ISOLATION AND MOLECULAR CHARACTERIZATION OF PHOSPHATE SOLUBILIZING FILAMENTOUS FUNGI FROM SUBTROPICAL SOILS IN OKINAWA

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Abstract. Phosphorus (P) is an essential nutrient element required for plant growth and development. Low phosphorus availability in soil is one of the major constrains for crop production. Phosphate solubilizing fungi enhance available phosphorous released from soils and contribute to fulfill the plants phosphorous requirement. This study aimed to isolate and identify potential phosphate solubilizing fungi from subtropical soils for environment friendly biofertilizer development. Sixteen fungal strains were isolated and identified as *Aspergillus* spp., *Penicillium* spp. and *Talaromyces* spp. from subtropical dark red soil, red soil and grey soil based on phosphate solubilization index, morphological studies and the sequences of β-tubulin and/or Calmodulin. Subsequently, fungal isolates having excellent phosphate solubilization efficiency were selected by their potential in broth containing insoluble $Ca_3(PO_4)_2$, $AlPO_4$ and $FePO_4$. Interestingly, *Aspergillus niger* isolates (strain SI-10URAgr, SI-11URAgr and SI-12URAgr) have marked phosphate solubilization ability regardless of the substrates followed by *Panicillium oxalicum* and *Talaromyces pinophilus.* In addition, there was inverse proportion between the pH and phosphate solubilizing capacities. These excellent properties of strains suggested that they have a great potential for agricultural utilization as environmentally sound biofertilizer. In this study, phosphate solubilization by filamentous fungi is reported for the first-time in subtropical Okinawa.
Keywords: *phosphorous, Aspergillus niger, biofertilizer, eco-friendly nutrient management, subtropical soil*

Introduction

Phosphorus (P) is one of the major nutrients for crop production (Reena et al., 2013). This nutrient play important physiological and bio chemical activities of plants, like, photosynthesis, energy and sugar production, nucleic acid synthesis, and promotes nitrogen fixation in legume plants (Saber et al., 2005). Phosphorus promotes the strength of grain crop straw, flower initiation and fruit settings, root development and seed formation (Sharma et al., 2013) and disease resistance capacity of plants (Richardson et al., 2007). In soils, only 0.1% of the total P exists in a soluble form available for plant uptake because of its fixation into an unavailable form (Zhou et al., 1992; Khan et al., 2010). In order to provide this nutrient, farmers use chemical fertilizers. The most widely used fertilizers are obtained from the acidification of rock phosphates with strong acids which not only represent a major cost of agricultural

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production but also impose adverse environmental impacts on overall soil health, terrestrial, freshwater and marine resources (Sing et al., 2011; Tilman et al., 2001).

The major soils in sub-tropical Okinawa are, red soil, dark-red soil and grey soil which are phosphorous deficient (Oshiro et al., 2016). The red soil and dark red soil have low pH value (5.4 and 6.6 respectively), one the other hand grey soil has high pH value (8.4). Large amount of soluble P fertilizers is widely used in order to increase agricultural production world widely (Bo et al., 2011). Moreover, the efficiency of applied P fertilizers in chemical form rarely exceeds 30% due to its fixation, either in the form of iron/aluminium phosphate in acidic soils or in the form of calcium phosphate in neutral to alkaline soils (Norish et al., 1983; Lindsay et al., 1989). According to the latest estimates, the global reserve of P could become depleted within 50-100 years (Heppel et al., 2016). Besides the efficient use of P reserves, it is also important to reduce the current wastage of P fertilizers and to recover applied P. The realization of all these potential problems associated with chemical P fertilizers has led to the search for environmentally compatible and economically feasible alternative strategies for improving crop production in low or P-deficient soils (Zaidi et al., 2009).

The microbial inoculants (biofertilisers) function as key player in sustainable agriculture by improving soil fertility and crop productivity (Deepak et al., 2014). Especially, fungi are able to penetrate in to deep underground and show good attachment to insolubilized P particles as results of its hyphal structure compared to bacteria and actinomycetes. Furthermore, fungi are good acid producer and consequently show greater phosphate solubilization activity than bacteria (Deepak et al., 2014; Jose et al., 2010). Among these, *Aspergillus* spp., *Penicilium* spp., *Talaromyces* spp. and *Eupenicilium* spp. are considered “key organisms” in the P cycle (Jose et al., 2010). However most of the fungal species solubilize inorganic calcium phosphate and have a limited capacity to solubilize aluminium or iron phosphate. There are few in vitro studies concerning the solubilization of other phosphates by fungal species. To address this limitation, the present study aims to isolate and identify new isolates of indigenous phosphate solubilizing filamentous fungi which could be potential to solubilize both tricalcium phosphate, aluminium phosphate and iron phosphate.

