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Original Research

The Impact of Phosphate Solubilizing Bacteria Modified Biochar on Maize Seedling Growth and Rhizosphere Phosphate Availability

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Abstract

In light of the environmental pollution resulting from the excessive use of chemical fertilizers, several methods, including the analysis of morphological and cultural characteristics as well as ITS rDNA sequencing, were utilized to identify the strain and evaluate its phosphate solubilizing capacity. A controlled greenhouse pot experiment was then conducted to assess the effects of the phosphate solubilizing bacteria strain Y-5 on crop growth and soil fertility. The identification results revealed that the phosphate solubilizing strain, Y-5, belongs to *Pseudomonas donghuensis*. Under liquid culture for 5 days, the ratio of the phosphate solubilizing ring's diameter to the colony's diameter (D/d) was 3.51. Notably, strain Y-5 exhibited exceptional phosphate solubilization efficiency, with a maximum phosphate solubilizing amount of calcium phosphate reaching 432.25 mg/L. Furthermore, the highest phosphate soluble content of lecithin was 116.37 mg/L, highlighting its robust phosphate solubilizing capacity. The pot experiment results revealed that the modified biochar of strain Y-5 effectively enhances the content of available phosphate and organic matter in soil. Additionally, the modified biochar of the Y-5 strain exerted a significant growth-promoting effect on corn seedlings. Based on the indices of maize seedling growth and soil property changes, it was determined that the optimal application ratio of *Pseudomonas donghuensis* modified biochar was approximately 10 g/kg. This research result significantly reduced the cost of popularizing and applying phosphate solubilizing bacteria modified biochar and served as a new agricultural material with good prospects for popularization and application, potentially contributing to the green and sustainable development of agriculture in the future.

Keywords: phosphate solubilizing bacteria modified biochar; maize; seedlings; promoting growth; rhizosphere phosphate availability

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Introduction

Phosphorus is one of the three essential mineral nutrients for plant growth and a critical limiting factor in agricultural production [1]. Studies have shown that 74% of arable soil in China lacks phosphorus, with 95% of the nutrient being in an inert form, making it difficult for plants to absorb and utilize. Phosphate fertilizer represents a vital material to guarantee grain yield increases and food security, as its application can significantly raise soil phosphate content and supply capacity, thereby improving agricultural production efficiency [2]. However, the utilization rate of phosphate fertilizer applied to the soil in China is low, with utilization efficiencies ranging between 5%-25% in this season. A large proportion of the phosphorus combines with Ca^{2+}, Fe^{2+}, Fe^{3+}, and Al^{3+} in the soil to form insoluble phosphates. The long-term inefficient use of chemical fertilizers often results in the excessive accumulation of phosphorus and other elements, altering soil physical and chemical properties and causing environmental pollution, which has adverse effects on food safety and human health. The phenomenon of soil phosphate accumulation caused by the overuse of phosphate fertilizer is a common occurrence worldwide [3], with the situation being particularly severe in China. Soil phosphorus accumulation in China is increasing at a rate of 11% [4], leading to an increasing trend of soil available phosphorus in different regions [5]. This not only significantly reduces the utilization rate of phosphate fertilizers but also wastes limited phosphate resources and increases the risk of pollution in water environments [6, 7].

Phosphate solubilizing bacteria have the remarkable ability to transform insoluble or poorly soluble phosphate, which is difficult for plants to absorb and utilize, into forms that are easily accessible to plants. This conversion, in the absence of available phosphate, significantly improves the availability of phosphate in soil. It is recognized as a safe, economic, and efficient biological method for activating insoluble soil phosphate and has great development and application potential. Since Staltrom's discovery [8] of the existence of phosphate solubilizing microorganisms in soil, countries around the world have conducted subsequent research. Up to now, 36 genera of phosphate solubilizing microorganisms have been reported, with 89 species of microorganisms and tens of thousands of phosphate solubilizing strains [9, 10]. Strains with strong abilities to dissolve insoluble phosphate include Bacillus, Pseudomonas, and Penicillium [11-13].

