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ANTIMICROBIAL ACTIVITY OF STREPTOMYCES SP. PJ90 ISOLATED FROM SOIL IN NORTHEAST THAILAND

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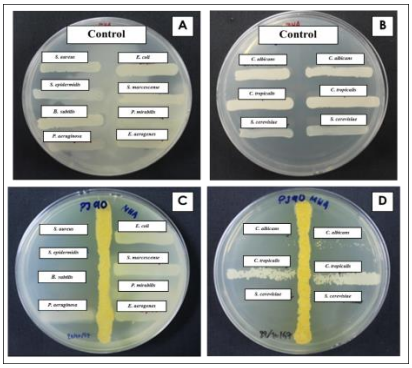
Graphical abstract



Abstract

In this study, an antimicrobial-producing Actinomycetes PJ90 was isolated from forest soil in Suranaree University of Technology, Nakhon Ratchasima, Thailand. The morphological characteristics and 16S rRNA gene analysis revealed that isolate PJ90 could be classified as *Streptomyces triostinicus*. The isolate PJ90 exhibited antimicrobial activity against *Staphylococcus aureus* TISTR1466, *Staphylococcus epidermidis* TISTR518, *Bacillus subtilis* TISTR008, *Candida albicans* TISTR5779, *Candida tropicalis* TISTR5174 and *Saccharomyces cerevisiae* TISTR5049. To our best knowledge, this study constitutes the first anti-bacterial and anti-yeast activities of *Streptomyces triostinicus* isolated from soil in Thailand.

Keywords: Isolate, Antimicrobial, Actinomycetes, *Streptomyces*

1.0 INTRODUCTION

Infectious disease caused by opportunistic pathogens including drug-resistant strains have been a global healthcare problem for decades [1]. Therefore, the search for the novel potent drugs becomes an importance subject. Since, the discovery of penicillin-producing mold in 1928 by Alexander Fleming, microorganisms have become a target source for the production of antimicrobial agents [2]. A number of antibiotic drugs have been discovered from soil-inhabiting microorganisms, which include fungi (20% of currently available antibiotics), Actinomycetes (70%) and eubacteria (10%) [3-4]. It had been shown that bacteria belonging to the order Actinomycetes are a potential source for bioactive secondary metabolites including antimicrobial agents. Actinomycetes are widely distributed groups in soil environments which play a major role in the recycling of organic matters and nutritional materials [5]. They are filamentous Gram-positive bacteria belonging to the phylum Actinobacteria.

They represent one of the largest taxonomic units currently recognized within the domain Bacteria [6]. Approximately 80% of the world's antibiotics are derived from Actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora* [7-8]. During 1940 to 1970, antibiotics such as streptomycin, tetracyclines, chloramphenicol, vancomycin and cephalosporins were discovered [9-10]. However, the finding of new compounds has substantially decreased since late 1980s which may be due to the decline in screening efforts. In 2001, Watvw and co-workers estimated that only 1-3% of all known antimicrobial compounds produced by genus *Streptomyces* alone has been isolated so far. Thus, there is a vast majority of antibiotics left to be discovered [11]. Moreover, Prakasham and colleague reported that only one fifth of the global soil has been used for the screening of antimicrobial-producing organisms which means that

there are plenty of space on earth left for the search of new antimicrobial-producing strains [12].

According to the World Bank report published in 2012, tropical forest cover approximately one third of Thailand's total land area. However, there have been a few studies on the isolation and characterization of Actinomycetes from Thai forest soil [13]. The present study described the isolation and identification of *Streptomyces* sp. PJ90 from forest soil by conventional and molecular methods. The antibiotic activities of PJ90 were also investigated.

2.0 EXPERIMENTAL

2.1 Site of Sample Collection

Soil samples were collected from several sites in Suranaree University of Technology, Nakhon Ratchasima, Thailand. The sampling area was located at a latitude of 14.8729 and a longitude of 102.0237. Soil samples were randomly taken at 10-15 cm depth from surface and aseptically transferred by sterile polyethylene bag to the laboratory.

2.2 Media and Culture Conditions

Isolation of Actinomycetes was carried out using starch casein agar (SCA): (g/l: soluble starch 10; casein 0.3; potassium nitrate 2; sodium chloride 2; dipotassium hydrogen phosphate 2; magnesium sulphate 0.05; calcium carbonate 0.02; ferrous sulphate 0.01; agar 15; pH 7.2). The cultivation temperature of Actinomycetes was 28°C. Mueller Hinton Agar (MHA) (Hi-media, India) was used for the determination of antimicrobial activity. The incubation temperature for antimicrobial activity test were 30°C or 37°C depending on the strains of test pathogens.