Materials and methods

Isolation

The study was carried out in the Mycology Laboratory, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan during August 2017–November 2018 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 of including toxic fungal species treated as BSL2 during the isolation.

The sampling area located at 26.5000°N and 128.0000°E (*Fig. 1*). Its climate is subtropical, temperatures range from 10 to 32 °C. Low temperature (10 to 26 °C) exists in winter season and higher temperature (27 to 32 °C) exists in summer with a humidity level near 100%. The major soil types are dark red soil, red soil and grey soil in this area.

Zero to fifty cm depth soil samples were collected from ten different locations of three type soils using sterile auger. One-hundred-gram soil was taken from each sampling point and it makes a total of 500 g composite sample (five points from each location make one composite sample). The samples were transferred to laboratory in

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sterile sealed polythene bag under aseptic condition and stored at room temperature. Then microbiological study was done as early as possible.

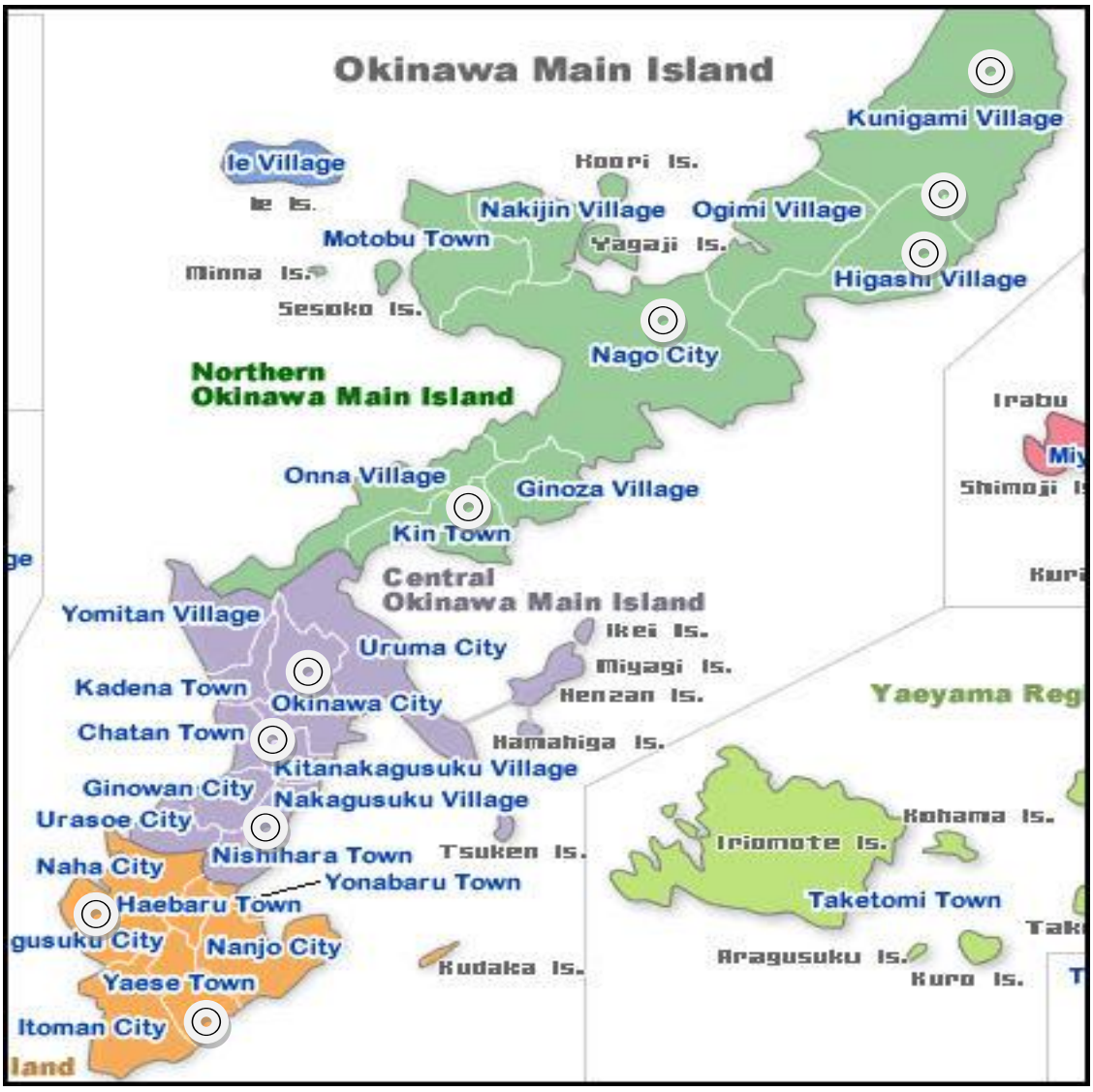

Figure 1. *Geographical map of the study area indicated sampling location*

For isolating phosphate solubilizing fungi Pikoveskaya's (PKV) agar medium was used. Pikoveskaya's (PKV) agar medium consisted of 10.0 g glucose, 5.0 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 .7H_2O$, 0.02 g NaCl, 0.02 g KCl, 0.003 g $FeSO_4 .7H_2O$, 0.003 g $MnSO_4 \cdot H_2O$ 0.5 g yeast extract, 15.0 g agar and 1000 mL distilled water (Rao, 1982). In this medium $Ca_3 (PO_4)_2$ was used as a source of insoluble phosphate. The medium was autoclaved at 121 °C for 15 min. About 20 ml of the sterilized medium poured into each petri dish and allowed to solidify before inoculation. Chloramphenicol was also used to avoid bacterial growth.

Isolation of phosphate solubilizing fungi using serial dilution plate technique. Five-gram soil sample was diluted in to 50 ml of sterile water. It was vigorously shaken until to get homogenous suspension and serially diluted to 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}. From each dilution, 200 µL was plated on Pikovskaya's agar. The phosphate solubilizing

