

## COMPARATIVE STUDY OF PHOSPHATE SOLUBILIZATION POTENTIAL OF *TALAROMYCES PINOPHILUS* STRAINS

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**Abstract.** Phosphorus (P) is one of the most essential macronutrients for plant growth and development. Most of the soils in the world contain insoluble P that cannot be utilized by the plants. Phosphate solubilizing fungi (PSF) including *Talaromyces pinophilus* possess more potential for providing available P in soil for plant nutrition. There is no report regarding P solubilization potential among the different strains of *T. pinophilus*. The article aimed to compare the Phosphorous solubilization capabilities of 17 *Talaromyces pinophilus* fungal strains, in order to determine the best options for environmentally friendly fertilizers. The P solubilization efficiency of the fungal strains was investigated in broth containing insoluble  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{AlPO}_4$  and  $\text{FePO}_4$  compounds. Result showed that  $\text{Ca}_3(\text{PO}_4)_2$  was solubilized the most followed by  $\text{AlPO}_4$  and  $\text{FePO}_4$ . The strains SI-17URAgr, NBRC 6345 and NBRC 100533 have strong abilities to solubilize  $\text{Ca}_3(\text{PO}_4)_2$ , but SI-19URAgr could solubilize both  $\text{AlPO}_4$  and  $\text{FePO}_4$ . These results also imply that the strains SI-4URAgr and JCM 22801 have the potential to solubilize  $\text{AlPO}_4$  and  $\text{FePO}_4$ , respectively. Although the P solubilization by different strains were source dependent, the strains SI-17URAgr, NBRC 6345 and NBRC 100533 were proved to be potential for solubilizing P from three sources tested. Solubilized P was negatively correlated with pH of the medium. This study suggested that these strains have a great potential as eco-friendly biofertilizers for sustainable soil management and crop production.

**Keywords:** *fungal strain, phosphorus, insoluble phosphate, eco-friendly biofertilizers, sustainable soil management, soil pH*

### Introduction

Phosphorus (P) is the second most important limiting element required for plant growth and development (Chai et al., 2011; Ram et al., 2015). The soluble soil P concentration varies from 0.05 to 10 ppm, and more than 80% of the P becomes fixed and unavailable for absorption by plants due to adsorption, precipitation or conversion to organic forms (Holford, 1997). The fixed form of P in alkaline soils is tricalcium phosphate,  $\text{Ca}_3(\text{PO}_4)_2$ , whereas in acidic soils, it is mainly found as  $\text{FePO}_4$  and  $\text{AlPO}_4$  (Subba Rao, 1999).

The application of plant growth promoting microorganisms in agriculture could reduce the use of agrochemicals and support eco-friendly crop production (Herrera et al., 1993; Glick, 1995; Requena et al., 1997). It has been reported that a wide range of

microorganisms, especially bacteria, solubilize insoluble phosphate (Badr El-Din et al., 1986; Gaind et al., 1991) although fungi are more efficient than bacteria (Venkateswarlu et al., 1984). A promising biotechnological strategy in the management of P fertilization is the use of phosphate-solubilizing fungi to solubilize rock phosphates and allow the recovery of unavailable P fixed in the soil.

*Talaromyces* (Trichocomaceae) is an important fungal genus which is derived from the Greek word for 'basket', which capably describes the body in which ascospores are formed. In the past, species producing sexual stages with *Penicillium* anamorphs have been classified in *Eupenicillium* spp. and *Talaromyces* spp. After July 2011, species formally reclassified as the *Penicillium* subgenus *Biverticillium* were classified in *Talaromyces*. The situation is complicated by the fact that many species now classified in *Talaromyces* will continue to be sought as *Penicillium* species in identifications (Pitt, 2014).

The habitats of *Talaromyces pinophilus* are global in environment, *T. pinophilus* (Samson et al., 2011); anamorph: *Penicillium pinophilum* (Thom, 1910) and *P. allahabadense* (Mehrotra et al., 1962) currently designated by *T. allahabadensis* (Samson et al., 2011) were prevalently isolated from soil, compost (El-Naggar et al., 2015), seeds grains (Ismail et al., 2016), phyllosphere (Lindow et al., 2002), phylloplane (Abdel-Hafez et al., 2015; Abdel-Gawad et al., 2017) and some medicinal plants (Koul et al., 2016; Yao et al., 2017).

