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The Ability of Streptomyces spp. Isolated from Iranian Soil to Solubilize Rock Phosphate

Nazila Biglari[1]*, Hasnuri Mat Hassan[1], Javid Amini[2]

1. School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia
2. Department of Microbiology, Academic and Scientific Research Center, Kerman, Iran

Abstract

Many agricultural soils are deficient in plant available phosphate and hence not able to sustain optimal crop productivity. The problem is due to the ability of many soils to fix phosphate in a wide range of soil pH and ecological conditions. There is a need to search for more environmental friendly alternatives to improve soil fertility and crop production in phosphate deficient soil. The aim of this study was to isolate, screen, and characterize phosphate solubilizing actinomycetes found in different types of soil with varied pH from various sites in Iran. Phosphate solubilizing ability of the actinomycetes was evaluated both on modified Pikovskaya's (PVK) agar and into broth media containing Christmas Island Rock Phosphate (CIRP). The abilities of each isolate to solubilize phosphorus was evaluated from day 1 to day 14 after inoculation. Streptomyces spp. were identified morphologically under scanning electron microscope (SEM). About 31% (22/70) isolates of actinomycetes were found to have the ability to solubilize (CIRP). Isolates IA15 and IA31 showed high solubilizing index (SI) on agar medium whereas isolates IA61, IA59, IA38, IA35, and IA31 were determined to have high CIRP solubilizing ability in broth medium. Isolates IA11, IA31, IA10, and IA61 had high pH decrease in broth medium after 14 days of inoculation. A gradual decrease in pH was observed over a 14 day period of incubation, suggesting a slow release of phosphate from CIRP. The mechanism of solubilization was related to pH decrease in broth medium. In general, majority of phosphate solubilizing actinomycetes revealed superior ability to solubilize CIRP.

Key words: rock phosphate; solubilizing phosphate; Streptomyces spp;

Introduction

Among phosphate solubilizing microorganisms (PSM), Actinomycetes are of prime interest since these filamentous sporulating bacteria are able to develop in diverse form of soils (Salcedo et al., 2014). However, they produce various substances including (anti-fungi, insecticides, anthelminthics, and phytohormone-like compounds that could be useful for plant growth (Castanheira et al., 2014). Among actinobacteria class, the Streptomyces genus leads the great value group of branching soil bacteria due to secondary metabolites

Corresponding author:
Nazila Biglari
School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia
Email: nazila_1979i@yahoo.com

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production, such as antibiotics and extracellular enzymes (Sharma et al., 2014). Streptomyces spp. are an economic producer for vitamins, enzymes, and antibiotics in both environment and soil as the major communities of microbes (Ban et al., 2014). Streptomyces spp. are able to distribute earthy smell and rain adores in environment due to microbial products called geosmins especially after rain (Tsao et al., 2014). Permanent and branched mycelium was seen in Streptomyces spp.

After nitrogen (N), phosphate (P) readily is a major nutrition element limiting plant growth in world-wide agricultural soils especially for crop production (Brännvall et al., 2014). Unfortunately, one of the least present and moving mineral nutrients for plants in the soil is P (Nesme et al., 2014). Therefore, about 0.1% of the total P is available to plants. Thus, P availability to plant is subjected to chemical fixation in soil with some metal cations which depend on soil pH (Viani et al., 2014). The largest deposit of phosphorus are in rocks and this substance should afford an interesting supply of phosphate fertilizer for yield production (Rodríguez and Fraga, 1999). De Oliveira Mendes et al. (2014) mentioned that in the fields, rock phosphate (RP) is the main phosphate fertilizers. They also suggested that natural RP has been found as a source of P in a wide area with different soil types specially acid soil which is a valuable alternative and less expensive natural source for phosphate fertilizer. The weakly soluble RP is used in agriculture as a natural slow releasing phosphate fertilizer (Rahman et al., 2014). The favorable effect of rock phosphate has made this material an attractive element for application in agriculture (Barea et al., 2002).

In this research study, we studied on P solubilizing ability of isolated Streptomyces spp. The significance of this finding may lead to a novel actinomycetes solubilizing rock phosphate that has the potential to be utilized to increase soil fertility and crop productivity.

