

Isolation and Molecular Characterization of Antifungal Production from Rice Fields Rhizosphere soil, Thailand

Thitiya Rattanakavil^{1,2}, Tantima Kumlung³, and Khanungkan Klanbut^{1,2,*}

Received May 19, 2020; Revised May 29, 2020; Accepted June 29 2020

Abstract

One hundred actinomycete strains were isolated from rice field soil Nong Bua, Nakonsawan, Thailand and twenty actinomycete strains were isolated from Happy Rice Organic Farm at Nong sua, Pathumthani, Thailand. A total of one hundred and twenty strains were tested for antifungal activities against rice blast disease (*Pyricularia* sp. CRI60007). The selected actinomycete strains were chosen from in vitro radial growth inhibition test against *Pyricularia* sp. CRI60007 that showed the radial growth inhibition percentage of the growth of the fungi (%RGI) more than 50%. The results showed eleven strains gave %RGI more than 50% and strain NK05201 gave the highest %RGI (100 ± 0.00%). Analysis of 16S rRNA gene sequence and blast analyses confirmed that the selected actinomycete, ten strains belong to the genus *Streptomyces* and one strain belong to the genus *Amycolatopsis* with similarity to *Streptomyces albidoflavus* (NK01101 (99.56%), NK05201 (99.78%), NK05203 (99.79%), NK05205 (99.79%), NK07201 (99.79%), and NK08202(99.79%)), *Streptomyces hydrogenans* (NK08203 (100%)), *Streptomyces jeddahensis* (NK08205 (99.17%)), *Streptomyces corchorusii* (NK21201 (99.86%)), *Streptomyces shenzhenensis* (HPF3303 (99.86%)), and *Amycolatopsis rhizosphaerae* (HPF2102 (99.72%)).

Keywords: Actinomycete, Antifungal, Rice blast disease, 16s rRNA gene

Introduction

Rice and wheat are two main cereal crops that supply the majority of the global daily carbohydrate intake as energy source of human. Any pathogen targeting these two crops supposed to have serious connotations for the economy (John and Jacqueline, 2003). Thailand is one of rice producers to export rice product about 45 percent of rice yield. The main effect that reducing rice yield caused of the outbreaks of rice blast disease that can damaged to rice plant in all stages of growth (Saruda et al.,2017). Rice blast disease caused by the plant pathogenic fungi *Magnaporthe grisea* (Hebert) Barr. (anamorph, *Pyricularia grisea* Sacc.) is one of the most economically harmful crop diseases (Seochang and Katherine, 2004). The disease is widespread and distributed about 85 countries around the world where rice is grown. The disease appearance and asperity depend-

ing on year, place, environmental conditions and crop management practices. Yield loss appraisals from other areas of the world have ranged from 1% to 50% (Jeffrey, 2016).

Potential use of microbes-based biocontrol-agents as supplement or displacement for agrochemicals has been reported in several reports (Shimizu et al., 2000). Actinomycetes well-known as the source of several drugs such as antibiotics, antiviral, antitumor, antiparasitic agents, and immunosuppressants, microorganisms are responsible for the production of about 23,000 bioactive secondary metabolites (Feitosa et al., 2014; Subbanna et al., 2018). Some strains of actinomycete from rice were found to have antagonism against *Magnaporthe grisea*, *Rhizoctonia solani*, *Xanthomonas oryzae* pv. *oryzae*. and *Fusarium moniliforme* (Tian et al., 2004).

¹ Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand

² Actinobacteria Research Unit, Department of Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand

³ Expert Centre of Innovative Agriculture, Thailand Institute of Scientific and Technological Research

* Corresponding author. E-mail: khanungkan@hotmail.com

The purpose of this present study to isolate actinomycetes from rhizosphere rice that can produce antifungal activity and searching for potential strains to control *Pyricularia* sp. CRI60007 that causes rice blast on rice leaves. Characterisation of the selected strains were done based on morphological, biochemical and physiological properties and studied of hydrolytic enzyme production against fungal cell wall. Identification analysis of 16S rRNA gene sequencing were performed. The results from this study can be used for pot experiment test in further study.

Material and methods

Collection of soil sample

Soil samples were collected from Happy Rice Organic Farm at Nong sua, Pathumthani, Thailand. Samples were randomised collecting at rhizosphere soil. These samples were kept in sterile polyethylene bags without sealed and transported immediately to the laboratory (Ruttanasutja and Pathomaree, 2015).