*T. pinophilus* has received increasing attention in mycological research for its ability to act as a fungal antagonist and plant-growth promoter (Nicoletti et al., 2004; Pandey et al., 2008; Wani et al., 2016). A few categorical studies on phosphate solubilizing abilities of *T. pinophilus* were reported previously by others (Abdul Wahid and Mehana, 2000; Sembiring and Fauzi, 2017) but no studies have been made on the in-depth p-solubilization potential and comparative performance of different *T. pinophilus* strains yet. Therefore, the study aimed to compare the phosphorous solubilization capabilities of 17 *T. pinophilus* fungal strains, in order to determine the best options for environmentally friendly fertilizers.

## Materials and Methods

### *Fungal Isolates and Culture Preparation*

The study was carried out in the Mycology Laboratory, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan during September 2017- March 2019 under a class II biohazard cabinet (BHC-1306IIA/3B, AIRTECH, Tokyo, Japan) followed to the biosafety classification by National Institute of Infectious Disease of Japan, because of possibilities including toxic fungal species treated as BSL2 during the working tenure. Seventeen *T. pinophilus* fungal strains were used in this study where 4 strains isolated from subtropical soils in Okinawa, Japan and 13 strains collected from different institutions in Japan (Table 1). The isolates were cultured on potato dextrose agar (PDA; Becton, Dickinson and Company, Sparks, MD, USA) slant at room temperature (25°C) for further study.

### *Morphological Studies*

Morphological studies were done according to the method (Watanabe, 2010). Pigment exudation were investigated on PDA slants. High-temperature resistance and

pathogenic potential of the isolates were examined. The isolates were cultured on PDA slants in duplicate and incubated at 35°C, 37°C and 42°C for 7 days to evaluate the growth of mycelia. Growth of isolates at 25°C (room temperature) treated as positive control.

### **Preparation of Spore Suspension**

Sporulated pure fungal cultured slants were selected for preparation of spore suspension followed by standard procedure. A total volume of 5 ml sterile water with 0.02% of tween 80 (Polyoxyethylene sorbitan monooleate, Nacalai Tesque, Inc, Kyoto, Japan) was poured on the culture slants and the fungal colony surface was lightly scraped by a sterile inoculation loop (Thermo Scientific™, Nunc™ Disposable Loops and Needles, Thermo Scientific™ 251586, Fisher Scientific, Tokyo, Japan). The cultures were passing through a syringe with a 4 x 4 cm sheet of a sterile absorbent cotton (Kyualet, Kawamoto Sangyo, Osaka, Japan). Spore count was done by a hemocytometer and the suspension was adjusted to approximately 10<sup>6</sup> spores mL<sup>-1</sup>.

**Table 1.** List of the fungal strains used in the study

| Isolates | Strain in GenBank | Source                     | Country of origin | Fungi                            | Institution      |
|----------|-------------------|----------------------------|-------------------|----------------------------------|------------------|
| 1        | SI-4URAgr         | Soil                       | Japan             | <i>Talaromyces pinophilus</i>    | Univ. Ryukyus    |
| 2        | SI-15URAgr        | Soil                       | Japan             | <i>Talaromyces pinophilus</i>    | Univ. Ryukyus    |
| 3        | SI-17URAgr        | Soil                       | Japan             | <i>Talaromyces pinophilus</i>    | Univ. Ryukyus    |
| 4        | SI-19URAgr        | Soil                       | Japan             | <i>Talaromyces pinophilus</i>    | Univ. Ryukyus    |
| 5        | IFM 64651         | Sputum                     | Japan             | <i>Penicillium pinophilum</i>    | Chiba University |
| 6        | IFM 57309         | Sputum                     | Japan             | <i>Penicillium pinophilum</i>    | Chiba University |
| 7        | NBRC 6345         | Radio set                  | Papua New Guinea  | <i>Talaromyces pinophilus</i>    | NITE BRC         |
| 8        | NBRC 100533       | Polyvinyl chloride plastic | France            | <i>Talaromyces pinophilus</i>    | NITE BRC         |
| 9        | NBRC 33285        | Polyvinyl chloride plastic | France            | <i>Talaromyces pinophilus</i>    | NITE BRC         |
| 10       | NBRC 106907       | Soil                       | Japan             | <i>Penicillium pinophilum</i>    | NITE BRC         |
| 11       | NBRC 9575         | Polyvinyl chloride plastic | France            | <i>Penicillium allahabadense</i> | NITE BRC         |
| 12       | JCM 9928          | Soil                       | India             | <i>Penicillium pinophilum</i>    | RIKEN            |
| 13       | JCM 5593          | Radio set                  | Papua New Guinea  | <i>Penicillium pinophilum</i>    | RIKEN            |
| 14       | JCM 22801         | Wood stakes                | Australia         | <i>Penicillium pinophilum</i>    | RIKEN            |
| 15       | JCM 22802         | Barley grain               | Australia         | <i>Penicillium pinophilum</i>    | RIKEN            |
| 16       | JCM 22803         | Moldy sorghum grain        | Australia         | <i>Penicillium pinophilum</i>    | RIKEN            |
| 17       | JCM 23043         | Radio set                  | Papua New Guinea  | <i>Penicillium pinophilum</i>    | RIKEN            |

