>15.79 ± 0.97 BC</td>
</tr>
<tr>
<td>D3</td>
<td>601.78 ± 109.55 A</td>
<td>11.85 ± 1.20 B</td>
<td>19.00 ± 2.57 A</td>
<td>11.47 ± 1.79 B</td>
<td>16.35 ± 0.75 C</td>
</tr>
<tr>
<td>D4</td>
<td>623.10 ± 92.02 A</td>
<td>12.00 ± 1.08 B</td>
<td>19.83 ± 1.17 AB</td>
<td>12.01 ± 2.18 AB</td>
<td>15.09 ± 1.12 A</td>
</tr>
<tr>
<td>D5</td>
<td>581.20 ± 141.90 A</td>
<td>12.20 ± 1.18 B</td>
<td>19.95 ± 2.15 AB</td>
<td>12.46 ± 2.02 B</td>
<td>15.59 ± 0.90 AB</td>
</tr>
</tbody>
</table>

Values within each column followed by different lowercase letters are significant at p < 0.05, and those followed by different capital letters are significant at p < 0.05 according to Duncan’s multiple range test. The T1~T5 treatments increased the cross and longitudinal diameters of clusters and showed values significantly different from those of CK1; the cross diameters increased by 12.02–26.07%, and the longitudinal diameters increased by 9.28–18.90%. Compared with CK1, the cross and longitudinal diameters of the clusters of CK2 were higher; the cross diameter significantly increased by 8.66%. The cluster cross diameters of the D1~D5 treatments were significantly higher than that of CK2, while there was no significant difference in the cluster longitudinal diameter.

The T1~T5 treatments significantly increased berry biomass compared with CK1. Among them, the T1 and T2 treatments increased berry biomass most significantly, at 33.7% and 29.8% higher than CK1. The berry biomass of CK2 was significantly heavier than that of CK1, and the former increased by 20.74% compared with the latter. The berry biomass of the D1~D5 treatments was not significantly different from that of CK2.
treatment, the D1–D5 treatments had no significant effect on the TSS content. Compared with CK2, the D3 treatment significantly increased the TSS content of fruit by 5.62%.

Table 6 shows that the T1–T5 treatments all promoted internode length, and the maximum value occurred in the T1 treatment (10.81 cm). There was no significant difference in internode length between CK1 and CK2. Other than the internode length of the D1 treatment, which increased by 13.80%, the other treatments had no marked difference from that of CK2.

Table 6. Effects of different treatments on shoot growth parameters in the first year.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot Internode Length/cm</th>
<th>Stem–Pith Ratio</th>
<th>Roundness of Primary Shoots/%</th>
<th>Shoot Internode Thickness/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK1</td>
<td>9.12 ± 1.03 ab</td>
<td>4.58 ± 0.36 a</td>
<td>91.04 ± 4.73 a</td>
<td>9.37 ± 3.74 a</td>
</tr>
<tr>
<td>T1</td>
<td>10.81 ± 1.57 c</td>
<td>5.29 ± 0.37 cd</td>
<td>92.61 ± 3.89 ab</td>
<td>9.72 ± 3.41 ab</td>
</tr>
<tr>
<td>T2</td>
<td>9.82 ± 2.11 abc</td>
<td>5.14 ± 0.35 bcd</td>
<td>93.78 ± 5.23 ab</td>
<td>9.75 ± 3.43 ab</td>
</tr>
<tr>
<td>T3</td>
<td>9.43 ± 1.43 ab</td>
<td>5.42 ± 0.41 d</td>
<td>93.79 ± 4.52 ab</td>
<td>9.57 ± 2.97 ab</td>
</tr>
<tr>
<td>T4</td>
<td>9.98 ± 1.69 bc</td>
<td>5.44 ± 0.25 d</td>
<td>93.9 ± 4.10 ab</td>
<td>10.01 ± 3.83 b</td>
</tr>
<tr>
<td>T5</td>
<td>9.62 ± 1.88 ab</td>
<td>4.93 ± 0.38 bc</td>
<td>94.61 ± 3.59 b</td>
<td>9.66 ± 3.67 ab</td>
</tr>
<tr>
<td>CK2</td>
<td>9.06 ± 1.32 ab B</td>
<td>4.65 ± 0.28 ab BC</td>
<td>95.27 ± 3.28 b A</td>
<td>9.46 ± 3.96 a A</td>
</tr>
<tr>
<td>D1</td>
<td>10.31 ± 1.60 C</td>
<td>4.32 ± 0.24 AB</td>
<td>95.28 ± 3.72 A</td>
<td>9.42 ± 3.68 A</td>
</tr>
<tr>
<td>D2</td>
<td>9.61 ± 1.66 BC</td>
<td>4.16 ± 0.25 A</td>
<td>94.72 ± 4.10 A</td>
<td>9.86 ± 4.16 AB</td>
</tr>
<tr>
<td>D3</td>
<td>9.26 ± 1.56 BC</td>
<td>4.73 ± 0.31 BC</td>
<td>94.01 ± 4.27 A</td>
<td>9.50 ± 3.36 A</td>
</tr>
<tr>
<td>D4</td>
<td>9.80 ± 2.26 BC</td>
<td>4.85 ± 0.35 C</td>
<td>93.42 ± 5.38 A</td>
<td>9.89 ± 4.13 AB</td>
</tr>
<tr>
<td>D5</td>
<td>8.76 ± 1.56 AB</td>
<td>5.11 ± 0.41 C</td>
<td>92.97 ± 7.21 A</td>
<td>10.06 ± 3.83 B</td>
</tr>
</tbody>
</table>

