Isolation and screening of Actinomycetes from soil for their enzymatic and antifungal activity

Kingchan Malisorn[1] and Kanokwan Nikhome[1]

ABSTRACT: Actinomycetes, slow growing gram positive bacteria, are known as an organism that is useful in the search for bioactive compounds. One hundred twenty-nine isolated of actinomycetes were isolated from soil samples collected in Phulungka National Park, Nakhon Phanom province. The isolates were identified as actimomycetes by morphological studies. All isolates were selected for their basis of their chitinolytic, amylolytic, cellulolytic activities. The ability to produce different enzymes was observed by measurement of clear zone around each colony. Results showed that 81, 75 and 41 isolates exhibited chitinolytic, amylolytic and cellulolytic activities, respectively. Antifungal test was conducted using selected phytopathogen as test strain and it was observed that 75 isolates showed antagonistic reaction with *Fusarium* sp. FT-04. The isolates of actinomycetes strains were identified as *Streptomyces, Microbispora* and *Microtetraspora*.

Keywords: Isolation; Screening, Actinomycetes, Enzymatic, Antifungal activity

Introduction

Actinomycetes are the most widely distributed group of microorganism in nature which primarily inhabit the soil. They are gram positive bacteria and tend to grow slowly as branching filaments (Holt et al., 1994). They are known for their economic importance as producers of biologically active substances, such as antibiotics, vitamins and enzymes (DeBoer et al., 2005). The recent advent of biotechnology, there has been a growing interest and demand for enzymes with novel properties. Extracellular hydrolytic enzymes from actinomycetes are interesting. It can breakdown complex sugar, for example, starch into simple sugars such as glucose, maltose and dextrin. Amylase production by actinomycetes has been reported, amylase from marine actinomycetes had been isolated from South coast of India for producing industrial enzyme (Reyad, 2013; Selvam et al., 2011). Cellulose is the most abundant polysaccharide in nature, as it is a principal constituent in the plant cell wall composition. Cellulase is group of hydrolytic enzymes with hydrolyze the glucosidic bonds of cellulose and related to cellodigosaccharide derivatives (Ito, 1997). Actinomycetes are one of the known cellulose producers has attracted considerable research interest (Arunachalam et al., 2010; Jang and Chenks, 2003). Cellulase is a useful biocatalyst and can be in many industries like bio-textile auxiliaries, cotton and linen products processing, bio-fertilizer processing, food technology, biofuel formation, and paper production (Reyad, 2013). Chitin is polymer of b-1,4-linked N-acetylglucosamine (GlcNAc) and is abundant natural polymer, coming only second after cellulose among the polysaccharides. It is

[1] Department of Biology, Faculty of Science, Udonthani Rajabhat University, Udonthani, 41000, Thailand

* Corresponding author: kingchanchoomponla@yahoomail.com, Ling_noi1516@hotmail.com

the main structural compound of cell walls of fungi, insect exoskeletons and the shells of crustaceans (Gohel et al., 2006). Chitinase is involved in the process of producing mono- and oligosaccharides from chitin. Furthermore, chitinase is a potential antifungal agent produced by actinomycetes that suppresses plant pathogenic fungi and mosquitos and are also used in mycolytic enzyme preparation, fungal protoplast technology, preparation of chito-oligosaccharides, glucosamine, and GlcNAc, cytochemical localization of chitin/chitosan using chitinase-chitosanase-gold complexes, production of single-cell protein, estimation of fungal biomass, morphogenesis, medical application and degradation of fish wastes, etc. (Dahiya et al., 2006). Most actinomycetes in soil belong to the genus *Streptomyces* and 60% of the sources of most biologically active compounds that have been developed for agricultural use are originated from them (Ilić et al., 2007). The commercial product, Mycostop, based on *S. griseoviridis* K16 and *S. lydicus* WYEC108 can control some root rots and wilt diseased caused by *Pythium* spp. *Fusarium* spp., *Rhizoctonia* spp. and *Phytophthora* spp. (Mahadevvan and Crowford, 1997). The aim of the present study was to isolate and screen of actinomycetes from soil that have ability to act as biodegradation agents or biocontrol agents.

Materials and Methods

Soil sample collection

Soil sample were collected about 5 cm below the surface of the soil. All the soil samples were collected randomly from Phulangka National Park, Nakhon Phanom province.

