# Screening of Phosphate Solubilizing and Siderophores Producing Actinomycetes from Rice Rhizosphere Soil

# การคัดแยกเชื้อแอคติโนมัยซีทที่สามารถละลายฟอสเฟตและสร้างสารไซเดอร์โรฟอร์ จากดินบริเวณรอบรากต้นข้าว

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**ABSTRACT**

Forty four isolates of actinomycetes from rice rhizosphere soil were screened for their ability to solubilize phosphate on Pikovaskaya's medium (PVK). The results showed that 40 isolates (90%) were able to solubilize 0.5% $Ca_3PO_4$ on PVK agar. Isolate HS5-4 produced the largest clear zone on PVK agar. However, quantitative analysis of available phosphorus showed that isolate S7-1 released the maximum phosphate of 32.34 µg/ml. Seven phosphate solubilizing isolates were also tested for siderophore production. Isolate HS5-4 gave the widest siderophore zone. Isolate RH1-5-14 had the highest percentage of inhibiton (75.62%) against rice fungus pathogen, *Fusarium monilifome*. All 7 isolates were classified as *Streptomyces* sp.

**บทคัดย่อ**

จากการคัดเลือกแอคติโนมัยซีทจากดินรอบรากข้าวจำนวน 44 ไอโซเลทเพื่อศึกษาความสามารถในการละลายฟอสเฟตบนอาหารแข็งสูตร Pikovaskaya's medium พบว่ามีแอคติโนมัยซีทจำนวน 40 ไอโซเลท (90%) สามารถละลายฟอสเฟตในรูป 0.5% $Ca_3Po_4$ บนอาหารแข็งสูตร PVK และไอโซเลท HS5-4 สามารถสร้างวงใสบนอาหารแข็งได้กว้างที่สุด อย่างไรก็ตามเมื่อทำการวิเคราะห์หาปริมาณฟอสเฟตที่ละลายได้พบว่าไอโซเลท S7-1 สามารถละลายฟอสเฟตได้ปริมาณสูงที่สุดเท่ากับ 32.34 µg/ml หลังจากนั้นนำไอโซเลทที่สามารถละลายฟอสเฟตได้ดีจำนวน 7 ไอโซเลทมาทดสอบการสร้างไซเดอร์โรฟอร์พบว่าไอโซเลท HS5-4 สร้างไซเดอร์โรฟอร์ได้ดีให้ขนาดวงสีกว้างที่สุด เมื่อนำไปทดสอบการเป็นปฏิปักษ์ต่อเชื้อรา *Fusarium moniliforme* พบว่าไอโซเลท RH1-5-14 มีความสามารถในการยับยั้งการเจริญของเชื้อราได้ดีที่สุดคิดเป็น 75.62% และแอคติโนมัยซีททั้ง 7 ไอโซเลทจัดอยู่ในจีนัส *Streptomyces* sp.

**Key Words:** Actinomycetes, Phosphate Solubilization , Siderophore

**คำสำคัญ:** แอคติโนมัยซีท การสลายฟอสเฟต การสร้างสารไซเดอร์โรฟอร์

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**Introduction**

Phosphorus (P) is an important nutrient for plant growth. Phosphorus is required for photosynthesis, reproduction and N- fixation (Brady and Weil, 2008; Mehrvarz et al., 2008). Most soils contain high amount of phosphorus both insoluble and soluble phosphate forms. However, soluble phosphate is very difficult to dissolve in soil (Vassilev et al., 2006). Uwasawa et al (1986) analyzed phosphorus content in soil and rice plant samples from paddy fields in Thailand and found that the contents of organic-P was very low (69 µg/g). Saengthong (1991) reported that Thailand soil comprised of 65% inorganic P and 35% organic P from 32 upland soils. From these conditions, the chemical fertilizers were used in agriculture. There are swiftly soluble and immobilize in the soil (Reddy et al., 2002), but plant cannot utilize for growth or eluviations (Shigaki et al., 2006). Indeed only 10% of P fertilizer is uptake in plant or animal cycle (De Freitas JR and et al., 1997). The available P in soil depends on pH and soil types. Otherwise, the chemical fertilizers are expansive and harmful for agriculturist. The green agricultures were developed. The benefits of microorganisms such bacteria and fungi increasing recognized. There could be concluding that it has been termed as plant growth promoting rhizobacteria (PGPR) (Glick, 1995). The microorganism plays important role compose insoluble phosphorus to soluble phosphorus by phosphatase. The interesting group is actinobacterium because it had capacity to release phosphorus into the soil. Actinomycetes are microorganism that found in indigenous soil and rhizosphrere soil. They are gram - positive bacteria in order Actinomycetales, predominantly found as *Streptomyces* spp. These have potential producer bioactive compounds including plant growth factors, antibiotics and other substances (Keiser et al., 2000, Omura, 19 86; Shahidi et al., 2004). Many reports have been done on phosphate solubilizing actinomycetes because they able to solubilize phosphorus. They can secret the organic acids to solubilize the bound phosphate in soil (Balakrishna et al., 2012). The *Streptomyces griseus* –related strains BH7 had the most efficiently stimulated aerial growth of wheat plant more than 70% *in vitro* and more than 30% in rock phosphate soil (Hamdali et al., 2008). Saldeco et al (2014) reported that 4 strains of actinomycetes had capacity to soluble tricalcium phosphate. Another secondary metabolite of actinomycetes is siderophore. It is a low molecular weight, ferric ion chelating agents (Neilands, 1995). All organisms need iron for a various functions, for example ATP synthesis, reduction of ribotide precursor of DNA, heme formation and other purposes. The applications of siderophore were used in anti-pathogenic microorganism such as rice pathogenic fungi. Chaiharn et al (2009) reported that *Streptomyces sp.* Strain A130 was effective inhibit *Alternaria* growth in *vitro.*