Materials and Methods

2.1 Screening of Streptomyces spp.

The strains isolated from soil sources according to P solubilization abilities were coded in IA1 to IA70. For screening Streptomyces spp. the Malt Extract Agar (MEA) medium at 28ºC temperature for 3 days incubation was used containing (per liter): maltose 12.75 g, dextrin 2.75 g, glycerol 2.35 g, peptone 0.78 g, and agar 15 g (Busarakam et al., 2014). Then, gram stain from cover slip culturing and acid-fast stain were used to identify the genus (Staneck and Roberts, 1974).

2.2 Qualification of Total Free Inorganic Phosphate

For study on P abilities, PVK agar medium was used at 28ºC temperature for 14 days (Mehta et al., 2014). The halo zone due to dissolving phosphate into inorganic phosphate which can used by microorganism was measured at 1st, 7th, and 14th day of incubation. All experiments were replicated in three times. The index of solubility was calculated using the formula:

Solubilization index (SI) = Colony diameter + Halo zone diameter/ Colony diameter (Khan et al., 2014).

2.3 Quantitation Total Free Inorganic Phosphate

Molybdenum blue technique was chosen for determination of available P (Ngosong et al., 2014).The fresh cultures of strains were inoculated into the modified Pikovskaya's (PVK) broth with RP (30%), followed by shaking at 180 rpm and a temperature range of 28-30ºC for 14 days. 10 ml of suspension of inoculate was taken out and centrifuged at 1500 rpm for 15 min (sigmacentrifuge, Model 4K15).The supernatant was reacted with the reagents to obtain blue colour. Concentration

determination of soluble inorganic phosphate pi was determined spectroscopically at 880 nm wavelength. All treatments were replicated in three times.

2.4 Measurement of pH During Phosphate Solubilizing Activity by Selected Isolates

The pH changes in the pi solubilizing process was measured to find the relationship between dissolved Pi and pH values. After taking out 5 ml of supernatant to react with reagents, the rest of aliquot were used to measure pH values with a pH meter (Orion, USA). The measurement of pH was concurrently performed with P determination of each aliquot (Salcedo et al., 2014).

2.5 Statistical Analysis

The results and data were evaluated by one-way analysis of variance (ANOVA) and significance of differences between means and SE were tested using Duncan's Multiple Range Test at $p<0.05$ by SPSS Statistics 20 IBM®.

Results

3.1 Isolation of Phosphate Solubilizing Actinomycetes

Actinomycetes were identified morphologically by microscopic observation based on the characteristics of spores (spore formation on the substrate and/or aerial mycelium and presence of single spores or sporangia), hyphae, pigmentation, and mycelia fragmentation. The morphological characteristics of 22 isolates based on elevation, edge, pigmentation, and surface appearances were recorded. From 22 isolates, 14 were irregular, 5 were filamentous, 2 were rhizoid, and 1 isolates was circular. 15 strains of actinomycetes were dry, rough, and penetrating agar media. In some cases, colonies were sticky, smooth, and glistening. Different pigmentation, edge, and elevation of colonies were observed among all isolates as well (Table 1).

3.2 Inorganic Phosphate (Pi) Release by Phosphate Solubilizing Streptomyces Strains

Qualification of Pi release: Isolates IA61 showed high Pi released on modified PKV agar medium while isolates IA22, IA15, and IA31 were significantly lower Pi release on 1st day of inoculation (Table 2). Whereas at 7th days after inoculation, IA22 showed the highest Pi released followed by IA15, IA61, and IA27 on modified PKV agar medium. Finally, at 14th day of inoculation, isolates IA15 and IA31 had the highest Pi released on modified PKV agar medium.

Quantitation of Pi release: At 1st day of incubation, IA61 released significantly highest Pi release. During 7 days of incubation, IA11 released more Pi and followed by strains IA31, IA8, IA38, and IA59. The highest Pi released was determined in isolate IA61 after 14 days of incubation at 28°C. Similarly, isolates IA59, IA38, IA35, and IA31 were also dissolved high amount of Pi at the same period of incubation at 28°C.

3.3 Relationship between Solubilized Pi Concentration and pH

A decrease in pH of the media was observed with all isolates of Actinomycetes (Table 4). It was found that isolates IA61, IA8, IA22, IA31, IA27, IA13, IA10, IA12, and IA59 had a high pH decrease in modified PVK broth medium after 1 day of incubation. Isolate IA10 had the highest pH decrease in modified PVK broth medium after 7 days of incubation. After 14 days of incubation, pH of isolates IA11, IA31, IA10, and IA61 was decreased in modified PVK broth medium. Generally, pH of isolates IA12, and IA13 was increased during incubation period and pH of isolate IA27 was increased from 1st day to 7th day of incubation and then pH was decreased at 14th days of incubation.