Isolation of Actinomycetes

The soil samples were pre-treated by air-drying method for 3-5 days at room temperature. Ten grams of soil sample was accurately weighed and mashed then transferred to 90 milliliters of 0.1% Tween80, mixed well (10-1). One milliliter of resultant solution was serially diluted with 9 milliliters of 0.85% Sodium Chloride (NaCl) up to 10⁻⁵. 200 µl of each intermediate dilution was spread on Modified Zhang's Soil Starch Extract (ZSSE) agar medium that supplemented with nystatin (50 micrograms /milliliter). The plates were incubated for the growth of actinomycetes colonies at 30 ± 2 °C and observed growth of actinomycete intermittently during incubation. After 7 days incubation, colony was picked by micro-needle then cross streak on International Streptomyces Project 2 (ISP2) medium. The pure colonies of actinomycete isolates were selected and maintained on ISP2 medium at 30 ± 2 °C for 7–14 days. Stocks were preserved in 20% glycerol (w/v), and stored at -80 °C for long time preservation. (Waksman et al., 1961).

In vitro radial growth inhibition test

The antifungal activity was analysed using dual culture method. Potato Dextrose Agar (PDA) medium

plates were used, inoculated with the actinomycete isolates by single streak far from edge of petri dish two centimeters and incubated at 30 ± 2 °C for 7 days then using cork borer size 5 millimeters plug the fungi (*Pyricularia* sp. CRI60007) and placed on opposite site of actinomycete far from edge of petri dish two centimeters and incubated at 28 ± 2 °C. The inhibition zone was measured after the fungal mycelia in the control plates reached the edges of the plates. The antagonism against fungi was recorded by radial growth inhibition percentage was obtained using the formula: %RGI = 100 x (R1-R2)/R1 where R1 was the furthest radial growth distance of the fungus (control) and R2 was the radial growth distance of the fungus in dual culture with the actinomycetes (Mahadtanapuk et al., 2007).

Morphological biochemical and physiological characterization of the selected isolate

The morphological characteristics were observed using light microscopy with long working distance lens (40X) by using 14 days cultures grown at 30 ± 2 °C on ISP2 medium. The colour of aerial mycelium, substrate mycelium and soluble pigment were examined by using ISCC–NBS colour system (Kelly, 1964). Growth at different temperature was test at 4, 10, 20, 28, 30, 37, 40, 50 °C on ISP2 agar plates and visible growth was determined. Growth at different pH range 4 to 12 in ISP2 broth medium was recorded after 14 days, and growth in presence of NaCl (%) concentration 0 to 10 were studied on ISP2 agar plates. All phenotypic properties were recorded after incubation for 14 days at 30 ± 2 °C. Biochemical properties were tested using the several standard methods. Carbon source utilisation was performed by using ISP 9 medium containing different sugar types (Arai, 1975; Shirling and Gottlieb, 1966; Williams and Cross, 1971). Production of melanin pigment was determined by using ISP 6 (Pridham and Lyons, 1969). Hydrolysis of tyrosine, xanthine, hypoxanthine, adenine, and cellulose was recorded positive result by clear zone formation around the colony. Starch hydrolysis was observed as described by Cowan (1974). Urease test was determined by the indicator change phenol red changing pH yellow to pink (Gordon et al., 1974).

DNA isolation, PCR amplification and 16s rRNA gene sequencing

DNA amplification, sequencing and phylogenetic analysis of selected actinomycete strains were identified by using 16S rRNA gene. The selected strains were grown on ISP2 agar medium for 4 days at 30 °C. The colony was picked up by a sterilized toothpick and resuspended in 40 µl of TE buffer pH 8.0 as DNA template. The 16S rRNA gene was amplified and sequenced by using primers 9F (5' GAGTTTGATCITIGCTCAG3') and 1541R (5'AAGGAGGTGATCCAGCC3'). The temperature for PCR amplification and sequencing reaction followed the method of Yukphan et al., (2005). Each PCR reaction of 50 microliters in total included 25 microliters AccuPower® Taq PCR Master Mix (Bioneer), 18 microliters dH₂O, 2.5 microliters the final concentration 10 picomole of each primer and 2 microliters DNA template. The cycling conditions for the amplification of the 16s rRNA gene region were as follow: 3 minutes at 94 °C, 25 cycles at 94 °C for 1 minute, at 50 °C for 1 minute and 2 minutes at 72 °C, then followed by a final extension step for 3 minutes at 72 °C. The sequences of 16s rRNA gene was aligned with the program BioEdit Sequence Alignment Editor (version 7.0.0. Distance matrices for the aligned sequences were calculated by using the two-parameter method of Kimura (1980). The Maximum Likelihood method based on the Kimura 2-parameter model of Kumar et al., (2016) was used to construct a phylogenetic tree with the program MEGA7.