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fungi were identified by the presence of a clear halo around the colonies after 7 days incubation at 25 °C (Rao, 1982). The experiment was performed in triplicate. Phosphate solubilizing fungi of the soil samples were isolated and purified by transferring into new plates. The pure cultures were preserved on potato dextrose agar slants at 4 °C for further study. Phosphate solubilisation index was measured using the following formula (Birhanu et al., 2017):

$$SI = [\text{Colony diameter} + \text{Halo zone diameter}] / \text{colony diameter}$$

Identification of phosphate solubilizing fungi

The genera of phosphate solubilizing fungal isolates were identified based on the taxonomic keys based on morphologies (Watanabe, 2010). The keys were the color and tint in colony overs and revers, presence of aerial hyphae, colony surface texture, colony margin and pattern of pigment exudations. Wet mounts prepared from micro culture were mounted in lacto phenol and lacto phenol cotton blue. Microscopic examination and photomicrography were performed with an OLYMPUS BX50 microscopy equipped with image Analysis system (Olympus Corporation, Tokyo, Japan).

DNA was extracted from one piece of fungal mycelia from a culture incubated at 25 °C for 48 h on Sabrouraud medium containing 2% glucose and 1% peptone using a DEXPAT kit (TaKaRa, Japan) to identify the isolates at genetic level (Yamaguchi et al., 2014). Beta- tubulin gene sequences amplified with primers bt2a and bt2b and calmodulin genes amplified with primers CMD5 and CMD6 were determined (Samson et al., 2014). Sequences were analyzed by the NCBI BLAST tool to classify and identify closely related fungal sequences. We identified the isolates to the certain species if the BLAST results showed similarity values of 98% or higher.

Preparation of spore suspension

Sporulated pure fungal cultures slants were selected for preparation of spore suspension by using standard procedure. A total volume of 5 ml sterile water with twin 80 was added in culture slants and the fungal colony surface was lightly scraped by sterile bamboo stick. The cultures were passing through a syringe with staff cotton. Spore count was done by a hemocytometer and the suspension was adjusted to approximately10^6 spores mL^{-1}.