Values within each column followed by different lowercase letters are significant at $p < 0.05$, and those followed by different capital letters are significant at $p < 0.05$ according to Duncan’s multiple range test.

The T1–T5 treatments increased shoot internode thickness, among which the T4 treatment had a marked effect, at 14.22% higher than CK1. There was no significant difference in shoot internode thickness between CK1 and CK2. Except for the internode thickness of the D5 treatment, which increased by 13.24%, the other treatments showed no marked difference from that of CK2.

The stem–pith ratio indicates the degree of lignification and maturity of the grape branches. The T1–T5 treatments significantly increased the stem–pith ratio, which was 7.64–18.78% higher than that of CK1. Compared with CK1, the stem–pith ratio of CK2 was improved, but the effect was not significant. The D1–D5 treatments had no significant difference compared with CK2 except D2, which significantly reduced the stem–pith ratio.

In this study, the T1–T5 treatments all improved the roundness of primary shoots, among which the T5 treatment showed the most significant improvement: 3.92% higher than that of CK1. Compared with CK1, the roundness of primary shoots in CK2 was also significantly improved: 4.65% higher than that of CK1. There was no difference in roundness between the D1–D5 treatments and CK2.

3.3. Effect of the Five PGPB in Combination with Cow Dung Organic Fertilizers on Grape Growth and Fruit Quality over Two Consecutive Years

The experimental results from 2019 to 2020 showed that the fruit quality using microbial inoculant combined with cow dung fertilizer was better than that of distillers’ grain fertilizer; additionally, the cost of microbial inoculant combined with cow dung fertilizer was lower and the benefit was greater, which was more in line with the needs of agricultural production. Therefore, during the second year, we continued to trace the effects of combining cow manure fertilizer with microbial inoculant.

After two years of continuous application of microbial inoculant with cow dung, the growth of grape seedlings was effectively promoted. Figure 4 indicates that the T1–T5 treatments significantly increased the SPAD value of leaves, which was 5.69%, 4.83%, 3.52%, 2.13%, and 2.77% higher than that of CK1, respectively. Compared with CK1, the
T1 and T3 treatments significantly increased the leaf thickness of grapes by 24.51% and 27.45%, respectively. The leaf area of the T1~T5 treatments also increased, and the leaf area effects of T1, T2, T4, and T5 were the most significant, increasing by 16.88%, 26.72%, 23.23%, and 14.41%, respectively, compared with CK1. The T1~T5 treatments increased the shoot internode length to different degrees, among which the T1 and T2 treatments increased shoot internode length most significantly, by 6.03% and 5.27% compared with CK1, respectively.

Figure 4. Effects of the five PGPB in combination with cow dung organic fertilizers on the (A) SPAD value; (B) leaf thickness; (C) leaf area; and (D) shoot internode length over two consecutive years. Different letters in the column show that values are significantly different ($p < 0.05$) from each other as evaluated by the DMRT (Duncan's multiple range test).

