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Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants

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ABSTRACT

Soil potassium supplementation relies heavily on the use of chemical fertilizer, which has a considerable negative impact on the environment. Potassium-solubilizing bacteria (KSB) could serve as inoculants. They convert insoluble potassium in the soil into a form that plants can access. This is a promising strategy for the improvement of plant absorption of potassium and so reducing the use of chemical fertilizer. The objectives of this study were to isolate and characterize tobacco KSB and to evaluate the effects of inoculation with selected KSB strains on tobacco seedlings. Twenty-seven KSB strains were isolated and identified through the comparison of the 16S ribosomal DNA. Among them, 17 strains belonged to *Klebsiella variicola*, 2 strains belonged to *Enterobacter cloacae*, 2 strains belonged to *Enterobacter asburiae*, and the remaining 6 strains belonged to *Enterobacter aerogenes*, *Pantoea agglomerans*, *Agrobacterium tumefaciens*, *Microbacterium foliorum*, *Myroides odoratimimus*, and *Burkholderia cepacia*, respectively. All isolated KSB strains were capable of solubilizing K-feldspar powder in solid and liquid media. *K. variicola* occurred at the highest frequency with 18 strains. Four isolates, GL7, JM3, XF4, and XF11, were selected for a greenhouse pot experiment because of their pronounced K-solubilizing capabilities. After being treated with the four KSB strains, plant dry weight and uptake of both K and nitrogen (N) by tobacco seedlings increased significantly. These increases were higher with the combination of KSB inoculation and K-feldspar powder addition. Isolate XF11 showed the most pronounced beneficial effect on plant growth and nutrient uptake by tobacco seedlings. Combining the inoculation of KSB and the addition of K-feldspar powder could be a promising alternative to commercial K fertilizer and may help maintain the availability of soil nutrients. Further studies are necessary to determine the effects of these bacterial strains on mobilization of potassium-bearing minerals under field conditions.

1. Introduction

Potassium (K) is essential to plant growth and development. It is involved in the adjustment of plant cellular osmotic pressure and the transportation of compounds in plants. It promotes the activation of enzymes, the utilization of nitrogen (N), and the syntheses of protein and sugar. It also boosts plant photosynthesis (Sparks, 1987). Plants can only take in K through the soil. In plants, K deficiency causes yellowing of the leaf edges, giving them a burned appearance. It can also cause slow growth and incomplete root development (Munson, 1985). Before the 1980s, almost all agricultural operations in China used only organic fertilizer, which did not contain sufficient K. Potassium fertilizer has been widely used since the 1980s, but the great increase in crop yield further exacerbated soil K deficiency, which stimulates the application of K fertilizer (Shi et al., 2008). The consumption of K exceeded 10.4 million tons in 2011, when 40% of all China's K fertilizer was imported (Qi et al., 2012). This indicates that soil K deficiency and K fertilizer deficiency have become important limiting factors for the development of agriculture in China (Sheng et al., 2002). The mass application of fertilizer can increase costs, decrease the efficiency of K fertilizer (Zhang et al., 2008), and damage the environment. An alternative to chemical K fertilizer is necessary for the sustainable development of agriculture.

One possible alternative could be to fully exploit the reservoir of K in the soil. Soil has rich reserves of K, among which only 1–2% can be directly absorbed by plants (Malinovskaya et al., 1990). About 90–98% of the soil K exists in silicate minerals such as K-feldspar and mica, which only release K slowly (Goldstein, 1994). This presents

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the paradox that soil is both K deficient and K rich. It may be more economically viable to transform the fixed slow-release K into available K that can be absorbed by plants. Studies have shown that a variety of soil microbes can release soluble K from K-bearing minerals such as K-feldspar, mica, and illite. These microbes release organic acid, which quickly dissolves rock and chelate silicon ions, releasing K ions into the soil (Bennett et al., 1998; Friedrich et al., 2004). It has been shown that *Bacillus mucilaginosus* and *Bacillus edaphicus* can generate polysaccharide and carboxylic acids, such as tartaric acid and citric acid, to solubilize K compounds (Richards and Bates, 1989; Lin et al., 2002). Using K-solubilizing microbes to increase the concentration of available K ions in the soil may mitigate K deficiency (Barker et al., 1998).

Tobacco is an economically important crop. One million acres of tobacco are planted each year in China (Zhu, 2008). Tobacco growth largely depends on the availability of K supply in the soil. Potassium is not only a crucial nutrient for tobacco; it also improves the flammability of tobacco leaves and so decreases the amount of tar produced during burning. Potassium content is one of the most significant indices of tobacco quality (Barshad, 1964; Mitchell, 1975).

