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Potassium-Solubilizing Activity of *Bacillus aryabhattai* SK1-7 and Its Growth-Promoting Effect on *Populus alba* L.

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Abstract: In the present study, the potassium-solubilizing characteristics of *Bacillus aryabhattai* SK1-7 and its growth-promoting effect on plants were evaluated to determine the biotechnological potential of this bacterium in alleviating soil potassium deficiency. The potassium-solubilizing activity of SK1-7 was determined by fermentation. Additionally, the fermentation broth was determined by flame spectrophotometry. The aluminum and silicon ion contents in SK1-7 fermentation broth were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) after digestion with nitric acid hydrogen peroxide hydrofluoric acid. Scanning electron microscopy (SEM)-based observations were performed to assess the morphological changes in potassium feldspar surfaces digested by potassium-solubilizing bacteria. In addition, the effects of SK1-7 on plant growth and soil physical and chemical properties were analyzed. After incubation for 7 days in a potassium-solubilizing medium, the concentration of potassium dissolved reached 10.8 µg/mL and the percentage of potassium released was 32.6%. The pH rapidly decreased from 7.2 to 4.321 within the first day and then further decreased to 3.90 after 7 days. After 7 days, the concentrations of aluminum and silicon in the fermentation broth were 1.01 and 24.19 µg/mL, respectively. The growth promotion assay results showed that SK1-7 has good growth-promoting effects on poplar and can effectively improve the available potassium content in poplar rhizosphere soil. The SK1-7 strain can effectively dissolve insoluble potassium to release soluble potassium ions and clearly promotes the growth of poplar after being applied to soil. Thus, the SK1-7 strain is a potassium-solubilizing microorganism with good application prospects.

Keywords: *Bacillus aryabhattai*; potassium-solubilizing bacteria (KSB); potassium-solubilizing characteristics; growth-promoting characteristics

1. Introduction

Potassium (K) is an essential element for plant nutrition and plays an important role in the growth and metabolism of plants. Potassium can improve cold, drought, and stress resistance and promote photosynthesis in plants [1–4]. The potassium content (K₂O) in general crops is 0.3% to 5% (dry weight), which is equivalent to nitrogen and higher than phosphorus content. Although the soil in China is rich in potassium, approximately 70% of cultivated land is deficient in potassium and approximately 45% of cultivated land is severely deficient in potassium. This deficiency results from more than 90% of the potassium in soil being in a slow-release state within silicate-rich minerals, such as potassium feldspar and mica, which cannot be directly absorbed and utilized by plants [5]. At present, China primarily relies on the application of chemical potash fertilizer to increase available potassium in potassium-deficient soil, but more than 90% of potash fertilizer is associated with various problems such as an excessive dependence on imports, high cost, short supply, and severe pollution [6].

Microbial fertilizer is a new type of biological fertilizer, developed in the past ten years, that has the advantages of being safe, highly sustainable, and less environmentally polluting, leading to this approach receiving increasing attention in agricultural and forestry production [7]. Potassium-solubilizing bacteria (KSB), also known as silicate bacteria, are a type of bacteria isolated from soil and plant rhizospheres that can dissolve aluminosilicate and apatite minerals and transform insoluble potassium, phosphorus, silicon, and other elements in soil into soluble forms, allowing these bacteria to be used as microbial fertilizer [8–11]. Potassium-solubilizing bacteria can not only transform potassium from insoluble into soluble forms that can be absorbed by plants in soil but also improve plant biomass by improving the soil's physical and chemical properties and secreting hormones that promote plant growth [12].

