Isolation and Identification of Potassium Releasing Bacteria from Sugarcane Rhizosphere Soil and Optimization of Fermentation Condition

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Abstract [**Objectives**] To isolate and optimize the potassium releasing bacteria from sugarcane rhizosphere soil in Baitu Town, Gaoyao District, Zhaoqing City, Guangdong Province, and then evaluate their potassium-releasing ability, and optimize the fermentation conditions of the strains with the best potassium-releasing ability, so as to provide a scientific basis for the development and production of potassium releasing bacteria fertilizer. [**Methods**] Potassium-solubilizing bacteria were isolated from sugarcane rhizosphere soil using potassium-feldspar powder as the potassium source of isolation medium by dilution coating method and plate streaking method. The isolated strains were identified by 16s rRNA sequence analysis. The potassium-releasing ability of each strain was determined by sodium tetraphenylborate (STPB) method, and the strain GK-37 with the optimal potassium-releasing ability was selected. The fermentation conditions of GK-37 strain were further optimized, and the effects of different carbon sources on its growth were mainly investigated. [**Results**] Seven strains of potassium-solubilizing bacteria were isolated and purified, and identified by 16s rRNA. They belonged to *Pseudomonas knackmussii*, *Pseudomonas*, *Pseudomonas insulaes* and *Caballeronia zhejiangensis*. Among the tested strains, strain GK-37 had the best potassium-releasing ability, and its potassium-releasing capacity was 26.99 mg/L. By optimizing the fermentation conditions of GK-37 strain, it was found that when the fermentation medium was sucrose as carbon source, the growth of the strain was the best. [**Conclusions**] In this study, the potassium releasing bacteria were successfully isolated and identified from the rhizosphere soil of sugarcane, and the strain GK-37 had high potassium-releasing ability. Through the optimization of fermentation conditions of GK-37 strain, sucrose was determined as the optimal carbon source. This study is expected to provide a valuable reference for the further development and production of potassium releasing bacteria fertilizer.

Key words Potassium releasing bacteria, Potassium-feldspar powder, Molecular identification, Sodium tetraphenylborate, Fermentation conditions

1 Introduction

Potassium is another important element for plant growth besides nitrogen and phosphorus. In plant cells, potassium mainly exists in the form of ions, which participates in the catalysis of enzymes; in soil, potassium exists in various forms, but plants can not directly absorb insoluble potassium in minerals, plants can only absorb soluble potassium [1-2]. Soil microorganisms can promote the transformation of mineral elements in the earth, and the use of potassium-releasing microorganisms to transform potassium resources in the soil is an effective way to solve the lack of available potassium in the soil [3]. As a kind of soil microorganism that can convert ineffective potassium into quick-acting potassium, potassium releasing bacteria also has the function of dissolving phosphorus [4]. In addition, potassium releasing bacteria can also produce a series of plant hormones that can promote crop growth and improve crop quality [5]. At present, potassium releasing bacteria mainly include *Bacillus mucilaginosus*, *Microbacterium zeae*, *Bacillus soilus*, *etc.*, among which *B. mucilaginosus* has received widespread attention because of its strong potassium release efficiency and strong environmental adaptability, and has been made into a microbial fertilizer [6].

In recent decades, a large number of studies at home and abroad have shown that potassium releasing bacteria have obvious potassium release effect, and different kinds of potassium releasing bacteria have different potassium release ability. Potassium releasing bacteria can convert insoluble potassium in potassium feldspar into soluble potassium, which can promote the growth and development of plants although it can not significantly improve the utilization rate of potassium fertilizer in plants [7]. Ren Jianguo *et al.* [9] isolated a strain from the soil of *Pseudostellaria heterophylla* with strong potassium release and nitrogen fixation, and identified it as *Paenibacillus castaneae*. Balasubramanian Bagyalakshmi *et al.* [10] isolated pseudomycete Bacillus from tea planting soil, and it was proved that pseudomycete could mobilize the bound form of potassium in the soil, which could be used as a biological fertilizer to solubilize potassium.

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Sugarcane, a crop grown mainly in temperate and tropical regions, can be used to make brown sugar and ethanol [11]. China is one of the countries with the largest production of sugarcane, but there are few reports on the isolation of potassium releasing bacteria from sugarcane rhizosphere soil in China. Potassium releasing bacteria has a niche effect, and its ability to decompose potassium varies greatly with soil conditions and plant roots [12]. In this study, seven strains of potassium releasing bacteria were isolated from sugarcane rhizosphere soil in Baitu Town, Gaoyao District, Zhaoqing City, Guangdong Province, and their potassium releasing ability was detected by sodium tetraphenylborate (STPB) method. A strain GK-37 with strong potassium releasing ability was screened out, and its fermentation conditions were optimized, which provided a reference for the preparation of potassium releasing bacteria fertilizer in the later stage.