NITE; National Institute of Technology and Evaluation, Biological Resource Center, NITE (NBRC), Japan. RIKEN; is a large scientific research institute in Japan

### **Determination of TCP Solubilization Index on Solid Medium**

All the isolates were tested under *in vitro* condition for their phosphate solubilization activity on Pikovskaya's agar medium containing 0.5% tricalcium phosphate (TSP) as an insoluble phosphorus source. A spot inoculation of each fungal isolate was made onto the plates in triplicate under aseptic condition and incubated at 28°C for 7 days in darkness. At 7<sup>th</sup> day of incubation, phosphate solubilization index was measured using the following formula (Premono et al., 1996):

$$\text{Solubilization Index (SI)} = [\text{Colony diameter} + \text{Halo zone diameter}] / \text{Colony diameter}$$

### Quantitative Estimation of Phosphate Solubilization

It was carried out using Erlenmeyer flask containing 40 ml Pikovskaya's broth medium supplemented with 0.5% tricalcium phosphate [ $\text{Ca}_3(\text{PO}_4)_2$ ], aluminium phosphate ( $\text{AlPO}_4$ ) and iron phosphate ( $\text{FePO}_4$ ). After sterilization, the medium of each flask was inoculated with the 5% (v/v) spore suspension of a particular fungal isolate containing  $10^6$  spores  $\text{mL}^{-1}$ . Sterile distilled water inoculated flasks were treated as control. Three replicates were maintained for each test isolate and mean value was recorded. Incubation was done at  $25^\circ\text{C}$  in an incubator shaker (EYELA Multi Shaker MMS-3010) at 120 rpm for 9 days. The samples were autoclaved and centrifuged (TOMY MX-301) at 5000 rpm for 25 minutes to remove any suspended solids and mycelial parts. Then the cultures were filtered through  $0.45\ \mu\text{m}$  pore size syringe filter unit (Advantech, Japan). The filtrates were used for analysis of soluble phosphate and pH value. The pH value of the culture supernatants was determined by a pH meter (HORIBA, Japan) equipped with a glass electrode. The amount of soluble phosphorus in culture supernatants was measured by molybdenum blue method (Murphy and Riley, 1962) and expressed as mg/L. Samples cultured for 3, 6 and 9 days were compared. After calculation of mean phosphate degradation ability from 17 isolates of each day, we selected the adequate period for the comparison depending on the substrate.

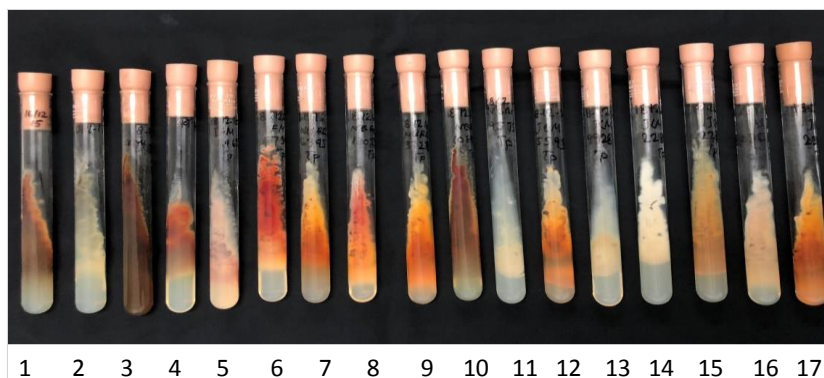
### Data Analysis

All experiments were conducted in triplicate and data were analyzed using Microsoft Excel program. The mean values were compared by Fisher test and significant differences were detected at  $p < 0.05$  level. Correlation between solubilized phosphate and pH of the medium was determined by using Pearson correlation studies.

## Results

### Pigment Exudation

All isolates were reconfirmed based on morphological studies. Morphological studies results are not shown. The isolates produced various colour in PDA slant, such as coffee, orange, yellow and cream (Fig. 1). Differential colour formation by *T. pinophilus* isolates might be attributed to the presence of bioactive metabolites.