Poplar (*Populus* spp.) is an important afforestation tree species with strong adaptability that is easy to cultivate and has a high survival rate [13]. However, with the continuous expansion of poplar plantation areas, many unreasonable practices are being implemented in the management process, such as continuous cropping and excessive rotation cutting, causing a decline in forestland quality that severely affects the healthy growth of poplar. With the decline of soil fertility in poplar plantations, a deficient potassium supply is a prominent and universal problem [14,15]. In our previous study, the potassium-solubilizing strain *Bacillus aryabhattai* SK1-7 was isolated from poplar rhizosphere soil [16], and we found that the SK1-7 strain has a certain potassium-resolving ability; other phenotypes of this strain, regarding the potassium-resolving process, have not been discussed in depth. However, in this study, we measured the changes in the content of aluminum and silicon ions in the fermentation broth and the dissolution effect on the surface morphology of potassium feldspar to gain a more comprehensive understanding of the potassium-solubilizing characteristics of the SK1-7 strain. At the same time, in our previous research, it was also clear that the SK1-7 strain has a certain effect on tomato germination and growth. However, compared with tomato, the growth-promoting mechanism of poplar is more complicated, and potassium bacteria that have the ability to promote the growth of herbaceous plants do not necessarily have the same growth-promoting effect on woody plants. Based on this, we inoculated the SK1-7 strain into poplar rhizosphere soil. By measuring some physiological indicators of poplar and the changes in soil pH and available potassium, we confirmed that the SK1-7 strain has a good growth-promoting effect on poplars. In general, these studies can undoubtedly help us to understand the potassium-releasing activity and growth-promoting ability of the SK1-7 strain more comprehensively. This lays the foundation for our subsequent in-depth research on the growth-promoting mechanism of SK1-7 and also provides the SK1-7 strain as a biological organism. The practical application of potash fertilizer provides a theoretical basis.

2. Materials and Methods

2.1. Strains, Plant Material, Soil Material

Bacillus aryabhattai SK1-7 was isolated from the rhizosphere of *Populus alba* L. and preserved at Nanjing Forestry University. The test plant was annual *Populus alba*, purchased from Suqian Zhenpin Co., Ltd. (Suqian, China). The soil was collected from the plow layer of soil (0–20 cm) in Xiashu forest farm, Zhenjiang, China (31°900 N, 119°100 E), and the basic physicochemical properties of soil were as follows: pH 7.66, organic matter of 15.8 g/kg, available N of 82.3 mg/kg, available P of 16.0 mg/kg, and available K of 106 mg/kg.

2.2. Culture Medium of the SK1-7 Strain

Luria–Bertani (LB) medium was composed of 10.0 g of tryptone, 5.0 g of yeast extract, 10.0 g of NaCl, and 1000 mL of deionized water (pH 7.2).

Potassium-solubilizing fermentation medium was composed of 10.0 g of sucrose, 1 g of Na_2HPO_4 , 1 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.0005 g of FeCl_3 , 0.5 g of $(\text{NH}_4)_2\text{SO}_4$, 0.2 g of yeast extract, 12 g of potassium feldspar powder, and 1000 mL of deionized water at pH 7.2.

Potassium feldspar was purchased from Rongshide Co., Ltd. (Hefei, China), ground and sieved, soaked in a hydrochloric acid solution for 24 h, washed with deionized water, and dried for later use.

2.3. Determination of the Amount of Dissolved Potassium and Potassium-Dissolving Rate

The tested strain was inoculated into LB broth and cultured at 30 °C, with shaking at 200 r/min until the logarithmic growth stage to obtain a seed culture. Then, the seed culture was inoculated into 20 mL of potassium fermentation medium at a 5% inoculum 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 r/min. Fermentation broth samples were collected at 0, 1, 2, 3, 4, 5, 6, and 7 days, centrifuged at 8000 r/min for 10 min, and then 5 mL of supernatant was reserved. The pH value of the fermentation broth was measured using a pH meter (FE28, Five Easy Plus, Shanghai, China), and the soluble potassium content was determined by flame spectrophotometry (FP6450, Shanghai, China) [17].

$$\text{Dissolved potassium amount} = \text{soluble potassium content of the test group} - \text{soluble potassium content of the control group.} \quad (1)$$

$$\text{Potassium dissolution rate (\%)} = (\text{soluble potassium content of the test group} - \text{soluble potassium content of the control group}) / \text{soluble potassium content of the test group} \times 100\%. \quad (2)$$

2.4. Determination of Aluminium and Silicon Ion Contents in Fermentation Broth

The tested strain was inoculated into LB broth and cultured at 30 °C, with shaking at 200 r/min until the logarithmic growth stage to obtain a seed culture. Then, the seed culture was inoculated into 20 mL of potassium fermentation medium at a 5% inoculum rate, with 3 replicates performed for each group. In addition, the same volume of LB broth was inoculated as a blank control and cultured at 30 °C, with shaking at 200 r/min for 7 days. Fermentation broth samples were collected at 1, 4, and 7 days, centrifuged at 8000 r/min for 10 min, and then 5 mL of supernatant was reserved. Using nitric acid-hydrogen peroxide-hydrofluoric acid for digestion, the aluminum and silicon ion contents in the culture supernatants were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