In vitro screening of the selected actinomycete isolates for hydrolytic enzyme production against fungal cell wall

Detection of Protease

Protease detection was performed using skim milk agar plate (peptonisation), skim milk broth (coagulation) was determined by following the methods described by Atlas (1997) and bouillon gelatin broth (gelatinisation).

Detection of Lipase

Lipase detection was observed by the production of clear zone in agar plates using Tween80 by following the methods described by Schoofs et al., (1997) and using phenol red by following the methods described by Singh et al., (2006).

Detection of Chitinase

Chitinase detection was detected by following the methods of Nawani et al., (2002). The positive result was determined by formation of clear zone around colony on chitin agar plates.

In vitro screening of the selected actinomycete isolates for plant growth-promoting (PGP) activities
Siderophore production

Siderophore production test of the selected actinomycete strains were screened on Chrom azurol S (CAS) agar medium. The selected actinomycete isolates were spot inoculated on the medium and incubated at 30 ± 2 °C for 7-14 days. A positive CAS reaction was observed by changing to be yellow color around the spot growth. (Alexander and Zuberer, 1991).

Phosphate solubilisation

Solubilization of Phosphate test was used Pikovskaya's agar plates. Plates were divided in four equal sectors and the selected actinomycete isolates were spot on the agar plates and incubated at 30 ± 2 °C for 3-14 days. The presence of clear zone around the spot growth indicated phosphate solubilisation activity (Gaur, 1990).

Hydrogen cyanide (HCN) production

Production of hydrogen cyanide, the selected isolates were screened by adapting the method of Lock (1948). Using ISP2 agar medium supplemented with 0.44% glycine. Actinomycetes were streaked on these modified agar plate. Whatman filter paper No.1 dipped in 2% sodium carbonate in 0.5% Picric acid solution placed in the top of the plate. Plates were sealed with parafilm and incubated at 30 ± 2 °C for 3-14 days. The orange to red color of filter paper was indicated a positive test for HCN production.

Ammonia production

The selected actinomycete isolates were tested for ammonia production in peptone water medium. The selected actinomycete isolates were inoculated into 10 milliliters of peptone water and incubated at 30 ± 2 °C for 7-14 days. After that Nessler's reagent (0.5 milliliter) was applied to each tube. The results showed brown to yellow color was determined to be a positive test for ammonia production (Cappucino and Sherman, 1992).

Results and discussion

Isolation of Actinomycetes

A total of 120 actinomycete isolates were isolated from rice field soil Nong Bua, Nakonsawan, Thailand (Thitiya and Khanungkan., 2019) 100 isolates and 20 isolates from Happy Rice Organic Farm at Nong sua, Pathumthani, Thailand.

In vitro radial growth inhibition test

Screening for antifungal activity of 120 isolates were tested against *Pyricularia* sp. CRI60007. The results of antifungal activity showed that 11 actinomycete strains were able to inhibit growth of *Pyricularia* sp. CRI60007 more than 50% including of NK01101 ($66.44 \pm 0.59\%$), NK05201 ($100 \pm 0.00\%$), NK05203 ($85.88 \pm 0.61\%$), NK05205 ($71.30 \pm 0.68\%$), NK07201 ($78.24 \pm 0.73\%$), NK08202 ($61.11 \pm 0.41\%$), NK08203 ($90.51 \pm 0.50\%$), NK08205 ($77.78 \pm 0.05\%$), NK21201 ($84.95 \pm 0.14\%$), HPF2102 ($99.53 \pm 0.03\%$), and HPF3303 ($51.39 \pm 0.31\%$) as shown in Figure 2.

Morphological biochemical and physiological characterization of the selected actinomycetes strains

The selected actinomycete strains were grouped by using ISCC–NBS colour system (Kelly, 1964) of aerial mycelium into beige yellow (NK01101, NK05201, NK05203, NK05205, NK07201, NK08202, and NK08203), light gray (NK21201 and HPF3303), white (HPF2102) and NK08205 strain was not produced aerial mycelium on ISP 2 agar medium. All strains were able to utilise galactose, glucose, mannose and arabinose while strain NK08205 and HPF3303 were able to utilise sucrose. Strain NK01101, NK05201, and NK05203 were unable to utilise raffinose and xylose while NK05205, and NK07201 were unable to utilise raffinose, and strain NK21201, and HPF2102 were unable to utilise mannitol as carbon source. The ability to use differential types of carbon source might be indicate that actinomycetes were able to survive in the different environment (Ridhi et al., 2017). Most of selected actinomycetes were able to grow at temperature 20 to 40 °C (Table 1). All of them could not grow at temperature 4 °C. The optimum temperature and pH were found to be 30 °C and pH7, respectively.