In recent years, biochar, an emerging material that serves as a fertilizer, adsorbent, soil improver, and heavy metal chelating agent, has increasingly been reported to improve the structure of the soil microbiome and serve as a carrier of microbial fertilizer [14-18]. Studies have shown that the unique physical and chemical properties of biochar can provide a place for the growth and reproduction of phosphate solubilizing bacteria and promote their phosphate solubilizing activity [19]. Despite the high amount of biochar required and its associated cost, its large-scale application in agricultural production remains challenging. To date, there are limited studies on the combined application of biochar and phospholytic bacteria. Therefore, effectively integrating the advantages of both biochar and phospholytic bacteria while significantly reducing the application cost is currently a hot and challenging topic.

To address the research gap regarding the dosage and impact of the combined application of phosphate solubilizing bacteria and biochar, we conducted a study in Guanzhong Plain, Shaanxi Province, and collected soil samples from various locations to analyze the local phosphate solubilizing bacteria. Using this approach, we established a resource bank of highly efficient phosphate solubilizing bacteria for the Guanzhong area of Shaanxi Province. Additionally, we developed a novel technique for modifying biochar using phosphate solubilizing bacteria, a first-of-its-kind innovation both domestically and internationally. Subsequently, we conducted maize seedling growth experiments using the modified biochar. These experiments aimed to investigate the synergistic effect of biochar and phosphate solubilizing bacteria on maize seedling growth and rhizosphere phosphate availability under conditions of phosphate deficiency. The results of this study provide a scientific basis for the development of biochar-based microbial fertilizers or seedling substrates for phosphate solutions. These innovations have the potential to significantly improve maize production through their widespread application.

Materials and Methods

Experimental Material

Soil Source. The soil samples were procured from the topsoil layer (0-20 cm) in the Yangling Agricultural High-Tech Industrial Demonstration Zone, located in Xianyang City, Shaanxi Province, in February 2023. The sampling coordinates were 34°11'35" latitude north and 108°2'51" longitude east. The cultivated land is characterized by a winter wheat-summer maize rotation and is classified as Lou soil. The region belongs to a temperate monsoon climate, with average annual temperatures ranging from 9 to 13.2ºC, average annual rainfall of 537 to 651mm, and a frost-free period spanning 211 days. The fundamental properties of the soil samples collected in this study were as follows: organic matter content of 1.62 g/kg, total N of 0.67 g/kg, available P of 3.45 mg/kg, bulk weight of 0.93 g/cm^3, and a pH of 8.02.

Culture Medium. The Meng Jinna organic medium comprises the following components: 10.0g glucose, 0.5 g $(NH_4)_2SO_4$, 0.3 g NaCl, 0.3 g $MgSO_4·7H_2O$, 0.03 g $MnSO_4·H_2O$, 0.3 g KCl, 0.03 g $FeSO_4·7H_2O$, 2.0 g lecithin, 15-20 g AGAR, and 1000ml purified water. The Mengjinna inorganic medium comprises the following components: 10.0g glucose, 0.5g $(NH_4)_2SO_4$, 0.3 g NaCl, 0.3 g $MgSO_4 \cdot 7H_2O$, 0.03 g $MnSO_4 \cdot H_2O$, 0.3 g KCl, 0.03 g $FeSO_4 \cdot 7H_2O$, 5.0 g $CaCO_3$, 15-20 g AGAR, and 1000ml purified wate. The LB medium comprises the following components: 10 g tryptone, 5 g yeast extract, 10 g NaCl, and 950 ml purified water.

Biochar. Biochar, sourced from the pyrolysis of discarded fruit tree trunks and branches in a controlled environment of oxygen limitation (450ºC), was ground to a particle size of 1mm for use. The test strain, bacteria Y-5, was isolated from soil samples obtained for this study. The maize variety tested in this experiment was Shanyu 501, provided by Northwest Agriculture and Forestry University.

Equipment and Instruments. The main equipment and instruments used were as follows: the total bacterial DNA extraction and identification were completed by Beijing Qingke Biotechnology Co., LTD., using a TSINGKE Plant DNA extraction kit (universal) with self-developed primer and an Applied Biosystems 3730XL Sequencer. The centrifuge was a Thermo Fisher Scientific Legend Micro 17, and the PCR instrument was an Applied Biosystems 2720 Thermal Cycler. The UV Spectrophotometer Evolution 350, produced by Thermo Fisher Scientific, was also employed.

