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Isolation and Identification of Actinomycetes from Termite's Gut against Human Pathogen

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ABSTRACT

Actinomycetes are a group of prokaryotic organisms belonging to Gram-positive bacteria and play an important ecological role in recycling substances in the nature. The objective of this study was to isolate and identify actinomycetes from termite's gut against human pathogen. Total isolates were examined for antimicrobial activity and selected isolate was identified using morphological characters and molecular technique. The results showed that eighty-three strains of actinomycetes were isolated from guts of *Termes* sp. Among these, 66, 67, 7, 9 and 3 isolated strains were active against the tested pathogenic microorganism of *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida utilis,* respectively. Furthermore, some isolate of actinomycetes was able to inhibit both Gram-positive and Gram-negative pathogen. The isolated actinomycetes strain FSPNRU 102 having broad spectrum of inhibition was selected. The morphological character of this strain showed aerial mycelium with longitudinal spirales-type spore chain and light black soluble pigment. The aerial spore color varied from white to gray. Moreover, this strain contained LL-diaminopimelic acid of the peptidoglycan in the whole-cell hydrolysate of chemotaxonomical characteristic. These results assigned strain FSPNRU 102 to genus *Streptomyces*. Based on its 16S rDNA sequence and phylogenetic tree analysis, this new isolate belong to the *Streptomyces niveoruber*.

Key words: *Streptomyces*, antimicrobial activity, termite, pathogen, screening, characterization

INTRODUCTION

Termites are a group of social insects that one of important factors in ecosystem by playing an important role in the carbon and nitrogen cycles (Robert *et al*., 2007; Khucharoenphaisan *et al*., 2011). Termite guts contained large number and wide variety of bacteria. Their bacteria play an essential role in the metabolism of organic matter such as carbon and nitrogen from termite food sources (Ramin *et al*., 2008). They are a source of a novel genera and species. Based on classical microbiology and 16S rDNA sequencing technique, over a dozen genera of bacteria have been identified in the subterranean termite as a new species (Leadbetter and Breznak, 1996; Schafer *et al*., 1996; Ohkuma and Kudo, 1996).

Actinomycetes are a group of Gram-positive bacteria with its DNA rich in mol G+C content (Lo *et al*., 2002). They were outstanding source for bioactive compound production. Actinomycetes produced large number of important secondary metabolite such as antibiotic compounds including streptomycin, actinomycin and tetracycline (Barrios-Gonzalez *et al*., 2005). Most antibiotics from actinomycetes have been reported by many researchers (Kavitha *et al*., 2009; Raja and

Prabakarana, 2011). Among actinomycetes predominantly *Streptomyces* sp. has been recognized as major producer of bioactive metabolite with broad spectrum of activities such as antibacterial and antifungal agents (Usha *et al*., 2011; Reddy *et al*., 2011; Atta and Ahmad, 2009), where as *Micromonospora* and *Norcardia* lesser than those. (Arifuzzaman *et al*., 2010). The isolates of *Nocardia levis* from soils (Kavitha *et al*., 2009), *Steptomyces* sp. from desert soil (Hozzein *et al*., 2011) and actinomycetes from mangrove sediments, soil (Baskaran *et al*., 2011; Dhananjeyan *et al*., 2010; Arifuzzaman *et al*., 2010) against Gram-negative and Gram-positive pathogenic bacteria. However, isolated actinomycetes from animal and insect against human pathogenic bacteria have not been reported. They may have more inhibited capacity to human pathogen than other sources such as soils or sediments.

The aim of this present study was to isolate and identify the isolated actinomycete from termite gut samples in genus *Termes*. Furthermore, they were determined their antagonistic activity against selective human pathogenic microorganism such as some Gram-negative, Gram-positive pathogenic bacteria and pathogenic yeast.

MATERIALS AND METHODS

This research project was conducted from September 2010 to June 2011 at Faculty of Science and Technology, Phranakhon Rajabhat University, Thailand.

Isolation and screening of actinomycetes: Actinomycetes were isolated from gut of *Termites* in genus of terms that was collected from Sakaerat Environmental Research Station in Nakhon Ratchasima province, Thailand. Each sample was grinded and diluted to go on serial dilution and plate on humic acid vitamin agar (Hayakawa and Nonomura, 1987) supplemented with 50 mg L^{-1} of cyclohexamide and 20 mg L^{-1} of nalidixic acid. The isolation plates were incubated at room temperature for 30 days. The number of total actinomycetes was evaluated after the colonies that appeared on the plates. The colonies had been transferred to yeast extract-malt extract (ISP medium No. 2) (Shirling and Gottlieb, 1966) plates for purity check.