After two years of continuous application of microbial inoculants with cow dung fertilizer, the yield of grapes was effectively increased (Figure 5). The T1~T5 treatments significantly increased the cluster biomass, which increased by 20.41~47.51% compared with CK1, and the T1, T2, and T5 treatments were significantly better than the other treatments. Compared with CK1, the cross diameter and longitudinal diameter of grape clusters in the T1~T5 treatments were significantly increased by 11.49~22.36% and 12.83~20.79%, respectively. Other than the T3 treatment, the berry biomass was significantly increased; the T1, T2, T4, and T5 treatments significantly increased the berry biomass by 12.70%, 13.22%, 12.49%, and 23.50%, respectively, compared with CK1.

After two years of continuous application of microbial inoculants with cow dung fertilizer, the quality of grapes effectively improved (Figure 6). The content of TSS in fruits that received the T1~T5 treatments increased significantly, by 14.58%, 12.22%, 10.63%, 10.44%, and 8.40%, respectively, compared with the control. The content of RS also increased to some extent, among which the T1 and T3 treatments showed the most significant increases, at 33.72% and 41.79%, respectively, compared with CK1. Compared with CK1, the titratable acid content of all treatments decreased, especially those of the T3 and T4 treatments, which decreased by 21.28% and 27.66%, respectively. The results of the solid-acid ratio showed that T4 > T3 > T2 > T1 > T5 > CK1.
Invasive roots that help plants uptake large volumes of nutrients and absorb water [30]. In

Effects of the five PGPB in combination with cow dung organic fertilizers on the (A) cluster biomass; (B) cross diameter of grape clusters; (C) longitudinal diameter of grape clusters; and (D) berry biomass over two consecutive years. Different letters in the column show that values are significantly different (p < 0.05) from each other as evaluated by the DMRT (Duncan’s multiple range test).

Figure 5. Effects of the five PGPB in combination with cow dung organic fertilizer on the (A) cluster biomass; (B) cross diameter of grape clusters; (C) longitudinal diameter of grape clusters; and (D) berry biomass over two consecutive years. Different letters in the column show that values are significantly different (p < 0.05) from each other as evaluated by the DMRT (Duncan’s multiple range test).

4. Discussion

Many PGPB synthesize IAA, which is responsible for increasing numbers of adventitious roots that help plants uptake large volumes of nutrients and absorb water [30]. In
In our study, *P. aryabhattai* SK1-7 had the strongest IAA-producing capacity. Similarly, Pallab Kumar Gosh et al. [31] found that *P. aryabhattai* MCC3374 has the capacity to produce IAA efficiently and could be exploited for stress amelioration and plant growth enhancement in rice cultivars. Moreover, Li et al. [32] from the Laboratory of Forest Protection Pathology of Nanjing Forestry University, showed that *R. aquatilis* JZ-GX1 could secrete indole acetic acid and directly and effectively promote seed germination and root length of maize and could secrete phytase and indirectly promote the growth of corn. Therefore, the other strains used in this study could also directly promote grape growth by producing IAA.

The availability of phosphorus in soil is usually limited, so the presence of phosphorus-solubilizing bacteria may be essential [33]. The growth of phosphorus-increasing bacteria often leads to soil acidification, which leads to the solubilization of phosphorus. However, microorganisms dissolve phosphorus and improve its uptake from the soil. Therefore, a continuous action of this type will lead to a reduction in the presence of phosphorus in the soil so it must be replenished on an ongoing basis from other sources (such as organic fertilizers) as a means of cycling nutrients through the soil. Torres et al. [34] found that *B. velezensis* XT1 could dissolve soil organic phosphorus and inorganic phosphorus and promote the growth of tomato stem and root biomass, plant height, and leaf number. Xiang et al. [35] showed that *B. megaterium* DSM3228, which is salt-tolerant and has phosphate-solubilizing functions, can effectively improve the uptake of phosphorus from soil by plants. The five PGPB tested in this study have strong phosphorus-solubilizing abilities and can be used as good PSB during agricultural production.

More than 90% of the potassium in soil exists in a slow-release state within silicate-rich minerals such as potassium feldspar and mica and cannot be directly absorbed and utilized by plants, resulting in poor growth of plants due to potassium deficiencies [36]. Potassium-solubilizing bacteria (KSB) can dissolve aluminosilicate and apatite minerals and transform insoluble potassium, phosphorus, silicon, and other elements in soil into soluble forms, thus promoting absorption and utilization by plants [37]. Li et al. [38] found that *B. velezensis* BA-26 can effectively improve soil nutrients and increase the content of available potassium in soil, thus increasing potato growth and reducing the use of chemical fertilizers. Chen et al. [20] confirmed that SK1-7 has good growth-promoting effects on poplar and can effectively improve the available potassium content of poplar rhizosphere soil. In this study, the good potassium-releasing functions of the five PGP strains provided strong assurance of their ability to promote plant growth.