Quantitative estimation of phosphate solubilization

It was carried out using Erlenmeyer flask containing 40 ml Pikoveskaya's (PKV) broth medium supplemented with 0.5% tricalcium phosphate [$Ca_3 (PO_4)_2$], aluminium phosphate ($AlPO_4$) and iron phosphate ($FePO_4$). 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 mL^{-1}. Sterile distilled water inoculated flaks was treated as control. Three replicates were maintained for each test isolate and mean value was recorded. Incubation was done at 25 °C in an incubator shaker at 120 rpm up to 9 days. The samples were autoclaved and centrifuged at 5000 rpm for 25 min to remove any suspended solids and mycelial parts. Then the cultures were filtered through 0.45 µm 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

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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 and expressed as mg/L (Morphy and Riley, 1962). Samples cultured for 3, 6 and 9 days were compared. After calculation of mean phosphate degradation ability from 16 isolates of each day, we selected the adequate period for the comparison depending on the substrate.

Effect temperature on the growth and survival of isolates

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

Statistical 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

Screening and identification of phosphate solubilizing fungi

A total of 16 fungal isolates showed phosphate solubilizing activities. The isolates were 6 *Aspergillus* spp., 6 *Penicillium* spp. and 2 *Taleromyces* spp. identified based on colony morphology, microscopic observation and Beta-tubulin and/or calmodulin sequences (*Table 1*).

Table 1. *List of fungal strains with gene bank accession number isolated from dark red, red and grey soils of subtropical environment*

Isolates	Strain in gene bank	Soil types	Sampling places	Organisms	Accession number	
					Beta tubulin gene	Calmodulin gene
1	SI-1URAgr	Dark red soil	Nishihara, Okinawa	*Penicillium sp.*	LC425316	Not done
2	SI-2URAgr	Dark red soil	Nishihara, Okinawa	*Aspergillus floccosus*	LC425317	Not done
3	SI-3URAgr	Dark red soil	Nishihara, Okinawa	*Aspergillus niveus*	LC425318	LC425334
4	SI-4URAgr	Grey soil	Nishihara, Okinawa	*Talaromyces pinophilus*	LC425319	LC425335
5	SI-5URAgr	Grey soil	Nishihara, Okinawa	*Aspergillus niveus*	LC425320	LC425336
6	SI-6URAgr	Grey soil	Nishihara, Okinawa	*Penicillium oxalicum*	LC425321	Not done
7	SI-7URAgr	Red soils	Kunigami, Okinawa	*Penicillium sp.*	LC425322	Not done
8	SI-8URAgr	Red soils	Kunigami, Okinawa	*Penicillium sp.*	LC425323	Not done
9	SI-9URAgr	Red soils	Kunigami, Okinawa	*Penicillium sp.*	LC425324	Not done
10	SI-10URAgr	Red soils	Kunigami, Okinawa	*Aspergillus niger*	LC425325	LC425337
11	SI-11URAgr	Red soils	Yanbaru forest, Okinawa	*Aspergillus niger*	LC425326	LC425338
12	SI-12URAgr	Red soils	Yanbaru forest, Okinawa	*Aspergillus niger*	LC425327	LC425339
13	SI-13URAgr	Dark red soil	Nishihara, Okinawa	*Penicillium sp.*	LC425328	LC425340
14	SI-14URAgr	Dark red soil	Nishihara, Okinawa	*Aspergillus floccosus*	LC425329	Not done
15	SI-15URAgr	Grey soil	Nishihara, Okinawa	*Talaromyces pinophilus*	LC425330	Not done
16	SI-16URAgr	Dark red soil	Nishihara, Okinawa	*Penicillium oxalicum*	LC425331	Not done

