



Isolation and Characterization of Potassium Solubilizing Bacteria in some Vietnamese Soil Samples

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ABSTRACT: Potassium (K) is the third essential macroelement for plant growth after nitrogen and phosphorous. However, over 90% K exists in the soils in insoluble mineral form that is unavailable for plants uptake. Soil bacteria can dissolve potassium from insoluble K-bearing minerals. This study was carried out with aims to characterize potassium solubilizing bacteria isolated from soils. From different soil samples collected from 4 different provinces where eleven bacterial strains of potassium solubilizing bacteria were isolated on Aleksandrov containing potassium aluminium silicate media. Among them, two strains namely C1.1 and YN3 showed significant potassium solubilizing capacity based on the potassium solubilizing index. They caused maximum potassium solubilization when they were cultured in medium with pH.7.0, temperature 30°C, and KCl was used as a potassium source. Strain C1.1 was identified as *Burkholderia* sp. based on the nucleotide sequence of 16S rRNA, and named as *Burkholderia* sp. C1.1. The results have provided useful information on the cultural conditions of potassium solubilizing bacteria for sustainable agriculture system.

Keywords: Potassium, Potassium-solubilizing bacteria (KSB), solubilisation index, solubilisation effect.

Abbreviations: IAA, Indole-3-acetic acid; K, Potassium; N, Nitrogen; P, Phosphorus; BS: Bacterial strain; CD: Colony Diameter; SE: Solubilization efficiency.

I. INTRODUCTION

Among the essential nutrients needed for plants, Potassium (K) is of great importance, just only after Nitrogen (N) and Phosphorus (P). K is necessary for the activity of more than 80 enzymes involved in biological processes in plant and animal cells, such as energy exchange, nitrate reduction [1-2], maintenance of cellular pressure, hydrolysis, sugar and starch transportation, improvement of crop quality through enhancing photosynthesis, protein synthesis, plant resistance to insects and diseases, tolerating adverse conditions [3]. In soils, K exists in the form of K ion (K⁺) as exchangeable cations; non-exchangeable K is fixed on the surface of clay minerals and organic matter or fixed by other minerals and presents in some minerals containing K [4]. The total K content in soil ranges from 0.5 to 3%, but only 2-10% of K content in the soil is at bioavailable state with plants. The rest, approximately 90-98%, often associates with other minerals and they are difficult to absorb by plants [4-6].

Currently, in agricultural production, many high yielding crops and numerous kinds of chemical fertilizers are exploited, thus usable K stored in soil is depleted very quickly. Soil microorganisms affect the bioavailability of minerals in the soil, participate in the circulation of ions and improve soil fertility [7, 3]. Many worldwide studies showed that a diverse group of ubiquitous

microorganisms including arbuscular mycorrhizae, yeast, fungal and rhizobacterial species are capable of solubilizing potassium minerals with high efficiency such as *Acidithiobacillus ferrooxidans*, *Pseudomonas*, *Burkholderia*, *B. mucilaginosus*, *B. circulans*, *B. Edaphicus*, *B. megaterium*, and *Paenibacillus* sp, *Rhizobium pusense*, *Aspergillus terreus*, *Glomus mosseae*, *G. intraradices*, *A. niger* [8-12].

They can either help plants absorb more nutrients or strengthen abiotic and biotic stresses [13]. Isolation and selection of strains capable of solubilizing potassium minerals contribute to the conservation of resources, minimizing the risk of environmental pollution by using chemical fertilizers. Therefore, this study aimed to isolate and evaluate some biological characteristics of the bacteria that solubilize potassium in the soil

II. MATERIALS AND METHODS

Isolation of potassium solubilizing bacteria. All soil samples were collected from the farming areas of Hanoi, Nam Dinh, Thai Binh and Bac Giang provinces, were kept in plastic bags and transferred to the laboratory. After treatment, the soil samples were diluted and bacteria strains were isolated on Aleksandrov's medium containing insoluble potassium minerals. This medium includes 5.0 g of glucose; 0.5 g of MgSO₄.7H₂O; 0.1 g of CaCO₃; 0.005 g of FeCl₃; 2.0 g of CaPO₄; 2.0 g of potassium aluminum silicate and 0.5

g of yeast extract per one liter. The results were observed after three days, bacterial strains which were able to solubilize K would create a transparent ring around their colonies. These strains were purified and spotted on Aleksandrov's medium to calculate: (1) Potassium solubility index (SI) by the formula of Setiawati and Mutmainnah [4]; Jabin and Ismail [14]: $SI = \text{Colony diameter} + \text{potassium solubilizing ring diameter, mm} / \text{Colony diameter, mm}$; (2) Potassium Solubilization Efficiency (SE) = $(\text{Solubilization ring diameter, mm} / \text{colony diameter, mm}) \times 100$.