**Figure 1.** *Talaromyces pinophilus* isolates grown on Potato Dextrose Agar (PDA) slant for 7 days at  $25^\circ\text{C}$  [Coffee: 1, 3, 10; Cream: 2, 5, 11, 13, 14, 16; Orange: 4, 6; Yellow=7, 8, 9, 12, 15, 17]

### Qualitative Phosphate Solubilization

All isolates showed different levels of phosphate solubilization from inconspicuous to a substantial in Pikovskaya's agar medium using tricalcium phosphate as the substrate. Six isolates produced clear zone, 5 developed translucent and the remaining isolates had inconspicuous zones. All isolates also showed significant phosphate solubilization in Pikovskaya's agar medium using tricalcium phosphate as the substrate. The phosphate solubilization index (PSI) ranged from 1.05 to 1.46 (Table 2).

**Table 2.** TCP solubilization in Pikovskaya's agar by 17 fungal strains

| Isolate | Fungal strain | Fungi                            | PSI                     | Type of zone |
|---------|---------------|----------------------------------|-------------------------|--------------|
| 1       | SI-4URAgr     | <i>Talaromyces pinophilus</i>    | 1.31±0.01 <sup>b</sup>  | C            |
| 2       | SI-15URAgr    | <i>Talaromyces pinophilus</i>    | 1.46±0.03 <sup>a</sup>  | C            |
| 3       | SI-17URAgr    | <i>Talaromyces pinophilus</i>    | 1.09±0.01 <sup>d</sup>  | T            |
| 4       | SI-19URAgr    | <i>Talaromyces pinophilus</i>    | 1.12±0.01 <sup>d</sup>  | T            |
| 5       | IFM 64651     | <i>Penicillium pinophilum</i>    | 1.07±0.02 <sup>d</sup>  | I            |
| 6       | IFM 57309     | <i>Penicillium pinophilum</i>    | 1.09±0.03 <sup>d</sup>  | I            |
| 7       | NBRC 6345     | <i>Talaromyces pinophilus</i>    | 1.11±0.02 <sup>d</sup>  | T            |
| 8       | NBRC100533    | <i>Talaromyces pinophilus</i>    | 1.21±0.08 <sup>bc</sup> | C            |
| 9       | NBRC33285     | <i>Talaromyces pinophilus</i>    | 1.14±0.08 <sup>cd</sup> | C            |
| 10      | NBRC 106907   | <i>Penicillium pinophilum</i>    | 1.24±0.03 <sup>b</sup>  | T            |
| 11      | NBRC 9575     | <i>Penicillium allahabadense</i> | 1.07±0.03 <sup>d</sup>  | I            |
| 12      | JCM 9928      | <i>Penicillium pinophilum</i>    | 1.09±0.06 <sup>d</sup>  | T            |
| 13      | JCM 5593      | <i>Penicillium pinophilum</i>    | 1.19±0.09 <sup>bc</sup> | C            |
| 14      | JCM 22801     | <i>Penicillium pinophilum</i>    | 1.05±0.05 <sup>d</sup>  | I            |
| 15      | JCM 22802     | <i>Penicillium pinophilum</i>    | 1.08±0.05 <sup>d</sup>  | I            |
| 16      | JCM 22803     | <i>Penicillium pinophilum</i>    | 1.09±0.05 <sup>d</sup>  | T            |
| 17      | JCM 23043     | <i>Penicillium pinophilum</i>    | 1.06±0.05 <sup>d</sup>  | I            |

PSI: Phosphate solubilization index, Values given are the mean ± standard deviation of three independent replicates, Same letter in the column are not significantly different at p<0.05 by Fisher's test, C = clear, T = translucent, I= inconspicuous

### Quantitative Phosphate Solubilization

Phosphate solubilization by all isolates were tested in Pikovskaya's broth medium using three substrates of recalcitrant phosphate compounds: tricalcium phosphate  $\text{Ca}_3(\text{PO}_4)_2$ , aluminium phosphate ( $\text{AlPO}_4$ ) and iron phosphate ( $\text{FePO}_4$ ). The phosphate solubilizing ability of fungal isolates varied with incubation period and substrates. The best period of observation was 6 days considering their mean P solubilization for the three substrates (Table 3).