2.3 Test Pathogens

Pathogenic microbial strains used in this study were obtained from Thailand Institute of Scientific and Technological Research (TISTR). They were *Staphylococcus aureus* TISTR1466, *Staphylococcus epidermidis* TISTR518, *Bacillus subtilis* TISTR008, *Pseudomonas aeruginosa* TISTR781, *Escherichia coli* TISTR780, *Serratia marcescens* TISTR1354, *Proteus mirabilis* TISTR100, *Enterobacter aerogenes* TISTR 1540, *Candida albicans* TISTR5779, *Candida tropicalis* TISTR5174 and *Saccharomyces cerevisiae* TISTR5049.

2.4 Isolation of Actinobacteria from Soil Samples

One gram of soil sample was suspended in Erlenmeyer flask containing 99 ml sterile water and incubated at room temperature without shaking for 30 min. The soil suspension was serially diluted and spread on SCA plate. The plates were then incubated at 28°C for 5 days. After incubation, the suspected Actinomycetes colonies were selected and used for further study.

2.5 Antimicrobial Activity Test

The antimicrobial activity of soil isolate was determined by perpendicular-streak method. Soil isolate was inoculated on MHA plate by single streaking at the center of a petridish. The plates were incubated at 28°C for 5 days in order to allow the organisms to produce antimicrobial substances and release to an agar medium. The plates were then seeded with test pathogens by streaking perpendicular to the line of soil isolate colonies. The inhibition zone is the area where no growth of bacteria and yeasts are observed.

2.6 16S rRNA Gene Sequencing and Sequence Analysis

Genomic DNA of bacterial strains was isolated from cell grown in 5 ml of Mueller Hinton Broth (MHB) (Himedia, India) at 28°C for 3 days. The cell was obtained by centrifugation and suspended with 180 µl of 50 mM NaOH. The cell suspensions were incubated at 95°C for 10 min and neutralized by adding 20 µl of 1M Tris-HCl (pH 8.0). Cells were pelleted by centrifugation at 13,000 rpm for 5 min. The supernatant was used as DNA template for PCR amplification of 16S rRNA gene. PCR amplification of the 16S rDNA of PJ90 was performed by using specific universal primers, 243F (5'-GGATGAGCCGCGGCCTA-3') and A3R (5'-CCAGCCCCACCTTCGAC-3') [14]. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 60 s, annealing at 55°C for 60 s, extension at 72°C for 60 s and a final elongation at 72°C for 7 min. The amplified fragments were purified from 0.8% agarose gel by using NucleoSpin® Gel and PCR clean-up kit (MACHEREY-NAGEL, Germany). The purified product was submitted for sequencing at Macrogen, Korea. The 16S rRNA gene sequence of soil isolate was compared for similarity with the reference species available at NCBI GenBank database.

3.0 RESULTS AND DISCUSSION

Antibiotic drugs are the most important bioactive compounds for the treatment of infectious diseases. Nowadays, widespread and application use of antibiotics have led to the development of antibiotic-resistant microorganisms all over the world [15-16]. The emergence of drug resistances has compromised the treatment of infectious diseases. Therefore, there is an urgent need for the search of the new, safe and effective drugs to replace the invalidated ones. Actinomycetes have been proven as a potential and richest source of secondary metabolites such as antibiotics, pesticide and herbicide [17]. In this study, we attempted to isolate antimicrobial-producing Actinomycetes from soil in Suranaree University of Technology. Soil from this area has been known for its poor in nutrients and highly acidic which could establish somewhat extreme condition. An extreme

environmental condition has been known to activate the protective mechanisms of soil-inhabiting microorganisms by inducing the production of antibiotics and other defense compounds [18]. Therefore, the forest soil in Suranaree University of Technology could be served as a potential source for the screening of the novel antimicrobial drugs.

3.1 Isolation and Classification of Soil Isolate PJ90

In this study, we were able to isolate Actinomycetes strain PJ90 from forest soil in Suranaree University of Technology by using serial dilution and plating technique. The colony morphology of PJ90 appeared yellow, rough and powdery after cultured on SCA medium at 28°C for 10 days. The Macroscopic and microscopic morphologies of PJ90 were summarized in Table 1. From all of these results, it could be concluded that PJ90 might belong to the genus *Streptomyces* [19].