2.5. Determination of Potassium Feldspar Dissolution by Surface Morphology Analysis

The tested strain was inoculated into LB broth and cultured at 30 °C, with shaking at 200 r/min until the logarithmic growth stage to obtain a seed culture. The seed culture was inoculated into 20 mL of potassium fermentation medium at a 5% inoculum rate, with 3 replicates performed for each group. The same volume of LB broth was inoculated into a potassium-dissolving fermentation medium as a blank control. After cultivation at 30 °C, with shaking at 200 r/min for 7 days, samples were collected, and the potassium feldspar powder in the fermentation liquor was recovered by filter paper filtration and dried completely at 50 °C in an oven. Subsequently, scanning electron microscopy (SEM; Quanta 200, Hillsboro, OR, USA) was used to observe the surface morphology of the potassium feldspar.

2.6. Effect of the SK1-7 Strain on Plant Growth

One-year-old potted *P. alba* seedlings with consistent growth were transplanted into flowerpots (210 × 160 mm) filled with 2500.0 g of soil, with one seedling in each pot. The tested strains were inoculated into LB broth and cultured to the logarithmic phase at 30 °C, with shaking at 200 r/min. After centrifugation, the cells were washed with sterile water 3 times and then resuspended. Then, the tested strain was inoculated into LB broth and cultured to the logarithmic phase at 30 °C, with shaking at 200 r/min. After culturing, the cells were centrifuged, washed with sterile water 3 times, and then resuspended in sterile water. Subsequently, the suspension was adjusted to a final density of 10^7 cfu/mL.

Then, 50.0 mL of the bacterial suspension was poured into the soil of each pot, and the same amount of sterile water was added as a blank control (CK). Each treatment was repeated 10 times, and the plants were placed in a greenhouse and cultivated under natural light for 45 and 100 days.

The height and diameter of the poplar plants were measured after 100 days with a tape measure and Vernier caliper, respectively, and the growth rate was calculated. To determine fresh weight, the plants were removed from the pots, washed, and weighed. Then, the plants were heated at 105 °C for 30 min, dried at 75 °C to a constant weight, and measured after 100 days to determine the dry weight. The relative amount of chlorophyll present in the leaves was determined after 100 days by a hand-held soil plant analysis development (SPAD) meter (SPAD-502). Root activity was determined after 100 days using the α -naphthylamine oxidation method [18], while the total potassium content of poplar was determined after 100 days using the H_2SO_4 - H_2O_2 digestion method and flame spectrophotometry [19].

2.7. Determination of Soil pH and Available Potassium

The pH of the soil after 45 and 100 days of culture was determined using a pH meter (FE28, Five Easy Plus, Shanghai, China), and the available potassium content in the soil was determined using the ammonium acetate extraction-flame photometer method [20].

2.8. Statistical Analyses

One-way analysis of variance (ANOVA, Duncan) was performed using SPSS; different letters indicate significant differences ($p < 0.05$).

3. Results

3.1. Solubilization of Potash Feldspar by the SK1-7 Strain

The SK1-7 strain was inoculated into a potassium fermentation medium, and the changes in potassium ion concentrations in the medium were regularly evaluated. As shown in Figure 1A, the potassium ion concentration in the fermentation broth of the inoculated SK1-7 strain rapidly increased from 3–4 days and tended to steadily increase thereafter, reaching 33.1 $\mu\text{g/mL}$ after 7 days, compared with those in the blank control group. According to the previously described formula, the amount of potassium dissolved by the SK1-7 strain was 10.8 $\mu\text{g/mL}$ and the potassium-dissolving rate was 32.6%. On days 1, 2, 3, 4, 5, 6, and 7, the contents of aluminum ion and silicon ion in the fermentation broth of the inoculated SK1-7 strain were significantly different from the contents of aluminum ion and silicon ion in the fermentation broth of the blank control group