All *T. pinophilus* strains were tested for their ability to solubilize hardly soluble phosphate sources [ $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{AlPO}_4$ , and  $\text{FePO}_4$ ]. All of the *T. pinophilus* strains showed potential P solubilizing in the medium containing  $\text{Ca}_3(\text{PO}_4)_2$  followed by  $\text{AlPO}_4$  and  $\text{FePO}_4$  (Table 4). The solubilized P ranged between 83.7-574.8  $\text{mgL}^{-1}$ , 50.5-192.4  $\text{mgL}^{-1}$  and 50.5-192.4  $\text{mgL}^{-1}$  from  $\text{Ca}_3(\text{PO}_4)_2$ ,  $\text{AlPO}_4$ , and  $\text{FePO}_4$ , respectively. Among 17 isolates, 6 isolates (SI-4URAgr, SI-17URAgr, SI-19URAgr, NBRC 6345, NBRC 100533 and JCM 22801) were considered as outstanding isolates because solubilized P was higher than the sum of the mean and standard deviation of P solubilized by 17 isolates. The highest amount of P solubilization from  $\text{Ca}_3(\text{PO}_4)_2$  was shown in NBRC6345 (574.8  $\text{mgL}^{-1}$ ) followed by NBRC100533 (540.4  $\text{mgL}^{-1}$ ) and SI-17URAgr (537.2  $\text{mgL}^{-1}$ ). The highest amount of P solubilization from  $\text{AlPO}_4$ , was

shown in SI-4URAg (192.4 mgL<sup>-1</sup>) followed by SI-19 (153.7 mgL<sup>-1</sup>). The highest amount of P solubilization from FePO<sub>4</sub> was shown in SI-19URAg (145.2 mgL<sup>-1</sup>) followed by JCM 22801 (126.1 mgL<sup>-1</sup>). The strain SI-19URAg showed outstanding performance in both AlPO<sub>4</sub>, and FePO<sub>4</sub> solubilization (Table 4). In case of total solubilized phosphate (mgL<sup>-1</sup>) from three substrates (TCP, AL-P and Fe-P), the highest amount of P solubilization was shown in NBRC 6345 (829.6 mgL<sup>-1</sup>) followed by SI-17URAg (789.2 mgL<sup>-1</sup>) and NBRC100533 (770.4 mgL<sup>-1</sup>). The tested isolates produced various colours in broth medium using three P substrates during incubation period (data not shown).

**Table 3.** Selection for the best period of phosphate solubilization by 17 isolates

| Solubilized phosphate (mg/L) |              |             |           |             |           |           |            |           |
|------------------------------|--------------|-------------|-----------|-------------|-----------|-----------|------------|-----------|
| TCP                          |              |             | Al-P      |             |           | Fe-P      |            |           |
| 3 days                       | 6 days       | 9 days      | 3 days    | 6 days      | 9 days    | 3 days    | 6 days     | 9 days    |
| 196.2±93.0                   | 354.3±136.1* | 343.2±125.1 | 80.6±29.8 | 111.6±40.7* | 63.6±22.2 | 55.3±16.3 | 95.8±28.1* | 69.4±20.1 |

TCP: tricalcium phosphate, Al-P: aluminium phosphate, Fe-P: iron phosphate, Values given are the mean ± standard deviation of P solubilized by 17 fungal isolates, An asterisk (\*) indicated the best period of phosphate solubilization

