

## EVALUATION OF SOME BIOPHYSICAL CHARACTERISTICS OF FIVE SILICATE SOLUBILIZING BACTERIA ISOLATED FROM DIFFERENT ECOSYSTEMS

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### SUMMARY

Silicate solubilizing bacteria (SSB) are key microorganisms to solubilize silicate minerals in the soil. Silicon helps to increase the growth and yield of plants and to enhance the environmental stress tolerance capability of plants. The aim of this study was to evaluate the effect of several factors like pH, salinity, and temperature on silicate solubilizing capacity of five selected SSB. Moreover, phosphorus solubilizing, nitrogen-fixing and indole-3-acetic acid (IAA) synthesizing capacity of these five bacteria were also tested. Liquid soil extract medium containing 0.25%  $Mg_2O_8Si_3$  was used in this study. Abilities of bacteria in phosphorous solubility, nitrogen fixation, and IAA synthesis were tested in NBRIP, Burk's and NBRIP containing 100 mg  $L^{-1}$  tryptophan media, respectively. The results of the study indicated that five SSB showed their high capacity in silicate solubilization at pH 7.0, NaCl 0.0% and 35°C. However, at a concentration of NaCl 0.5%, these five SSB still solubilized well silicate mineral. Moreover, they also solubilized effectively three different insoluble phosphate sources of  $Ca_3(PO_4)_2$ ,  $AlPO_4$  and  $FePO_4$  with a range varied between 105.8 and 928.7 mg  $P_2O_5 L^{-1}$ , 33.9 and 49.6 mg  $P_2O_5 L^{-1}$ , and 1.94 and 34.1 mg  $P_2O_5 L^{-1}$ , respectively. They also fixed biologically nitrogen with a range from 1.37 to 5.09 mg  $NH_4^+ L^{-1}$  after 2 incubation days. Finally, they also showed their ability in IAA synthesis with an amount between 4.85 and 51.5 mg IAA  $L^{-1}$ . In short, these five SSB in this study not only had the ability in silicate solubilization but also had other functions in plant growth promotion.

**Keywords:** *biophysical properties, IAA synthesis, nitrogen fixation, phosphate solubilization, salt concentration, silicate solubilizing bacteria*

### INTRODUCTION

Silicon (Si) has been proven to have many benefits for the growth and development of many plant species (Liang *et al.*, 2007). Although the amount of Si is abundant naturally in the earth's crust, it mostly presents in insoluble forms that cannot be readily absorbed by plant roots. In nature, silicates are solubilized and released into the soil from rocks, minerals by weathering process, the biological activity of plant roots and microorganisms. Among them, silicate solubilizing bacteria (SSB) can play an efficient role in solubilizing insoluble forms of silicates into a soluble form ( $H_4SiO_4$ ) to increase soil fertility, plant growth and yield as well as defense mechanisms of plants against pests and diseases (Vasanthi *et al.*,

2012). Moreover, nitrogen fixation, phosphorus solubilization and IAA synthesis by microorganisms play other important roles in various physiological processes of plants and importantly contribute to enhance plant growth and productivity (Khan *et al.*, 2009). A study of Osman (2009) showed that the vitally important impact of environmental factors like pH, temperature and salt concentration on silicate solubilization of two isolated silicate solubilizing bacteria, *Bacillus circulans* and *Bacillus mucilaginosus*. In detail, they were able to grow in a wide range of pH, temperature and NaCl concentration including 7-9, 4-45°C and 0.25-1.0%, respectively. Furthermore, many studies have focused on the efficient application of silicate solubilizing, nitrogen fixing, phosphate solubilizing and IAA producing bacteria on crop growth and

yield, however, the studies about the capacity in nitrogen fixation, phosphate solubilization and IAA synthesis of the silicate solubilizing bacteria has still limited. Driving from these matters, the present study was carried out to evaluate influences of some environmental factors on silicate solubilizing ability of five selected bacteria, simultaneously, to test of their nitrogen fixing, phosphate solubilizing and IAA synthesizing capacity.

## MATERIALS AND METHODS

### Source of bacteria

Five isolated silicate solubilizing bacteria including *Ochrobactrum ciceri* TCM\_39 (TCM\_39), *Microbacterium neimengense* MCM\_15 (MCM\_15), *Klebsiella aerogenes* LCT\_01 (LCT\_01), *Olivibacter jilunii* PTST\_30 (PTST\_30) and *Citrobacter freundii* RTTV\_12 (RTTV\_12) were isolated from intensive bamboo, sugarcane and rice soil samples, as well as earthworm intestine and earthworm feces in sandy soil were used in this study (Tran Vo Hai Duong, Nguyen Khoi Nghia, 2018).

### Impact of some environmental factors on the silicate solubilizing capability of five selected SSB under the laboratory conditions

#### pH

**Bacterial source:** bacterial strains were enriched in 100 mL flasks containing 20 mL TSB medium in 3 days. The TSB medium composition in 1 L included 30 g tryptone soya broth in 1 L distilled water. The bacterial broth culture suspension after incubation was aseptically transferred into sterilized 50 mL Falcon tube, centrifuged at 6,000 rpm in 5 mins, discarded the supernatant, refilled with 20 mL sterilized distilled water and repeated this whole procedure for another 3 times. Finally, bacterial pellet was adjusted to  $OD_{600nm}=0.7$  with sterilized MQ water by Multiskan Spectrum spectrophotometer (Thermo Scientific). Additionally, bacterial source prepared for evaluating salt content, temperature, phosphorous solubility, nitrogen fixation and IAA synthesis was carried out as the same manner.