Table 1 Morphological characteristics of the PJ90 strain

Characteristics	Observation
Gram staining	Positive
Spore chain morphology	Rectiflexible
Color of aerial mycelium	White
Color of substrate mycelium	Yellow
Color of colony	Yellow
Texture of colony	Powdery
Earthy odor	Present

An identification of PJ90 in the species level was done by 16S rRNA gene analysis. The 16S rRNA gene was amplified by using specific universal primers, 243F and A3R [14]. The amplified fragment was compared to the nucleotide sequences of known species from NCBI GenBank database. The homology search by BLAST program showed that 16S rDNA of PJ90 contained 99% similarities to *Streptomyces triostinicus* (Figure 1). Thus, isolate PJ90 could be classified as *Streptomyces triostinicus*.

3.2 Determination of Antimicrobial Activity of PJ90

The perpendicular-streak method was used to determine the antimicrobial activity of PJ90 (Figure 2). As shown in Figure 2C and 2D, *Staphylococcus aureus* TISTR1466, *Staphylococcus epidermidis* TISTR518, *Bacillus subtilis* TISTR008, *Candida albicans* TISTR5779 and *Saccharomyces cerevisiae* TISTR5049 could not grow on MHA plate when co-cultured with PJ90. Whereas, the growth of PJ90 on MHA plate did not affect the growth of *Pseudomonas aeruginosa* TISTR781, *Escherichia coli* TISTR780, *Serratia marcescens* TISTR1354, *Proteus mirabilis* TISTR100 and *Enterobacter aerogenes* TISTR1540 (Figure 2C). In addition, the growth of *Candida tropicalis* TISTR5174 in the presence of PJ90 colonies (Figure 2D) was slightly decrease compared to the negative control (Figure 2B)

The antimicrobial activity of PJ90 against test pathogens is summarized in Table 2. These results indicated that PJ90 exhibits anti-bacterial activity against Gram-positive bacteria but not Gram-negative bacteria.

The strain PJ90 also shows anti-yeast activities which are strongly active against *Candida albicans* TISTR5779 and *Saccharomyces cerevisiae* TISTR5049 and weakly active against *Candida tropicalis* TISTR5174.

In Thailand, the anti-bacterial and anti-yeast activities were studied from *S. sporoclivatus*, *S. iranensis, S.sundarbansensis, S. termitum, S. mycarofaciens*, *S. albospinus*, *S. aureoversilis, S. aureofaciens, S. xanthocidicus*, *S. roseocinereus*, *S. hygroscopicus* and *S. spectabilis* isolated from soil in Rayong, Samut Song Khram, Songkhla, Pattaloong, Nan, Chaiyaphum and Chiangrai, respectively [20-22]. An anti-mold activity of *S. triostinicus* isolated from Chanthaburi was also reported [23]. Our study shows the first successful isolation of *Streptomyces triostinicus* from soil in Nakhon Ratchasima province, Thailand. To our best knowledge, this is the very first report of anti-bacterial and anti-yeast activities of *Streptomyces triostinicus* isolated from Thai soil so far. Thus, forest soil in Suranaree University of Technology has proven to be the valuable sources for the screening of the antibiotic-producing bacterial strains. In addition, the antimicrobial properties of PJ90 are currently under investigated in our laboratory which includes the purification and characterization of its bioactive compounds. The screening of other antimicrobial-producing Actinomycetes strains from soil in Suranaree University of Technology are also being conducted in our laboratory.