Identification of actinomycetes against human pathogen: The 16S rDNA amplification of actinomycetes was prepared by PCR using universal primer 9F (5'-GAGTTTGATCCTGGCTCAG-3') and 1541R (5'-AAGGAGGTGATCCAGCC-3'). The PCR products were purified and directly sequence using a Big Dye® Terminator V3.1 cycle sequencing kit (Applied Biosystems) and the universal primers 9F(5'-GAGTTTGATCCTGGCTCAG-3'),785F(5'-GGATTAGATACCCTGGTAGTC-3'), 802R (5'-TACCAGGGTATCTAATCC-3') and 1541R(5'-AAGGAGGTGATCCAGCC-3'). The nucleotide sequences were compared with other bacteria using the Genetyx version 5.0 program. The phylogenetic tree was constructed by using the neighbor-joining method in MEGA version 4 software. The topology was evaluated by bootstrap analysis based on 1000 resamplings (Felsenstein, 1985).

Diaminopimelic acid isomer from whole-cell of isolated actinomycetes culturing on ISP medium No. 2 were determined according to standard procedures (Cuesta *et al*., 2010). Spore-chain morphology under light microscopy, aerial spore colour and diffusible pigment on ISP No. 2 medium were recorded after 14-day cultivation at 30±2°C.

Determination of antibacterial activity: Anitibacterial activities of isolated strains were tested against Gram-positive (*Staphylococcus aureus, Bacillus cereus*), Gram-negative (*Pseudomonas*

aeruginosa, Escherichia coli) and yeast (*Candida utilis*). The isolated strains were inoculated on ISP no 2 agar (Khucharoenphaisan and Sinma, 2011) along the middle line of the plate by streaking. After 7-day incubation, the fresh test microorganism were streaked at 90° angle as close as possible to the streak hne of actinomycetes on both sides. Three replicates were obtained for each isolated actinomycetes.

RESULTS AND DISCUSSION

Isolation of actinomycetes and antibacterial activity: Eighty-three actinomycetes were isolated from termite. All isolated strains were analyzed their antimicrobial activities and some of them were showed in Table 1. They were 66, 67, 7, 9 and 3 isolates were active against the tested pathogenic microorganism of *B. cereus*, *S. aureus*, *P. aeruginosa, E. coli* and *C. utilis,* respectively. Among these, some isolate of actinomycetes was able to inhibit both Gram-positive and Gram-negative pathogen. The result indicated that antimicrobial activities obtained from actinomycetes were effective against Gram-posotive bacteria (*B. cereus*, *S. aureus*) more that Gram-negative bacteria (*P. aeruginosa, E. coli*) and yeast (*C. utilis*). The isolated actinomycetes strain FSPNRU 102 having broad spectrum of inhibition as shown in Table 1 was selected for further study. The finding agreed with the report of Hozzein *et al.* (2011) that *Streptomyces* sp. isolated from desert soil have effective activity against Gram-posotive bacteria more that Gram-negative bacteria. Moreover, Ghadin *et al.* (2008) reported that isolate, namely SUK 06 showed killing activity against one or more pathogenic bacteria containing *B. subtilis*, *P. aeruginosa* ATCC 27853 and *B. cereus*. Raja *et al.* (2010) also isolated *Intrasporangium* sp., *Dactylsporangium* sp., *Micromonospora* sp., *Streptoverticilium* sp. and two *Streptomyces* sp. from hill soil and showed antibacterial activity against *Streptococcus mutans* and *Streptococcus oralis*.

Table 1: The antimicrobial activities of actinomycetes isolated from Termite's guts

Isolated code	Antibacterial activity				
	Bs	Sa	Pa	Ec	Cu
FSPNRU 1	+	+	-	-	-
FSPNRU 2	-	-	-	-	-
FSPNRU 11	-	+	-	-	-
FSPNRU 22	+	+	-	+	-
FSPNRU 30	+	+	-	-	-
FSPNRU 34	-	-	-	-	-
FSPNRU 37	-	+	-	-	-
FSPNRU 40	+	+	-	-	-
FSPNRU 49	-	-	-	-	-
FSPNRU 60	-	+	-	+	-
FSPNRU 67	-	+	-	-	-
FSPNRU 72	-	+	-	-	-
FSPNRU 79	+	-	-	-	-
FSPNRU 81	+	+	+	-	-
FSPNRU 84	-	+	-	-	-
FSPNRU 91	-	-	-	-	-
FSPNRU 99	+	-	-	-	-
FSPNRU 102	+	+	+	+	+
FSPNRU 107	+	+	-	-	-