**Objective of this study**

The aims of this study were to screen actinomycetes from rice rhizosphere soil for phosphate solubilizing activity and to examine the siderophore production.

**Methodology**

**Screening of phosphate solubilizing actinomycetes**

Rice rhizosphere soils were collected from Sansai district, Chiang Mai, Thailand for phosphate solubilizing actinomycetes isolation. Ten grams of

soil samples were placed in polyethylene bags, closed tightly and transported to the laboratory. One gram of soil samples was serially diluted in distilled water from $10^{-1}$ to $10^{-3}$ and 0.1 ml of each dilution were spread on the Pikovskaya (PVK) agar (PVK agar per liter: 10 g glucose; 5 g $Ca_3(PO_4)_2$; 0.2 g KCl; 0.5 g $(NH_4)_2SO_4$; 0.2 g NaCl; 0.002 g $MnSo_4.H_2O$; 0.002 g $FeSo_4.7H_2O$ and 15 g agar, pH 7.0 ) (Chaiharn and Lumyong, 2011). Plates were incubated at 37°C for 7 - 10 days. Actinomycete colonies with clear zone were purified by re-streak on ISP2 agar. Phosphate solubilizing activity of pure culture was determined and confirmed on PVK agar in triplicate. Plates were incubated at 37°C for 7 days and diameter of clear zone was measured.

**Quantitative analysis of available phosphorus solubization in culture broth**

The selected isolates of actinomycete (halo diameters 0.4 – 0.7 cm) were tested the quantitative estimation of available phosphorus and cultured on ISP2 agar for 7 days and then 5 discs were placed into 50 ml of PVK broth in 250 ml Erlenmeyer flasks (triplicate), incubated at room temperature for 5 days on orbital shaking incubator at 150 rpm. The cultures were harvested supernatant by centrifugation at 6,000 rpm for 15 min. To estimate the available phosphate was detected by using Fiske and Subbarow method (1925). The supernatant was mixed with 10% (w/v) trichloroacetic acid for 1:1 (v/v), added 4 ml of color reagent and incubated at room temperature for 15 min. The developing blue color was measured at 820 nm by spectrophotometer. The pH was determined by pH meter.

**Siderophore production**

Siderophore production was detected by CAS plate assay (Schwyn and Neilands, 1987). Actinomycete isolates were placed on CAS agar and incubated at room temperature for 7 days in the dark. The colonies with yellow-orange halo zones were considered as positive for siderophore production. The diameter was measured in term of centrimeter.

**Antagonistic activity against rice pathogenic fungus**

Actinomycete isolates which produced siderophore were inoculated on ISP2 agar and the pathogenic fungus, *Fursarium moniliforme*, was inoculated on PDA for 5-7 days at room temperature. Then, transferred the actinomycete and pathogenic fungus to PDA for antifungal screening test by dual culture method (Chaiharn et al., 2009). The fungus disc was inoculated on PDA individually as control. Plates were incubated at room temperature for 7 days and percentage of fungal growth inhibition was detected and calculated from the following formula

Inhibition (%) = [(a–b) / a] x 100

When; a = Fungal growth radius of control

b = Fungal growth radius in the direction of actinomycetes

All data were analyzed by using the SPSS 11.0 for Windows® software with a one way Analysis of Variance (ANOVA) and Duncan's test to determine any significant difference at $p< 0.05$.

**Characterization of actinomycetes**

Actinomycete isolates were characterized based on their morphology (Kenneth, 1958).

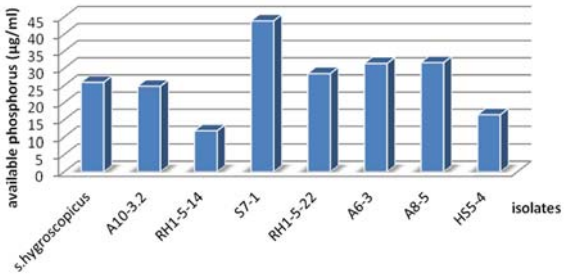
**Results**

A total of 44 actinomycete isolates from rhizospere soils were used in this study. These isolates were white -gray in spore mass color. These

isolates were classified as *Streptomyces* sp. For phosphate solubilization screening showed that 40 isolates (90%) were able to solubilize 0.5% tricalcium phosphate with diameter range from 0.11±0.02 cm. to 0.76±0.24 cm. S*treptomyces* isolate HS5-4 had the highest zone of P-Solubilization (0.76±0.24).