Actinomycetes isolation

Soil sample collected were pretreated by drying them in open air for 2 days. Samples of 25 g each were mixed with 225 ml of sterile distilled water. The soil suspension was initially carried out by using serial dilution (Machin, 1999; Waksman, 1927) and spread plate method on starch casein agar (SCA) (Madigan and Martinko, 2006; McCormick and McCormic, 1997). The plates were incubated at 37±2°C for 7 days. Actinomycetes colonies were picked and purified by streak plate technique at the same isolation medium.

Enzymatic screening

Actinomycetes that were grown on SCA were transferred by sterilized needle and stabbed into chitin agar plates (grams per liter: 3g chitin precipitated, 1.1g $Na_2HPO_4.2H_2O$, 0.7g KH_2PO_4, 0.2g $MgSO_4.7H_2O$, 0.01g $FeSO_4.7H_2O$, 0.01g $MnSO_4.H_2O$, 18g agar, pH 7.0), starch agar plate (grams per liter: 3g Beef extract, 5g peptone, 10g soluble starch, 18g agar, pH 7.0) and cellulose agar plate (grams per liter: 0.5g $(NH_4)_2SO_4$, 1g K_2HPO_4, 0.2g $MgSO_4$, 0.5g KCl, 0.1g $CaCl_2$, 0.5g yeast extract, 10g carboxy methyl cellulose, 18g agar, pH 7.0). The plates were incubated at 30±2°C for 7 days. The chitinase production was observed by appearance of clear zone around their colonies. The amylase production was observed by flooding the agar plates with gram's iodine solution then produced zone of clearance or decolorization against the blue color ground. The cellulase activity was observed by flooding the agar plates with 2% congo red and decoloried by 1M NaCl then the yellow zone in respect to the red background was considered as hydrolysis of cellulose.

Antimicrobial screening

Assay plates were prepared using potato dextrose agar for *Fusarium* sp. FT-04. Actinomycetes were grown on SCA, they were transferred to agar assays by dual culture method Oldenburg et al. (1996) on a solid medium. The isolates of actinomycetes were streak on PDA at the distance of 1.5 cm from the edge of the Petri dish. The plates were incubated at 30±2°C for 7 days to allow growth and sporulation of the isolates of actinomycetes. Afterwards a 5 mm diameter freshly growing mycelium plug of *Fusarium* sp. FT-04 was placed on the centre of a plate and incubated for another 3 days. The pathogen alone was used as a positive control and the experiments were repeated three times. A non-fungal growth area surrounding the isolates of actinomycetes culture indicated antagonistic activity.

Morphology identification

Actinomycetes were streaked on SCA, cover slip method Kawato and Shinobu (1959) was employed for microscopic. Identification of actinomycetes to genus level was then carried out based on the Bergey's manual of Systematic Bacteriology. 9th edition (Holt et al., 1994).

Result

Isolation

Soil samples were randomly selected from rhizosphere soil of orchid, antill soil, rhizosphere soil of tree and rhizosphere soil of mushroom. One hundred and twenty nine isolates of actinomycetes were obtained from screening. The colors of colonies were grey, white, brown, black, yellow and cream. Result showed 55 (43%) of total isolates in greyish, 32 (25%) in white, 31 (24%) in brownish, 5 (4%) in black, 3 (2%) in yellowish and 3 (2%) in creamy (**Figure 1**).

Enzyme screening

In vitro of actinomycetes for enzymatic reaction showed 81 (63%) of total isolates were able to be hydrolysed chitin, 74 (58%) hydrolysed starch and only 50 (39%) hydrolysed cellulose (Figure 2).

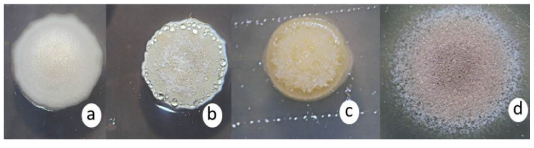

Figure 1 Colony color of isolated actinomycetes, (a) white, (b) creamy, (c) yellowish and (d) greyish

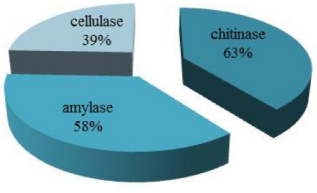

Figure 2 Number of actinomycetes isolates that were able to be hydrolysed chitin, starch and cellulose

Antimoicrobial screening

All 129 isolates of actinomycetes were then tested for antagonist reaction with plant pathogenic fungi selected. Seventy four (57%) of the isolates showed positive reaction towards *Fusarium* sp. FT-04.

Morphology identification

One hundred twenty five isolates were identified as a species of the genus *Streptomyces*

(**Figure 3**). They were found to be Gram positive and produced aerial mycelium abundantly. The spores were produced in spiral long chain. Isolate A14-1, C5-2 and E5-1 were identified as a species of the genus *Microbispora*, they were produced two spores in chain. Only isolate A11-1 was identified as genus *Microtetraspora*, it produced four spores in chain.