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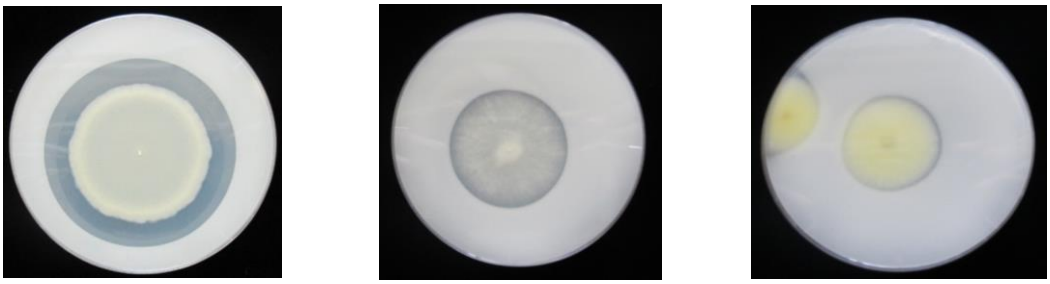
Qualitative phosphate solubilization

Sixteen fungal isolates showed significant phosphate solubilization in Pikovskaya agar medium using tricalcium phosphate as the substrate. The phosphate solubilization index (PSI) ranged from 1.42 to 2.24 (*Table 2*). Isolate SI-16URAgr (*Penicillium oxalicum*) produced highest PSI; 2.24 (*Fig. 2*), whereas; the smallest PSI of 1.42 was achieved from SI-3URAgr (*Aspergillus nevius*)

Table 2. *In vitro phosphate solubilization in solid medium by 16 fungal strains*

Sl. No.	Fungal strain	Type of fungi	PSI
1	SI-1URAgr	*Penicillium sp.*	1.6 ± 0.03^{d}
2	SI-2URAgr	*Aspergillus floccosus*	1.67 ± 0.08^{d}
3	SI-3URAgr	*Aspergillus niveus*	1.42 ± 0.02^{e}
4	SI-4URAgr	*Talaromyces pinophilus*	1.8 ± 0.04^{c}
5	SI-5URAgr	*Aspergillus niveus*	1.67 ± 0.05^{d}
6	SI-6URAgr	*Penicillium oxalicum*	1.78 ± 0.03^{c}
7	SI-7URAgr	*Penicillium sp.*	1.5 ± 0.05^{d}
8	SI-8URAgr	*Penicillium sp.*	1.56 ± 0.08^{d}
9	SI-9URAgr	*Penicillium sp.*	1.42 ± 0.04^{e}
10	SI-10URAgr	*Aspergillus niger*	1.91 ± 0.03^{b}
11	SI-11URAgr	*Aspergillus niger*	1.66 ± 0.04^{d}
12	SI-12URAgr	*Aspergillus niger*	1.64 ± 0.04^{d}
13	SI-13URAgr	*Penicillium sp.*	2.02 ± 0.24^{b}
14	SI-14URAgr	*Aspergillus floccosus*	1.62 ± 0.09^{d}
15	SI-15URAgr	*Talaromyces pinophilus*	1.72 ± 0.04^{d}
16	SI-16URAgr	*Penicillium oxalicum*	2.25 ± 0.06^{a}

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



A B C

Figure 2. *Clear halo formation by representative fungal isolates in Pikovskaya agar plates (A: Penicillium oxalicum, B: Aspergillus niger and C: Talaromyces pinophilus)*

Quantitative phosphate solubilization

Phosphate solubilizations by the isolated fungi were analyzed in Pikovskaya broth medium using three substrates of recalcitrant phosphate compounds: tricalcium phosphate [$Ca_3(PO_4)_2$], aluminium phosphate ($AlPO_4$) and iron phosphate ($FePO_4$). The

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P-solubilizing ability of fungal isolates varied with incubation period and substrates. The best period of observation was selected considering their mean P solubilization, which 9 days for $Ca_3(PO4)_2$ and 6 days for both $AlPO_4$ and $FePO_4$ (*Table 3*).

Table 3. *Selection for the best period of phosphate solubilization by fungal strains*

Solubilized phosphate (mg/L)								
Tricalcium phosphate (TCP)			**Aluminium phosphate (Al-P)**			**Iron phosphate (Fe-P)**		
3 days	**6 days**	**9 days***	**3 days**	**6 days8***	**9 days**	**3 days**	**6 days***	**9 days**
192.2±106.2	245.4±101.5	303.4±216.3	81.4±31.6	236.0±167.6	194.2±192.5	93.6±93.7	173.4±212.0	156.5±171.7

Values given are the mean ± standard deviation of P solubilized by 16 fungal isolates
An asterisk (*) indicated the highest solubilization day