Experimental Method

Isolation and Screening of Phosphate Solubilizing Bacteria Strains. To isolate phosphate solubilizing bacteria strains, follow these steps: taking 3 g of fresh soil, add sterile ultra-pure water to 30 ml, shake the mixture well at 28±1ºC, 150-180 r/min for 20 minutes; Stand the mixture at room temperature for 10 minutes, then dilute to 10^{-3}, 10^{-4}, and 10^{-5}, and inoculate with organic phosphate solid medium and inorganic phosphate solid medium using the coating method, respectively, and culture in an incubator at 28±1ºC for 3-5 days. By observing the phosphate solubilizing ring, the phosphate solubilizing bacteria were purified by the coating plate method 2-3 times. Fresh purified bacteria were inoculated on LB liquid medium, cultured at (28±1)ºC, 150 r/min~180 r/min for 48 hours, mixed with 60% sterilized glycerin solution at a ratio of 1:1, and stored in the refrigerator at -80ºC.

Identification of Phosphate Solubilizing Bacteria Strains. The pure bacterial cultures were sent to Beijing Qingke Biotechnology Co., Ltd. for further analysis. The total DNA of the strain was extracted using a bacterial DNA kit and then amplified using bacterial universal primers. The PCR amplification products were then sequenced. The obtained 16SrDNA sequences were then queried and compared in the NCBI database (blast.ncbi.nlm.nih.gov). Additionally, the morphology of the spores and mycelium was observed under a section microscope, and their physical and chemical properties were tested.

Assessment of Phosphate Solubilizing Efficiency. In the plate qualitative experiment, the phosphate solubilizing bacteria were inoculated in the center of the solid medium, with three replicate plates set up. The culture was conducted at 28ºC for 3 to 5 days. The phosphate dissolution rate was calculated using the formula: phosphate dissolution rate (D/d) = diameter of transparent ring (D)/colony diameter (d). In the shake flask quantitative experiment, the purified phosphate solubilizing bacteria were added to the liquid medium, and three replicates were set up. The culture was shaken at 28ºC and 200 rpm for 5 days. The content of soluble phosphate in the shake flask was determined by the molybdenum-antimony method.

Preparation of Modified Biochar by Phosphate Solubilizing Bacteria. The purified phosphate solubilizing bacteria were inoculated in LB liquid medium and cultured at (28±1)ºC and 150 to 180 rpm for 24 hours to reach the logarithmic growth phase. The biochar was then mixed with the phosphate solubilizing bacteria solution at a ratio of 1g biochar to 2 ml solution, and the mixture was incubated at (28±1)ºC for 48 hours. After soaking, the mixture was dried and set aside for further use.

Experiment Design

Test Setup. The plastic pots used in the test had a size of 18 cm×15 cm×15 cm, and each pot contained 1 kg of soil. A total of 6 treatments were established, with each treatment being repeated 4 times. The treatments were as follows: (CK) - no phosphate solubilizing bacteria were added to modified biochar; (BC5) - adding 5 g phosphate solubilizing bacteria modified biochar; (BC10) : adding 10g phosphate solubilizing bacteria modified biochar; (BC20)-adding 20 g phosphate solubilizing bacteria modified biochar; (BC40)- adding 40g phosphate solubilizing bacteria modified biochar; (BC80) - adding 80 g phosphate solubilizing bacteria modified biochar. The objective of this experiment was to investigate the influence of various dosages of modified biochar infused with phosphate solubilizing bacteria on the growth of maize seedlings and the content of available phosphate in the soil.

Test Procedure. Select whole and mature maize seeds and rinse them repeatedly with distilled water to remove any floating seeds. Soak in 15% sodium hypochlorite for 15 minutes, then rinse with distilled water 5 to 7 times. The treated corn seeds were placed in a petri dish covered with filter paper and cultured in an incubator at 28ºC until the rice seeds turned white. The germinated seeds were then placed in a plastic bowl using tweezers and covered with a thin layer of soil. Culture conditions: photoperiod was 12 hours of light, 12 hours of darkness, temperature was 28ºC, light intensity was 2500 lx, and the same amount of sterile water was added every 3 days for 20 days.