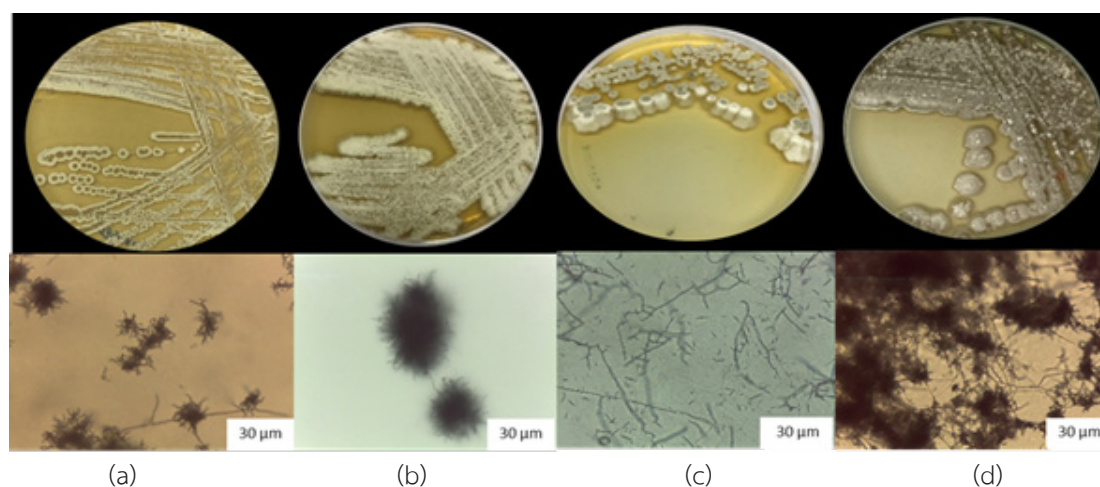


Figure 1. Morphological appearance colonies on ISP2 agar medium and spore arrangement of some selected actinomycete strains NK05201 (a), NK08203 (b), NK21201 (c), and HPF3303 (d).

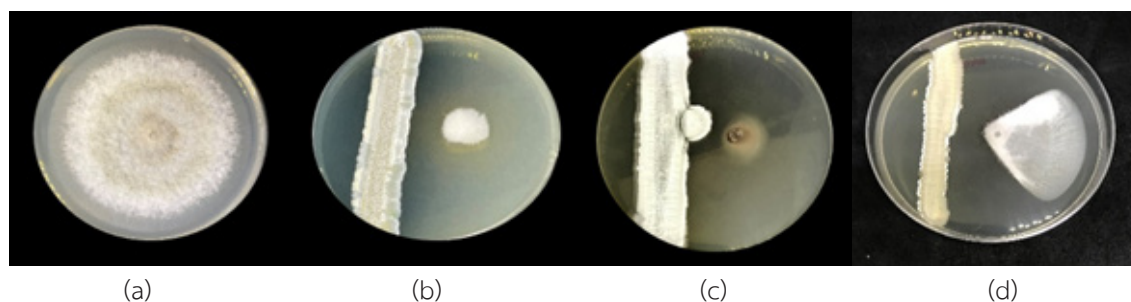


Figure 2. Antifungal activity evaluation of some actinomycete strains (a) control, (b) NK07201, (c) NK21201, and (d) HPF 3303 against *Pyricularia* sp. CRI60007.

Table 1 (Continue)

Characteristics	Actinomycete strains										
	1	2	3	4	5	6	7	8	9	10	11
Hydrolysis of											
Tyrosine	-	-	-	-	-	-	-	-	-	-	-
Xanthine	-	-	-	-	-	-	-	-	-	-	-
Hypoxanthine	+	+	+	+	+	+	+	+	+	+	+
Adenine	+	+	+	+	+	+	+	+	+	+	+
Cellulose	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	+	+	+	+	+	+	+	-
Urea	-	+	-	+	-	-	w	-	-	-	-

Strains; 1. NK01101, 2. NK05201, 3. NK05203, 4. NK05205, 5. NK07201, 6. NK08202, 7. NK08203, 8. NK08205, 9. NK21201, 10. HPF2102, and 11. HPF3303.

The symbol, + means the positive reaction or appearance of growth while symbol – means the negative or absence of growth.

Molecular identification

The 16S rRNA gene sequences and Blast analyses confirmed that selected ten strains of actinomycete belong to the genus *Streptomyces* spp. with similarity of 99.56-100 % including of *S. albidoflavus* (NK01101, NK05201, NK05203, NK05205, NK07201, and NK08202), *S. hydrogenans* (NK08203), *S. jeddahensis* (NK08205), *S. corchorusii* (NK21201), *S. shenzhenensis* and (HPF3303) with similarity percentage of 99.56, 99.78 99.79, 99.79, 99.79, 100, 99.17, 99.86 and 99.86, respectively. One strain belongs to the genus *Amycolatopsis* spp. As *A. rhizosphaerae* (HPF2102) with similarity 99.86 %. These sequences from selected actinomyces strains have been deposited in the GenBank with accession number as showed in Table 2 and the maximum likeli-

hood phylogenetic tree construction showed in Figure 2.