Effects of temperature, pH and K source. The selected bacterial strains were cultured on Aleksandrov's medium at 25°C, 30°C, 35°C, 40°C and 45°C; pH: 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, respectively. K sources: KCl, KNO₃ and K₂SO₄. Diameters of colonies and potassium solubilizing ring were measured after 3 days to calculate the potassium solubility index.

Investigation of IAA synthesizing ability. Selected bacteria were cultured in LB broth (containing w/v: 1.0% peptone; 0.5% high yeast and 1.0% NaCl; pH 7.0; solid medium supplemented with 2% agar) added 10 g/l of L-tryptophan kept in dark and shaken for 180 rpm. After 3 days, bacterial culture media were centrifuged at 10,000 rpm, 10 min at 4°C. The supernatant was collected, added Salkowski's reagent and left for 15 min at room temperature. The concentration of IAA synthesized by each strain was determined by measuring OD value on the UV-VIS spectrophotometer at 530 nm wavelength and calculating the concentration of IAA synthesized based on the calibration equation [15].

Biological characteristics of strains. The selected isolates were investigated for cell shape, catalase, gram reaction, citrate, urea, gelatin following the method described by Schaad *et al.* [16].

DNA extraction and isolates sequencing. Total genomic DNA was performed and purified following the method reported by Pospiech & Neumann [17]. In order to confirm the samples, the purified PCR samples were directly sequenced by 16s RNA region from two types of primers combined and analysed by the service of Phu Sa company (Applied Biosystems). The length of

product was approximately 1500 bp. The base sequences of each sample were used by BLAST search for classification on the National Center for Biotechnology Information (NCBI).

Statistical analyses. The statistical analysis of data and mean comparisons were performed by using Excel 2016

III. RESULTS AND DISCUSSION

Isolation of potassium solubilizing bacteria in vitro condition. The soil samples after being treated and diluted to a concentration of 10⁻⁵-10⁻⁶ were spread onto Aleksandrov's agar medium containing potassium aluminium silicate and placed in the incubator at 30°C, concentration was at 10⁻⁵ – 10⁻⁶. After 3 days, the results were observed, bacterial strains capable of solubilizing potassium created a ring around their colonies as shown in Fig. 1.

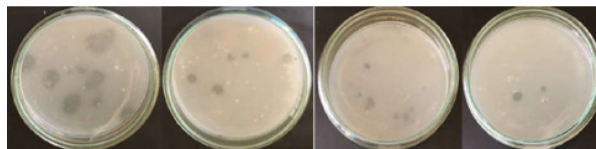


Fig. 1. Bacteria capable of resolving potassium on the Aleksandrov's medium.

As results shown in Table 1, 11 strains of potassium solubilizing bacteria were isolated, purified, and then inoculated on Aleksandrov's medium to evaluate potency and potassium solubility index. After 3 days of culture, all bacterial strains generated bright rings around their colonies on Aleksandrov's medium. The diameter of colonies and potassium solubilization rings of 11 bacterial strains are shown in Table 1. Strain C1.1 had the largest solubilization ring (13mm), followed by strain YN3 (12mm). The solubility index and potassium solubilization efficiency of these two strains (C1.1 and YN3) were the highest, respectively 3.25 and 225.0%, 3.0 and 200.0% respectively.

Table 1: Potassium dissolution ability of isolated bacterial strains.