Isolated colony	Form of whole colony	Elevation	Edge	Pigmentation	Surface	Gram stain	Species identification
IA6	Filamentous	Raised	Filamentous	White-light pink	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA7	Irregular	Raised	Undulate	White	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA8	Filamentous	Raised	Filamentous	White-light pink	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA10	Rhizoid	Flat	Undulate	Pale-yellow	Sticky, smooth, glistening	+	*Streptomyces* sp.
IA11	Circular	Umbonate	Entire	Orange	Smooth, glistening	+	*Streptomyces* sp.
IA12	Irregular	Convex or dome	Undulate	Yellow	Smooth, glistening	+	*Streptomyces* sp.
IA13	Filamentous	Raised	Filamentous	White-light pink	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA14	Irregular	Convex or dome	Entire	Yellow	Sticky	+	*Streptomyces* sp.
IA15	Irregular	Flat	Entire	Pale-yellow	Smooth, glistening	+	*Streptomyces* sp.
IA17	Irregular	Raised	Undulate	Brown-black	Dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA18	Rhizoid	Flat	Undulate	Pale-yellow	Smooth, glistening	+	*Streptomyces* sp.
IA19	Filamentous	Raised	Filamentous	White-light pink	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA22	Irregular	Raised	Undulate	White-red	Dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA24	Irregular	Raised	Undulate	White	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA27	Irregular	Umbonate	Curled	Gray-white	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA28	Irregular	Raised	Undulate	White	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA31	Irregular	Umbonate	Curled	Gray-brown	Dry, rough, powdery, penetrating agar media	+	*Streptomyces* sp.
IA35	Filamentous	Raised	Filamentous	White-light pink	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA38	Irregular	Raised	Entire	White	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA56	Irregular	Raised	Entire	White-yellow	Powdery, dry, rough, penetrating agar media	+	*Streptomyces* sp.
IA59	Rhizoid	Flat	Undulate	Pale-yellow	Smooth, glistening	+	*Streptomyces* sp.
IA60	Irregular	Convex or dome	Undulate	Yellow	Smooth, glistening	+	*Streptomyces* sp.
IA61	Irregular	Raised	Undulate	White	Dry, rough, powdery, penetrating agar media	+	*Streptomyces* sp.

Table 1: The colony characteristics based on substrate mycelium colour and colony morphology of 22 different actinomycetes colonies isolated on MEA agar plates for 3 days.

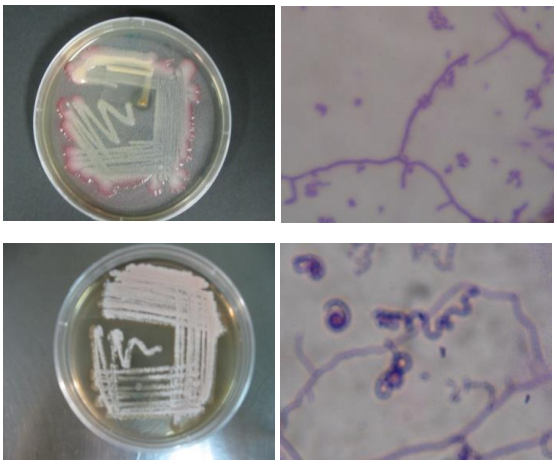

Figure 1: Colony Morphology Observation
Description: Streptomyces. sp with glistening colony on MEA medium (top, left side) Streptomyces. sp. with aerial mycelium with acid fast staining in purple colour (top, right side, 10µm) Streptomyces. sp with powdery/ dry/ white-pink colony on MEA medium (bottom, left side) Streptomyces. sp. with spiral mycelium with acid fast staining in purple colour (bottom, right side, 10µm). After 3 days of inoculation at 28°C (Table 3).