Several strains of the selected actinomycetes have been reported their antifungal activities such as strain NK01101, NK05201, NK05203, NK05205, NK07201, and NK08202 tend to be *Streptomyces albidoflavus*. Ahmed et al., (2018) reported that *Streptomyces albidoflavus* AS25 produced antifungal against. Strain NK 08203 tend to be *Streptomyces hydrogenans*. Rajesh and Talwinder, (2016) emphasized *Streptomyces hydrogenans* DH16 and its culture metabolites can be developed as biofungicides. Strain NK21201 tend to be *Streptomyces corchorusii*. Tamreihao et al., 2016 reported that *Streptomyces corchorusii* strain UCR3-16 showed antifungal activities against rice fungal pathogens.

Table 2 Molecular identification of the selected actinomycetes strains determined by 16s rRNA gene sequencing.

Strains	Source of isolation	Identification as	Similarity (%)	Accession
NK01101	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.56	LC516418
NK05201	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.78	LC516413
NK05203	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.79	LC516409
NK05205	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.79	LC516410
NK07201	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.79	LC516411
NK08202	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces albidoflavus</i>	99.79	LC488882
NK08203	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces hydrogenans</i>	100	LC516412
NK08205	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces jeddahensis</i>	99.17	LC477341
NK21201	Rice field soil, Nakhonsawan, Thailand.	<i>Streptomyces corchorusii</i>	99.86	LC516414
HPF2102	Rice rhizosphere soil, Pathumthani, Thailand.	<i>Amycolatopsis rhizosphaerae</i>	99.86	LC516416
HPF3303	Rice rhizosphere soil, Pathumthani, Thailand.	<i>Streptomyces shenzhenensis</i>	99.72	LC516417

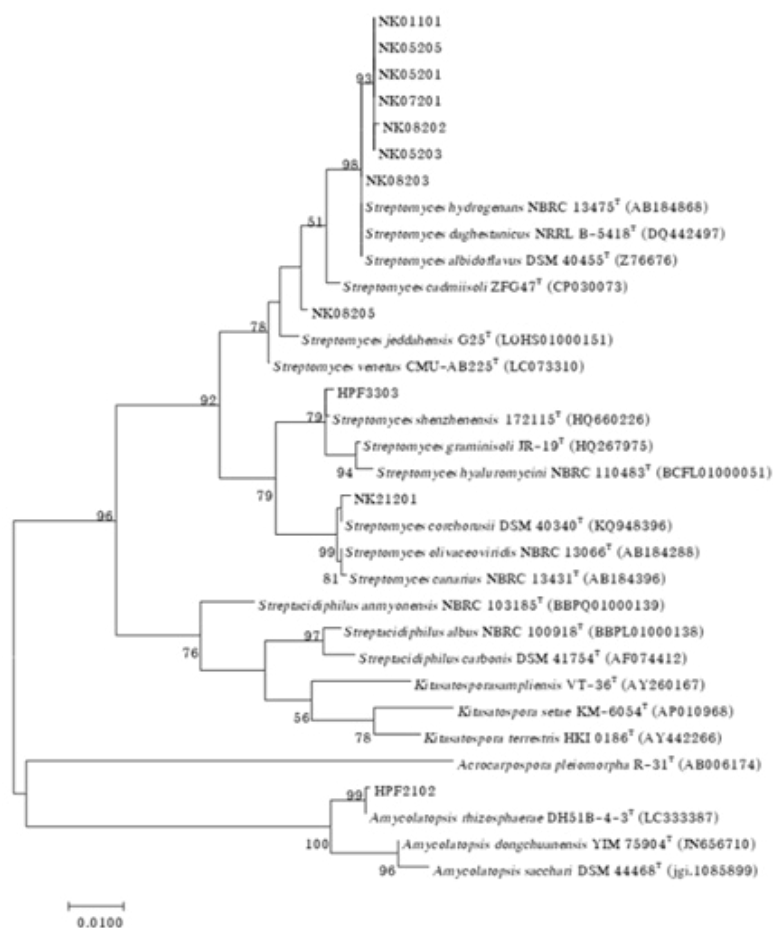


Figure 2. Maximum likelihood phylogenetic tree based on the 16S rRNA gene sequences of selected actinomycetes 11 strains and their closely related type strains.

