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Research Report

Identification of *Streptomyces* sp-MWS1 Producing Antibacterial Compounds

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ABSTRACT

An actinomycete, designated Streptomyces sp-MWS1, was isolated from mangrove ecosystem soil in the eastern coast of Surabaya. This organism was capable of producing a series of antibiotics that strongly inhibit the growth of Gram-positive and Gram-negative bacteria. Furthermore, culture morphological and physiological characteristics of the isolated strain, Streptomyces sp-MWS1 were compared to other reference strains belong to Streptomyces species. The analysis of nucleotide sequence of the 16S rDNA indicated similarity binary 98% with Streptomyces species.

***Key words**: Streptomyces, mangrove, antibiotic, 16S rDNA*

INTRODUCTION

The"strike back" of pathogens has revitalized the research for new drugs (Lemonick, 1994; Jaroff, 1994). Novel antibiotics are required to counter drug-resistant bacteria, fungi, and viruses. Only about 10% of the estimated total number of microbial species are known-there is an extensive and diverse resource that can be tapped for useful products, such as antibiotics, and processes, such as novel mechanisms of action (Bull *et al*., 1992). In this respect, natural antibiotics (particularly those from the genus *Actinomycetes*, the most abundant microbial source of antimicrobial compounds (Miyadoh, 1993) are as important as those, which are derived from chemical modification of existing antibiotics.

In our screening program for bioactive compounds, an actinomycete (which we designated *Streptomyces* sp-MWS1) was isolated from mangrove ecosystem soil in the eastern coast of Surabaya. This actinomycete is capable of producing antibiotics that strongly inhibit the growth of Gram-positive and Gram-negative bacteria. We present the identification of *Streptomyces* sp-MWS1 through a study of its biological properties.

MATERIAL AND METHODS

Microorganisms and Culture Conditions

Streptomyces sp-MWS1 was isolated from mangrove ecosystem soil in the eastern coast of Surabaya. Isolation and enumeration of actinomycetes colonies performed by soil dilution plate technique using ISP-4 Agar medium. One gram of soil was suspended into test tube containing 9 ml steril phosphat buffer pH 7.3 solution and heated at 50° C for 10 min. Different dilutions, 10^{-3}, 10^{-5}, 10^{-7} of the suspension were plated onto agar medium. The plates were incubated for 7 to 10 days at 28° C. Selected colonies were transfered from mixed culture of the plates onto respective agar plates and incubated onto at 28° C for other 7 days. After incubation, typically pigmented, dry, powdery colonies were selected from mixed plate culture and maintained on fresh medium to get pure cultures (Shirling and Gottlieb, 1966). Plates containing pure cultures were stored at 4° C until further examination. The letter in an isolates name designate what location is came from.

Cultural and Morphological Characterization

Cultural characteristics of *Streptomyces* sp-MWS1 were determined according to the International *Streptomyces*

Project (ISP) (Shirling and Gottlieb, 1966). The general criteria used for *Streptomyces* spp. identifications are morphology, production of diffusible pigments, utilization of various carbon sources and antimicrobial activity (Arment *et al*., 2004; Simon *et al*., 1999). Strain was maintained as spore suspensions in 20% glycerol at –80° C and/or as agar plugs cut from actively growing plates and stored at –80° C.

Morphology was examined by light microscope (Model SE; Nicon) by using the methode of Shirling and Gottlieb (1966). Active purified isolate of actinomycetes were identified up to the species level by comparing their morphology of spore bearing hyphae with entire spore chain and structure of spore chain with the actinomycetes morphologics, as described in Bergey's manual. This was done by using cover-slip method Cross (1989) in which individual cultures were transferred to the base of cover slips burried in ISP-4 medium.

Carbon utilization was determined on plates containing Nutrien Agar medium to which separately-sterilized carbon sources were added to a final concentration 1%. The plates were incubated at 28° C and growth was noticed after 7 days.

Carbohydrate Utilization

Utilization of carbohydrates was investigated with a basal carbon nutrient medium (Pridham and Gottlieb, 1948, Waksman, 1967). Methods and media used for physiological tests were as described by Waksman (1967) Luedemann and Brodsky (1964). The cutural broth was tested for its antimicrobial activity using the cup or the paper disc diffusion methods (Wu, 1984).

Total DNA Isolation

Streptomyces sp-MWS1 was inoculated in 25 ml of the ISP broth medium and incubated at 28° C with agitation speed 200 rpm overnight. After that genomic DNA of the strain was isolated as described by Pospiech and Newmann (1995). The collected pellets were left to dry and dissolved in a suitable volume (100 μl) of TE buffer (100 mM NaCl, 1 mM EDTA, 100 mM tris-HCl, pH 8) or deionized water and storage at –20° C.