Actinomycete	(Mean ± SE) Solubilizing index (SI)		
	Day 1	Day 7	Day 14
IA6	2.07 ± 0.03 ^{c}	2.12 ± 0.08 ^{de}	2.25 ± 0.05 ^{ef}
IA7	2.11 ± 0.01 ^{c}	2.10 ± 0.00 ^{de}	2.10 ± 0.00 ^{f}
IA8	2.03 ± 0.02 ^{c}	2.03 ± 0.00 ^{e}	2.09 ± 0.07 ^{f}
IA10	2.15 ± 0.05 ^{c}	1.94 ± 0.09 ^{e}	2.19 ± 0.08 ^{ef}
IA11	2.20 ± 0.00 ^{bc}	2.30 ± 0.01 ^{de}	2.22 ± 0.08 ^{ef}
IA12	2.05 ± 0.01 ^{c}	2.29 ± 0.09 ^{de}	2.20 ± 0.00 ^{ef}
IA13	2.13 ± 0.01 ^{c}	2.12 ± 0.06 ^{de}	2.06 ± 0.00 ^{f}
IA14	2.14 ± 0.05 ^{c}	2.09 ± 0.01 ^{de}	2.14 ± 0.06 ^{ef}
IA15	3.18 ± 0.02 ^{bc}	3.98 ± 0.22 ^{a}	6.01 ± 0.37 ^{a}
IA17	2.10 ± 0.00 ^{c}	2.10 ± 0.06 ^{de}	2.10 ± 0.00 ^{f}
IA18	3.15 ± 0.35 ^{bc}	2.97 ± 0.09 ^{c}	2.99 ± 0.06 ^{bc}
IA19	2.03 ± 0.26 ^{c}	2.27 ± 0.01 ^{de}	2.20 ± 0.07 ^{ef}
IA22	3.30 ± 0.15 ^{bc}	4.93 ± 0.26 ^{bc}	3.26 ± 0.20 ^{b}
IA24	2.24 ± 0.13 ^{bc}	2.18 ± 0.07 ^{de}	2.18 ± 0.07 ^{ef}
IA27	2.92 ± 0.28 ^{b}	3.13 ± 0.07 ^{c}	3.02 ± 0.19 ^{bc}
IA28	2.21 ± 0.00 ^{bc}	2.24 ± 0.04 ^{de}	2.21 ± 0.01 ^{bc}
IA31	3.17 ± 0.11 ^{a}	2.92 ± 0.35 ^{c}	4.06 ± 0.26 ^{ef}
IA35	2.20 ± 0.06 ^{bc}	2.14 ± 0.00 ^{de}	2.14 ± 0.00 ^{ef}
IA38	2.11 ± 0.00 ^{c}	2.13 ± 0.03 ^{de}	2.11 ± 0.00 ^{f}
IA56	2.05 ± 0.05 ^{c}	2.12 ± 0.06 ^{de}	2.08 ± 0.01 ^{f}
IA59	2.06 ± 0.06 ^{c}	2.03 ± 0.01 ^{de}	2.04 ± 0.02 ^{f}
IA60	2.06 ± 0.02 ^{c}	2.07 ± 0.03 ^{de}	2.11 ± 0.07 ^{ef}
IA61	4.18 ± 0.25 ^{a}	3.21 ± 0.17 ^{bc}	3.18 ± 0.19 ^{b}

Table 2: Solubilizing index (SI) of 22 solubilizing actinomycetes on D1, D7, and D14 of inoculation at 28°C.

*Mean values in the same column indicated by different superscript letters are significantly different (Duncan test, p < 0.05).