**Experimental design:** Experiment was performed in sterilized 250 mL Erlenmeyer flask containing 100 ml mineral salt medium supplying 0.25% magnesium trisilicate (medium composition contained 1.0 g glucose, 0.5 g  $K_2HPO_4 \cdot 3H_2O$ , 100 mL soil extract, 900 mL distilled water, and pH of 7.0-7.2. Besides, soil extract solution was prepared

by collecting approximately 40 g of soil and then added 50 mL of water and stirred for 20 mins. The slurry was allowed to settle down, then filtered firstly through a Kimwipe tissue, and after that, the filtrate was passed through a Number 1 Whatman filter paper placed on a funnel (Bold, 1949). Four treatments including pH3, pH5, pH7 and pH9 were established. Each treatment had 3 replicates and each pH level treatment had a control treatment without microbial inoculation. To prevent fungi growth and contamination, the liquid medium was added with 10 mg L<sup>-1</sup> Vimetatin 56 fungicides after autoclaving. An aliquot of one ml of prepared bacterial suspension (10<sup>8</sup> CFU mL<sup>-1</sup>) was transferred into 250 mL sterilized Erlenmeyer flask. The flasks were incubated at 30 ± 2°C on rotary shaking incubator (120 rpm) for 10 days in the dark as described by Sheng *et al.* (2008). Soluble Si concentration in liquid medium was determined at day 0, 2, 4, 6, 8 and 10. The method for determining soluble concentration of Si in liquid medium was adopted from Hallmark *et al.* (1982) as following procedure: firstly, transferred one ml of bacterial suspension into 50 mL Falcon tube, after that 2.5 mL of 20% ammonium acetate were added into each tube, capped tightly, and shook vigorously for 10 sec, uncapped and added 1.0 mL of 0.3 M ammonium molybdate into each tube. After 5 minutes for standing, an amount of 0.5 ml 20% tartaric acid was added into each tube, capped tightly and shook vigorously to thoroughly mix reagents, uncapped, added 0.5 mL reducing solution including 2 g Na<sub>2</sub>SO<sub>3</sub>, 0.4 g C<sub>10</sub>H<sub>9</sub>NO<sub>4</sub>S and 25 g NaHSO<sub>3</sub> in 250 mL distilled water, capped tightly and again shook vigorously. Finally, samples were diluted to a final volume of 7.5 mL with 20% acetic acid solution, capped and shook well. To develop the color of samples, samples were left for 60 minutes at room temperature and tubes were shaken vigorously to mix well prior to determining absorbance using a spectrophotometer calibrated at 815 nm. A series of the diluted standard solution of Si were prepared by adding 0, 40, 80, 120, 160 and 200 µL of 100 mg L<sup>-1</sup> Si standard solution to 7.5 mL polyethylene tubes and all reagents were added into the standard solution as the same manners as the real samples as described above.

#### NaCl concentration

To evaluate the impact of the salt content of liquid medium on Si solubilization capacity of five selected SSB, an experiment was conducted in the

same manner as the pH experiment. However, liquid minimal salt medium including different NaCl concentration (0%, 0.15%, 0.3% and 0.5% NaCl) were considered as different treatments.

#### **Temperature**

To assess the impact of different ambient air temperature on Si solubilization capacity of five selected SSB, an experiment was conducted as the manner as the pH experiment. However, different ambient air temperatures of 25, 35 and 45°C used to incubate the bacteria were considered as different treatments.

#### **Phosphorous solubilizing, nitrogen-fixing and IAA synthesizing capacity of five selected SSB**

##### ***Phosphate solubilizing capability***

Phosphate solubilizing capability of five SSB was performed with 3 different insoluble phosphate mineral sources including  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{FePO}_4$  and  $\text{AlPO}_4$  as considered as three different treatments. An aliquot of one ml already prepared bacterial solution ( $10^8$  CFU  $\text{mL}^{-1}$ ) was transferred into sterilized 250 mL Erlenmeyer flask containing 100 mL liquid NBRIP medium. The NBRIP medium composition in 1.0 l ( $\text{g.l}^{-1}$ ) contained 10 g glucose; 5.0 g  $\text{Ca}_3(\text{PO}_4)_2$ ; 5.0 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ; 0.25 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ; 0.2 g KCl and 0.1 g  $(\text{NH}_4)_2\text{SO}_4$  and pH = 7.0. All samples were incubated at  $30 \pm 2^\circ\text{C}$  on an orbital shaker (120 rpm) for 10 days in the dark under the laboratory conditions. Soluble phosphate concentration in liquid medium was determined as phosphomolybdate method at day 0, 2, 4, 6, 8 and 10. An aliquot of five ml bacterial suspension was filtered through a syringe with a  $0.45 \mu\text{m}$  membrane filter before phosphate analysis. Two mL of bacterial suspension were transferred into 2 mL Eppendorf, centrifuged at 14,000 rpm in 5 mins, harvested the bacterial pellet, performed 10 fold diluted solution series with deionized water, after that, an aliquot of 5 mL of solution was put into sterilized 30 mL test tube, added 1 mL B solution (Sample: B solution with a ratio of 5:1 (v/v)), mixed well in 1 min with vortex, left the samples at room temperature for 30 mins, and finally soluble phosphate concentration was determined at 880 nm by spectrophotometer.