Test Sampling. After 20 days of maize growth, destructive sampling was carried out, and three seedlings with similar growth were selected in each treatment. The plant height, dry matter mass, soil pH, organic matter, and available phosphate content were measured. Use a ruler to measure the highest point from the root of the corn seedling to the top and take the average value. To determine the dry weight of the plants, three rice seedlings were taken, and the plants were green cut at 105ºC for 30 min. Then, dry them at 75ºC until they reach a constant weight, and weigh them. The content of available phosphate in soil was determined using molybdenum-antimony resistance colorimetry [20].

Statistical Analysis Method for Data

The data were analyzed using both EXCEL 2013 and SPSS 20.0 software. A one-way analysis of variance (ANOVA) was conducted to assess statistical significance ($P<0.05$). For multiple comparisons, the least significant difference (LSD) method was utilized. All measured results were reported as the mean±standard deviation.

Results and Analysis

Isolation and Screening of Phosphate Solubilizing Bacteria

Strain Y-5 was cultured on an organophosphate plate for 5 days. The phosphate solubilizing ring had a maximum diameter of 1.23cm (Fig. 1), while the diameter of the colony was between 0.35 and 0.38 cm. The maximum ratio of the diameter of the phosphate solubilizing ring to the diameter of the colony was 3.51. Shaking flask quantitative experiments revealed that when strain Y-5 was cultured at 30ºC for 5 days in the laboratory, the maximum soluble phosphate content of calcium phosphate was 432.25 mg/L, while the maximum soluble phosphate content of lecithin was 116.37 mg/L. This indicates a strong ability to solubilize phosphate and the potential for further study.

Identification of Phosphate Solubilizing Bacteria

The Y-5 strain was inoculated into Mengjinna organic medium and incubated at 28ºC for 36 hours. After this period, the mycelium became visible to the naked eye. The colony exhibited an irregularly round and flat shape, with a moist, milky appearance and a slightly yellow color on the reverse side (Fig. 1). Microscopically, the strain was Gram-negative with obligate aerobic staining, and the main body appeared as a thick and short rod. The optimal growth temperature for the strain ranged between 28 and 32ºC, with an optimum pH value of 6.8 to 7.6. The obtained 16SrDNA sequence (Table 1) was uploaded to GenBank (entry number NR_136501.2). A homology comparison between the sequences of Blast and GenBank was performed, revealing that the homology between strain Y-5 and *Pseudomonas donghuensis* was 100%. Based on these results, the Y-5 strain was identified as *Pseudomonas donghuensis*.

The Impact of Phosphate Solubilizing Bacteria Modified Biochar on Growth Promotion of Potted Maize Seedlings

The pot experiment results (Fig. 2, Fig. 3 and Table 2) showed that modified biochar by different proportions of Y-5 phosphate solubilizing bacteria (*Pseudomonas donghuensis*) significantly impacted the growth of maize seedlings. Notably, there were significant differences in both the fresh weight and plant height of the seedlings. In terms of seedling fresh weight, the average fresh weight of the control (CK), BC5, BC10, BC20, BC40, and BC80 treatments at 20 days was 25.65 g, 42.60 g, 53.90 g, 36.55 g, 28.38 g, and 18.33 g, respectively ($P<0.05$). Compared to the control (CK), the fresh weight of BC5, BC10, BC20, and BC40 seedlings increased by 66.08%, 110.14%, 42.50%, and 10.64%, respectively. However, the fresh weight of BC80 seedlings decreased by 28.54% compared with the control (CK). Regarding seedling height, the average plant height at 20 days for the control (CK), BC5, BC10, BC20, BC40, and BC80

