Screening of rhizosphere growth promoting bacteria and their growth promoting ability of sunflower in cold black soil area

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Abstract. High efficient growth promoting bacteria were screened from the rhizosphere soil of sunflower in the cold black soil area, and their growth promoting ability was tested to obtain the strains with comprehensive growth promoting ability. The phosphate solubilizing bacteria were screened out by inorganic phosphate bacteria culture medium, the phosphate solubilizing ability of the strain was determined by mo sb anti chromogenic method, the indoleacetic acid producing ability of the strain was determined by salkowski method, and the growth promoting ability of the strain was evaluated comprehensively. Eleven strains of phosphate solubilizing bacteria were isolated and screened from soil samples. Among them, strain WP2 had the strongest phosphate solubilizing ability, and the concentration of phosphate radical was 12.75 μg/mL; strain WP1 had the strongest indoleacetic acid producing ability, and the concentration of indoleacetic acid was 25.37 μg/mL; strain WP1 had the strongest iron producing ability, with Su = 72.76%, and the strength was 4+. It was found that WP1, WP2 and WP10 had strong ability of promoting growth and had strong potential of development and utilization. The above results can provide excellent strains for the development and promotion of special bio fertilizer suitable for the cold black soil.

1. Introduction

There are only three cold black soils in the world. The organic carbon content of the cold black soil accounts for about 5%-8%. The soil is of good quality and is an important food production base. The black soil area in Northeast China has a long history of planting. Since the reclamation, part of the soil has been fertilized and matured, but the more common situation is the decline of black soil production capacity. The application of a large number of chemical fertilizers and pesticides leads to the imbalance of soil ecology, which is manifested in the deterioration of physical and chemical properties of black soil, the enhancement of resistance of pathogens, and the decline of crop yield and quality, which is not conducive to the sustainable utilization of black soil in the Northeast cold region. Therefore, it is very important to develop bio fertilizer and bio pesticide with multiple control mechanisms to improve soil fertility and enhance plant resistance. The root exudates of plants carry on the transmission and exchange of information flow, energy flow and material flow with microorganisms, and form the intraspecific and interspecific relationships such as parasitism, reciprocity, competition, symbiosis and antagonism in the rhizosphere microecological environment,
which significantly affect the bioavailability of various nutrients in the soil. Studies have shown that plants increase the survival pressure of microorganisms through allelopathy to select the microorganisms that contribute the most to their growth [1]. Bacteria are the most abundant microorganisms in the rhizosphere, especially considering their competitiveness in root colonization, so plant growth promoting rhizobia (PGPR) will largely affect the physiological function of plants [2]. PGPR can promote plant growth directly and indirectly [3]. Some strains directly regulate plant physiological growth by simulating the synthesis of plant hormones. At present, it has been found that about 80% of plant rhizosphere growth promoting bacteria have the ability to secrete auxin [4]. Other strains increase the available minerals and nitrogen in the soil to promote plant growth; some strains produce bacteriostatic substances or induce plant to produce systemic resistance, which is beneficial for plant root system occupation to reduce or inhibit the occurrence of plant diseases. The production of iron carriers is considered to be a major mechanism of PGPR for plant disease control. These PGPR may show more than two or three mechanisms to promote plant growth [5]. So far, researchers at home and abroad have made some explorations and researches on the growth promoting ability of plant rhizosphere bacteria, such as nitrogen fixation, iron production carriers. [6-7]. However, there are few researches on the growth promoting ability of rhizosphere bacteria of plants in the black soil region of the cold region, and only the corresponding researches on the composition, growth conditions and growth promoting effects of soybean rhizosphere bacteria community in the black soil region of the cold region. In this study, 11 growth promoting strains were screened from the rhizosphere soil of sunflower in the cold black soil area. The IAA, iron carrier and phosphorus dissolving ability of the strains were quantitatively analyzed, in order to obtain the plant rhizosphere growth promoting strains with multiple control mechanisms, and to provide theoretical basis and technical guidance for the development and utilization of bio fertilizer and bio pesticide in the cold black soil area.