2 Materials and methods

2.1 Soil sample The soil samples were collected from sugarcane rhizosphere in Baitu Town, Gaoyao District, Zhaoqing City, Guangdong Province (22°99′73″ N, 112°57′10″ E). According to the topographic conditions of the sugarcane field, 5 points were randomly selected for soil sampling (the distance between points is 1 m). When sampling, properly removed the dead branches and fallen leaves on the ground surface, used a soil drill to collect the rhizosphere soil of sugarcane, put the root system and its adhered soil samples into sterilization bags, labeled them, took them back to the laboratory to mash large pieces of soil, removed stones and residual roots of plants, packed them into sterilization bags, and stored them in a refrigerator at 4 ℃.

2.2 Culture medium Isolation medium of potassium releasing bacteria: 5.0 g/L of sucrose; 0.1 g/L of $CaCO_3$; 0.005 g/L of $FeCl_3$; 2.0 g/L of $Na_2HPO_4 \cdot 12H_2O$; 0.5 g/L of $MgSO_4 \cdot 7H_2O$; 5.0 g/L of potassium-feldspar powder; 18.0 g/L of agar powder; 1 L of distilled water; pH 7.0 – 7.2.

Fermentation medium for potassium releasing bacteria: 0.1 g/L of NaCl, 1.0 g/L of $CaCO_3$, 2.0 g/L of $Na_2HPO_4 \cdot 12H_2O$, 1.0 g/L of $(NH_4)_2SO_4$, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 10.0 g/L of sucrose; 0.5 g/L of yeast extract; 10.0 g/L of potassium-feldspar powder (soaked in deionized water overnight, rinsed 8 times and dried in the shade); 1 L of distilled water; pH 7.4.

LB liquid medium: 10.0 g/L of tryptone, 5.0 g/L of yeast extract, 10.0 g/L of NaCl; 1 L of distilled water; pH 7.0 – 7.2.

2.3 Isolation and purification of potassium releasing bacteria Weighed 10 g of fresh soil sample into a conical flask containing 90 mL of sterile water. The soil suspension was prepared by shaking culture for 30 min. Transferred 1 mL of the soil suspension to a test tube containing 9 mL of sterile water and diluted to 10^{-6} in a gradient. Took 0.1 mL of each concentration gradient from 10^{-3} to 10^{-5}, spread it on the potassium releasing bacteria isolation medium with a spreader (3 repeated groups for each concentration), and cultured it upside down at 30 ℃ for 3 – 5 d. Single colonies with different morphologies were selected and purified four times by plate streaking method, and then the single colonies were inoculated on LB slant medium and stored at 4 ℃.

2.4 Determination of potassium-releasing capacity Sodium tetraphenyl borate turbidimetry was used to determine the potassium-releasing ability of the strain [10]. 10 mL of the fermentation broth was centrifuged (6 000 r/min; 10 min), and the supernatant was collected and filtered through a 0.22 μm membrane. Pipetted 4.5 mL of the filtrate, added 3.0 mL of formaldehyde-EDTA masking agent, mixed well, added 3.0 mL of mL sodium tetraphenyl borate solution, shook well immediately, placed for 15 min, shook well again, and determined at the wavelength of *OD* 420 nm. The potassium releasing bacteria liquid medium without inoculation was used as the blank control.

2.5 Molecular biological identification of potassium releasing bacteria We extracted that potassium releasing bacteriaDNA according to the step of the bacterial DNA extraction kit. Using 27F 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R (5'-GGTTACCTTGTTACGACTT-3') as primers for 16S r DNA identification. The sequence of the sequenced strain was uploaded to the NCBI database to obtain the accession number. Then the genetic sequence were compared and analyzed by BLAST. The strains with higher similarity were downloaded, multiple sequence comparisons were performed using Clustal X, and the phylogenetic tree was constructed using the Neighbor joining program in MEGA 7.0 software.

2.6 Optimization of fermentation medium for potassium releasing bacteria The strain GK-37 with the best potassium-releasing ability was selected to optimize the fermentation conditions. On The basis of potassium releasing bacteria fermentation medium, 10 g/L sucrose, 10 g/L glucose, 10 g/L mannitol and 10 g/L fructose were added as the carbon source of the medium, respectively, and 2% seed solution was inoculated after high pressure steam sterilization. The culture was continued at 30 ℃ and 180 r/min for 96 h. Three replicates were set for each treatment, and the growth of each strain was evaluated based on the absorbance value at the wavelength of *OD* 600 nm, and the amount of potassium released from the supernatant was determined by sodium tetraphenylborate (STPB) method and flame spectrophotometer, respectively.