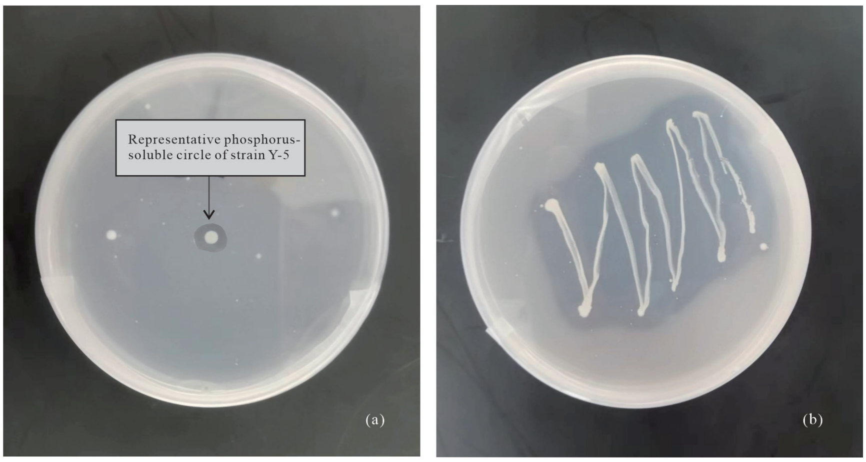

Fig. 1. Phosphate solubilizing ring a) and phosphate solubilizing effect photos b) of Y-5 phosphate solubilizing bacteria.

Table 1. 16SrDNA sequence of Y-5 (*Pseudomonas donghuensis*).

Contig Length	1415 bases
CCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCGACATTCTG ATTCGCGATTACTAGCGATTCCGACTTCACGCAGTCGAGTTGCAGACTGCGATCCGGACTACGATC GGTTTTGTGAGATTAGCTCCACCTCGCGGCTTGGCAACCCTCTGTACCGACCATTGTAGCACGTGT GTAGCCCAGGCCGTAAGGGCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGG CAGTCTCCTTAGAGTGCCCACCATAACGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGG ACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTCAGAGTTCCCG AAGGCACCAATCCATCTCTGGAAAGTTCTCTGCATGTCAAGGCCTGGTAAGGTTCTTCGCGTTGCT TCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGC GGCCGTACTCCCCAGGCGGTCAACTTAATGCGTTAGCTGCGCCACTAAAATCTCAAGGATTCCAAC GGCTAGTTGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTC GCACCTCAGTGTCAGTATGAGCCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGC ATTTCACCGCTACACAGGAAATTCCACCACCCTCTGCCCTACTCTAGCTCGCCAGTTTTGGATGCA GTTCCCAGGTTGAGCCCGGGGATTTCACATCCAACTTAACGAACCACCTACGCGCGCTTTACGCCC AGTAATTCCGATTAACGCTTGCACCCTCTGTATTACCGCGGCTGCTGGCACAGAGTTAGCCGGTGC TTATTCTGTCGGTAACGTCAAAATACTCACGTATTAGGTAAGTACCCTTCCTCCCAACTTAAAGTG CTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCA ATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCC TCTCAGACCAGTTACGGATCGTCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATCCGACCTA GGCTCATCTGATAGCGCAAGGCCCGAAGGTCCCCTGCTTTCTCCCGTAGGACGTATGCGGTATTAG CGTTCCTTTCGAAACGTTGTCCCCCACTACCAGGCAGATTCCTAGGTATTACTCACCCGTCCGCCGC TGAATCGAAGAGCAAGCTCTCTCATCCGCTCGAC	

treatments was 30.80 cm, 41.75 cm, 51.78 cm, 34.35 cm, 28.09 cm, and 22.73 cm, respectively (P<0.05). The fresh weight of the BC5, BC10, and BC20 treatments increased by 35.55%, 68.12%, and 11.53%, respectively, compared with the control. However, the fresh weight of the BC40 and BC80 treatments decreased by 8.80% and 26.20%, respectively, compared with the control.

The Impact of Phosphate Solubilizing Bacteria on Modified Biochar on Potting Soil Properties

Compared with the control group (CK), the modified biochar produced by different proportions of phosphate solubilizing bacteria had a more significant impact on the soil, leading to significant differences in the available phosphate content (Table 2). At 20 days, the available phosphate content in the soil of the CK,

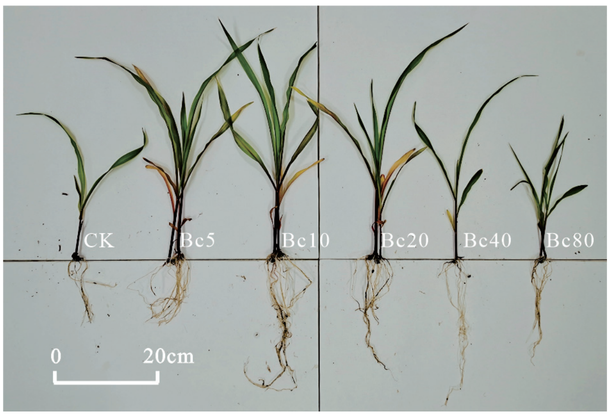

Fig. 2. Effect of biochar modified by Y-5 phosphate solubilizing bacteria on the growth of maize seedlings (20d).