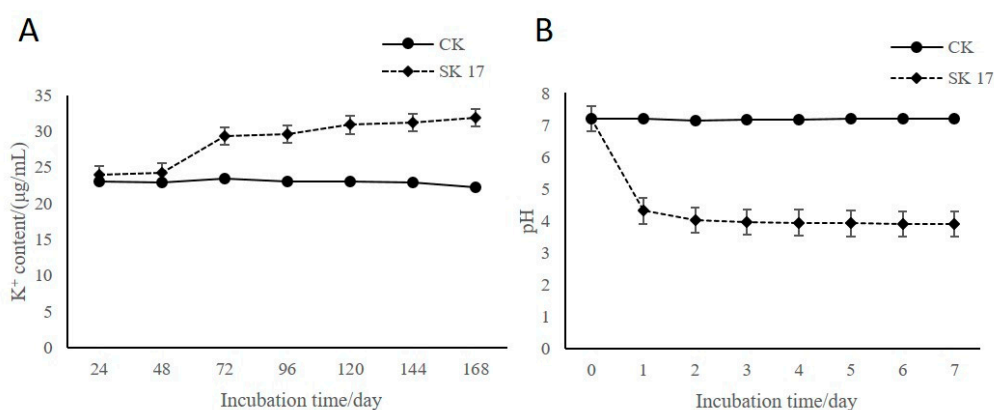


Figure 1. Solubilization of potassium feldspar by *Bacillus aryabhattai* SK1-7. CK: added with LB medium and water. SK1-7: inoculated with strain *B. aryabhatta*. (A) Changes in the potassium ion concentration in the potassium-solubilizing fermentation medium after 7 days of cultivation. (B) Changes in the pH of the potassium-decomposing fermentation medium after 7 days of cultivation. The error bars indicate standard errors.

During the 7-day culture period, after inoculating the SK1-7 strain into the potassium-solubilizing fermentation medium, the pH significantly changed with increasing culture time (Figure 1B). After inoculation, the pH of the potassium-solubilizing fermentation medium rapidly dropped to 4.32 within 0–1 day and then remained stable at approximately 3.9–4.0 after 3 days. In contrast, the pH of the blank control group, added with the same volume of LB broth, showed no obvious change. On days 1, 2, 3, 4, 5, 6, and 7, the pH values of the fermentation broth of the inoculated SK1-7 strain were significantly different from that of the CK fermentation broth.

At 7 days after inoculating the potassium-solubilizing fermentation medium with the SK1-7 strain, the concentrations of aluminum and silicon ions in the medium notably increased, increasing at 1, 4, and 7 days and reaching 1.01 and 24.1 $\mu\text{g/mL}$ at 7 days, respectively, values that were significantly higher than those of the control group (Figure 2). At 1, 4, and 7 days, the contents of aluminum ion and silicon ion in the fermentation broth of the inoculated SK1-7 strain were significantly different from the contents of aluminum ion and silicon ion in the fermentation broth of the control group.

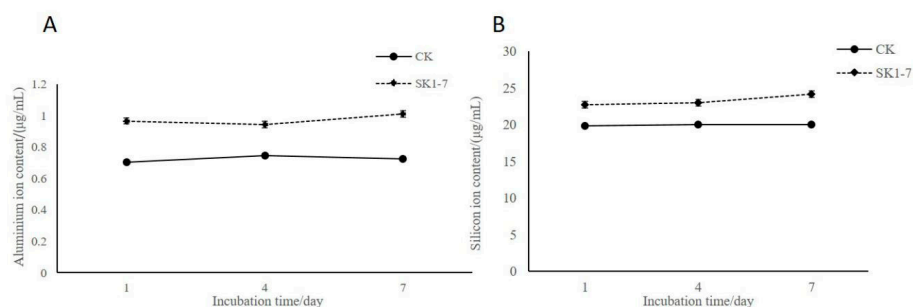


Figure 2. The aluminum and silicon ion contents in potassium solution fermentation broth after *B. aryabhatai* SK1-7 inoculation. CK: added with LB medium and water. SK1-7: inoculated with strain *B. aryabhatai*. (A) Changes in the aluminium ion concentration of potassium-solubilizing fermentation medium after 7 days of cultivation. (B) Changes in the silicon ion concentration of potassium-solubilizing fermentation medium after 7 days of cultivation. The error bars indicate standard errors.