BS	CD (d,mm)	K Solubilizing (D, mm)	SI (D/d)	SE (SF, %)
TT1	3.0	7.0	2.33	133.3
TT2	2.0	5.0	2.50	150.0
TT3	3.0	8.0	2.67	166.7
TT4	3.0	6.0	2.00	100.0
TT5	4.0	11.0	2.75	175.0
YN1	3.0	8.0	2.67	166.7
YN2	3.0	8.0	2.67	166.7
YN3	4.0	12.0	3.00	200.0
YN4	3.0	8.0	2.67	166.7
C1.1	4.0	13.0	3.25	225.0
C1.2	7.0	11.0	1.57	57.1

BS: Bacterial strain, CD: Colony Diameter, K: Solubilizing, SI value, SE: Solubilization efficiency

According to the report of Setiawati and Mutmainnah [4], the potassium solubility of bacteria was classified as a low rate if SI was lower than 2.00, the medium was higher than 2.0 to 4.0 and high if SI was over than 4.0. The bacterial strains in our experiment had medium potassium solubility, except strain C1.2 (SI<2.0). Many

studies on isolation and selection of potassium solubilizing bacterial strains in soil have been published. Of which, Jabin and Ismail [14] selected *Pseudomonas* sp. (KSB-PD-1-A) with a potassium solubility index of 3.57 and solubilization efficiency of 257.32%. Pirhadi *et al.*, (2016) selected *Enterobacter cloacae*, *Bacillus*

pumilus, *Pseudomonas* sp. whose potassium solubility index was in the range of 0.25 to 2.00, respectively [18]. Bashir *et al.*, (2017) described that the soil samples were taken from un-fertilized fields isolated *Pseudomonas* sp. KSB-5 with SI index of 4.66 [3]. Pratama *et al.*, [5] reported selecting 6 strains of bacteria from agricultural and mine soil with SI index from 2.05 to 6.77. Similarly, the strains Burkholderia, Serratia and *Pseudomonas* putidain research of Mursyida *et al* had SI index from 0.31 to 3.73, respectively [7]. In the study published by Setiawati and Mutmainnah [4], most bacterial strains had moderate potassium solubility ($2.0 < SI < 4.0$), only approximately 13.33% of experimented strains possessed strong potassium solubility with $SI > 4.0$. In our experimental results, SI values of the soil samples were ranged from 1.57 to 3.24 which were slightly lower than the results of the above authors. It may explain possibly due to soil structure, properties and nutrient composition at areas where the sample was collected. Also, IS variations are significantly affected by the speed of microbial growth and the ability of microbial metabolism [4]. The two strains C1.1 and YN3 showed the highest SI and SE indices among experimented bacterial strains, therefore, they were chosen for further studies.

Effect of temperature and pH of culture medium. pH and temperature of culture medium are directly affected by metabolic activities of cells. Each microorganism strain has a favorable temperature and pH. If these values are different from the favourite values, cells grow poorly or stop metabolism-related activities or die. Two selected bacterial strains were grown on Aleksandrov's medium containing 0.2% potassium aluminium silicate at different temperatures 25°C, 30°C, 35°C, 40°C and 45°C. After 3 days of culture, the diameter of potassium solubilizing rings and colonies were measured to calculate SI value (Fig. 2). Both strains showed the strongest solubility of potassium at 30°C, the SI index of strain C1.1 was 3.23 and YN3 was 3.4. Moreover, at 35°C, the solubility indices of C1.1 and YN3 strains were 3.08 and 3.25, respectively, that were approximate to the indices at 30°C. When the temperature was increased to 40°C to 45°C, potassium solubilizing efficiency of two strains tended to decrease, at 45°C, the potassium solubility of strain C1.1 decreased strongly while strain YN3 was no longer solubilized potassium.

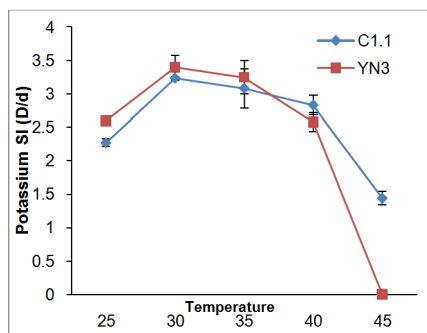


Fig. 2. Effect of temperature on potassium solubility of strain C1.1 and YN3.