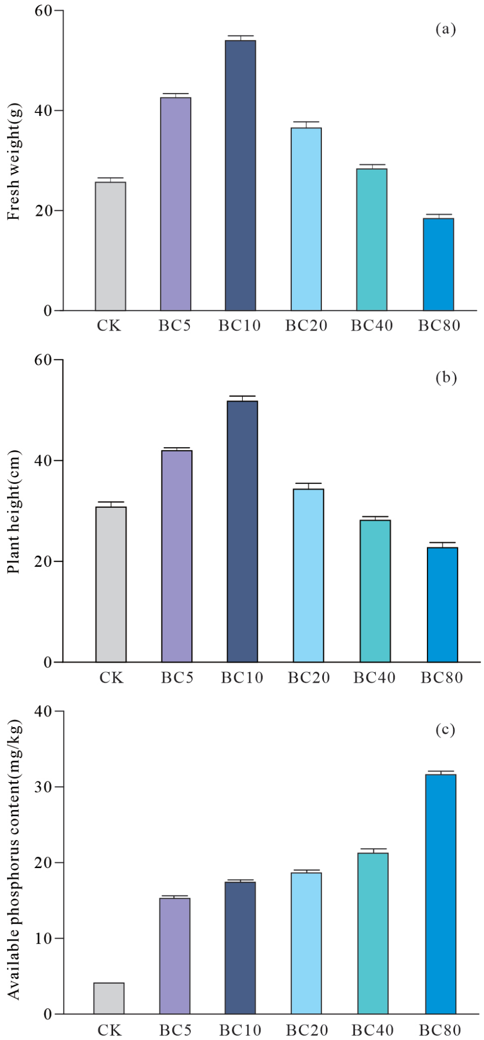

Fig. 3. Effects of Y-5 phosphate solubilizing bacteria modified biochar on seedlings (20d).

Table 2. Effects of biochar modified by Y-5 phosphate solubilizing bacteria on fresh weight, plant height and soil available phosphate of maize seedlings.

Treatment	Fresh weight		Plant height		Available phosphate content	
Measurement value (g)		Increased rate	Measurement value (cm)	Increased rate	Measurement value (mg/kg)	Increased rate
CK	25.65 d	—	30.80 c	—	4.13 c	—
BC5	42.60 b	66.08%	41.75 b	35.55%	15.29 b	270.22%
BC10	53.90 a	110.14%	51.78 a	68.12%	17.37 b	320.58%
BC20	36.55 c	42.50%	34.35 c	11.53%	18.64 b	351.33%
BC40	28.38 d	10.64%	28.09 d	-8.80%	21.22 b	413.80%
BC80	18.33 e	-28.54%	22.73 e	-26.20%	31.68 a	667.07%
Signif (*P*)	0.05	—	0.05	—	0.05	—

* The letters indicate the significant difference at 5% level.

BC5, BC10, BC20, BC40, and BC80 treatments was 4.13 mg/kg, 15.29 mg/kg, 17.37 mg/kg, 18.64 mg/kg, 21.22 mg/kg, and 31.68mg/kg, respectively ($P<0.05$). Compared with the CK control group, the soil available phosphate content of BC5, BC10, BC20, BC40, and BC80 increased by 270.22%, 320.58%, 351.33%, 413.80%, and 667.07%, respectively. Furthermore, significant changes were observed in soil pH and organic matter content. At 20 days, the soil pH values of CK, BC5, BC10, BC20, BC40, and BC80 were 8.12, 8.35, 8.54, 8.61, 8.84, and 9.26, respectively. The corresponding organic matter contents were 2.31 g/kg, 9.57 g/kg, 15.33 g/kg, 17.45 g/kg, 22.16 g/kg, and 39.26 g/kg, respectively.