Seven actinomycetes were tested for available phosphorus. The result showed that *Steptomyces* isolate S7-1 had the maximum P solubilization of 32.34µg/ml compared to other isolates, A8-5 (31.68 µg/ml), A6-3 (31.42 µg/ml), RH1-5-22(28.49 µg/ml) and *Streptomyces hygroscopicus* (25.92 µg/ml) as positive control (Figure 1) The final pH of the medium decreased from 7.0 to range of 5.79 – 4.90.

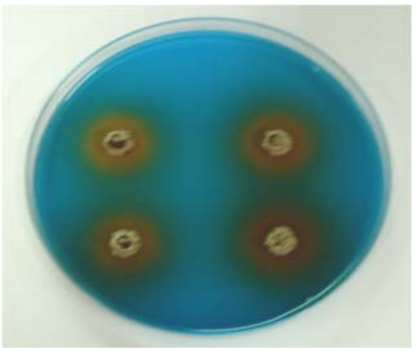
**Figure 1** Phosphate solubilization of actinomycete in




PVK broth

Siderophore production test were screened by CAS assay on Chrome azurol S (CAS) agar based on color changes from blue to yellow or red - orange color. The result showed that 5 isolates (75%) produced siderophore (Figure 2). Isolate HS5-4 produced the widest zone 1.93±0.05cm. Three isolates (37.5%) were not performed the siderophore.

A



B

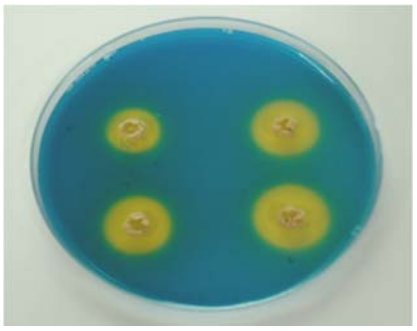


**Figure 2** CAS assays of siderophores produced by actinomycetes (A) *Streptomycetes* isolate HS5-4 performed the red orange halo and (B) *Streptomycetes isolate* RH1-5-22 performed the yellow halo.

Antagonistic activity of siderophore producing actinomycete was tested with rice pathogenic fungi, *Fusarium moniliforme*, and causative agent of Bakane disease by dual culture method on PDA agar for 7 days (Table 2). The result showed that *Streptomyces* isolate RH1-5-14 gave the highest inhibition of 75.62% (Figure 3)

**Figure 3** Dual cultures of actinomycete isolates against *Fusarium moniliforme*. Left: center disc of *Fuasrium moniliforme* agar (control), Right: *Streptomycetes* isolate RH1-5-14

**Table 2** Antifungal activity of actinomycete isolates.

| Isolate | Dual culture assay % mycelia inhibition in PDA *Fusarium moniliforme* |
|---|---|
| RH1-5-22 | 71.62 |
| *S.hygroscopicus* | 65.07 |
| RH1-5-14 | 75.62 |
| S7-1 | 67.98 |
| A10-3.2 | - |
| A8-5 | 68.34 |
| HS5-4 | - |

Antagonistic activity was not relevant to siderophore production. S*treptomyces* isolate RH1-5-14 had the highest ability to inhibit the mycelia growth of fungal but showed smaller in sideophore zones than isolate HS5-4. In contrast, isolate HS5-4 gave the highest siderophore zones but no activity to inhibit the fungal growth. These results indicated that the inhibition percentage was varied depend on actinomycetes strain and not related with siderophore production activity.

**Discussion and conclusions**

Our study showed that actinomycetes from rice rhizosphere were able to solubilize phosphate, produce siderophore and inhibit growth of rice pathogenic fungi. Forty actinomycetes were able to solubilize phosphate with *Streptomyces* isolate HS5-4 showed the largest clear zone on PVK agar. However, analysis of available phosphorus showed that *Streptomyces* isolate S7-1 released the maximum phosphate and gave the lowest pH in broth assay. This result was similar to a study of Salcedo (2004) who reported that *Streptomyces* sp. MCR24 lowered pH and solubilized significantly phosphorus in the liquid culture. The decreasing of pH in the broth suggested that production of acid or phosphatase by microorganisms to solubilize (Gargova, 1997; Khan et al, 2006). These results support the view that microorganisms play important role in phosphorus cycle. Hence, phosphate solubilizing actinomycetes are good candidate for plant growth promoting agent as biofertilizer. For siderophore production, *Streptomyces* isolate HS5-4 gave the biggest zone of production. However, it showed no antagonistic activity against fungal pathogen. *Streptomyces* isolate RH1-5-14 showed the highest inhibition percentage. This finding suggested that siderophore production was not likely involved in the antifungal activities.

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