##### ***Nitrogen-fixing capacity***

An aliquot of one ml already prepared bacterial suspension ( $10^5$  CFU  $\text{mL}^{-1}$ ) was transferred into 250 mL sterilized Erlenmeyer flask containing 100 ml Burk's medium. N-free Burk's medium containing

following ingredients ( $\text{g L}^{-1}$ ) 20.0 g sucrose; 0.64 g  $\text{K}_2\text{HPO}_4$ ; 0.16 g  $\text{KH}_2\text{PO}_4$ ; 0.20 g  $\text{MgSO}_4$ ; 0.20 g NaCl; 0.05 g  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ; 5.0 mL  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (0.05%); 5.0 mL  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (0.3%) and pH = 7.0. All samples were incubated at  $30 \pm 2^\circ\text{C}$  on an orbital shaker (120 rpm) for 10 days in the dark under the laboratory conditions. Nitrogen amount fixed by bacteria in liquid medium was determined at day 1, 2, 3, 4, and 5. One mL of bacterial suspension was transferred into 2 mL Eppendorf, centrifuged at 12,000 rpm in 5 mins, took an amount of 0.5 mL supernatant into 50 mL Falcon, added successively 2 mL of deionized water, 0.5 mL EDTA, 1 mL Nitroprusside and 2 mL Sodium hypochloride into each Falcon tube, mixed well the samples by vortex in 1 min and then left the samples at the room temperature for 30 mins and finally, the amount of nitrogen concentration was determined at 636 nm by a spectrophotometer.

##### ***Indole-3-Acetic Acid (IAA) synthesizing capacity***

The experiment was conducted in a 100 mL flask with 3 replicates corresponding to 3 incubation flasks. An aliquot of one mL of prepared bacterial suspension ( $10^8$  CFU  $\text{mL}^{-1}$ ) was transferred into flasks containing 29 mL liquid NBRIP medium with supplementation of  $100 \text{ mg L}^{-1}$  tryptophan. All samples were incubated at  $30 \pm 2^\circ\text{C}$  in a rotary shaker at 120 rpm for 10 days in the dark and under the laboratory conditions. Synthesized IAA concentration by bacteria in liquid medium was determined at day 0, 2, 4, 6, 8, 10, and 12. An aliquot of bacterial suspension (1.5 mL) was transferred into 2 mL Eppendorf, centrifuged at 13,000 rpm in 10 mins, then transferred 1 mL supernatant into test tubes containing 2 mL R2 reagent, mixed well the solution with vortex, left the samples at room temperature for 10 min in the dark, finally the synthesized IAA concentration in liquid medium was determined at 530 nm by spectrophotometer. The composition of one liter of R2 reagent containing 1 L  $\text{H}_2\text{SO}_4$  10.8 M and 4.5 g  $\text{FeCl}_3$ .

#### **Data analysis**

The data was processed with Microsoft Office Excel 2013 and SPSS 22.0 software.

## **RESULTS**

### **Impact of different pH levels of liquid medium on silicate solubilizing capacity of five silicate solubilizing bacteria**

The impact of pH of the medium on silicate

solubilization capacity of five selected SSB (PTST\_30, LCT\_01, RTTV\_12, TCM\_39 and MCM\_15) presented in Fig. 1 indicated that the silicate concentrations of all pH treatments of five bacteria had the same pattern as the concentration of Si in the liquid medium increased steadily at the stage of 0-4 days of incubation then dramatically increased on day 4, and lasted until day 6. The highest peak of Si concentration in liquid medium was detected at day 6 and then decreased quickly afterward until the end of the experiment. For all bacteria tested, soluble silicate concentrations were found to be highest almost on day 6. Moreover, all five bacteria showed their best silicate solubilizing

capability in the treatments with pH 7 where the highest silicate concentrations reached between 32.3 and 54.9 mg L<sup>-1</sup>. Furthermore, the concentration of Si dissolved by five bacteria was still high in treatments with pH of 3, 5, and 9. The soluble silicate concentrations dissolved by five SSB in treatments with pH 3, 5 and 9 were varied between 9.86 and 23.9 mg L<sup>-1</sup>, 20.2 and 35.7 mg L<sup>-1</sup>, and 13.2 and 1.5 mg L<sup>-1</sup>, respectively. As taking into account to compare treatments having the same pH level in the liquid medium but different bacteria with each other, the result showed that there was not much difference about silicate concentrations at any sampling time.