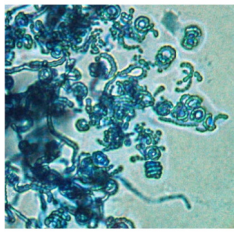

Figure 3 Spiral spore chain morphology of *Streptomyces* sp. E1-7.

Discussion

Actinomycetes are characterized by the ability to produce a large variety of secondary metabolites, such as vitamins, enzymes and antibiotic. Actinomycetes or their enzymes have array of biological industrial and environmental applications, like polymer hydrolysis, synthesis of chemicals, soil decontamination, biological control of diseases, and decomposition of organic matter (Minotto et al., 2014). The soil samples were collected in Phulangka National Park, Nakhon Phanom province. There were rhizosphere soils and antill soil. The sample was plated on starch casein agar for 7 days at 37±2°C temperature. The isolates under investigation belonged to three genera that *Streptomyces, Micromonospora* and *Microtetraspora.* Majority of the isolates in this study possessed spiral long chain, two spores in chain and four spores in chain, respectively. Spore morphology is considered as one of the important characteristic features in actinomycetes identification and it varies among the genus and species (Meena et al., 2013). Moreover, the results acquired in this study have been outlined in Bergey's Manual of Determinative Bacteriology. According to Usha et al. (2010) indicated actinomycetes isolates could be isolated from Pichavaram mangrove soil in Tamilnadu, India. The observation showed chains of conidia on aerial mycelium. The isolates under investigation belonged to the genus *Streptomyces*. *Streptomyces* are an important source of enzyme and bioactive products. Most produce secondary metabolites that have antibacterial, anti-fungal, anti-tumor or antiprotozoal activity making them a target for isolation in large-scale screening programs in industries (Karanja et al., 2010). The present study investigated hydrolytic enzymes from actinomycetes isolates. They have ability to produce amylolytic, cellulolytic and chitinolytic enzymes. Variation in the production of enzyme by actinomycetes was reported by Minotto et al. (2014) The study, endophytic actinomycetes observed the enzymatic activity amylase, caseinase, pectinase and cellulase. The results confirmed variation, showing that the degradation of different specific substrates is associated with incubation condition. The study of chitinolytic enzyme from *Streptomyces* sp. J12 was established by varying different conditions under solid state fermentation, reported by Choomponla & Upadhyay. In present study i nvestigated the antagonistic action of actinomycetes isolates on *Fusarium* sp. FT-04. Fungal growth inhibition by antifungal compound and/or hydrolytic enzymes produced by the actinomy-

cetes isolates. According to hydrolytic enzymes may have a roll in the activity of the antagonist when controlling fungal mycelium (Loliam et al., 2013). The in vitro results do not necessarily translate to what occurs in planta (Zivkovic et al., 2010). Nonetheless, this study and the results are particularly useful for identifying likely candidates for biocontrol and for making educated guesses concerning the mechanisms by which the reduce pathogen damage.

Conclusion

In present study, 129 isolates of actinomycetes were isolated from soil that randomly collected soil from Phulangka National Park, Nakhon Phanom province. Actinomycetes isolated have more potential in hydrolysing chitin, starch and cellulose, respectively. The antimicrobial reaction showed positive reaction towards *Fusarium* sp. FT-04 in 57% for all isolates. The isolates of actinomycetes were identified as *Streptomyces, Microbispora* and *Microtetraspora*. Majority of actinomycetes are free living saprophytic bacteria found widely distributed in soil, water and colonizing plants. The population of actinomycetes has been identified as one of the major groups of soil population, which may vary with the soil type Actinomycetes from soil are interesting isolates which show the potential to produce bioactive compound that may be useful in the control plant pathogenic fungi.

Acknowledgenment

Authors are thankful to Higher Education Research Promotion (HERP) and Udonthani Rajabhat University for providing facilities research work.

References

Arunachalam, R., E. G. Wesley, J. George, and G. Annaduri. 2010. Novel approaches for identification of *Streptomyces nobortoensis* TBGH-V20 with cellulose production. Curr. Res. Bacteriol. 3(1): 15-26.

Dahiya, N., R. Tawari, and G. H. Sigh. 2006. Biotechnological aspects of chitinolytic enzymes: a review. Appl. Microbiol. Biotechnol. 71: 773-782.