Actinomycete	(Mean ± SE) Pi release (µg/l)		
	Day 1	Day 7	Day 14
IA6	3.1 ± 0.04 ^{defg}	4.1 ± 0.13 ^{bcde}	4.9 ± 0.05 ^{bcdef}
IA7	4.3 ± 0.04 ^{bcdefg}	4.7 ± 0.15 ^{bcde}	4.6 ± 0.14 ^{cdefg}
IA8	5.5 ± 0.16 ^{bcd}	5.4 ± 0.11 ^{abce}	3.7 ± 0.02 ^{defgh}
IA10	5.8 ± 0.07 ^{bcd}	4.1 ± 0.15 ^{bcde}	3.5 ± 0.10 ^{defgh}
IA11	4.2 ± 0.01 ^{bcdefg}	6.0 ± 0.01 ^{abe}	3.9 ± 0.03 ^{defgh}
IA12	3.9 ± 0.14 ^{bcdefg}	3.6 ± 0.01 ^{bcde}	2.2 ± 0.04 ^{gh}
IA13	5.5 ± 0.17 ^{bcd}	2.9 ± 0.00 ^{bcde}	2.8 ± 0.02 ^{efgh}
IA14	5.2 ± 0.11 ^{bcde}	4.1 ± 0.16 ^{bcde}	3.1 ± 0.03 ^{efgh}
IA15	3.7 ± 0.01 ^{bcde}	4.5 ± 0.13 ^{bcde}	4.3 ± 0.05 ^{cdefg}
IA17	2.4 ± 0.02 ^{efg}	4.9 ± 0.02 ^{bcde}	2.3 ± 0.07 ^{fgh}
IA18	2.9 ± 0.03 ^{dcfg}	4.2 ± 0.05 ^{bcdc}	3.6 ± 0.02 ^{dcfgh}
IA19	3.1 ± 0.09 ^{defg}	3.6 ± 0.02 ^{bcde}	3.5 ± 0.04 ^{defgh}
IA22	5.3 ± 0.03 ^{bcde}	2.5 ± 0.12 ^{cde}	3.1 ± 0.01 ^{efgh}
IA24	1.9 ± 0.04 ^{fg}	2.9 ± 0.03 ^{bcde}	3.0 ± 0.02 ^{efgh}
IA27	4.9 ± 0.07 ^{bcdef}	2.5 ± 0.03 ^{cde}	4.3 ± 0.03 ^{cdefgh}
IA28	4.6 ± 0.08 ^{bcdefg}	3.8 ± 0.04 ^{bcde}	4.8 ± 0.05 ^{bcdefg}
IA31	6.2 ± 0.04 ^{bc}	5.5 ± 0.13 ^{abce}	5.0 ± 0.06 ^{bcde}
IA35	4.5 ± 0.02 ^{bcdefg}	3.7 ± 0.03 ^{bcde}	5.1 ± 0.04 ^{bcde}
IA38	4.4 ± 0.08 ^{bcdefg}	5.0 ± 0.12 ^{bcde}	5.4 ± 0.04 ^{bcde}
IA56	3.9 ± 0.05 ^{bcdefg}	4.3 ± 0.06 ^{bcde}	3.6 ± 0.03 ^{defgh}
IA59	6.9 ± 0.08 ^{ab}	5.0 ± 0.17 ^{bcde}	5.9 ± 0.10 ^{bcd}
IA60	6.9 ± 0.18 ^{ab}	3.3 ± 0.04 ^{bcde}	4.6 ± 0.18 ^{cdefg}
IA61	9.0 ± 0.13 ^{a}	4.0 ± 0.00 ^{bcde}	7.2 ± 0.20 ^{ab}

Table 3: Inorganic P Release (µg/l) by Phosphate Solubilizing Actinomycetes.

*Mean values in the same column indicated by different superscript letters are significantly different (Duncan test, p < 0.05).

Actinomycete	(Mean ± SE) pH decrease		
	Day 1	Day 7	Day 14
IA6	5.36 ± 0.08 ^{cdefgh}	5.01 ± 0.07 ^{h}	4.14 ± 0.03 ^{bcde}
IA7	5.63 ± 0.32 ^{efgh}	4.72 ± 0.07 ^{defgh}	4.36 ± 0.05 ^{bcde}
IA8	4.56 ± 0.22 ^{ab}	4.14 ± 0.12 ^{abcd}	4.07 ± 0.13 ^{bcd}
IA10	4.88 ± 0.25 ^{ab}	3.95 ± 0.03 ^{ab}	3.92 ± 0.04 ^{bc}
IA11	5.82 ± 0.29 ^{gh}	4.29 ± 0.12 ^{abcdefg}	3.63 ± 0.37 ^{ab}
IA12	4.91 ± 0.11 ^{abcde}	6.50 ± 0.20 ^{j}	6.49 ± 0.03 ^{f}
IA13	4.87 ± 0.05 ^{abcd}	5.80 ± 0.31 ^{i}	6.10 ± 0.11 ^{f}
IA14	5.55 ± 0.24 ^{defgh}	4.21 ± 0.26 ^{abcde}	4.22 ± 0.27 ^{bcde}
IA15	5.32 ± 0.05 ^{cdefgh}	4.83 ± 0.10 ^{fgh}	4.18 ± 0.24 ^{bcde}
IA17	5.93 ± 0.47 ^{h}	4.39 ± 0.15 ^{bcdefg}	4.35 ± 0.43 ^{bcde}
IA19	5.67 ± 0.05 ^{fgh}	4.25 ± 0.03 ^{abcdefg}	4.82 ± 0.12 ^{cde}
IA22	4.76 ± 0.39 ^{abc}	4.38 ± 0.13 ^{bcdefg}	4.39 ± 0.20 ^{bcde}
IA24	6.59 ± 0.29 ^{i}	4.38 ± 0.03 ^{bcdefg}	5.02 ± 0.04 ^{e}
IA27	4.86 ± 0.07 ^{abcd}	5.02 ± 0.54 ^{h}	4.91 ± 0.99 ^{de}
IA28	5.22 ± 0.06 ^{bcdefgh}	4.72 ± 0.04 ^{defgh}	4.27 ± 0.14 ^{bcde}
IA31	4.80 ± 0.19 ^{hij}	4.42 ± 0.05 ^{bcdefg}	3.72 ± 0.21 ^{ab}
IA35	5.61 ± 0.09 ^{efgh}	4.49 ± 0.12 ^{bcdefgh}	4.10 ± 0.10 ^{bcde}