3 Results and analysis

3.1 Morphological identification of potassium releasing bacteria Fifty strains (DK1-DK50) were isolated from the rhizosphere soil of sugarcane. The shape of the colony is close to round or oval, white or off-white, translucent or opaque, and most of them show regular edges, which is in line with the morphological characteristics of the colony of potassium-releasing bacteria. Five strains (GK-22, GK-25, GK-34, GK-39, GK-41) of potassium releasing bacteria with good growth were obtained by further purification of 50 strains (Fig. 1), and subsequent experiments were carried out.

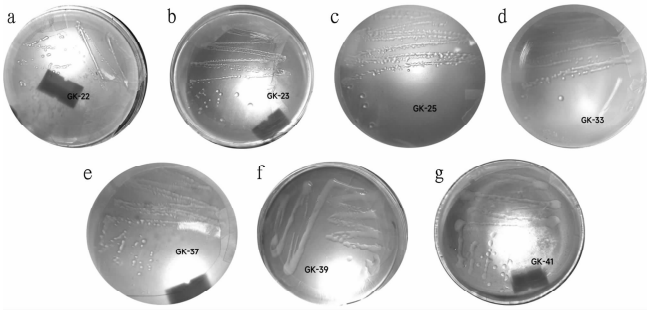

Fig. 1 Potassium releasing bacteria strains numbered GK-22, GK-23, GK-25, GK-33, GK-37, GK-39, and GK-41

3.2 Molecular biological identification of potassium releasing bacteria DNA of the potassium releasing bacteria was extracted according to the steps of the bacterial DNA extraction kit, and then amplified. The amplified products were run by electrophoresis. The specific results are shown in Fig. 2. Band 1 is Marker 2000, and bands 2 to 6 are GK-22, GK-25, GK-34, GK-39 and GK-41 in turn. The molecular size was about 1 500 bp.

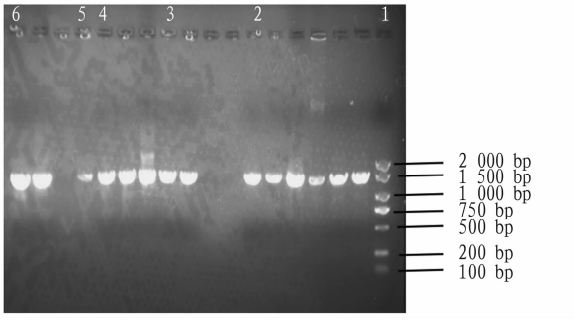

Fig. 2 Electropherogram of PCR amplification product

The sequences of 5 strains of potassium releasing bacteria were submitted to NCBI, and the sequences with higher homology were downloaded. The phylogenetic tree of potassium-dissolving bacteria was further constructed using the Neighbor – Joining method of MEGA 7.0 (Fig. 3). The results showed that GK-22 and GK-33 were located in the same clade as *P. knackmussii*. GK-25, GK-34 and GK-37 were in the same clade as *Pseudomonas*; GK-39 was located in the same clade; GK-41 was located in the same clade as *C. zhejiangensi*, and both had a high homologous expression of 99%.

3.3 Determination of potassium releasing ability of potassium releasing bacteria The sodium tetraphenylborate (STPB) method was used to determine the potassium-releasing ability of 7 strains of potassium-releasing bacteria, and the results are shown in Table 1. There was a significant difference in the amount of potassium released ($P < 0.05$). The strain GK-37 had the best potassium-releasing ability with the potassium-releasing capacity of 26.99 mg/L, while the strain GK-22 had the worst potassium-releasing ability with the potassium-releasing capacity of 1.63 mg/L.

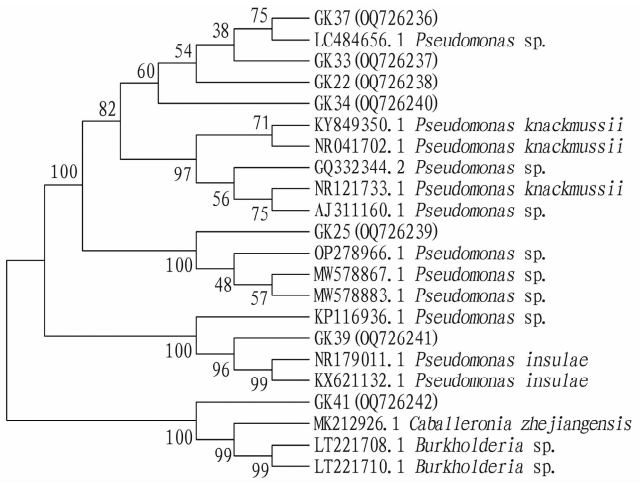

Fig. 3 Phylogenetic tree of potassium releasing bacteria based on 16s rRNA genetic sequence