Our findings have consisted of the Results of Verma *et al.*, [19] and Prajapati and Modi [20] who confirmed that

potassium solubilizing bacterial strains from soil exhibit the strongest potassium solubility at a temperature range of 30-35°C and a sharp decrease at 45°C. Two bacterial strains C1.1 and YN3 were grown on Aleksandrov's medium added 0.2% of potassium aluminum silicate at pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, at 30°C. The result of potassium solubility index was shown in Fig. 3.

Two experimented bacterial strains exhibited the ability to solubilize potassium at pH values from 5.0 to 9.0. SI index of strain C1.1 ranged from 2.67 to 3.23, strain YN3 from 2.2 to 3.5. SI indices of these two strains were the highest at pH 7.0 and gradually decreased when pH was increased to 8.0 and 9.0, respectively.

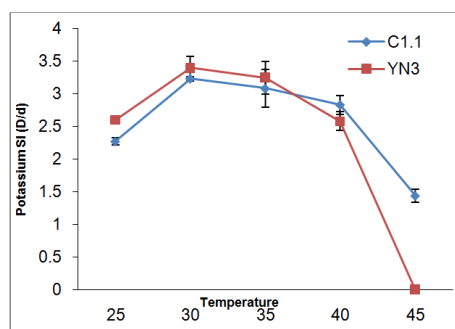


Fig. 3. Effect of pH on potassium solubility of strain C1.1 and YN3.

At pH 4.0, two strains were no longer be capable of solubilizing potassium. Some other researchers also reported the differences in the ability of microorganisms to solubilize potassium at different pH conditions. Parmar and Sindhu (2013) isolated two bacterial strains WPS73 and NNY43 which were able to solubilize potassium the best at pH 7.0 when pH was increased, the solubility of two strains decreased [21]. Similar results were published in 2016 by Verma *et al.*, [19].

Effect of potassium sources. To investigate the effect of different potassium sources on potassium solubility of strains C1.1 and YN3, we cultured these two strains in Aleksandrov's medium supplemented with KCl, KNO₃ or K₂SO₄. The control was set as Aleksandrov's medium containing KAlO₆Si₂.

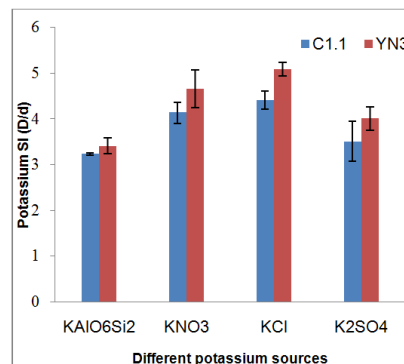


Fig. 4. Effect of potassium sources on potassium solubility of strains C1.1 and YN3.

After 3 days of culture, the obtained results showed that SI indices of two strains C1.1 and YN3 when cultured on medium containing experimental potassium sources

were all higher than that of $KAlO_6Si_2$ (Fig. 4). These two strains disclosed the maximum potassium solubility when the potassium source in the environment was KCl, followed by KNO_3 . Verma *et al.*, [19] reported that KCl was a favorable potassium source for potassium solubilizing microorganisms due to their highest SI index. Parmar and Sindhu (2013) [21] confirmed that bacterial strain WPS73 and NNY43 solubilize potassium the most when the potassium source was KCl.

Investigation of IAA synthesizing ability of selected strains. The rhizosphere microorganisms in addition to the ability to mineralize insoluble chemical compounds in soil to provide nutrients for plants, they also synthesized phytohormone stimulating plant growth such as IAA, gibberellin to help plants develop. To determine the content of Indole-3-acetic acid (IAA) synthesized by strains of bacteria, a calibration curve between the OD and IAA content ($\mu\text{g/l}$) in the environment is required. Thus, the ability to synthesize IAA of bacterial strains could be assessed.

Based on the calibration curve (Fig. 5), the correlation coefficient $R^2 = 0.9918$, very close to the value 1. The correlation between IAA concentration ($\mu\text{g/ml}$) in solution and OD value at 530 nm was very tight, could be used to determine IAA concentration synthesized by selected strains.