IA38	5.66 ± 0.17 ^{fgh}	4.80 ± 0.15 ^{efgh}	4.01 ± 0.07 ^{bcd}
IA56	5.47 ± 0.28 ^{cdefgh}	4.40 ± 0.05 ^{bcdefg}	4.41 ± 0.10 ^{bcde}

	(Mean ± SE) pH decrease		
	Day 1	Day 7	Day 14
IA60	5.21 ± 0.14 ^{bcdefgh}	4.60 ± 0.03 ^{cdefgh}	4.34 ± 0.14 ^{bcde}
IA61	4.49 ± 0.20 ^{a}	4.10 ± 0.01 ^{abc}	3.93 ± 0.09 ^{bc}

Table 4: pH Decrease of Media Containing Phosphate Solubilizing Actinomycetes with CIRP

*Mean values in the same column indicated by different superscript letters are significantly different (Duncan test, $p < 0.05$).

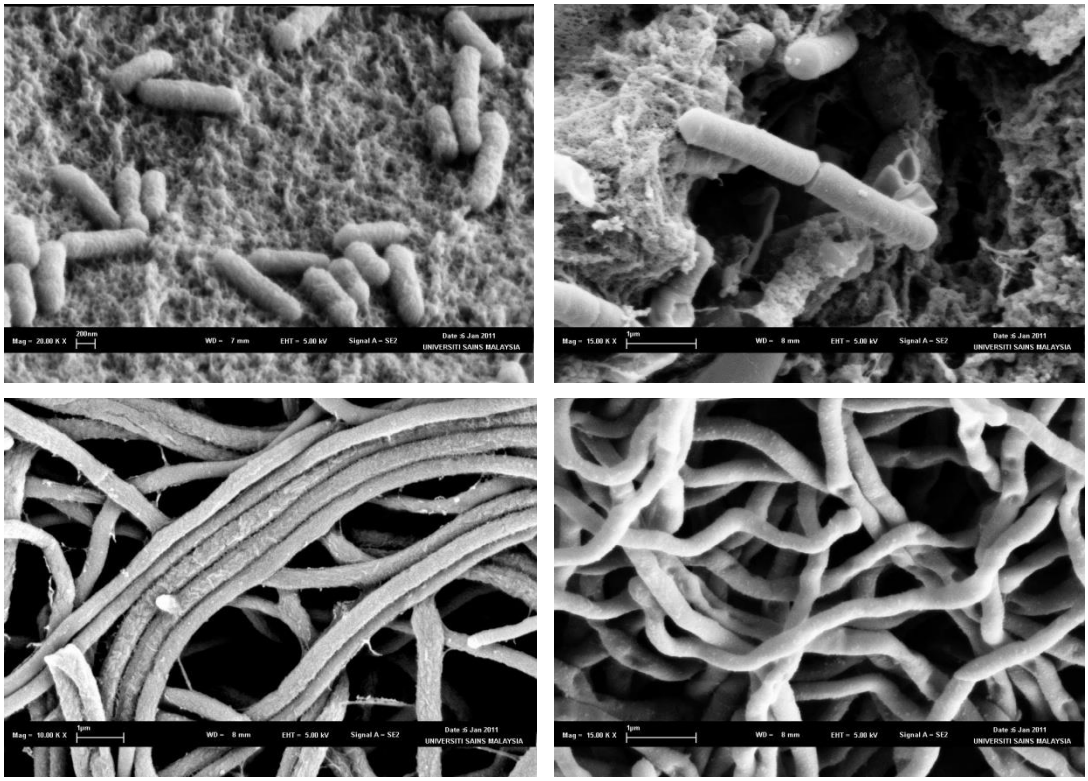

Plate 2: Scanning Electron Microscope (SEM) Observation

Description: Colony morphology of actinomycetes isolates under scanning electron microscope (SEM) (top, left side) isolate IA59, bacilli form, Streptomyces sp. (top, right side) isolate IA10, bacilli form, Streptomyces sp. (bottom, left side) isolate IA8, extensively branching filament, Streptomyces sp. (bottom, right side) isolate IA35, extensively branching filament, Streptomyces sp.