**Table 4.** Phosphate solubilization from different substrates by *T. pinophilus* fungal strains

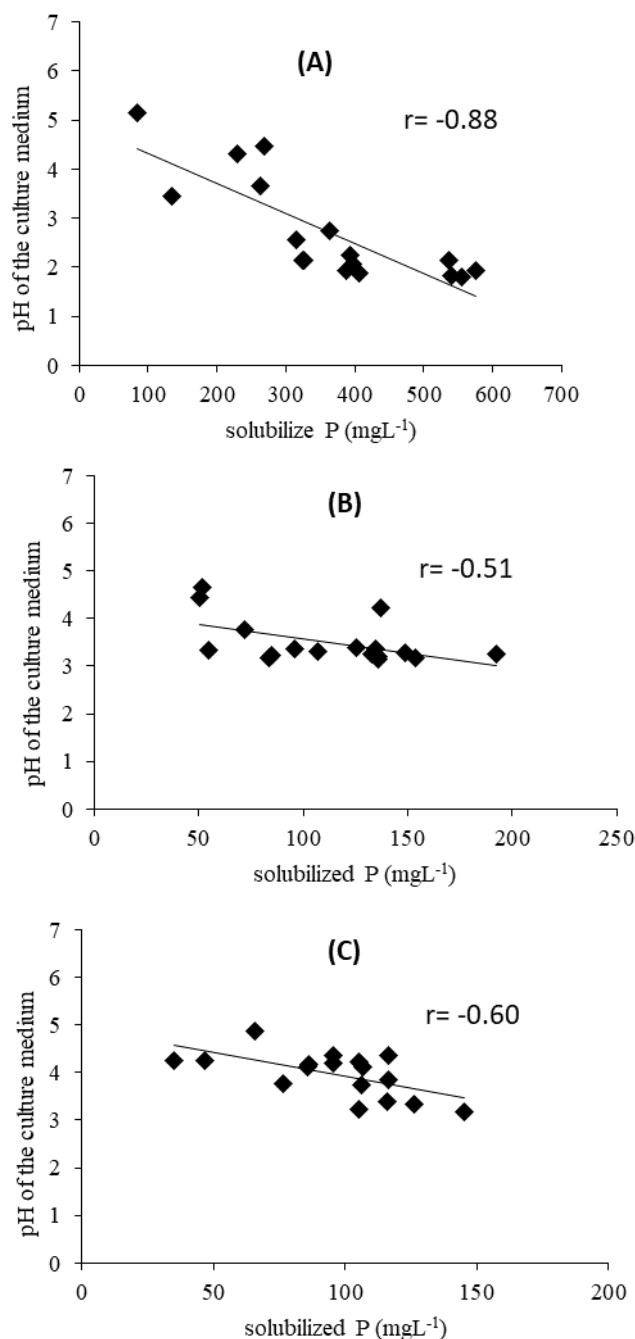
| Sl. No | Strain     | Fungi                            | Solubilized phosphate (mg/L) |            |           | Total P (mg/L) |
|--------|------------|----------------------------------|------------------------------|------------|-----------|----------------|
|        |            |                                  | TCP                          | AL-P       | Fe-P      |                |
| 1      | SI-4URAg   | <i>Talaromyces pinophilus</i>    | 268.4                        | 192.4*     | 46.5      | 507.3          |
| 2      | SI-15URAg  | <i>Talaromyces pinophilus</i>    | 363.7                        | 132.9      | 34.8      | 531.4          |
| 3      | SI-17URAg  | <i>Talaromyces pinophilus</i>    | 537.2*                       | 135.7      | 116.3     | 789.2*         |
| 4      | SI-19URAg  | <i>Talaromyces pinophilus</i>    | 314.0                        | 153.7*     | 145.2*    | 612.8          |
| 5      | IFM 64651  | <i>Penicillium pinophilum</i>    | 324.3                        | 50.5       | 85.6      | 460.5          |
| 6      | IFM 57309  | <i>Penicillium pinophilum</i>    | 228.5                        | 51.4       | 65.6      | 345.5          |
| 7      | NBRC 6345  | <i>Talaromyces pinophilus</i>    | 574.8*                       | 148.6      | 106.2     | 829.6*         |
| 8      | NBRC100533 | <i>Talaromyces pinophilus</i>    | 540.4*                       | 134.7      | 95.3      | 770.4*         |
| 9      | NBRC 33285 | <i>Talaromyces pinophilus</i>    | 263.5                        | 135.8      | 95.4      | 494.7          |
| 10     | NBRC106907 | <i>Penicillium pinophilum</i>    | 482.5                        | 107.2      | 106.5     | 696.3          |
| 11     | NBRC 9575  | <i>Penicillium allahabadense</i> | 83.7                         | 96.0       | 116.5     | 296.2          |
| 12     | JCM 9928   | <i>Penicillium pinophilum</i>    | 135.2                        | 85.1       | 105.3     | 325.6          |
| 13     | JCM 5593   | <i>Penicillium pinophilum</i>    | 387.7                        | 137.2      | 105.3     | 630.2          |
| 14     | JCM 22801  | <i>Penicillium pinophilum</i>    | 405.7                        | 54.8       | 126.1*    | 586.6          |
| 15     | JCM 22802  | <i>Penicillium pinophilum</i>    | 392.2                        | 83.9       | 85.9      | 562.1          |
| 16     | JCM 22803  | <i>Penicillium pinophilum</i>    | 397.1                        | 125.2      | 116.1     | 638.4          |
| 17     | JCM 23043  | <i>Penicillium pinophilum</i>    | 325.2                        | 71.7       | 76.6      | 473.5          |
|        |            | Mean±SD                          | 354.3±136.1*                 | 111.6±40.7 | 95.8±28.1 | 561.8±157.8    |

TCP: tricalcium phosphate, Al-P: aluminium phosphate, Fe-P: iron phosphate, Values given are the mean ± standard deviation of P solubilized by 17 fungal isolates, An asterisk (\*) indicated outstanding values of solubilized phosphate, It was higher than sum of mean and standard deviation of P solubilized by 17 fungal isolates

### Correlation Between pH and Soluble P

The pH of the culture medium exhibited the opposite changes. It decreased with the increased amount of soluble P in the medium. Correlation studies showed a significant inverse relationship between soluble P and pH of the culture medium (Fig. 2A, 2B, 2C).