The strongest phosphate (P) solubilization effect was found in the medium containing $Ca_3(PO_4)_2$ followed by $AlPO_4$ and $FePO_4$ (*Table 4*). The solubilized P ranged between 73.2-759.4 mg/L, 85.6-599.6 mg/L and 36.6-663.8 mg/L from $Ca_3(PO_4)_2$, $AlPO_4$ and $FePO_4$ respectively. Among the isolates the highest amount of P was solubilized by *Aspergillus niger* followed by *Penicillium oxalicum* and *Talaromyces pinophillus.* Finally, *Aspergillus niger* strain SI-10URAgr, SI-11URAgr and SI-12URAgr were considered as outstanding isolates because solubilized P was higher than sum of the mean and standard deviation of P solubilized by 16 isolates. The amount of solubilized P from $Ca_3(PO_4)_2$ was 759.5, 647.8 and 670.5 mg/L; from $AlPO_4$ was 388.0, 558.3 and 599.7 mg/L and from $FePO_4$ was 663.8, 555.9 and 517.0 mg/L respectively (*Table 4*). SI-10URAgr showed outstanding performance in both $Ca_3(PO_4)_2$ and $FePO_4$ solubilization but in case of $AlPO_4$, it was very close to the outstanding.

Table 4. *Comparison of phosphate solubilization from different substrate by phosphate solubilizing fungal strains*

Sl. No.	***Fungal strain***	***Type of fungi***	***Solubilized phosphate (mg/L)***		
			TCP	**Al-P**	**Fe-P**
1	SI-1URAgr	*Penicillium sp.*	295.5	166.7	176.0
2	SI-2URAgr	*Aspergillus floccosus*	83.5	190.3	42.4
3	SI-3URAgr	*Aspergillus niveus*	73.3	96.3	41.4
4	SI-4URAgr	*Talaromyces pinophilus*	175.9	194.9	36.1
5	SI-5URAgr	*Aspergillus niveus*	126.1	102.6	39.4
6	SI-6URAgr	*Penicillium oxalicum*	240.5	370.0	41.9
7	SI-7URAgr	*Penicillium sp.*	157.9	108.0	40.5
8	SI-8URAgr	*Penicillium sp.*	84.2	256.7	38.4
9	SI-9URAgr	*Penicillium sp.*	207.7	90.6	37.4
10	SI-10URAgr	*Aspergillus niger*	759.5*	388.0	663.8*
11	SI-11URAgr	*Aspergillus niger*	647.8*	558.3*	555.9*
12	SI-12URAgr	*Aspergillus niger*	670.5*	599.7*	517.0*
13	SI-13URAgr	*Penicillium sp.*	305.4	101.8	86.9
14	SI-14URAgr	*Aspergillus floccosus*	321.9	333.2	227.5
15	SI-15URAgr	*Talaromyces pinophilus*	308.0	134.8	48.0
16	SI-16URAgr	*Penicillium oxalicum*	397.2	85.7	182.9
		Mean±Sd	303.4 ± 216.3	236.0 ± 167.6	173.4 ± 212.0

TCP: tricalcium phosphate; Al-P: aluminium phosphate and Fe-P: iron phosphate
An asterisk (*) indicated outstanding values of solubilized phosphate. It was higher than sum of mean and standard deviation of P solubilized by 16 fungal isolates
Values given are the mean ± standard deviation of P solubilized by 16 fungal isolates

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pH value of the culture medium

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. 3*). The strongest negative correlation was observed in all fermented broth culture and correlation coefficient (r) was -0.88, -0.74 and -0.84 in TCP, Al-P and Fe-P respectively.