+: Positive; -: Negative; Bs: *Bacillus cereus*; Sa: *Staphylococcus aureus*; Pa: *Pseudomonas aeruginosa*; Ec: *Escherichia coli*; Cu: *Candida utilis*

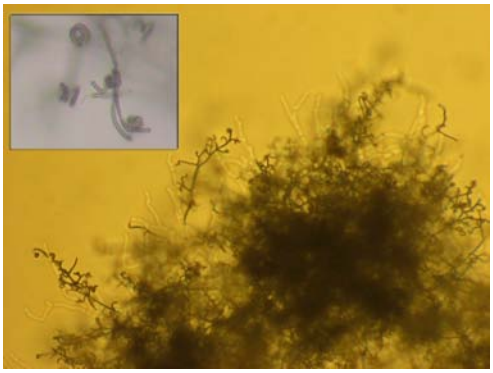

Fig. 1: Light microscopic micrograph of strain FSPNRU 102 showing substrate mycelium, aerial mycelium and spore chain forming coil

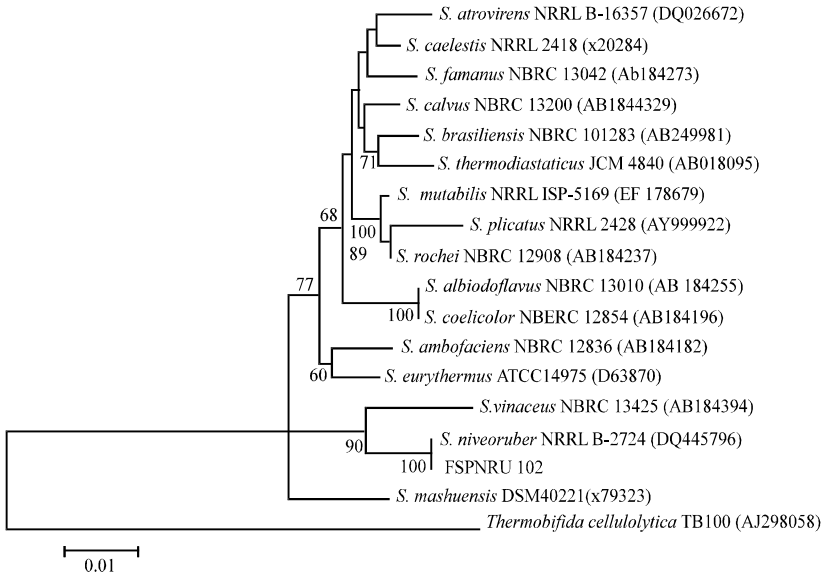

Fig. 2: Phylogenetic tree of nucleotide sequence analysis of 16S rDNA of strain FSPNRU 102 with related species of *Streptomyces* constructed by Neighbor-joining method from MEGA4 program. The tree is rooted by the nucleotide sequence of *Thermobifida cellulolytica* TB100. Scale bar shown distance values under the tree means 0.01 substitutions per nucleotide position. Bootstrap analyses were performed with 1000 re-samplings and percent values (>50) are shown at the branching points. The isolated strains in this study are bold letter.

Morphological and chemotaxonomical characteristic: Strain FSPNRU 102 was aerobic, Gram-positive, non-acid alcohol-fast actinomycetes that forms extensively branched substrate mycelia. Selected strains has morphological character under microscopic and agar slant as shown in Fig. 1. This strain produced brown aerial mycelium with longitudinal spirales-type spore chain and light black soluble pigment. The aerial spore color varied from white to gray. Moreover, this strain contained LL-diaminopimelic acid of the peptidoglycan in the whole-cell hydrolysate. This result assigned strain FSPNRU 102 to genus *Streptomyces* (Williams *et al.*, 1989).

Molecular identification: The 16S rDNA sequence was generated for strain FSPNRU 102 (1416 nucleotides). Comparison of this nucleotide sequence with members of actinomycetes clearly showed that this strain belong to the genus *Streptomyces* (Fig. 2). The close relationship to *Streptomyces niveoruber* is supported both by treeing algorithms and by a high bootstrap value (Fig. 2).

CONCLUSION

The strain FSPNRU 102 that isolated from termite gut is belonging to *Streptomyces niveoruber*. This strain produced bioactive compound with broad spectrum activity against Gram-positive and Gram-negative bacteria. Further study needs to be undertaken to analyze the mechanism for the antimicrobial activity of this bioactive compounds. It might be considered as a candidate source for dug production.

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