3.2. Dissolution of the SK1-7 Strain on the Surface of Potash Feldspar

SEM analysis was performed to observe the changes in the surface potash feldspar after interacting with the SK1-7 strain. As shown in Figure 3A, the surface of potash feldspar in the control group without SK1-7 was smooth and angular, while in the group inoculated with the SK1-7 strain (Figure 3B), the surface was severely corroded, with fuzzy angular and rough surfaces observed.

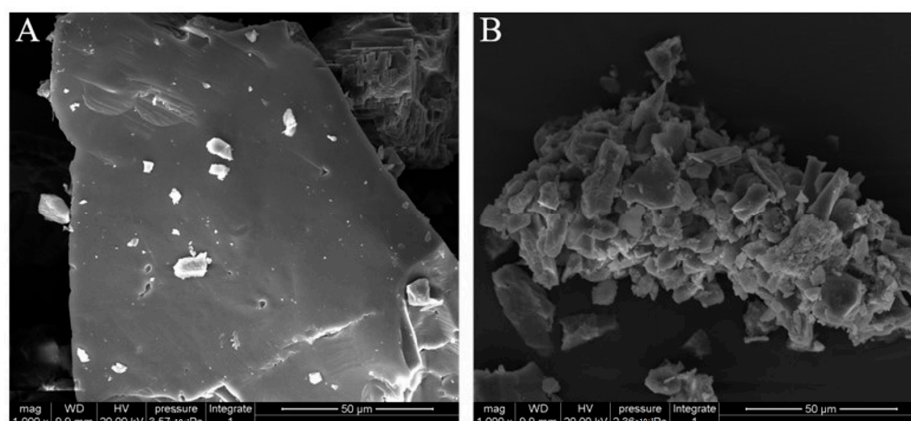


Figure 3. Scanning electron micrograph of dissolution of potassium feldspar after inoculation with *B. aryabhatai* SK1-7. (A) Potash feldspar surface in the blank control treatment. (B) Potash feldspar surface in the group treated with *B. aryabhatai* SK1-7.

3.3. Effects of Applying the SK1-7 Strain to Soil on Poplar Growth, Soil pH, and Available Potassium

The results of analyses evaluating the growth-promoting effect of the SK1-7 strain on poplar are shown in Table 1; the poplar growth indexes were notably improved compared to those in the control group. Fresh and dry weights increased by 38.3 and 22.7%, chlorophyll contents increased by 39.8%, root activity increased by 31.7%, and total potassium and chlorophyll content values of the plants were increased compared with those of the control plants.

Table 1. Effect of *Bacillus. aryabhatai* SK1-7 at 100 days after inoculation on the growth indexes of *P. alba*. Different letters above the bars indicate significant differences ($p < 0.05$).

Treatment	Rate of Plant Height Increase (%)	Rate of Plant Diameter Increase (%)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Relative Chlorophyll Content	Plant Total Potassium (%)	Root Activity ($\mu\text{g/mL}\cdot\text{g}\cdot\text{h}$)
CK ¹	1.91 \pm 0.82 b	4.88 \pm 2.13 b	68.6 \pm 10.8 b	43.5 \pm 9.35 b	32.7 \pm 2.41 b	0.43 \pm 0.02 b	68.7 \pm 2.01 b
SK17 ²	21.1 \pm 7.6 a	9.7 \pm 3.42 a	94.9 \pm 9.8 a	53.4 \pm 9.69 a	45.7 \pm 2.31 a	0.6 \pm 0.01 a	90.6 \pm 2.36 a

¹ Control: added with LB medium and water. ² SK1-7: inoculated with strain *B. aryabhatai*.

The available K content in poplar rhizosphere soil was higher than that of the blank control group (Figure 4A) by 45.9% and 49.6% at 45 and 100 days after the bacterial strain treatment, respectively, increasing from 156.8 at 45 days to 164 mg/kg at 100 days.