2. **Materials and Methods**

2.1. **Experimental Materials**

2.1.1. **Sample Collection.** The healthy and morbid rhizosphere soil of sunflower in kangjinjing experimental base of Heilongjiang Academy of Agricultural Sciences was selected 12 sample points were set up along the diagonal of each plot. Four samples were taken at intervals on each plot. The nylon bag and the sunflower root with soil were taken out together. The soil was washed and the complete root system was obtained. The aseptic sample collection bag was put into the bag, and the sampling information was marked on the label and stored in the 4 ℃ refrigerator.

2.1.2. **Culture Medium.** Inorganic phosphorus bacteria medium, LB liquid medium containing L-tryptophan (100 mg /L), PKO liquid medium, beef extract peptone liquid medium.

2.1.3. **Molybdenum-antimony anti-color developing agent.** Weigh 1.5g of ascorbic acid, add 100mL of molybdenum-antimony stock solution, stir to dissolve, and prepare on the day of use; phosphorus standard working solution: weigh 0.4390g of potassium dihydrogen phosphate (KH₂PO₄, analytically pure, bake at 105℃ in 2h). Dissolve in 200mL distilled water, add 5mL concentrated sulfuric acid, transfer to a 1.0L volumetric flask and shake to a constant volume. This is 100 μg/mL phosphorus standard storage solution, which can be stored for a long time. Pipette 5mL phosphorus standard stock solution into a 200mL volumetric flask, dilute to volume with pure water, and shake it up. This is 2.5 μ/mL phosphorus standard working solution; molybdenum antimony stock solution: take a 1000mL glass beaker, add about 800mL pure water, and then slowly add 181.0mL of concentrated sulfuric acid, stir while adding, then add 10.0g of ammonium molybdate and 0.5g of potassium antimony tartrate, stir thoroughly to dissolve, after cooling to room temperature, bring the volume to 1000mL, shake well for use; salkowski reagent: contains 0.5 15mL of mol/L FeCl₃ solution, 300mL of concentrated H₂SO₄
isolation and screening of phosphate solubilizing bacteria. Take 1.0g of the air dried soil sample, mix it well and evenly, dissolve it in 9mL of sterile water, shake it for 10min to make it mix evenly, leave it for 1min, transfer 1mL of supernatant to a centrifuge tube filled with 9mL of sterile water, make a soil suspension with a concentration of 10^-2, and so on to make a 10^-2-10^-7 dilution bacterial solution. Take 0.1mL diluent of different gradients respectively and spread it on the inorganic phosphorus bacteria culture medium, repeat each treatment for 3 times, and culture it in 28 ℃ constant temperature incubator for 3 days. The results showed that the larger the ratio of H / C to h / C, the stronger the ability of P-solubilizing bacteria to degrade inorganic phosphorus. The pure colony of phosphate solubilizing bacteria was isolated and purified, and stored at 4℃.

2.2.2. Determination of growth promoting characteristics of strains. (1) Determination of phosphorus dissolving ability. The single colony of the strain was inoculated in the liquid medium of beef extract peptone. After incubation at 28 ℃ for 2 days, the liquid was taken and centrifuged in a centrifuge tube at 4000R / min for 3 minutes. The bacteria were suspended in sterile water for sedimentation. In the experimental group, 1mL of bacterial supernatant was inoculated into a 100mL conical flask containing 50mL of PKO medium, and in the control group, the same amount of sterile water was taken. The conical flask was shaken at 30 ℃ and 180r / min for 3 days, the culture medium was centrifuged at 4000R / min for 30 minutes, the supernatant was taken, and the content of soluble phosphorus in the supernatant was determined by Mo sb anti colorimetry, unit: μg / mL. In this part, three repeats were set for the determination of phosphorus dissolving ability.