Discussion

In this study, a high-strength phosphate solubilizing bacteria called Y-5 (*Pseudomonas donghuensis*) was successfully isolated in Yangling, Shaanxi Province. This bacterium can effectively increase the soil's available phosphate content. The phosphate solubilizing bacteria modified biochar to achieve the maximum application dose of 80g/kg. Nevertheless, there exists a notable difference between modified biochar and unmodified biochar, necessitating consideration of the actual crop growth and development when determining the optimal dosage. The monitoring results of fresh weight and plant height indexes of corn seedlings treated by six groups in this study revealed that when the addition amount of phosphate solubilizing bacteria modified biochar was about 10g/kg, it exhibited the strongest growth-promoting effect on corn seedlings. Compared to the blank control group, the growth rates of fresh weight and plant height reached 110.14% and 68.12% on day 20.

At the same time, we found that excessive use of modified biochar by phosphate solubilizing bacteria could potentially inhibit crop production. This study's findings showed that, when compared to an application dose of 10 g/kg, maize seedlings' plant height and fresh weight took a downward turn at an application dose of 20 g/kg. Notably, the fresh weight at 40 g/kg was close to that of the blank control. However, at 80 g/kg, the fresh weight was 28.54% lower than the blank control, while the plant height at 40 g/kg and 80 g/kg was 8.80% and 26.20% lower than that of the blank control, respectively. Based on these results, we believe that the optimal application dose of modified biochar by *Pseudomonas donghuensis* is approximately 10 g/kg. Excessive application could be counterproductive, while insufficient application may not yield the optimal effect.

Studies have shown that pseudomonas could effectively improve the utilization efficiency of nutrients such as N, P, Zn, and Fe [21-23]. As a soil amendment, biochar could improve the colonization rate of phosphate solubilizing bacteria and maintain its high biological activity, resulting in improved soil properties and enhanced nutrient absorption by plants. Consequently, the biomass and yield of green beans, maize, and other crops can be improved [24-25]. Earlier research has shown that, the optimal dosage of biochar is 80 g/kg [26], while the optimal dosage of phosphate solubilizing bacteria modified biochar in tomatoes is 50 g/kg [27]. When applying these dosages, biochar can significantly promote plant growth and improve the activity of antioxidant enzymes in leaves, ultimately increasing crop yield. However, an application dose of 80 g/kg is costly and not amenable to large-scale promotion. In a word, this study effectively demonstrates that the modified biochar enhanced by phosphate solubilizing bacteria significantly improves soil properties and corn yield. Additionally, the study innovatively determines the optimal application ratio, providing valuable guidance for future research.

Although this paper has discovered suitable phosphate solubilizing bacteria for maize planting in Yangling, Shaanxi Province, and proposed the production method and optimal application amount of phosphate solubilizing bacteria modified biochar, it should be noted that the composition of cultivated land flora varies in different countries and regions of the world. Therefore, appropriate optimization can be carried out on the basis of the proposed method during the production of phosphorus-soluble bacteria modified biochar. For example, conducting adequate pot tests to further accurately determine the optimal dosage and the best phosphate solubilizing bacteria. Additionally, it is essential to comprehensively consider the safety, activity, and production cost of modified biochar by phosphate solubilizing bacteria.

Conclusions

The results of solid medium, liquid medium, and phosphate solubility tests showed that strain Y-5 phosphate solubilizing bacteria exhibited strong phosphate solubility. Through ITS sequence analysis and morphological observations, the strain was identified as *Pseudomonas donghuensis*.

The modified biochar produced by strain Y-5 phosphate solubilizing bacteria effectively increased the levels of available phosphate and organic matter in soil. With an increase in the application dosage to 80g/kg, the levels of available phosphate and organic matter in the soil continued to increase, along with an increase in soil pH.

The modified biochar produced by strain Y-5 phosphate solubilizing bacteria exhibited a significant growth-promoting effect on maize seedlings, with the application dose of BC10 yielding the best results. The growth rate of fresh weight and plant height ratio of the control (CK) could reach 110.14% and 68.12%, respectively. However, excessive application (40 g/kg or 80 g/kg) had a significant inhibitory effect on the growth of maize seedlings.

Taking into consideration the growth indicators of maize seedlings and changes in soil properties, it was concluded that the optimal application ratio of modified biochar produced by phosphate solubilizing bacteria (*Pseudomonas donghuensis*) was approximately 10 g/kg.

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Conflict of Interest

The authors declare no conflicts of interest.

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