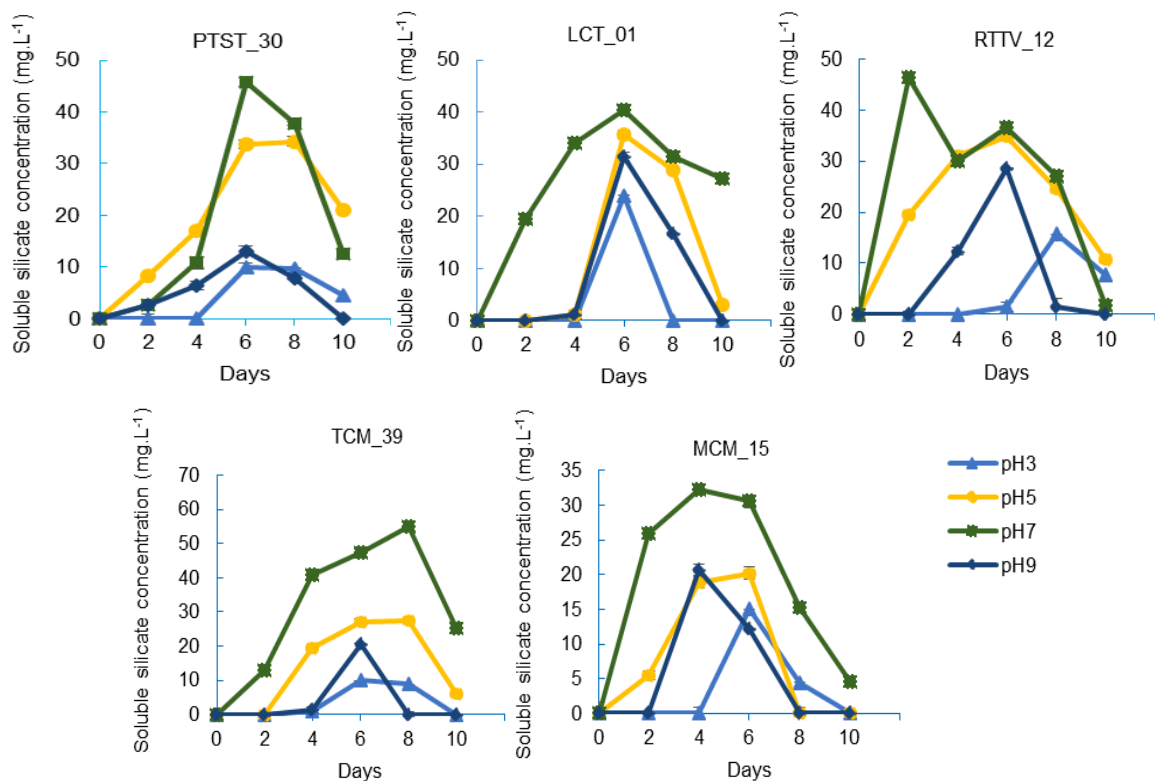


Figure 1. Silicate concentrations solubilized by five bacteria in different pH treatments.

**Influence of different NaCl concentrations of liquid medium on silicate solubilization capacity of five silicate solubilizing bacteria**

Silicate concentrations solubilized by five bacteria, PTST\_30, LCT\_01, RTTV\_12, TCM\_39, and MCM\_15, in different treatments containing different salt contents (0, 0.15, 0.3 and 0.5%) were presented in Fig. 2. The results showed that there

was a rapid increase of the silicate concentrations in the liquid medium in the time period of 0-6 days of incubation, reached the highest peak of the concentration on day 6 and after this day, the concentration of silicate was dropped strongly until the end of the experiment. In addition, most of the five bacteria showed their significant highest capacity in silicate solubilization in the treatments without NaCl addition where silicate concentrations

varied between 37.5 and 53.7 mg L<sup>-1</sup>. Moreover, a negative correlation between the soluble silicate concentrations and the concentration of NaCl in liquid medium was also found. This means that the concentrations of silicate in the liquid medium dissolved by bacteria from Si mineral were reduced when increasing concentration of NaCl in the liquid medium. However, these five bacteria still had good

silicate solubilization capacity in liquid medium containing NaCl up to 0.5% and the highest soluble silicate concentration of the five bacteria varied between 12.5 and 17.7 mg L<sup>-1</sup>. Moreover, no much difference about silicate concentrations in the liquid medium at any sampling time among treatments owning the same concentration of NaCl in the liquid medium regardless of bacteria.

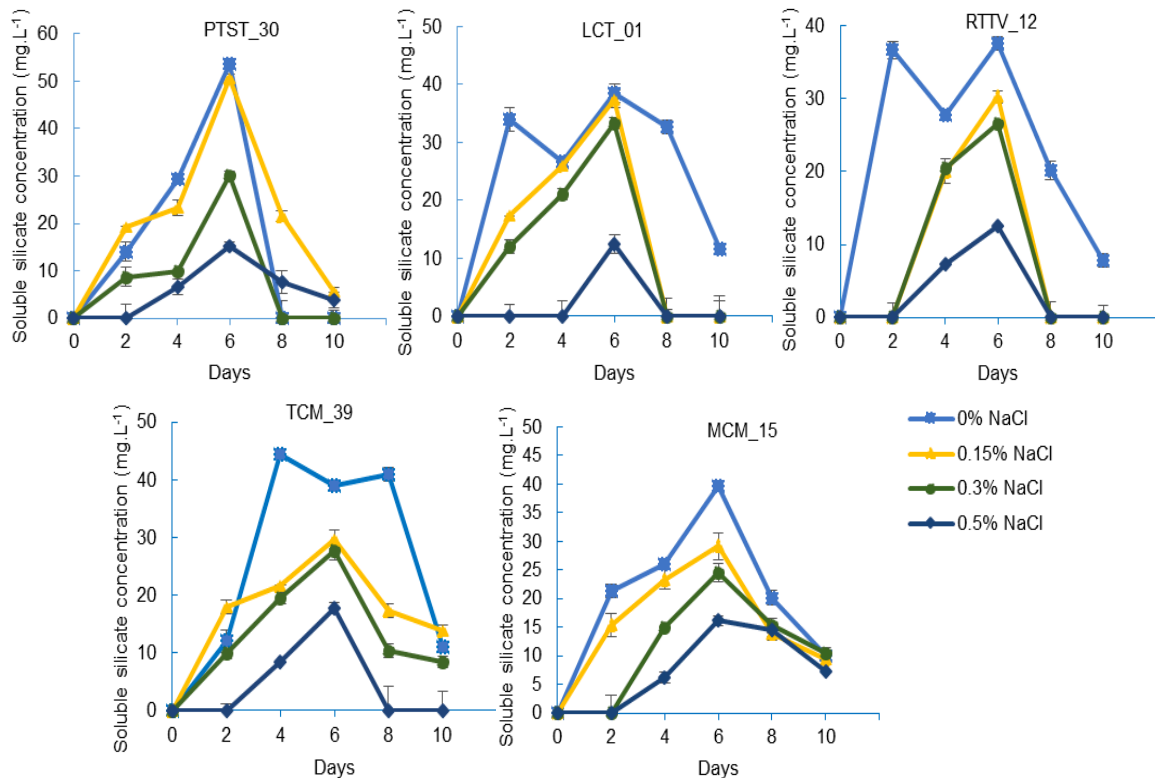


Figure 2. Silicate concentrations solubilized by five bacteria in different NaCl concentration treatments

**Impact of different ambient air temperatures on silicate solubilization capacity of five silicate solubilizing bacteria**