3.4 Morphology Observation under Scanning Electron Microscope (SEM)

Vegetative cell, extensively branching filament and bacilli form of 8 Streptomyces spp. (IA8, IA10, IA35, and IA59) were observed under scanning electron microscope (SEM) in Plate 2.

Discussion

4.1 Phosphate Solubilizing Index (SI) of Phosphate Solubilizing Actinomycetes

Actinomycetes isolates in the present study showed different ability of phosphate solubilization as shown in the value of SI (Table 2). One third of the isolates were obtained with decrease in SI value with continues incubation until 14th days. The decrease in SI of strains from 1st to 7th day of incubation were

reported due to consumption of released P by the strains (Hamdali et al., 2008). Gupta et al. (2010) found that five Streptomyces species isolated from the phyllosphere of Heritiera fomes, showed optimum solubilization at 15 days of incubation which gradually declined upon extension to 21 days. This could be the explanation on why SI were decreased after some incubation period.

The lower value of SI in the current study (1.94 to 6.01) could be due to the type of insoluble phosphate which was used i.e. Christmas Island Rock Phosphate (CIRP) and/ or species of actinomycetes. Previous study was done by Sahu et al. (2007) using Kuster's agar medium supplemented with CaHPO4 (5 g/l) as the sole source of phosphate. The SI of actinomycetes strains were in the range of 10.82 ± 0.7 to 41.66 ± 2.3 (Sahu et al., 2007). In Turkey, the SI of Streptomyces spp. inoculated on PVK agar media supplemented with tri-calcium phosphate incubated at 28°C after 3 days was in the range of 3.6 to 28.5 (Tallapragada and Seshachala, 2012). In another study among 10 phosphate solubilizing isolate, the phosphate solubilization index ranged 12 to 32 (Kaviyarasi et al., 2011).

4.2 Pi Release (µg/l) of Phosphate Solubilizing Actinomycetes

The present study demonstrated that the ability of PSA to solubilize CIRP varied between isolates of actinomycetes from various sampling sites in Iran, some being more efficient than others. This findings suggest that isolates IA61 had high abilities for enrichment of agriculture soils with inorganic phosphorus. The release of phosphate by phosphate solubilizing actinomycete isolates was 9.00 µg/l as being the most and 1.90 µg/l as being the least. The small release was in accordance with the need of phosphate by the growing actinomycetes in the solution culture. This is one of the important characteristics of a bio-fertilizer. A bio-fertilizer should have the ability to slowly release nutrients from a source. Other studies also reported varied amount of phosphate solubilized in solution cultures. In earlier study, strains isolated from research in Colombia had phosphorus solubilizing capacities between 396 and 525 µg/ml with tri-calcium phosphate (Salcedo et al., 2014).Whereas the 22 isolates were able to solubilize the tri-calcium phosphate ranged from 315.46 µg/mg to 50.14 µg/mg (Sujatha and Ammani, 2014). Another study done in India, selected isolates could dissolved inorganic phosphate of Ca3(PO4)2 within National Botanical Research Institute Phosphate (NBRIP) liquid medium with highest concentration of 164.1±4.1 µg/ml and 145.1±2.7 µg/ml respectively. Although, the lowest concentration in same study was found in Streptomyces sp. with 89.3±3.1 µg/ml (Dastager and Damare, 2013). While Streptomyces spp. showed 92 mg/l Pi released from the solubilization of tri-calcium phosphate in culture medium (Sadeghi et al., 2012). In another study, phosphate solubilized in PVK liquid medium ranged between 55.60 and 168.30 mg/L (Karagöz et al., 2012). Release of Pi by different isolates of actinomycetes was between 0.08 to 0.01 mg/g in broth medium (Sahu et al., 2007). Hamdali et al. (2012) reported five selected actinomycetes strains showed different abilities to release phosphate i.e. from 4.38 to 25.87 µg/ml. In India, the quantitative estimation of phosphate solubilization by Strepmtomeces spp. reported diverse concentration of phosphate solubilizing ability ranging from 4.80 to 26.50 mg/100ml (Gangwar et al., 2012). The maximum level of phosphorus solubilization potential of isolates selected from tropical soils was established about 1727 µg/ml of Ca-P and 48.0 µg/ml of RP. In same experimental work, the other group of isolates obtained 1703 µg/ml solubility of Ca-P and 34.5 µg/ml of RP (Asuming-Brempong and Aferi, 2014). In contrast, fungus (Trichoderma spp.) isolated from rhizosphere of pine, deodar,