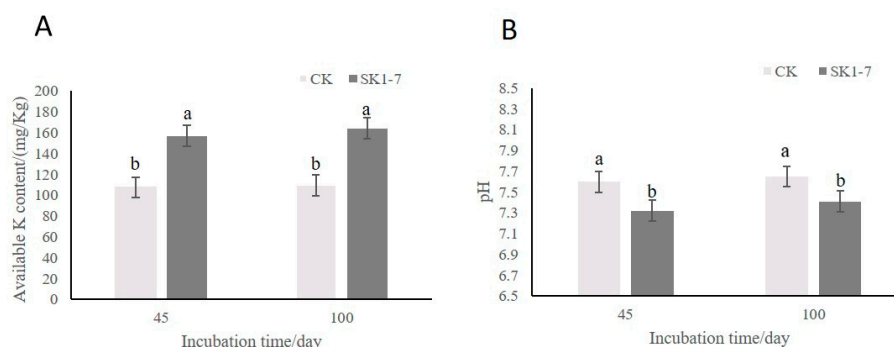


Figure 4. Soil pH and available potassium effects of applying *B. aryabhatai* SK1-7 to soil. CK: added with LB medium and water. SK1-7: inoculated with strain *B. aryabhatai*. (A) Effects of the SK1-7 strain on available potassium contents in poplar rhizosphere soil. (B) Effect of the SK1-7 strain on the pH of poplar rhizosphere soil. Different letters above the bars indicate significant differences ($p < 0.05$).

In addition, the pH of the inter-poplar root soil after inoculation of the SK1-7 strain was also lower than that of the control group. The pH values of poplar inter-tree roots at 45 and 100 days were 7.32 and 7.41, respectively, which were lower than the control values of 0.28 and 0.24.

4. Discussion

Potassium-solubilizing bacteria, also known as potassium-dissolving bacteria and silicate bacteria, can solubilize aluminosilicate minerals in soil; the solubilized nutrients can be used for plant growth [10,21,22]. Elizabeth et al. (2007) showed that when potassium mineral powder and soil were used as potassium sources, the available potassium increased by 2.7%–40.5% and 1.6%–21.6%, respectively, compared to the control levels, and the released potassium was all derived from insoluble potassium [23]. Wu et al. (2020) showed that the available potassium content in the supernatant of the ZMD02 strain reached 1.5 $\mu\text{g/mL}$ after 7 days of fermentation [24]. In the present study, the potassium-solubilizing characteristics of *Bacillus* sp. SK1-7 showed that the amount of potassium dissolved by the SK1-7 strain was 10.8 $\mu\text{g/mL}$ and the potassium-solubilizing rate was 32.6%, which were consistent with the values observed for highly efficient potassium-solubilizing bacteria reported by most researchers.

Buragohain et al. (2018) showed that the pH value of fermentation broth was 4.62–4.86 after 7 days of cultivation with potassium-solubilizing bacteria [25]. Moreover, Wang et al. (2016) showed

that when biotite was used as the potassium source, the pH value of *Aspergillus niger* dropped from 6.4 to 2.9 after inoculation with the potassium-solubilizing fungus *A. niger*; the reason for the decrease in pH was the secretion of many acidic metabolites during the growth of the strain [26]. This result is consistent with the observed change in the pH value caused by SK1-7 during potassium solubilization fermentation in the present study, with pH showing a significant downward trend, remaining at 3.90 after 7 days of culture. These results indicate that the SK1-7 strain may have secreted organic acids as secondary metabolites during growth, which decreased the pH of the fermentation broth. Sheng et al. (2002) noted that organic acids, such as cell metabolites, all contain complex functional groups that can form complexes with silicon and aluminum ions in minerals, dissolve minerals, and release potassium and silicon plasmas [27]. Huang et al. (2012) inoculated two strains of potassium-solubilizing bacteria into potassium-solubilizing medium and observed maximum silicon ion values of 3.39 and 3.21 mg/L after 50 days and maximum aluminum ion values of 0.51 and 0.74 mg/L after 30 days for the two strains [28]. Potassium feldspar ($K_2O \cdot Al_2O_3 \cdot 6SiO_2$) is an aluminosilicate mineral, and the SK1-7 strain solubilized the feldspar to release soluble potassium, aluminum, and silicate within the fermentation broth. SEM was performed to observe the changes in the potassium feldspar surface after interacting with the SK1-7 strain. The potassium feldspar surface was seriously corroded and uneven after interacting with SK1-7, which may also be related to the dissolution of potassium feldspar by secondary metabolites, such as organic acids and capsular polysaccharides secreted by bacteria during growth. The specific mechanism of potassium dissolution in the later stage requires further studies.