The results of the experiment in testing the influence of different ambient air temperature levels on silicate solubilizing capability of PTST\_30, LCT\_01, RTTV\_12, TCM\_39, and MCM\_15 were presented in Fig. 3 and showed that the silicate concentrations of most treatments increased sharply in the time period of 0-6 days of incubation, achieved the highest peaks on day 6, and then decreased strongly afterward until the end of the experiment. When comparing treatments among the

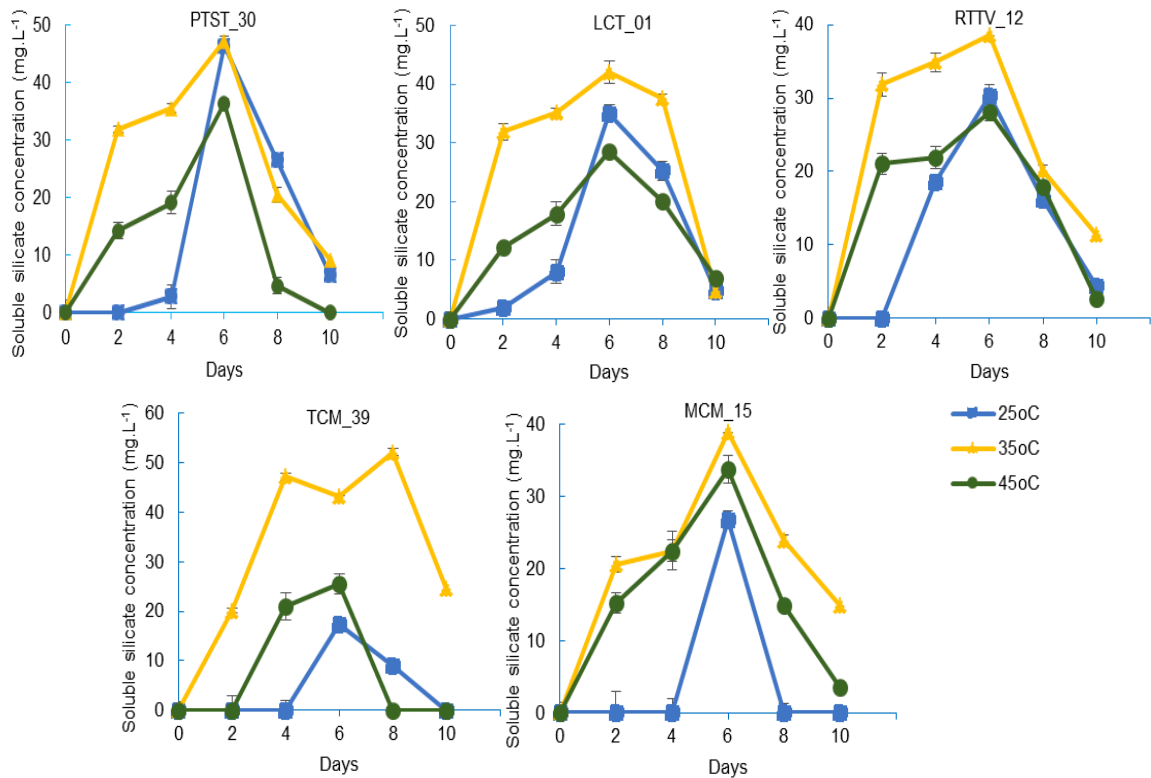
same bacteria, the results showed that the highest and significant silicate concentrations were found in treatments with temperature of 35°C and the highest concentrations of silicate in liquid medium on day 6 of PTST\_30, LCT\_01, RTTV\_12, TCM\_39, and MCM\_15 were 47.2, 42.0, 38.5, 52.1, and 39.0 mg L<sup>-1</sup>, respectively. Moreover, these five strains still had fairly good solubilizing capacity either under the environmental temperature of 25°C or 45°C with a range between 17.3 and 46.3 mg L<sup>-1</sup> and between 25.5 and 36.4 mg L<sup>-1</sup>, respectively. The lowest concentration of soluble Si in liquid medium was found in the treatment with 45°C. It was found that there was not much difference in silicate

concentrations at any sampling time among treatments having the same ambient air temperature regardless of bacteria.

**Phosphate solubilizing capability of five silicate solubilizing bacteria**

The results from the experiments in testing phosphate-solubilizing capacity of PTST\_30, LCT\_01, RTTV\_12, TCM\_39, and MCM\_15 were presented in Fig. 4 and indicated that for most of the bacteria, there was a rapid increase of phosphorus concentrations in liquid medium of treatments containing  $\text{Ca}_3(\text{PO}_4)_2$  in a time period 0-6 days of incubation, reached the highest concentrations at day 4 or day 6, depending on bacteria, but mainly day 6 and then decreased gradually until end of the experiment. Almost five bacteria had a low capacity in phosphate solubilization for  $\text{AlPO}_4$  and  $\text{FePO}_4$

phosphate sources. The concentration of soluble phosphorus in liquid medium was found to be varied between 33.9 and 49.6  $\text{mg L}^{-1}$  and between 1.94 and 34.1  $\text{mg L}^{-1}$  for  $\text{AlPO}_4$  and  $\text{FePO}_4$  phosphate sources, respectively, after 6 days of incubation. The highest phosphorous concentrations were found in treatments containing  $\text{Ca}_3(\text{PO}_4)_2$  as phosphorous source with a big variation of phosphorous concentrations among bacteria (between 106 and 929  $\text{mg L}^{-1}$  after 6 days of incubation). There was a significant difference ( $p < 0.05$ ) among bacteria about phosphorus-solubilizing capacity in treatments containing  $\text{Ca}_3(\text{PO}_4)_2$  as phosphorous source. The phosphate solubilization capacity of five tested bacteria in liquid medium containing  $\text{Ca}_3(\text{PO}_4)_2$  as phosphorous source was ranked as following orders: LCT\_01 > TCM\_39 > MCM\_15 > RTTV\_12 > PTST\_30 with a corresponding amount of 929, 781, 651, 565, and 106  $\text{mg L}^{-1}$ , respectively.