Chemical K fertilizer is the largest source of available soil K. Using larger amounts of K fertilizers and promoting the availability of K can increase K content in tobacco leaves (Ma, 2006; Li et al., 2007). However, in addition to increasing costs, the extensive use of fertilizers can destroy soil structures, decrease the amount of organic matter in the soil, and aggravate environmental pollution. In recent years, the importance of food security and environmental sustainability has gained more attention, contributing to the rapid development of organic farming in China. The production of organic tobacco increased when strict limits were placed on the use of chemical fertilizer and pesticides. To complement the requirements for K in organic tobacco planting, minerals containing K, such as K-feldspar and mica, have been used. It is the largest and the most widely distributed reservoir of non-soluble K resources in China (Hu et al., 2005). Potassium feldspar provides K for plant growth through chemical and biological transformations. One type of transformation, the conversion of K-feldspar to K fertilizer, is difficult and costly. Another option, converting K-feldspar into plant available forms, can be accomplished using bacteria (Nishanth and Biswas, 2008). Some studies have shown that the application of K-solubilizing bacteria (KSB) and K bearing minerals increases the amount of available K in the soil and promotes plant uptake of K (Sheng et al., 2002; Sheng, 2005; Basak and Biswas, 2009; Abou-el-Seoud and Abdel-Megeed, 2012).

Using KSB to increase the concentration of K available in the soil has considerable potential for practical application. However, reports of KSB in tobacco rhizosphere and the effects of KSB on tobacco growth are lacking in the literature. Therefore, the goals of this study are to (1) isolate and characterize tobacco KSB and (2) study the effects of KSB on the release of K from K-feldspar and their effects on tobacco growth.

2. Materials and methods

2.1. Materials

2.1.1. Soil sampling

Fifteen rhizospheric soil samples of approximately 500 g each were collected from three tobacco fields (five samples per field) covering a total area of 16,667 m^2 in June 2010. The three tobacco fields were located at Gulan County in Luzhou City, Sichuan Province, Jimo County in Qingdao City, Shandong Province, and Xianfeng County in Enshizhou Region, Hubei Province. The rhizosphere soil was sampled in June, which is peak growing season for tobacco. The soil samples were taken within 10 cm of tobacco plants. The top 5 cm of the topsoil was removed before the collection of soil samples from 6 to 10 cm soil layer, where roots were concentrated. From about 0 to 2.5 mm away from the root surface, a zone of soil is located that is significantly influenced by living roots and is referred to as the rhizosphere. Rhizosphere soil and roots were separated from the bulk of the soil by hand. Ten plant samples were taken randomly in every 667 m^2 (1 μm) and 10 g of rhizosphere soil was collected around each plant. The mixture obtained from 10 plant samples constituted a sample. All soil samples were sealed in sterilized ziplock bags, stored in an ice chest, and used within 8 h of collection. Some physicochemical properties of the experimental soils were determined by using the following methods and the results were shown in Table 1. Total organic C was determined by potassium dichromate method (Lu, 1999). Alkali-hydrolyzable N was determined by alkali solution method (Lu, 1999). Available P was determined by molybdenum blue colorimetric method after extraction by sodium bicarbonate (Lu, 1999). Available K was determined by ammonium acetate extraction and flame photometric method (Lu, 1999).

2.1.2. Mineral samples

K-feldspar powder (containing ≈11% K_2O; manufactured by Qingdao Xinyun Metallurgical Materials Co., Ltd) was purchased and passed through a #100 sieve. The sieved powder was submerged in sterilized water for three days to eliminate soluble K.

2.1.3. Culture substrates

Aleksandrov medium (Hu et al., 2006): 0.5% glucose, 0.05% magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), 0.0005% iron (III) chloride ($FeCl_3$), 0.01% calcium carbonate ($CaCO_3$), 0.2% calcium phosphate ($CaPO_4$), and 0.2% potassium aluminum silicate ($KAlSi_3O_8$), pH 7.0–7.5.

Solid nutrient agar: 0.3% beef extract, 0.5% peptone, 18% agar, pH 7.0–7.5.

Solid Gauze's medium no. 1: 2% soluble starch, 0.0005% sodium chloride (NaCl), 0.1% potassium nitrate (KNO_3), 0.0005% dipotassium hydrogen phosphate trihydrate ($K_2HPO_4{\cdot}3H_2O$), 0.0005% magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), 0.00001% iron (II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), pH 7.4–7.6.

2.2. Isolation of KSB from tobacco rhizosphere

Modified solid Aleksandrov medium (K-feldspar powder was added to the solid Aleksandrov medium as the sole source of K) was used to screen KSB in the soil. Five grams of soil sample from the rhizosphere was diluted to 10^{-5}–10^{-6} suspension using 100 mL sterilized water. The soil suspension of 50 μL was then spread over a petri dish containing culture medium for KSB. The petri dishes were placed in the incubator at 30 °C for 72 h. Each of fast-growing isolates that showed solubilization zones on the agar plates were retested in triplicate using the methods described above. The diameter of the solubilization zone was measured in cm and the values are reported as mean ± standard deviation for each sample. Screened strains were transferred to a slant with solid nutrient agar medium for bacteria, or to solid Gauze's medium no. 1 for actinomycete, which were then stored at 4 °C for future use.

2.3. Assessment of the solubilization ability of soil KSB from tobacco rhizosphere

2.3.1. Incubation of KSB on shaker

K-feldspar powder was added to liquid Aleksandrov medium as the sole source of K to test the ability of the isolates to solubilize the mineral. To further analyze the ability of KSB in solutions to solubilize K, 50 mL of Aleksandrov solution was added into 250 mL