Table 1 Potassium releasing ability of potassium releasing bacteria strain

Strain No.	Potassium releasing capacity//mg/L
GK-22	1.63 ± 1.33 e
GK-25	23.85 ± 1.71 b
GK-33	5.39 ± 0.49 cd
GK-34	6.13 ± 2.49 c
GK-37	26.99 ± 1.14 a
GK-39	3.34 ± 0.55 de
GK-41	5.05 ± 0.65 cd

NOTE Lowercase letters indicate that the growth of GK-37 with different carbon sources is significantly different at the 5% level.

3.4 Optimization of fermentation medium for potassium releasing bacteria On the basis of the potassium releasing bacteria fermentation medium, sucrose, glucose, fructose and mannitol were separately used as carbon sources for culture of 96 h, and the absorbance value at *OD* 600 nm and the amount of potassium released from the supernatant were measured. The results are shown in Fig. 4. The growth status of potassium releasing bacteria in four different carbon sources was sucrose > glucose > fructose > mannitol. When the strain GK-37 was cultured for 4 d with sucrose as the carbon source, the absorbance value (OD) of the strain GK-37 was the highest, reaching 1.077, indicating that the strain GK-37 grew best. At this time, the content of soluble potassium was 12.1 mg/L. However, when mannitol was used as carbon source, the growth of the strain was the worst, the absorbance value was only 0.790, and the soluble potassium content was 8.7 mg/L. When sucrose was used as carbon source, the content of soluble potassium was the highest, followed by glucose, and the worst was mannitol, which was basically consistent with the growth of the strain. By comparing and analyzing the experimental data, the optimal carbon source of potassium releasing bacteria GK 37 was determined to be sucrose on the basis of potassium fermentation medium.

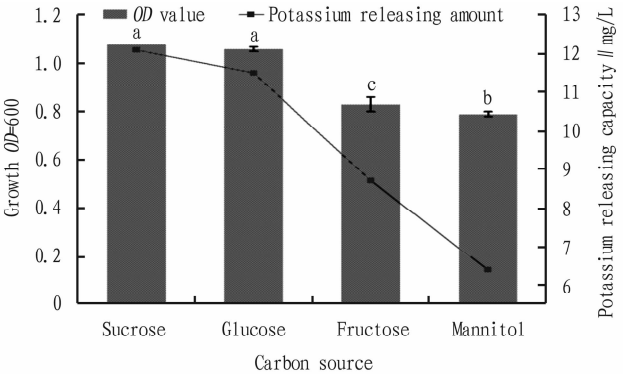

Fig. 4 The growth of potassium releasing bacteria GK-37 with different carbon sources and the amount of potassium dissolved by flame spectrophotometer

4 Discussion and conclusions

The potassium releasing bacteria is an important part of soil microorganism, is a high-efficiency and environment-friendly microbial fertilizer which can convert slow-acting potassium in soil into quick-acting potassium, and the development and utilization of the microbial fertilizer can improve the recovery rate of plants to stress, To maintain their normal physiological and chemical processes. Luo Wen *et al.* [13] screened and identified a strain K3 with strong potassium-dissolving ability from farmland soil, which was identified as *Pseudomonas*. Sun Huizhong *et al.* [14] isolated an LJP21strain with high water-soluble and available potassium content in fermentation broth from the rhizosphere soil of "Fengdan" peony, and identified it as *Exiguobacterium*. The sugarcane rhizosphere soil in Baitu Town, Gaoyao District, Zhaoqing was used as the sample, and the potassium releasing bacteria containing potassium feldspar powder was used as the isolation medium. *P. knackmussii*, *P. insulaes* and *C. zhejiangensi* were identified as seven strains of potassium releasing bacteria.

At present, there are many studies on the optimization of potassium releasing bacteria fermentation conditions, and most of them focus on the optimization of medium components and culture conditions. Li Xinxin [15] isolated a highly efficient potassium releasing bacterium G4 from red soil in Jiangxi, and optimized its fermentation conditions, which is helpful for the development of potassium fertilizer. Zhang Yuanyuan *et al.* [16] took the rhizosphere potassium-releasing bacteria of *Camellia oleifera* as the research object, and optimized the fermentation conditions of the strains. In this study, the strain GK-37 with the best potassium-releasing ability was selected to optimize the fermentation conditions, and the results showed that sucrose was the best carbon source for potassium releasing bacteria GK-37, to improve the potassium-releasing capacity and provide support in the preparation of the biological bacterial fertilizer.

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