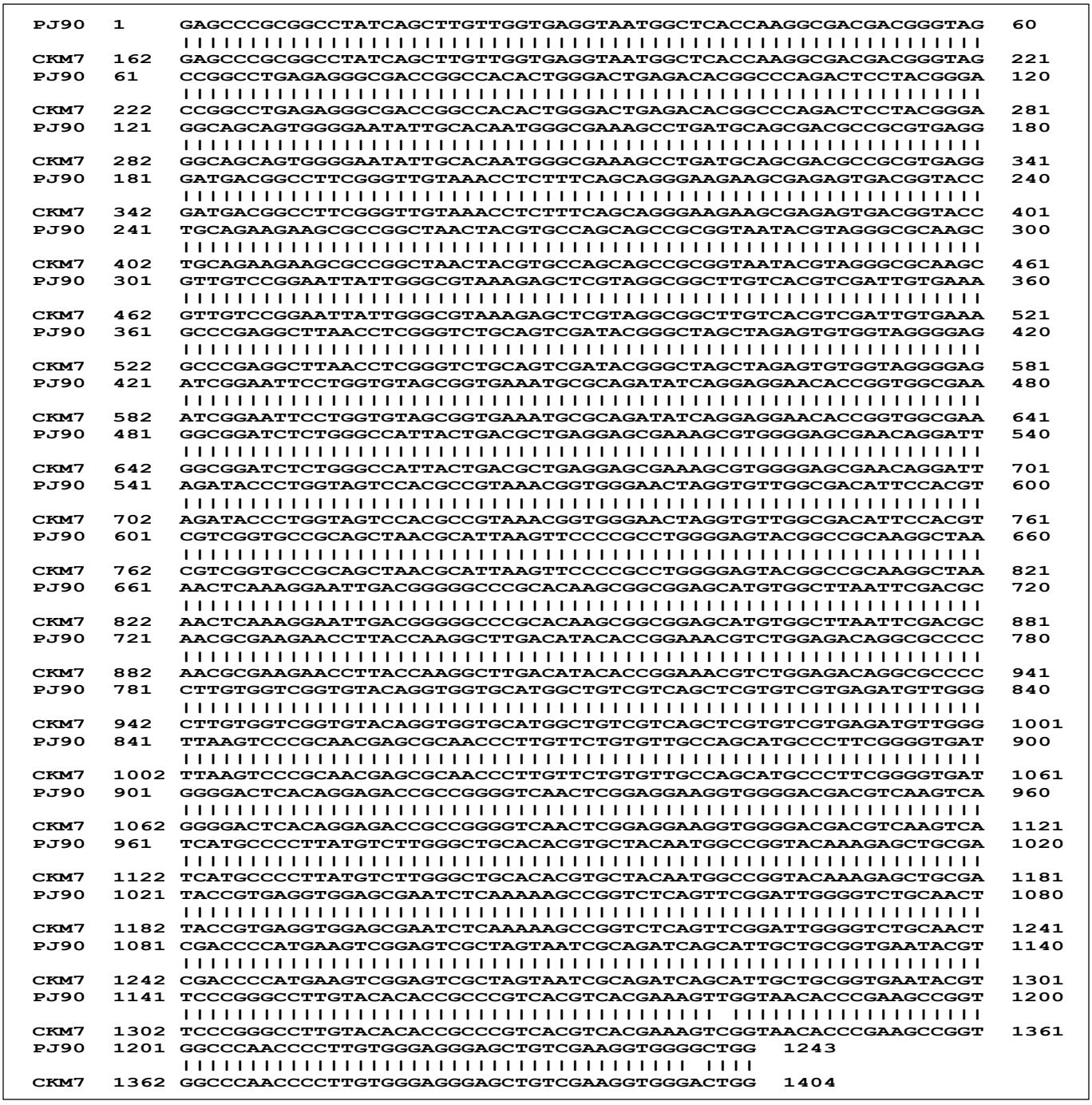


Figure 1 Alignment between 16S rRNA gene sequence of isolate PJ90 and nucleotide sequence of *Streptomyces triostinicus* CKM7