The negative correlation was observed in all fermented broth culture and correlation coefficient ( $r$ ) was -0.88, -0.51 and -0.60 in TCP, Al-P and Fe-P, respectively. The strong negative correlation was observed in TCP containing medium.



**Figure 2.** Pearson's correlation between soluble phosphate and pH of the culture medium supplemented with TCP (2A), AlPO<sub>4</sub> (2B) and FePO<sub>4</sub> (2C); inoculated by 17 fungal strains. [SI-4URAgr, SI-15URAgr, SI-17URAgr, SI-19URAgr, IFM 64651, IFM 57309, NBRC 6345, NBRC 100533, NBRC 33285, NBRC 106907, NBRC 9575, JCM 9928, JCM 5593, JCM 22801, JCM 22802, JCM 22803, JCM 23043]

### Temperature Effects on Strains

Survival of the strains at different temperature was investigated (Table 5). Most of the strains could grow and survive at 25°C-37°C. Strain SI-4URAg, SI-15URAg, SI-17URAg, SI-19URAg and NBRC 106907 could grow at 25°C-42°C. These strains are summer tolerant and they could survive both acidic (low pH) and alkaline environment (high pH).

**Table 5.** The growth and survival of *T. pinophilus* strains at different temperature

| SL No. | Strain     | Fungi                            | Growth temperature |      |      |      |
|--------|------------|----------------------------------|--------------------|------|------|------|
|        |            |                                  | 25°C               | 35°C | 37°C | 42°C |
| 1      | SI-4URAg   | <i>Talaromyces pinophilus</i>    | +                  | +    | +    | +    |
| 2      | SI-15URAg  | <i>Talaromyces pinophilus</i>    | +                  | +    | +    | +    |
| 3      | SI-17URAg  | <i>Talaromyces pinophilus</i>    | +                  | +    | +    | +    |
| 4      | SI-19URAg  | <i>Talaromyces pinophilus</i>    | +                  | +    | +    | +    |
| 5      | IFM 64651  | <i>Penicillium pinophilum</i>    | +                  | +    | +    | -    |
| 6      | IFM 57309  | <i>Penicillium pinophilum</i>    | +                  | +    | +    | -    |
| 7      | NBRC 6345  | <i>Talaromyces pinophilus</i>    | +                  | +    | +    | -    |
| 8      | NBRC100533 | <i>Talaromyces pinophilus</i>    | +                  | +    | +    | -    |
| 9      | NBRC 33285 | <i>Talaromyces pinophilus</i>    | +                  | +    | +    | -    |
| 10     | NBRC106907 | <i>Penicillium pinophilum</i>    | +                  | +    | +    | +    |
| 11     | NBRC 9575  | <i>Penicillium allahabadense</i> | +                  | +    | +    | -    |
| 12     | JCM 9928   | <i>Penicillium pinophilum</i>    | +                  | +    | +    | -    |
| 13     | JCM 5593   | <i>Penicillium pinophilum</i>    | +                  | +    | +    | -    |
| 14     | JCM 22801  | <i>Penicillium pinophilum</i>    | +                  | +    | +    | -    |
| 15     | JCM 22802  | <i>Penicillium pinophilum</i>    | +                  | +    | +    | -    |
| 16     | JCM 22803  | <i>Penicillium pinophilum</i>    | +                  | +    | +    | -    |
| 17     | JCM 23043  | <i>Penicillium pinophilum</i>    | +                  | +    | +    | -    |

(+) indicated growth of fungi, (-) indicated no growth.

### Discussion

Morphological studies reconfirmed that all the 17 strains used in this study belong to *Talaromyces pinophilus*. All fungal isolates produced different colours in the medium regardless of the P substrates, but the colour seemed not to have any relation with P solubilization.