It has been suggested by Saha et al. [39] that the production of siderophores is an important factor in phytopathogen antagonism and for the developmental growth of plants. In our study, we found that the five PGP strains were positive for siderophore production, and *B. megaterium* ZS-3 had the highest productivity. Similarly, Chakraborty [40] found that *B. megaterium* DE BABY TRS-4 can reduce the occurrence of brown root rot in tea plants and promote the growth of tea plants by producing siderophores, antifungal metabolites and other active compounds. In addition, Kong et al. [41] confirmed that inoculation with *R. aquatilis* JZ-GX1 can induce camphor leaves to show higher levels of active iron and enhance rhizosphere acidification capacity and iron chelate reductase activity, thus treating iron deficiency chlorosis in camphor trees.

The results of the field trials showed that the effect of using only distillers’ grain fertilizer, which contained high organic matter content, on grape growth and fruit quality was significantly better than that of using only cow manure fertilizer, which contained low organic matter content. Grapes need sufficient amounts and various types of nutrients during their process of growth and development [42]. The nutrients present in the highly decomposed distillers’ grain fertilizer are easily absorbed and utilized by vines, while the nutrients present in cow dung fertilizer, which is not highly decomposed, are not easily absorbed by vines [43], resulting in large differences in tree growth and fruit quality. Therefore, the selection of high-quality organic fertilizer can reduce the excessive soil accumulation of fertilizer materials that cannot be degraded and absorbed and can also prevent root burn and seedling damage.
In this study, inoculation of cow manure fertilizer with PGPB was effective in enhancing tree vigor, increasing grape leaf area, and improving both fruit yield and quality. This is consistent with the findings of Wei et al. [44], who showed that inoculation of biochar with *Pseudomonas putida* improved grape fruit quality and altered bacterial diversity. Their results showed that inoculation with live microorganisms played a key role. The mechanisms by which the PGPB enhanced the morphological and yield parameters of grape are hypothesized to be their PGP traits [45], which include nitrogen fixation [46], phosphorus solubilization [47], potassium dissolution, IAA, and siderophore production [48]. Unlike the study by Wei et al. [44], who used biochar as the carrier for PGPB, our study directly inoculated PGPB into organic fertilizer, using the organic fertilizer itself as the carrier. When PGPB are inoculated, the living microorganisms contact the available carbon and nitrogen sources in organic fertilizer, and they do not starve quickly because nutrients are readily available; this is conducive to bacterial reproduction, prolonged survival times, and improved colonization rates of strains in plant roots [49,50]. Notably, it is advantageous to have an initial high N content that promotes bacterial multiplication, but in subsequent stages with high N availability, the efficiency of atmospheric N fixation will be low. And free-living bacteria only release plant-available N after their death. The introduced microorganisms transform the nutrients that are difficult to absorb by plants in cow dung fertilizer and soil into nutrients that can be directly absorbed and utilized by plants via their PGP properties, which greatly increases the contents of organic matter and various bioactive substances in the soil, improves the fertilizer efficiency and utilization rate of organic fertilizer [51], improves the soil ecological environment and flora structure, and provides sufficient nutrients and good habitats for plants [52]. Previous research conducted by our team has also demonstrated that the inoculation of strains investigated in this study can improve soil nutrients and soil microecology. Additionally, there exists a significant positive correlation between soil nutrients and plant growth as documented in previous studies [20,53,54]. This is in accordance with the research conducted by Asghar et al. [55] wherein various PGPB they identified demonstrated increased wheat yield, improved nutritional and quality parameters, and enhanced availability of soil nutrients when employed in conjunction with organic and chemical fertilizers.

However, the effects of cow dung fertilizer inoculated with the five PGPB on the yield and quality of grapes were different, reflecting that different PGPB have different PGP abilities in the field and different fertilizer release abilities. Different strains might also have different colonization abilities in the rhizosphere, resulting in different final growth-promoting effects [56,57]. The five cow dung fertilizer inoculation treatments were more effective and less costly than treatment with distillers’ grain fertilizer alone. In practice, it may be possible to reduce the amount of organic fertilizer (with medium degrees of decomposition) needed by inoculating the fertilizer with PGPB.