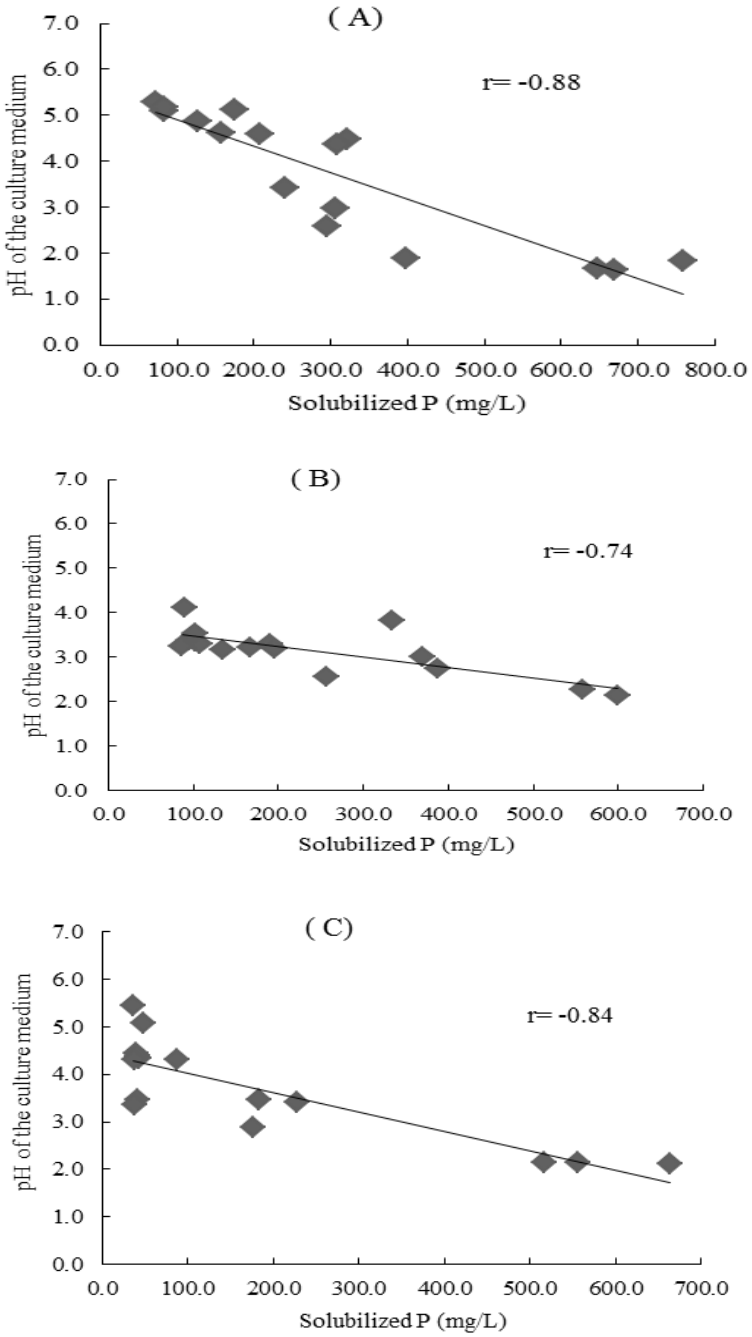

Figure 3. *Pearson's correlation between soluble phosphate and pH of the culture medium supplemented with [$Ca_3(PO_4)_2$] (A), $AlPO_4$ (B) and $FePO_4$ (C); inoculated by 16 fungal strains*

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Temperature effects on isolates

Survival of the isolates at different temperature was tested (*Table 5*). SI-7URAgr, SI-8URAgr and SI-9URAgr could grow and survived up to 35 °C while SI-1URAgr and SI-13URAgr survived up to 37 °C and other isolates were capable to grow at 42 °C.

Table 5. *The growth and survival of isolated phosphate solubilizing fungal strains at different temperature*

Sl. No.	*Fungal strains*	*Name of the fungi*	*Growth temperature*			
			Positive control 25 °C	**35** °C	**37** °C	**42** °C
1	SI-1URAgr	*Penicillium sp.*	+	+	+	-
2	SI-2URAgr	*Aspergillus floccosus*	+	+	+	+
3	SI-3URAgr	*Aspergillus niveus*	+	+	+	+
4	SI-4URAgr	*Talaromyces pinophilus*	+	+	+	+
5	SI-5URAgr	*Aspergillus niveus*	+	+	+	+
6	SI-6URAgr	*Penicillium oxalicum*	+	+	+	-
7	SI-7URAgr	*Penicillium sp.*	+	+	-	-
8	SI-8URAgr	*Penicillium sp.*	+	+	-	-
9	SI-9URAgr	*Penicillium sp.*	+	+	-	-
10	SI-10URAgr	*Aspergillus niger*	+	+	+	+
11	SI-11URAgr	*Aspergillus niger*	+	+	+	+
12	SI-12URAgr	*Aspergillus niger*	+	+	+	+
13	SI-13URAgr	*Penicillium sp.*	+	+	+	-
14	SI-14URAgr	*Aspergillus floccosus*	+	+	+	+
15	SI-15URAgr	*Talaromyces pinophilus*	+	+	+	+
16	SI-16URAgr	*Penicillium oxalicum*	+	+	+	-

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

Discussion

The sixteen P solubilizing fungal strains were isolated from the dark red, red and grey soils of subtropical Okinawa. The isolates were belonging to the genera of *Aspergillus, Penicilium* and *Talaromyces*.