*T. pinophilus* has various applications based on the production of enzymes (Hansen et al., 2015; Li et al., 2017) as well as a renewable source of pigments, colorants (Caro et al., 2017) and bioactive compounds (Wang et al., 2013; Nicoletti et al., 2016) and degrading ability of agricultural waste (El-Naggar et al., 2015). *T. pinophilus* is able to produce a variety of bioactive metabolites, including alkaloids, peptides, lactones, polyketides and miscellaneous structure type compounds, with different chemical and biological activities. *T. pinophilus* produces biomass-degrading enzymes such as  $\alpha$ -amylase (Xian et al., 2015), cellulase (Visser et al., 2013), endoglucanase (Pol et al., 2012), xylanase (Visser et al., 2013), laccase (Dhakar et al., 2014) and  $\alpha$ -galactosidase (Visser et al., 2013). *T. pinophilus* produces a variety of medically useful metabolites such as 3-O- methylfunicone, which is used to inhibit mesothelioma cell motility, and talaromycolides 1-3,5 and 11 which inhibit the growth of the human pathogen methicillin resistant *Staphylococcus aureus* (Buommino et al., 2012).

In the present study, all isolates showed better P solubilizing activity in liquid medium compared with solid medium. The possible reason for these anomalous behaviors of isolates on liquid and solid media could be attributed to nutrient availability, varying diffusion rate of different organic acids secreted by fungi, and growth requirement of fungi (Jain et al., 2014).

Alam et al. (2002) reported that some isolates having little clear zone on solid agar medium exhibited higher phosphate solubilization efficiency in liquid medium. Some fungal isolates showed larger clear zones on solid agar medium but low phosphate solubilization in liquid medium. This shows that higher PSI on solid agar medium does not necessarily show solubilization efficiency in liquid medium. Thus, the plate technique is insufficient to screen the best P solubilizers and to detect all phosphate solubilizers mentioned by Nautiyal (1999).

The strongest P solubilization was found in  $\text{Ca}_3(\text{PO}_4)_2$  followed by  $\text{AlPO}_4$  and  $\text{FePO}_4$  because  $\text{AlPO}_4$  and  $\text{FePO}_4$  have complex structure than  $\text{Ca}_3(\text{PO}_4)_2$  while fungi exhibited low P solubilizing ability in media containing  $\text{AlPO}_4$  and  $\text{FePO}_4$  (Son et al., 2006; Zhang et al., 2018). The mechanisms of phosphate solubilization by microorganisms are very complex and are not completely known yet. The very common mechanisms are acidification, chelation and exchange reactions (Chai et al., 2011). Organic acids play an important role in phosphate solubilization processes, which can help the release of P by providing protons and complexing anions, or ligand exchange reactions or complexation of metal ions release to solution. Organic acids production depends on the interaction of P source and fungi (Scervino et al., 2013; Zhang et al., 2018).

In the present study, the isolates (SI-4URAg, SI-17URAg, SI-19URAg, NBRC 6345, NBRC 100533, and JCM 22801) showed the highest efficiency in P solubilization by decreasing pH of the culture medium, which indicated higher amount of organic acid production. Solubilization of the different P sources mostly depended on the amount of organic acids production by fungi (Zhang et al., 2018). Tricarboxylic acids and other lower molecular weight organic acids are considered to be the main contributors to phosphate solubilization and a decrease in pH of the medium (Chai et al., 2011).

It was observed that phosphate solubilization was negatively correlated with pH of the medium. There are several reports where such correlation was documented (Pandey et al., 2008; Jain et al., 2012; Wani et al., 2016). The activities in lower pH indicated that the increase of organic acids in the medium (Pradhan and Sukla, 2005; Saxena et al., 2013). However, soluble P was increased without changing pH in some occasion because of other mechanism (Jain et al., 2012, 2017) such as; chelation and exchange reactions (Chai et al., 2011).

## Conclusions

The article aimed to compare the phosphorous solubilization capabilities of 17 *Talaromyces pinophilus* fungal strains, in order to determine the best options for environmentally friendly fertilizers. Findings showed that  $\text{Ca}_3(\text{PO}_4)_2$  was solubilized the most followed by  $\text{AlPO}_4$  and  $\text{FePO}_4$ . Among the strains tested SI-17URAg, NBRC 6345 and NBRC100533 were potential for  $\text{Ca}_3(\text{PO}_4)_2$  solubilization but SI-19URAg could solubilize both  $\text{AlPO}_4$  and  $\text{FePO}_4$  whereas SI-4URAg and JCM 22801 were potential for solubilization of Al-P and Fe-P, respectively. Overall findings suggest that the strains SI-17URAg, NBRC 6345 and NBRC 100533 could be potent for solubilizing P from different sources. Thus these strains might be used as inoculant in the soil containing various inorganic P. However, further trials are needed to determine the capability of these strains for P solubilization in field experiment, sustainable soil management as well as crop production.

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