DeBoer, W., L. B. Folman, R. C. Summerbell, and L. Boddy. 2005. Living in an fungal world: impact of fungi on soil bacterial niche developement. FEMS Microbial Rev. 29: 795-811.

Gohel, V., A. Singh, M. Vimal, P. Ashwini, and H. S. Chhatapar. 2006. Review: bioprospecting and antifungal potential of chitinolytic microorganisms. Afr. J. Biotechnol. 5: 54-72.

Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams. 1994. Bergey's manual of Systematic Bacteriology. 9th ed. Williams & Wilkins: USA.

Iliĉ, S. B., S. S. Konstantinoviĉ, Z. B. Todoroviĉ, M. L. Laziĉ, V. B. Veljkoviĉ, N. Jokoviĉ, B. C. Radovanoviĉ. 2007. Characterization and antimicrobial activity of the bioactive metabolite in Streptomycete isolates. Mikrobiologia. 76(4): 480-487.

Ito, S. 1997. Alkaline celluloses from alkaliphilic of *Bacillus*: enzymatic properties, genetics, and application to detergents. Extreamophiles. 1: 61-66.

Jang, H.D., and Chenks. 2003. Production and characterisation of thermostable cellulose from *Streptomyces transformant* T3-1. World J. Microbiol. Biotechnol. 19: 263-268.

Karanja, N. E., I. H. Boga, W. A. Muigai, F. Wamunyokoli, J. Kinyua, J., and O. Nonoh. 2010. Optimization of growth conditions and characterization of enzymatic activity of selected novel *Streptomyces* species from Kenya soils. Proceeding of 2010 JKUAT Scientific Technological and Industrialization Conference, Kenya.

Kawato, M., and R. Shinobu. 1959. On Streptomyces herbaricolor nov. sp. Supplement: a simple technique for the microscopical observation. Memories of Osaka University library art and education, 114.

Loliam, B., T. Morinaga, and S. Chaiyanan. 2013. Biocontrol of Phytium aphanidermatum by the cellulolytic actinomycetes *Streptomyces rubrolavendulae* S4. ScienceAsia. 39: 584-590.

Machin, B.D. 1999. Serial Dilution. University of Manchester, School of Biological Science.

Madigan, M. T., and J. M. Martinko. 2006. Biological of Microorganism. 11th ed. Upper Saddle River NJ: Pearson Prentice Hall.

Mahadevvan, B., and D. L. Crowford. 1997. Properties of the chitinase of the antifungal biocontrol agent *Streptomyces lydicus* WYEC108. Enzyme Microb. Technol. 20(70): 489-493.

McCormick, T. R. S., and T. McCormic. 1995. Essentials Microbiology. Research & Education Association.

Meena, B., A. L. Rajan, V. N. Vinithkumar, and R. Kirubagaran. 2013. Novel marine actinobacteria from emerald Andaman & Nicobar Islands: a prospective source for industrial and pharmaceutical byproducts. BMC. Microbiology. 13 (145): 1471-2180.

Minotto, E., L. P. Milagre, M. T. Oliveira, and S. T. Van Der Sand. 2014. Enzyme characterization of endophytic actinobacteria isolated from tomato plants. J. Adv. Sci. Res. 5(2): 16-23.

Oldenburg, R. K., T. K.Vo, B. Ruhland, J. P. Schatz, and Z. Yuan. 1996. A dual culture assay for detection of antimicrobial activity. J. Biomolecular Screening. 1(3): 123-130.

Reyad, M. A. 2013. Diverse of enzymatically active actinomycetes associated with mangrove rhizosphere in Janzan coast. Ann. Biol. Res. 4(4): 100-108.

Selvam, K., B. Vishnupriya, B. Subhash, and V. Bose Chandra. 2011. Screening and quantification of marine actinomycetes producing industrial enzymes amylase, cellulose and lipase from South coast of India. IJPBA. 2(5): 1481-1487.

Usha, R., P. Ananthaselvi, C. K. Venil, and M. Palaniswamyn. 2010. Antimicrobial and antiangiogenesis activity of *Streptomyces parvulus* KUAP106 from mangrove soil. European J. of Biol. Sci. 2(4): 77-83.

Waksman, S.A. 1927. Principle of Soil Microbiology. Baltimore, USA: Williams and Wilkins Co.

Zivkovic, S., S. Stojanovic, Z. Ivanovic, V. Gavrilovic, T. Poppvic, and J. Balaz. 2010. Screening of antagonistic activity of microorganisms against *Collectotrichum acutatum* and *Collectotrichum gloeosporioides*. Arch. Biol. Sci. Belgrade. 62(3): 611-623.