After distillers’ grain fertilizer reaches high degrees of decomposition, it forms stable humus [58]. There was no significant difference in grape yield and quality when distillers’ grain fertilizer was used in combination with PGPB compared with when distillers’ grain fertilizer was applied alone. This differs from the results of a study by da Silva, who showed that humic substances combined with PGP bacteria can enhance crop growth [59]. It is also different from the results of the present study in which the treatments of cow dung fertilizer inoculated with PGPB significantly improved grape growth and fruit quality. We speculate that because the nutrients in the distillers’ grain fertilizer could be directly absorbed and used by plants, there was little room for material transformation via the added exogenous microorganisms. Grapes, on the other hand, are a very fertilizer-demanding species, and most of their nutrients come from applied fertilizers. The nutrients directly available from the fertilizer are much greater in quantity than those transformed by PGP bacteria, so inoculation with PGPB does not produce a significant efficiency boost. While cow dung fertilizer is not fully fermented, inoculation of PGPB can transform the unavailable fertilizing effect of the fertilizer into a large number of nutrients that can be directly absorbed by plants, thus significantly promoting the growth of grapes. One reason may be that distillers’ grain
fertilizer is not suitable as the substrate for microbial inoculants, and the two cannot play a synergistic role. The residual alcohol, organic acids, and phenolic compounds in distillers’ grain fertilizer may exert inhibitory effects on inoculated PGPB, thereby restricting the growth of microorganisms. There may be an additional reason; when used in conjunction with high-nutrient-content distillers’ grain fertilizer, grapevines may experience nutrient excess and physiological imbalance, thereby limiting the performance of PGPB. However, in this study, field soil nutrients and soil physical and chemical properties were not determined, so the nutrient and improvement status of the soil could not be evaluated. Subsequently, a more in-depth investigation can be conducted into the nutrient changes following the inoculation of organic fertilizers with PGPB, aiming to better understand its underlying mechanisms.

Factors such as the climate and environment in fields can lead to unstable test results, so experiments are often carried out for many years in a row. In our study, it was found that cow manure fertilizer inoculation with the same PGPB for two consecutive years continued to significantly improve vine growth and fruit quality during the second year, which is consistent with the findings of Gong [60] and others, who showed that a continuous application of bioorganic fertilizer significantly increased cucumber biomass and yield. Grape fruit qualities, including TSS, RS, and TA, are most important for fruit growers and are key factors that promote consumer purchases. During the second year, in addition to effective increases in fruit yield and fruit size, there were significant improvements in TSS and RS, reductions in TA, and enhancements in fruit taste, which makes for very good fruit for growers to sell. Fruit trees store nutrients in the fall and winter through their woody structure and root distribution, which work together to allow the tree to grow more effectively the following year [61]. This also indicates that continuous soil fertility management is important for maintaining quality production and land health and that inoculation with PGPB improves soil microbial community structure and soil fertility, contributing to sustainable orchard management [62,63]. The use of organic fertilizers in combination with PGPB can be an option for sustainable agriculture.

5. Conclusions

In summary, the five PGPB have good abilities to fix nitrogen, dissolve phosphorus, dissolve potassium, and produce IAA and siderophores in vitro. Furthermore, the results show that a combination of the five PGPB and organic fertilizer can effectively enhance the growth of leaves and branches and improve the yield and quality of fruit. The effect was good after two consecutive years of use.

From the growth conditions and fruit quality of grapevines in the first year, it can be observed that the effects of T1, T2, and T3 are the most optimal. Examining the impact of two years of consecutive inoculation with microbial fertilizers on grapevines, T1, T2, and T5 show the best performance. In terms of long-term planting effects, it is recommended to inoculate with \textit{B. velezensis} 18, \textit{B. velezensis} 20, or \textit{R. aquatilis} 5 when applying cow dung fertilizer to promote robust seedlings and increase the yield of grapes. However, in this study, only the plant growth-promoting characteristics and field effects of five PGPB were studied, and the growth-promoting mechanism of these five PGPB co-applied with organic fertilizers needs further study.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14030491/s1, Figure S1: (A) Microbial inoculant; (B) field image; (C) organic fertilizer inoculation image; and (D) grapevine fruit morphology under different treatments.

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