In vitro screening of the selected actinomycete strains for hydrolytic enzyme production against fungal cell wall

The possible mechanisms of antifungal activity from actinomycetes to fungal pathogens is usually related to the production of antifungal compounds and extracellular hydrolytic enzymes including of protease, lipase, chitinase, and β -1,3-glucanase are considered to be important hydrolytic enzymes in the lysis of fungal cell walls (Prapagdee et al., 2008). The result of hydrolytic enzyme production against fungal cell wall showed all most of the selected actinomycete strains could be produced enzyme protease and lipase (Table 3).

In vitro screening of the selected actinomycete strains for plant growth promoting (PGP) activities

Despite their preliminary track record as BCAs and plant growth promoting (PGP) activities actinomy-

cetes. have been scarcely reported in the literature. Some reports exist for their ability to solubilize phosphate, and production of indole acetic acid (IAA), siderophores, 1-amino cyclopropane-1-carboxylic acid (ACC) deaminase (Sadeghi et al., 2012; Passari et al., 2015; Qin et al., 2015). The result of plant growth promoting (PGP) activities showed strain NK05205, NK07201, and NK08205 could be produced siderophore while strain NK05201, NK05203, NK05205, NK07201, NK08202, and NK08203 were determined to be a positive test for ammonia production. Strain HPF3303 showed positive result of hydrogen cyanide production. Strain NK21201 and HPF2102 showed positive result of phosphate solubilisation (Table 4).

Table 3 In vitro screening of the selected actinomycete strains for hydrolytic enzyme production against fungal cell wall.

Characteristics	Actinomycete strains										
	1	2	3	4	5	6	7	8	9	10	11
Protease Production											
Peptonization	+	+	+	+	+	+	+	-	w	-	-
Coagulation	+	-	-	+	+	+	+	-	w	-	-
Gelatinization	-	+	-	-	+	-	w	-	-	-	-
Lipase production											
Tween80	+	+	+	+	+	+	+	-	+	+	+
Phenol red	-	-	-	-	-	+	-	-	+	-	+
Chitinase	-	-	-	-	-	-	-	-	-	-	-

Strains.; 1. NK01101, 2. NK05201, 3. NK05203, 4. NK05205, 5. NK07201, 6. NK08202, 7. NK08203, 8. NK08205, 9. NK21201, 10. HPF2102, and 11. HPF3303. The symbol, + means the positive reaction while symbol, – means the negative reaction and w, weak.

Table 4 In vitro screening of the selected actinomycete strains for plant growth-promoting (PGP) activities

Characteristics	Actinomycete strains										
	1	2	3	4	5	6	7	8	9	10	11
Production of											
Siderophore	-	-	-	+	+	-	-	+	-	-	-
Ammonia	-	+	+	+	+	+	+	-	-	-	-
HCN	-	-	-	-	-	-	-	-	-	-	+
Phosphate solubili- sation	-	-	-	-	-	-	-	-	+	+	-

Strains.; 1. NK01101, 2. NK05201, 3. NK05203, 4. NK05205, 5. NK07201, 6. NK08202, 7. NK08203, 8. NK08205, 9. NK21201, 10. HPF2102, and 11. HPF3303.

The symbol, + means the positive reaction while symbol, – means the negative reaction and w, weak.

Conclusion

In this study the selected actinomycetes strains were chosen from in vitro radial growth inhibition test against *Pyricularia* sp. CRI60007 that showed the radial growth inhibition percentage of the growth of the fungi (%RGI) more than 50%. The results showed eleven strains gave %RGI more than 50%. Strain NK05201 gave highest %RGI ($100 \pm 0.00\%$) and followed by strain HPF2102 ($99.53 \pm 0.03\%$), NK08203 ($90.51 \pm 0.50\%$), NK05203 ($85.88 \pm 0.61\%$), NK21201 ($84.95 \pm 0.14\%$),

NK07201 ($78.24 \pm 0.73\%$), NK08205 ($77.78 \pm 0.05\%$), NK05205 ($71.30 \pm 0.68\%$), NK01101 ($66.44 \pm 0.59\%$), NK08202 ($61.11 \pm 0.41\%$), and HPF3303 ($51.39 \pm 0.31\%$) respectively. Molecular identification of the selected actinomycetes strains belong to the genus *Streptomyces* spp. with similarity of 99.56-100 % and one strain belong to the genus *Amycolatopsis* spp. with similarity 99.86%. The results from this study can be used for further study.

Acknowledgements

This research was supported by Faculty of Science, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand, Actinobacteria Research Unit, Department of Biology, Faculty of Science, King

Mongkut's Institute of Technology Ladkrabang, Bangkok, 10520, Thailand, and Expert Centre of Innovative Agriculture, Thailand Institute of Scientific and Technological Research. Special thank to Dr. Nonglak Parinthewong for providing fungal pathogen in this study.