**Figure 3.** Silicate concentrations solubilized by five bacteria in different ambient air temperature treatments.

**Nitrogen-fixing capacity of five silicate solubilizing bacteria**

The results from the experiment in testing the

biological nitrogen-fixing capacity of PTST\_30, LCT\_01, RTTV\_12, TCM\_39, and MCM\_15 were presented in Fig.5 and indicated that all five bacteria had a capacity to fix nitrogen biologically and the

available nitrogen concentrations fixed by five bacteria tended to increase gradually in the first two or three days, but mainly within two days, reached the highest points on day 2 or day 3 and then dropped strongly afterwards. Additionally, the highest fixed nitrogen concentrations of five bacteria

(PTST\_30, LCT\_01, RTTV\_12, TCM\_39 and MCM\_15) at day 2 or day 3 were ranked as following orders: PTST\_30 > TCM\_39 > MCM\_15 > RTTV\_12 > LCT\_01 with an corresponding amount of 5.09, 3.87, 2.75, 1.75 and 1.37 mg L<sup>-1</sup>, respectively.

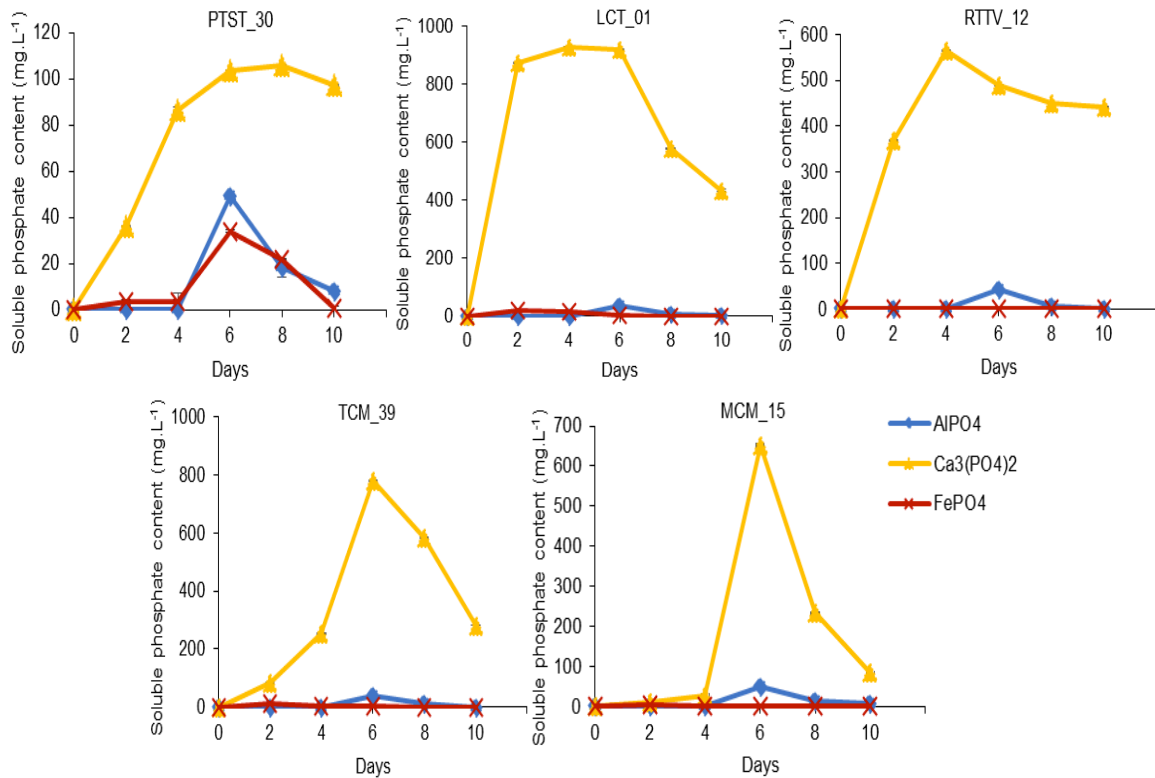


Figure 4. Phosphate concentrations solubilized by five silicate-solubilizing bacteria in different phosphorous source treatments.