According to Zang et al. (2018), Mendes et al. (2014) and Ruangsanka (2014), there were diversities on the isolation rate of phosphate solubilizing fungi depending on area. They reported that the most dominant genera of P solubilizing filamentous fungi are *Aspergillus, Penicilium* and *Talaromyces*, however, there were large variations in phosphate solubilizing abilities among fungal species (Barrooso et al., 2006; Surange et al., 1985). At the present studies, strains SI-10URAgr, SI-11URAgr and SI-12URAgr identified as *A. niger*, showed excellent P solubilizing abilities regardless of the phosphate substrates. It suggested that phosphate solubilizing abilities in *A. niger* is an universal property.

Among the filamentous fungi *Aspergillus* spp. are widely used for the production of fermented foods, organic acids and enzymes (Wongwicharn et al., 1999). Especially, *A. niger* has a long history of industrial usage, which means many strains already have a GRAS ("generally regarded as safe") status (Wongwicharn et al., 1999). It has been used for commercial production of many enzymes, e.g. pectinase, glucose oxidase,

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glucoamylase, hemicellulase, glucanases, acid proteinase, catalase (Aguilar and Huitron, 1993; Liu et al., 1999; Garhartz, 1990) and citric acid (Friedrich et al., 1989; Gokhale et al., 1991; Lee et al., 1989).

Interestingly, beside *A. niger* species, *P. oxalicum* (SI-14URAgr) also showed an excellent phosphate degradation ability on halo assay. According to Jain et al. (2014), Johri et al. (1999), Alam et al. (2002), Elias et al. (2016) and Jain et al. (2017) solid medium using agar plates performed better phosphate degradation ability than those in liquid media. Thus, it is impossible to ignore the *P. oxalicum* isolate SI-16URAgr. *Penicillium* spp. is important in the natural environment as well as food and drug production. Some members of the genus produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain bacteria spp. Other species are used in cheese industries (https://en.wikipedia.org/wiki/*Penicillium*). *Penicillium oxalicum* produces secalonic acid D, chitinase and oxalic acid (https://en.wikipidia.org/wiki/Penicillium_oxalicum).

The solubilization of $Ca_3(PO_4)_2$ was the highest, followed by $AlPO_4$ and $FePO_4$ because $AlPO_4$ and $FePO_4$ have complex structure than $Ca_3(PO_4)_2$. Zang et al. (2018) and Son et al. (2006) reported that fungi exhibited low P solubilizing ability in media containing $AlPO_4$ and $FePO_4$. The mechanisms of phosphate solubilization by microorganisms are very complex and are not completely known yet (Bo et al., 2011). The very common mechanisms are acidification, chelation and exchange reactions (Bo 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 complexion of metal ions release to solution. Zang et al. (2018) and Scervino et al. (2013) reported that organic acids production depends on the interaction of P source and fungi.

In this study, *A. niger* showed the highest efficiency in P solubilization by decreasing pH of the culture medium, which indicated higher amount of organic acid production. Silva et al. (2014), Li et al. (2016) and Barroso et al. (2006) reported that *A. niger* produce higher amount of organic acids and enhance phosphate solubilization. Zang et al. (2018) reported that solubilization of the different P sources mostly depended on the amount of organic acids production by fungi. Tricarboxylic acids such as citric acid, oxalic acid 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 (Bo 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; Xio et al., 2015). 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 and 2017), such as chelation and exchange reactions (Bo et al., 2011).

Conclusions

Isolates *A. niger* strain SI-10URAgr, SI-11URAgr and SI-12URAgr have unique capabilities to solubilized three insoluble phosphate compounds and may become an important bio resource for soil fertility management as well as sustainable crop production and pollution free environment.

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