References

- Ahmed Nafis, Najoua Elhida, Brahim Oubaha, Salah Eddine Samri, Timo Niedermeyer, Yedir Ouhdouch, Lahcen Hassani, Mustapha Barakate. (2018). Screening for Non-polyenic Antifungal Produced by Actinobacteria from Moroccan Habitats: Assessment of Antimycin A19 Production by *Streptomyces albidoflavus* AS25. *Int J Mol Cell Med Spring*. Vol 7 No 2:133-145.
- Alexander, D. B., and Zuberer, D. A. (1991). Use of Chrome Azurol S reagents to evaluate siderophore production by rhizosphere bacteria *Biol. Fertility Soils*. 12: 39–45.
- Atlas, R.M. (1997). *Handbook of Microbiological Media*. CRC Press, Boca Raton, Florida, USA
- Arai, T. (1975). *Culture Media for Actinomycetes*. Tokyo, Japan: The Society for Actinomycetes.
- Cappuccino, J. G., and Sherman, N. (2002). *Microbiology: A Laboratory Manual*, 6th Edn. California City, CA: Pearson Education
- Cowan, S. T. (1974). *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 2nd Edn. London: Cambridge University Press.
- Feitosa TR, Arruda FVF, Cavalcanti FJR, Baptista NMQ, Callou MJA, SILVA T, Miranda RCM, Gusmão NB. (2014). Antimicrobial activity of fungi isolated from the water of the sky high, Recife-PE supply against bacteria of clinical interest system. *Afr. J. Microbiol. Res.* 8:2999-3007
- Gaur, A. C. (1990). *Phosphate Solubilizing Microorganisms as Biofertilizer*. New Delhi: Omega Scientific Publishers.
- Gordon, R. E., Barnett, D. A., Handerhan, J. E., and Pang, C. H. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int. J. Syst. Bacteriol.* 24: 54–63.
- Jeffrey R. Ryan, in *Biosecurity and Bioterrorism (Second Edition)*, (2016). *Biosecurity and Bioterrorism* 185-216
- John L. Sherwood and Jacqueline Fletcher. (2003). *Crop Biosecurity: Are We Prepared?* Developed by the Public Policy Board of the American Phytopathological Society (APS)
- Kelly KL. (1964). *Inter-Society Colour Council–National Bureau of Standard Colour Name Charts Illustrated with Centroid Colours*. Washington, DC:US Government Printing Office.
- Kimura M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111-120.
- Kumar S., Stecher G., and Tamura K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.
- Lorck, H. (1948). Production of hydrocyanic acid by bacteria. *Physiol. Plant.* 1:142–146.
- Mahadnanapuk S, Cutler RW, Sanguanserm Sri M, Sardud V, College B, Anuntalabhochai S. (2007). Control of anthracnose caused by *Colletotrichum musae* on *Curcuma alis matifolia* Gagnep. Using antagonistic *Bacillus* spp. *American Journal of Agricultural and Biological Sciences*. 2(2):54–61.
- Nawani, N. N., Kapadnis, B. P., Das, A. D., Rao, A. S., & Mahajan, S. K. (2002). Purification and characterization of a thermophilic and acidophilic chitinase from *Microbispora* sp. V2. *Journal of Applied Microbiology*, 93(6):965–975.
- Passari, A.K., Mishra, V.K., Gupta, V.K., Yadav, M.K., Saikia, R., Singh, B.P., (2015). In vitro and in vivo plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with me-

dicinal plants. PLoS One 10, e0139468.