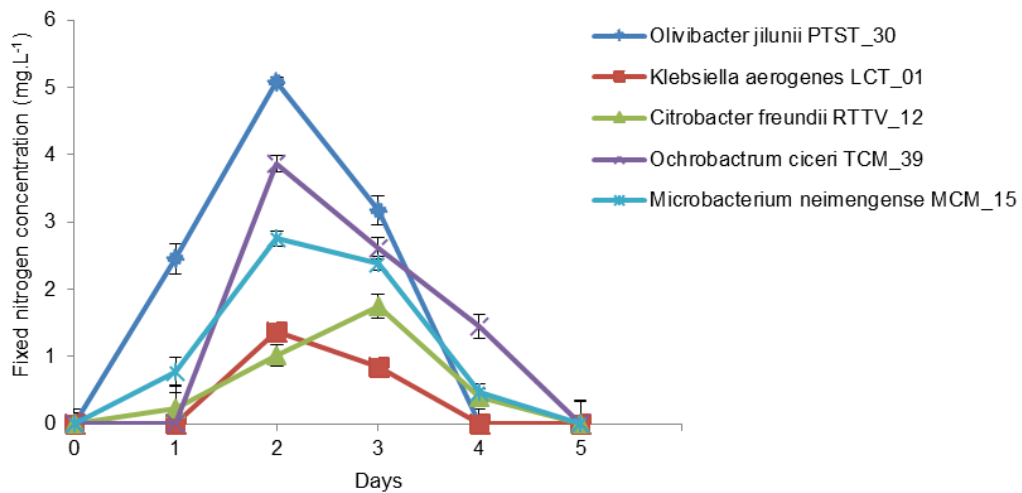
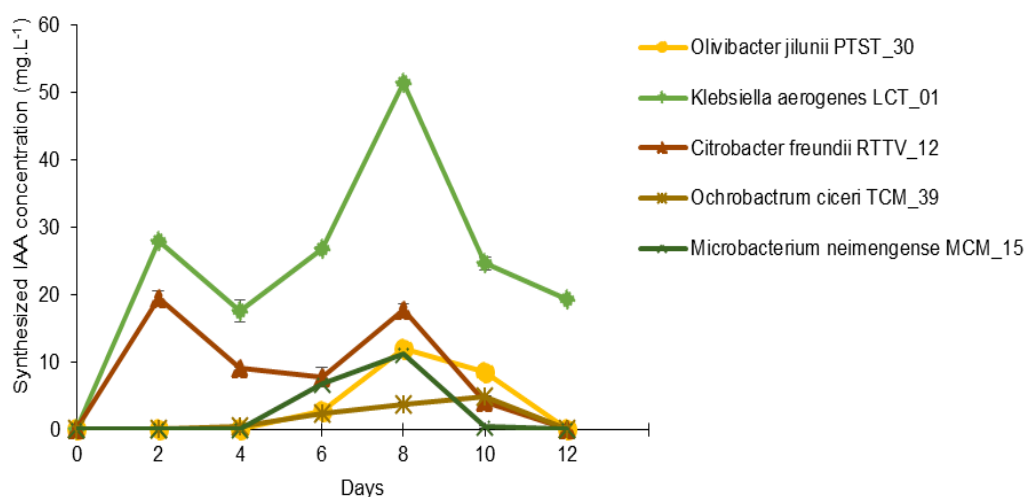


Figure 5. Available nitrogen concentrations fixed by five silicate solubilizing bacteria in Burk liquid medium.

### IAA-synthesizing capacity of five silicate solubilizing bacteria

Figure 6 presented the amounts of phytohormone IAA produced by five different bacteria in liquid medium containing 100 mg L<sup>-1</sup> tryptophan. All five bacteria named as PTST\_30, LCT\_01, RTTV\_12, TCM\_39, and MCM\_15 showed their capacity in IAA synthesis in the liquid medium. The highest concentrations of IAA produced by bacteria in liquid medium were detected on day 2 and day 8. At day 2 only two bacteria, LCT\_01 and RTTV\_12 could produce IAA with a concentration recorded as 27.9 and 19.6 mg L<sup>-1</sup>, respectively whereas three other bacteria did not produce any IAA production. On day

4, the concentrations of IAA produced by these two bacteria were decreased strongly. On day 6, three other bacteria started to produce IAA with an IAA production varied between 2.39 mg L<sup>-1</sup> IAA and 6.79 mg L<sup>-1</sup> IAA. At day 8 the concentrations of IAA produced by all five bacteria were recorded as the highest peak of IAA concentration during the experimental time. Among five bacteria tested, LCT\_01 strain produced highest amount of IAA production with an amount of 51.5 mg L<sup>-1</sup>, followed by RTTV\_12 with a concentration of 17.7 mg L<sup>-1</sup> while three other bacteria, PTST\_30, MCM\_15, and TCM\_39 synthesized lower amounts of IAA production of 12.1, 11.3 and 3.8 mg L<sup>-1</sup> IAA, respectively.



**Figure 6.** Phytohormone IAA concentrations synthesized by five different silicate-solubilizing bacteria in liquid medium.

### DISCUSSION

The results of this study revealed that under the conditions of environment like neutral pH 7, NaCl 0.0% and temperature of 35°C the silicate-solubilizing efficiency of all five SSB was outstandingly promoted. Moreover, these five bacteria were genetically identified as *Microbacterium neimengense* MCM\_15, *Olivibacter jilunii* PTST\_30, *Citrobacter freundii* RTTV\_12, *Klebsiella aerogenes* LCT\_01, and *Ochrobactrum ciceri* TCM\_39. From many previous studies, it was showed that *Microbacterium neimengense*, *Olivibacter jilunii* and *Citrobacter freundii* grew better under conditions of environment like pH range of 7.0-7.2, 3% salt content (NaCl), and at 37°C temperature (Gao *et al.*, 2013), pH of 6.0-9.0, 5.0%

NaCl and 4-42°C temperature (Chen *et al.*, 2013) and pH of 6.5, 3.0-4.5% NaCl and 37°C temperature (Keevil *et al.*, 1977; Jacob, Irshaid, 2012). However, there were not many studies about the influence of pH, salt content and temperature on *Klebsiella aerogenes* and *Ochrobactrum ciceri*. Moreover, in a study of Hadi *et al.* (2013) showed that *Ochrobactrum ciceri* grew better under the temperature of 30°C.

In addition, all five bacteria revealed their high capacity in phosphate solubility in the liquid medium. This could be due to these bacteria could produce organic acids such as gluconic acid, lactic acid, succinic acid, formic acid, malic acid, citric acid, oxalic acid, acetic acid or tartaric acid. These organic acids could help to dissolve efficiently not



only the insoluble silicate minerals but also insoluble phosphate minerals and become the soluble one for bacteria and plant to absorb for their growth (Khan *et al.*, 2014) and the outcome of the previous studies showed that *Citrobacter freundii* had another function in phosphate solubilization in liquid medium containing  $\text{Ca}_3(\text{PO}_4)_2$  as phosphorus source and *Klebsiella aerogenes* as well in phosphate solubilization (Toribio-Jiménez *et al.*, 2017; Samina *et al.*, 2010). However, these bacteria's function in phosphate solubilization for  $\text{AlPO}_4$  and  $\text{FePO}_4$  as phosphorous sources for bacteria has still not published yet. Moreover, the function in phosphate solubilization of *Microbacterium neimengense*, *Olivibacter jilunii* and *Ochrobactrum ciceri* has also still not published yet.