Previous studies have shown that potassium-solubilizing bacteria can solubilize silicate minerals such as mica and feldspar in soil into ionic states that can be absorbed by plants, while the strains simultaneously secrete plant hormones during growth, which promotes the growth and development of plants [29]. In addition, the main growing season of poplars is spring, and if SK1-7 is inoculated on poplars before spring, the growth-promoting effect of the SK1-7 strain on poplars can be observed more directly. In Ju's study (2016), after inoculating poplar with the potassium-solubilizing bacterium JW-7, the height, ground diameter, and fresh and dry weights of poplar seedlings increased by 37.64%, 19.44%, 65.82%, and 110.71%, respectively [30]. In the present study, the physiological and biochemical indexes of poplar inoculated with the SK1-7 strain significantly improved compared with those of the control. Studies have shown that potassium can not only promote the synthesis of chlorophyll in plant cells and improve the structure of chloroplasts but also promote the photosynthesis of plants under conditions of lower CO_2 concentration, enabling plants to use solar energy more effectively. In this study, compared with the control, the chlorophyll content of plants inoculated with SK1-7 increased significantly. The results of pot experiments performed by Wan et al. [31] (2016) showed that the available potassium content in tobacco rhizosphere soil increased by 4.72% after inoculation with potassium-solubilizing bacteria. In this experiment, after inoculation with the SK1-7 strain, the available potassium content in poplar rhizosphere soil was significantly higher than that of the control. In Zhu's research (2018), different potassium-solubilizing bacteria were applied to the rhizosphere soil of Xinjiang jujube; the pH value of the rhizosphere soil began to decrease gradually at 60 and 90 days after application of bacteria [32]. Moreover, the pH value of rhizosphere soil after inoculation was lower than that of the blank control. These results were consistent with previous findings on the potassium-solubilizing characteristics of the SK1-7 strain, indicating that the acidic substances secreted by the SK1-7 strain during growth and development led to a decrease in the pH value of the rhizosphere soil, resulting in a decrease in overall soil pH. Subsequently, mineral potassium was transformed into available potassium, which can be directly absorbed and utilized by plants, promoting the growth of poplar. Organic acids secreted by the strain during growth and metabolism cause the soil pH value to decrease, thus dissolving insoluble potassium in the soil and increasing chlorophyll content and plant total potassium content, which may be only a part of the mechanism of promoting growth by the SK1-7 strain; it may be that the SK1-7 strain secretes other secondary metabolites during the growth of plant rhizosphere soil, thus promoting the growth of poplar. The mechanism of promoting growth is a complex and comprehensive effect; for instance, K is

an activator of more than 60 enzymes such as synthetase and dehydrogenase, and it is involved in the synthesis and transport of various substances such as protein, starch, and sugar. Therefore, inoculating potassium-solubilizing bacteria SK1-7 in poplar rhizosphere soil can dissolve insoluble potassium in soil and increase the content of available potassium that can be directly absorbed by plants; this result can improve various physiological indexes of poplar. The growth-promoting mechanism of PGPR on plants is complex and comprehensive; we will further integrate the fermentation process of the SK1-7 strain with various research methods, such as molecular technology and the mechanism related to potassium solubilization and growth promotion, and conduct a comprehensive study on the potassium solubilization and growth promotion characteristics and mechanism of the SK1-7 strain.

5. Conclusions

The results of the present study show that *B. aryabhattai* SK1-7 can solubilize insoluble potassium in soil into available potassium and increase plant K concentration and growth. The mechanism of potassium solubilization by SK1-7 is not clear and requires further study. Furthermore, the fermentation process for SK1-7 and its use for bacterial fertilization and other aspects also need further research to provide theoretical support for the development and application of this strain as a biological potassium fertilizer in forest management.

Author Contributions: Y.C. performed the majority of the experiments and data analysis and drafted the link content of the manuscript in the manuscript. J.Y. participated in the planning of research work, interpretation of data, and supervision of manuscript writing. Q.K. was involved in the planning and execution of the research, analysis, and interpretation of the data. All authors have read and agreed to the published version of the manuscript.

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