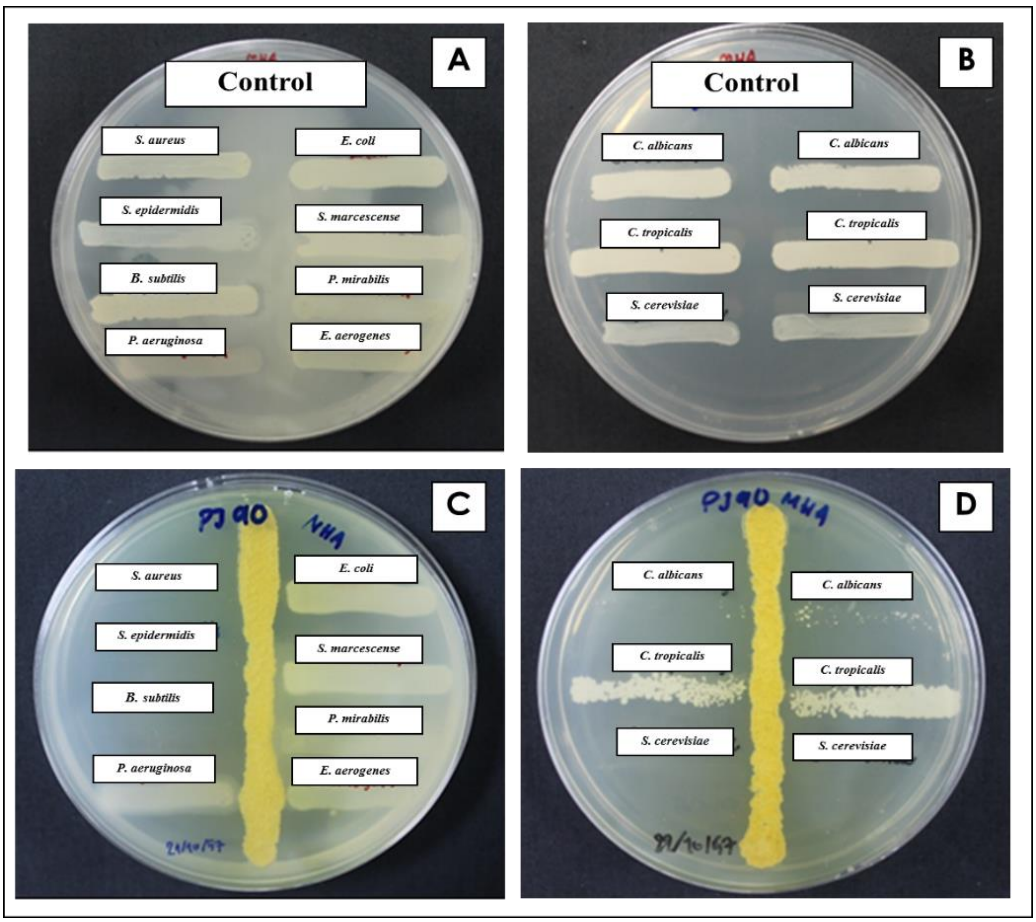


Figure 2 Antimicrobial activity test. (A) The growth of *Staphylococcus aureus* TISTR1466, *Staphylococcus epidermidis* TISTR518, *Bacillus subtilis* TISTR008, *Pseudomonas aeruginosa* TISTR781, *Escherichia coli* TISTR780, *Serratia marcescens* TISTR1354, *Proteus mirabilis* TISTR100 and *Enterobacter aerogenes* TISTR1540 on MHA plate. (B) The growth of *Candida albicans* TISTR5779, *Candida tropicalis* TISTR5174 and *Saccharomyces cerevisiae* TISTR5049 on MHA plate. (C) Anti-bacterial activity of PJ90 against of *Staphylococcus aureus* TISTR1466, *Staphylococcus epidermidis* TISTR518 and *Bacillus subtilis* TISTR008. (D) Anti-yeast activity of PJ90 against *Candida albicans* TISTR5779, *Candida tropicalis* TISTR5174 and *Saccharomyces cerevisiae* TISTR5049

Table 2 Antimicrobial activity of PJ90 by perpendicular-streak method

Test organisms	Isolate PJ90
Gram-positive bacteria	
Staphylococcus aureus TISTR1466	+
Staphylococcus epidermidis TISTR518	+
Bacillus subtilis TISTR008	+
Gram-negative bacteria	
Pseudomonas aeruginosa TISTR781	-
Escherichia coli TISTR780	-
Serratia marcescens TISTR1354	-
Proteus mirabilis TISTR100	-
Enterobacter aerogenes TISTR1540	-
Yeasts	
Candida albicans TISTR5779	+
Candida tropicalis TISTR5174	+
Saccharomyces cerevisiae TISTR5049	+

(+) inhibition; (-) no effect

4.0 CONCLUSION

In this study, PJ90 was isolated from forest soil in Suranaree University of Technology, Thailand. Based on morphological characteristics and 16S rRNA gene analysis, PJ90 might be classified as *Streptomyces triostinicus*. This strain has been shown to produce antimicrobial compounds which is mainly active against Gram-positive bacteria and yeasts. The study of this isolate could be further explored for the development of new antibiotic drugs to treat infectious diseases causing by pathogenic and drug-resistant strains.

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