For the nitrogen-fixing capability of these five strains, it was found that the nitrogen-fixing capacity of them was fairly high. The results from the previous studies have proved many species of the genus *Klebsiella* sp. showed their capacity in fixing nitrogen (Lin *et al.*, 2012). Similar to *Ochrobactrum* sp. (Ngom *et al.*, 2004) and *Citrobacter* sp. (Neilson, 1976). Besides, the nitrogen fixation capacity of *Microbacterium neimengense* and *Olivibacter jilunii* has not been found in any publication.

Finally, IAA production was detected via indole-3-pyruvic acid and indole-3-acetic aldehyde pathway with biochemical reactions from tryptophan (Patten, Glick, 1996). The results showed that five selected bacteria in this study showed their high capacity in IAA synthesis in the liquid medium containing tryptophan ( $100 \text{ mg L}^{-1}$ ). This result was similar to other previous studies, indicating that *Klebsiella* sp. (Lin *et al.*, 2012), *Citrobacter freundii* (Samina *et al.*, 2010) and *Ochrobactrum ciceri* (Imran *et al.*, 2015) produced high amounts of IAA in liquid medium containing tryptophan ( $100 \text{ mg L}^{-1}$ ). However, the previous studies in the IAA producing capacity of *Olivibacter jilunii* and *Microbacterium neimengenses* have not proved yet.

## CONCLUSION

Five silicate solubilizing bacteria in this study, *Microbacterium neimengense* MCM\_15, *Olivibacter jilunii* PTST\_30, *Citrobacter freudii* RTTV\_12, *Klebsiella aerogenes* LCT\_01, and *Ochrobactrum ciceri* TCM\_39 showed their most efficiency in silicate solubilization in liquid medium under following environmental conditions including pH 7,

NaCl 0.0% and temperature 35°C. Moreover, these five selected bacteria still had fairly good in silicate solubilization even under other environmental conditions of pH 3, 5 or 9, salt content (NaCl) of 0.5% and temperature at 25°C or 45°C. Beside the function in silicate solubilization, these five bacteria had also other functions like nitrogen fixation, phosphate solubilization mainly for  $\text{Ca}_3(\text{PO}_4)_2$  and IAA synthesis in liquid media. Therefore, these five collected bacteria have broad potential in producing bio-fertilizers to enhance crop growth and yield.

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## ĐÁNH GIÁ MỘT SỐ ĐẶC TÍNH SINH LÝ CỦA NĂM DÒNG VI KHUẨN HÒA TAN KHOÁNG SILIC ĐƯỢC PHÂN LẬP TỪ CÁC HỆ SINH THÁI KHÁC NHAU

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### TÓM TẮT

Vi khuẩn hòa tan khoáng silic (Si) đóng vai trò quan trọng trong hòa tan khoáng Si trong đất góp phần gia tăng sinh trưởng và năng suất cũng như tăng cường khả năng chống chịu với các điều kiện môi trường bất lợi của cây trồng. Mục tiêu của nghiên cứu này nhằm đánh giá một số đặc tính sinh lý như pH, nồng độ muối NaCl, nhiệt độ môi trường, khả năng hòa tan lân, cố định đạm và tổng hợp IAA trong môi trường lỏng của năm dòng vi khuẩn hòa tan khoáng Si tốt nhất. Môi trường dịch đất lỏng có chứa 0,25% magnesium trisilicate được sử dụng để đánh giá khả năng hòa tan khoáng Si của năm dòng vi khuẩn dưới ảnh hưởng của pH, nồng độ muối và nhiệt độ. Ngoài ra, khả năng hòa tan lân, cố định đạm và tổng hợp IAA của năm dòng vi khuẩn tuyển chọn được kiểm tra lần lượt trong các môi trường NBRIP, Burk's và NBRIP bổ sung 100 mg L<sup>-1</sup> tryptophan. Kết quả của nghiên cứu cho thấy năm dòng vi khuẩn hòa tan khoáng Si tốt nhất ở pH 7,0, nồng độ muối NaCl 0,0% và nhiệt độ 35°C. Hơn nữa, trong môi trường có nồng độ muối 0,5% các dòng vi khuẩn này vẫn hòa tan khoáng Si khá cao. Mặt khác, năm dòng vi khuẩn tuyển chọn cũng hòa tan hiệu quả 3 nguồn lân khó tan gồm Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, AlPO<sub>4</sub> và FePO<sub>4</sub> trong môi trường lỏng với hàm lượng lần lượt đạt 105,8-928,7 mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup>, 33,9-49,6 mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup> và 1,94-34,1 mg P<sub>2</sub>O<sub>5</sub> L<sup>-1</sup>, cố định đạm với hàm lượng dao động từ 1,37-5,09 mg NH<sub>4</sub><sup>+</sup> L<sup>-1</sup> sau 2 ngày nuôi cấy. Cuối cùng, chúng còn có khả năng tổng hợp IAA khá cao và dao động 4.85-51.5 mg IAA L<sup>-1</sup>. Như vậy, năm dòng vi khuẩn tuyển chọn trong nghiên cứu này không chỉ hòa tan hiệu quả khoáng Si mà còn có chức năng gia tăng sinh trưởng và năng suất cây trồng.

**Từ khóa:** cố định đạm, đặc tính sinh lý, hòa tan lân, nồng độ muối, tổng hợp IAA, vi khuẩn hòa tan khoáng silic