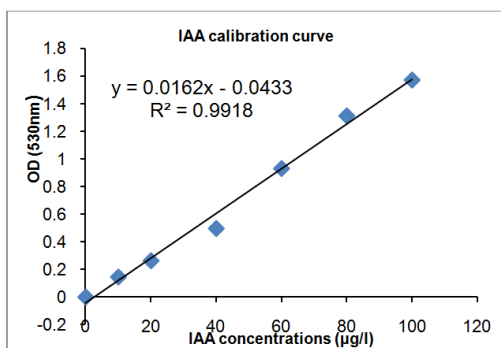


Fig. 5. Calibration curve of correlation between OD530 value and IAA content.

Bacterial samples were cultured in LB broth supplemented with L-tryptophan, shaken for 180 rpm, protected from light. After 3 days of culture, the culture medium was centrifuged, the microbial biomass was discarded, the supernatant was collected and added Salkowski's reagent, the sample containing IAA would turn pink, the intensity of color depends on the content IAA synthesized by strains of bacteria.

These supernatant samples were measured at wavelength $\lambda = 530$ nm, from the obtained OD values, based on the IAA calibration curve, IAA content synthesized by the bacterial strains was determined (Fig. 6). Two bacterial strains showed the ability of IAA biosynthesis since they both changed the reagent color to pink.

Biological characteristics of strains. Strain C1.1 and YN3 were able to synthesize 9.65 and 11.4 μg IAA/ml, respectively. After evaluating the ability of solubilizing potassium at different culture conditions, two strains C1.1 and YN3 were preliminarily investigated some biological characteristics such as cell morphology and cultural characteristics. The results are shown in Table 2.

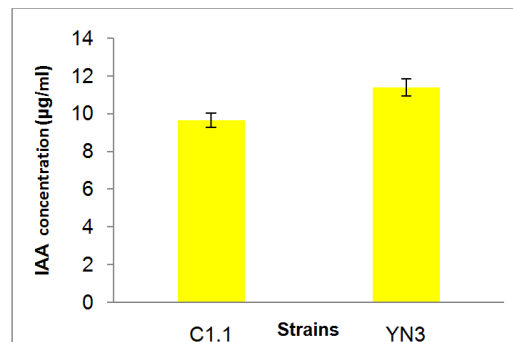


Fig. 6. IAA synthesizing ability of strain C1.1 and YN3.

Table 2: Biological characteristics of strain C1.1 and YN3.

Characteristic	Strain YN3	Strain C1.1
Cell shape	Rod	Rod
Gram	+	+
Catalase	+	+
Citrate	+	+
Urea	+	+
Gelatin	+	-
Mobility	+	+

Strain C1.1 was identified based on analysis of 16s rRNA nucleotide sequence and compared the results with BLAST software (blasthome). (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blastn). The results were strain C1.1 closely related to bacterial strains Burkholderia (Fig. 7), thus, this strain was namely given as Burkholderia sp. C1.1.

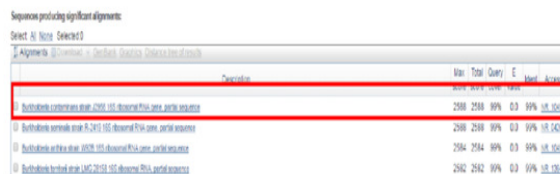
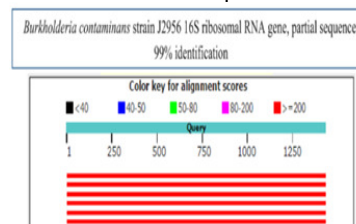


Fig. 7. Results of comparing 16s rRNA nucleotide sequence of C1.1 strain on Genbank.

IV. CONCLUSIONS

From various soil samples collected from different provinces (Nam Dinh, Thai Binh, Hanoi and Bac Giang), 11 bacterial strains which were able to solubilize K solubility were isolated, among them, strain C1.1 and YN3 possessed the strongest ability to solubilize K based on solubility index and solubilization efficiency. Two strains C1.1 and YN3 revealed the strongest potassium solubility when cultured in the medium supplied with KCl as K source, at pH 7.0 and 30°C. These two strains were also able to synthesize IAA. The

strain C1.1 was closely related to Burkholderia strain, hence, it was named as Burkholderia sp. C1.1.

V. FUTURE SCOPE

Further study should be examined or improved the performance and stability of the potassium solubilizing bacteria in the soil for enhanced the strains efficiency; and practically investigated the relationships the mechanism and interactions of these bacteria with the specific plant response and uptake of K.

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