(2) Determination of indoleacetic acid (IAA). The od530 values of each concentration (0, 0.5, 1.0, 5.0, 10.0, 15.0, 20.0 and 25.0 μ g / mL) of indoleacetic acid standard solution were measured, and the IAA standard curve was drawn. The content of IAA was determined by salkowski colorimetry. The strain was inoculated in LB liquid medium containing L-tryptophan, cultured at 28 ℃ and 150r / min for 2 days, centrifuged at 4000R / min for 45min, and then 1mL supernatant was added with 1mL pure water and 8mL salkowski reagent, and kept in dark at 38℃ for 30min to determine od530 value. The IAA content was calculated by substituting the standard curve.

(3) Determination of iron production carrier capacity
Take strain culture solution 4000R / min, centrifuged for 45min, take 1mL LB culture solution as blank, take 1mL supernatant and 2mL CAS detection solution to mix evenly, react at room temperature for 60min, measure od630, calculate the value of a / AR and Su to obtain strain iron production carrier capacity.

\[
Su = \left( \frac{AR-A}{AR} \right) \times 100\% \quad (1)
\]

Note: Su is the active unit of iron carrier, a is the test tube od630, AR is the blank tube od630.

3. Results and analysis

3.1. Preliminary screening of PGPR strain
There were 11 strains with obvious p-soluble ring and H / C value between 1.7-3.2. Among them, WP2 had a good growth and the most obvious p-soluble ring. The H / C value of WP2 was 3.2. It was found that the P-solubilizing bacteria with obvious P-solubilizing ring screened from soil lost the ability of P-solubilizing after purification and subculture found that 50% of P-solubilizing bacteria weakened or even disappeared in the process of transfer and passage[8]. Therefore, the transparent circle method can only be used as a preliminary screening index of phosphate solubilizing bacteria. The obtained strains need to be further screened to determine their phosphate solubilizing effect.

### Table 1. Preliminary screening results of strains

| Strain | Diameter of phosphorus dissolving ring (mm) | Colony growth diameter (mm) | H/C |
|--------|---------------------------------------------|-----------------------------|-----|
| WP1    | 6.0                                         | 2.1                         | 2.9 |
| WP2    | 9.9                                         | 3.1                         | 3.2 |
| WP3    | 2.0                                         | 1.0                         | 2.0 |
| WP4    | 3.9                                         | 1.9                         | 2.0 |
| WP7    | 3.4                                         | 1.9                         | 1.8 |
| WP8    | 5.7                                         | 2.1                         | 2.7 |
| WP9    | 7.0                                         | 3.0                         | 2.3 |
| WP10   | 9.0                                         | 2.9                         | 3.1 |
| WP5    | 1.7                                         | 0.8                         | 2.1 |
| WP6    | 1.9                                         | 1.0                         | 1.9 |
| WP11   | 0.0                                         | 1.0                         | 1.7 |

### 3.2. Activity test of strains

#### 3.2.1. Detection and analysis of phosphorus dissolving ability

11 strains with H / C value greater than 1.7 were detected by shake flask culture. It was found that 11 strains had better ability of dissolving phosphorus. There were differences in the amount of dissolving phosphorus in the fermentation broth of each strain, the content of which was between 2.35-12.75 μg / mL, among which, WP2 had the highest amount of dissolving phosphorus, reaching 12.75 μg / mL, followed by WP10 and WP1, 12.125 μg / mL and 10.325 μg / mL, respectively. Strains WP5, WP4, WP3, WP6 and WP7 were less effective in dissolving phosphorus. The amount of soluble phosphorus of strain WP11 was the least, only 2.35 μg / mL, which was significantly different from other strains.