- Prapagdee, B., Kuekulvong, C., & Mongkolsuk, S. (2008). Antifungal Potential of Extracellular Metabolites Produced by *Streptomyces hygroscopicus* against Phytopathogenic Fungi. *International Journal of Biological Sciences*, 330–337.
- Pridham, T. G., and Lyons, A. J. (1969). Progress in clarification of the taxonomic and nomenclatural status of some problem actinomycetes. *Dev. Ind. Microbiol.* 10: 183–221.
- Qin, S., Miao, Q., Feng, W.-W., Wang, Y., Zhu, X., Xing, K., (2015). Biodiversity and plant growth promoting traits of culturable endophytic actinobacteria associated with *Jatropha curcas* L. growing in Panxi dry-hot valley soil. *Appl. Soil Ecol.* 93: 47–55.
- Rajesh K. Manhas and Talwinder Kaur. (2016). Biocontrol Potential of *Streptomyces hydrogenans* Strain DH16 toward *Alternaria brassicicola* to Control Damping Off and Black Leaf Spot of *Raphanus sativus* *Front. Plant Sci.*
- Riddhi N. Dholakiya, Raghawendra Kumar, Avinash Mishra, Kalpana H. Mody, and Bhavanath Jha. (2017). Antibacterial and Antioxidant Activities of Novel Actinobacteria Strain Isolated from Gulf of Khambhat, Gujarat. *Front Microbiol.* 2017; 8: 2420.
- Ruttanasutja, P and Pathomaree, W. (2015). Selective Isolation of Cultivable Actinomycetes from Thai Coastal Marine Sediment. *Chiang Mai J. Sci.* 42(1): 88-103.
- Sadeghi, A., Karimi, E., Dahaji, P.A., Javid, M.G., Dalvand, Y., Askari, H., (2012). Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. *World J. Microbiol. Biotechnol.* 28:1503–1509.
- Saruda Hensawang, Angkool Wangwongchaia, Usa Humphries a, and Thidarat Bunsri. (2017). Simulation of severity of rice blast disease in Prachin Buri using plant disease epidemiological model: simulation of rice blast disease. The 22nd Annual Meeting in Mathematics (AMM 2017) Department of Mathematics, Faculty of Science Chiang Mai University, Chiang Mai, Thailand.
- Schoofs, A., F.C. Odds, R. Colebunders, M. Leven and H. Goussens. (1997). Use of specialized isolation media for recognition and identification of *Candida dubliniensis* isolates from HIV-infected patients. *Eur. J. Clin. Microbiol. Infect Dis.*, 16:296-300
- Seochan Kang and Katherine F. Dobinson. (2004). Molecular and Genetic Basis of Plant-Fungal Pathogen Interactions. *Applied Mycology & Biotechnology an International Series. Volume 4:59-97*
- Singh, R., N. Gupta and N. Kango. (2007). Production of lipase by hyper-lipolytic *Rhizopus oryzae* KG-10 on low value oil emulsions. *Res. J. Microbiol.*, 2: 671-677
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16:313–340.
- Shimizu, M., Nakagawa, Y., Sato, Y., Furuma, T., Igarosh, Y., Onaka, H., Yoshida, R. and Kunoh, H. (2000). Studies on endophytic actinomycetes and *Streptomyces* sp. isolated from *Rhododendron* and its antifungal activity. *J. Gen. Plant. Pathol.*, 66: 360-366.
- Subbanna ARNS, Rajasekhara H, Stanley J, Mishra KK, Pattanayak A. (2018). Pesticidal perspectives of chitinolytic bacteria in Molecular and Genetic Basis of Plant-Fungal Pathogen Interactions. *Applied Mycology & Biotechnology an International Series. Volume 4:59-97*
- Singh, R., N. Gupta and N. Kango. (2007). Production of lipase by hyper-lipolytic *Rhizopus oryzae* KG-10 on low value oil emulsions. *Res. J. Microbiol.*, 2: 671-677
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 16:313–340.

- Tamreihaoa, K., Debananda S. Ningthoujama, Salam Nimaichanda, Elangbam Shanta Singh, Pascal Reenac, Salam Herojeet Singh. (2016). Biocontrol and plant growth promoting activities of a *Streptomyces corchorusii* strain UCR3-16 and preparation of powder formulation for application as biofertilizer agents for rice plant. *Microbiological Research* 192:260–270
- Thitiya Rattanakavil and and Khanungkan Klanbut. (2019). Actinomycetes from rice field soil, Nakonsawan and their antimicrobial activity. The 31st Annual Meeting of the Thai Society for Biotechnology and International Conference. November 10-12, 2019 Duangjitt Resort & Spa, Patong, Phuket, Thailand, pp 482-495.
- Tian, X. L., Cao, L. X., Tan, H. M., Zeng, Q. G., Jia, Y. Y., Han, W. Q., & Zhou, S. N. (2004). Study on the communities of endophytic fungi and endophytic actinomycetes from rice and their antipathogenic activities in vitro. *World Journal of Microbiology and Biotechnology*, 20(3):303–309.
- Waksman SA. (1961). Baltimore: The Williams and Wilkins Company. The Actinomycetes, classification, identification and description of genera and species. 2: 61–292.
- Williams, S. T. & Cross, T. (1971). Actinomycetes. *Methods Microbiol* 4:295–334.
- Yukphan, P., T. Malimas, W. Potacharoen, S. Tanasupawat, M. Tanticharoen and Y. Yamada. (2005). *Neosasaia chiangmaiensis* gen. nov., sp. nov., a novel osmotolerant acetic acid bacterium in the α -Proteobacteria". *J. Gen. Appl. Microbiol.* 1(51): 301-311.