bamboo, guava and oak showed P solubilizing potential using NBRIP broth containing tri-calcium phosphate (TCP) as the sole P source. They recorded maximum amount of soluble phosphate about 404.07 µg/ml after 96 h of incubation at 30+1°C (Kapri and Tewari, 2010).

The concentration of Pi released in the PVK broth media indicated various efficiency of actinomycete isolates to solubilized CIRP. In general, the release of Pi concentration was higher in the beginning of incubation period due to P availability. The different source of phosphate and isolates as well as incubation period might be the factors that contribute to the difference in amount of Pi released. Regarding incubation period, no achievement by time even after 60 days of incubation on P equilibrium (Rajput et al., 2014). However, it was reported that actinomycete isolates showed optimum solubilization at 15 days of incubation which gradually declined upon extension to 21 days (Gupta et al., 2010). On the other hand, the concentration of Pi in solution culture also decreased or increased over incubation periods depends on enzyme mechanisms (Sahu et al., 2007). While a study by Coutinho, (2012) showed that the maximum concentration of Pi was obtained seven days after inoculation (Achal et al., 2007, Coutinho et al., 2012).

4.3 pH Decrease of Phosphate Solubilizing Actinomycetes

In this study, isolates that solubilized large amount of Pi were also showing high solubilizing index (SI) and pH decrease. The pH decreased from the initial value of 7.00 ± 0.02 to 3.63 within PVK broth culture after 14 days of incubation period. This decrease was more than 3 pH units. In most cases gradual P solubilization process of up to 14 d, indicated the slow action of actinomycetes on CIRP. Gradual change in pH observed in all isolates, confirmed this statement. Total phosphorus concentration of 360 mg/kg with only 118 mg/kg of available form obtained with pH around 5 or less (Salcedo et al., 2014). In a similar study Sahu et al. (2007) showed that pH change gradually decreased upon incubation. Earlier reports also indicated reduction in pH during P solubilization by microbes (Coutinho et al., 2012). Earlier study showed that maximum solubilization of phosphate occurred within 2 weeks period after inoculation under controlled conditions (Ogbo, 2010), which could be due to the release of acidic substances produced by the strain during incubation (Sahu et al., 2007).

The mechanisms of microbial solubilization of phosphate has been proposed as may be due to the excretion of organic acids which was indicated by acidification of the broth medium (Whitelaw, 1999) or to the excretion of chelating substances (such as siderophores) that form stable complexes with phosphorus adsorbents (aluminum, iron and calcium (Al-Halbouni et al., 2008). Earlier study showed the decrease in pH of culture filtrate confirmed the assertion that phosphate solubilizing microorganisms solubilize insoluble phosphates mainly by secreting organic acids into the medium (Gupta et al., 2010).

Conclusion

Seventy (70) actinomycetes of different morphological characteristics were isolated from various soils and water samples in Iran. About 31% of the total numbers was found to be phosphate solubilizing isolates. Half of PSA were able to solubilize CIRP was isolated from Shahdad desert of Iran. The mean solubilizing index, concentration of Pi in solution culture and pH decrease indicated small and gradual release of Pi from CIRP suggesting slow release of Pi by the isolates. This is an important finding because slow release of Pi from an insoluble source is one of the characteristics looked for in the preparation of bio-inoculants as bio-fertilizer. Slow and gradual release could avoid fixation and precipitation of Pi in many soil types at a wide range of pH.

Further studies on the biological, chemical and physical soil properties influencing rock phosphate solubilizing actinomycetes at the field would be necessary to evaluate the effectiveness of these potential bio-inoculants for crop improvement. Identification at the molecular level may reveal similarities and dissimilarities between the isolates. The ability of these isolates to also produce other secondary metabolites would give more value added property of the actinomycetes.

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