![Fig 1. Detection of phosphorus removal ability of 11 strains of bacteria](image1)

![Fig 2. Estimation of IAA producing abilities of different strains](image2)

#### 3.2.2. Detection and analysis of indoleacetic acid production capacity

Further test the IAA production capacity of WP1, WP2, WP8, WP9 and WP10, and find that they all have the IAA production capacity. The results are shown in Figure 2. The IAA concentration increment of WP1 fermentation broth is 25.37 μg / mL, and the IAA production capacity is strong.
Tab. 2 Siderophore producing abilities of 5 strains

| Strain | A/Ar | Su(%) | siderophore capacity |
|--------|------|-------|---------------------|
| WP1    | 0.272| 72.76 | ++++                |
| WP2    | 0.94 | 5.98  | +                   |
| WP5    | 0.967| 3.32  | +                   |
| WP8    | 0.977| 2.33  | +                   |
| WP10   | 0.874| 12.62 | +                   |

Note: The value of Su indicates the capacity of siderophore production, 0-0.2: +; 0.6-0.8: +++

Tab. 3 Summary of strain capacity

| Strain | Phosphorus content (μg/mL) | Content of IAA (μg/mL) | siderophore capacity |
|--------|-----------------------------|------------------------|---------------------|
| WP1    | 10.325                      | 25.37                  | ++++                |
| WP2    | 12.75                       | 6.88                   | +                   |
| WP5    | 5.1                         | 6.01                   | +                   |
| WP8    | 9.225                       | 2.85                   | +                   |
| WP10   | 12.125                      | 4.72                   | +                   |

3.2.3. Detection and analysis of iron production carrier capacity. The results showed that strains WP1 and WP10 had strong ability of producing iron carrier, among which the Su value of strain WP1 was 72.76%, and the intensity was ++++; the Su value of strain WP10 was less than 60%. The reason of influencing the results might be the difference of the characteristics of the strain itself, which led to the different amount of iron carrier secreted by the strain.

4. Discuss and conclusions

The ability of promoting growth and the comprehensive ability of promoting growth are closely related to the characteristics of the strains. Due to the restriction of culture conditions and other process factors, the number of microorganisms that are difficult to cultivate can not be ignored. This result can not fully reflect the existence of microorganisms in this test sample. The method of microbial environmental genomics can be used to study the genetic composition and community function of all microorganisms in the sample. The transparent circle size of the strain on the inorganic phosphorus bacteria medium is related to the type of enzyme or acid of the metabolite of the strain and the release speed. According to the test results, the available P content of the soluble P strain was 2.35-12.75 μg/mL. Yang Yan et al. isolated from the rhizosphere soil of Xishuangbanna, the strain b541 with the strongest P-soluble ability was 9.79mg/L. In contrast, the comprehensive phosphorus removal ability of this experiment was close to that of the phosphorus removal ability of the bacteria, which indicated that the bacteria in the rhizosphere soil of sunflower in the cold black soil area had great development value in dissolving inorganic phosphorus in the soil and promoting plant growth. Among the selected strains, WP1 has a strong ability to secrete indoleacetic acid, and the detection result is 25.37 μ g / mL, which is almost consistent with the highest value (55.71 ± 0.65mg/L) of IAA secreted, indicating that the rhizosphere soil bacteria of sunflower in the cold black soil area have a strong development and utilization potential to secrete indoleacetic acid to promote plant growth. The Su value of WP1 is more than 60% among the strains with the ability of producing iron carrier, which indicates that some populations of sunflower rhizosphere bacteria in the black soil area of the cold region have strong ability of producing iron carrier, can absorb iron, and can also provide nutrients for other plants with the same iron source. The strains with good ability of producing iron carrier can not only promote the vigorous growth of plants, but also achieve the biological control effect by competing for iron nutrition.

Five strains of growth promoting bacteria were screened from the rhizosphere soil of sunflower growing in the cold black soil area of Heilongjiang Province. After comprehensive analysis and detection results, it was found that strain WP1 had better abilities of phosphate solubilization, indoleacetic acid production and iron production carrier, and strains WP2 and WP10 had two kinds of growth promoting abilities. These three bacteria have great application potential, which can provide excellent bacteria and theoretical basis for the development and popularization of biological fertilizer in the cold black soil. To sum up, there are strains in the rhizosphere soil of Helianthus annuus in the black soil area of the cold region, and they have multiple control mechanisms, which have important research value and significance.
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