16S rDNA sequencing

The 16S rDNA analysis for this isolate was done by extracting DNA using a Qiagen Dneasy Plant Mini Kit according to the standard protocols, and made ready for DNA amplification. The 16S rDNA gene was then amplified by PCR using the Lyticase enzyme and the following pairs of primers : 9F (forwards: 5'-GAGTTTGATCCTGGC CAG-3') and 1541 R (reverse:5'-AAGGAGGTGATCCAGCC-3') (Zhang, *et al*., 2003). The PCR reaction mixture (50 μl) contained PCR beads 2 μl from each primer 9F and 1541 R and 10 µl template DNA up to finale volume 50 μl reached by distilled water. Amplification was performed with an initial denaturation step of 3 min at 94° C and then 35 cycles of (60 sec denaturation at 95° C, 60 sec at 60° C for pimer annealing and 60 sec at 72° C for primer extension) and kept at 72° C for 10 min to complete extension. Electrophoresis of the PCR products was carried out on 1% agarose gel containing ethidium bromide (0.5 μg/ml^{-1}), to ensure that fragment of the the correct size had been amplified and detected by Gel documentation system. Agarose gel analysis of the amplified PCR products showed that the amplified DNA has size of about 1600 bp. PCR product were purified before sequencing using the magnetic water (AMPure) and magnetic plate procedure according to the standard protocol. Purified PCR product was sequenced using Big Dye Terminator V3.1 Cycle Sequencing Ready Reaction Kit Original. Sequencing was performed in a total final of 10 μl. Products were then analized using a DNA sequencer (ABI Prism 3100).

Phylogenetic analysis of Streptomyces

Nukleotide sequences were compared with those maintained in the GenBank database through NCBI Blast. For phylogenetic analysis, sequences were aligned with those of reference strains with the program BioEdit version 7.0.4.01. The phylogenetic tree was derived from distance matrices using neighbor-joining method.

RESULTS AND DISCUSSION

Cultural and Morphological Characteristics

The characterization of *Streptomyces* species is mainly based on the aerial, substrate mycelia color, soluble pigment production, the shape and ornamentation of spore surface. Other additional testes are also considered to ascertain species classification of a new isolate. *Streptomyces* sp-MWS1 grew on ISP-4 media. The abundance and the color of aerial mycelium depended on the medium composition and the age of the culture. The results indicated of *Streptomyces* sp-MWS1 that the white aerial mass color and soluble pigment produced on ISP-4 media. For *Streptomyces* sp-MWS1, it was observed that the aerial hyphae bears spores of no spiral type as shown in Figure 1.

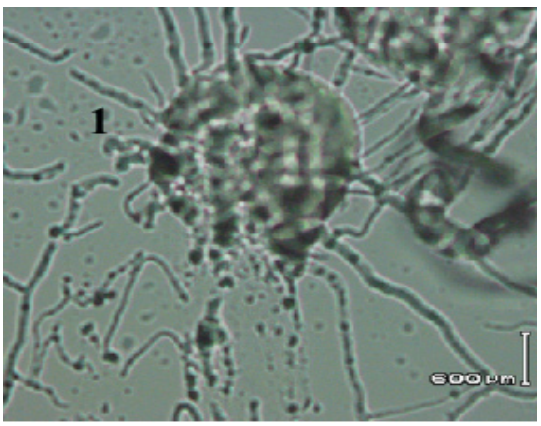

Figure 1. Morphology of spore-bearing aerial hyphae of *Streptomyces* sp-MWS1 after 14 days cultivation on ISP-4 Agar medium at 28° C showing sporechain no spiral (400×)

Carbohydrate Utilization

The utilization of various carbohydrates by *Streptomyces* sp-MWS1 suggests a very narrow pattern of carbon source assimilation. Amylum and glucose were utilized well, and L-arabinose, D-xylose, D-mannitol, Lactose, Saccaharose were not utilized.

Antimicrobial Activities

Streptomyces sp-MWS1 showed a broad antimicrobial spectrum against Gram-positive and Gram-negative bacteria (Table 1). Many such broad-spectrum antibiotics have been produced by *Streptomyces* sp. (Korzybski et al, 1967).

Table 1. Antimicrobial activities of antibiotics *Streptomyces* MWS-1

Indicators[a]	Diameter zone of inhibition (mm)	
	Streptomycin (control)	*Streptomyces* sp.MWS-1
Bacillus subtilis	20,6	17,9
Staphylococcus aureus ATCC 25923	14,4	21,9
Pseudomonas aeruginosa ATCC 7853	16,0	24,9
E. coli ATCC 25922	12,9	22,4
Salmonella typhimurium	13,4	25,7

[a] Bacteria were incubated on Nutrient Agar medium at 37° C for 24 hours

Comparison with the Known Strain

The characteristics of *Streptomyces* sp-MWS1 were compared with published descriptions of various *Streptomyces* species (Shirling and Gottlieb, 1968). In general, the taxonomic classification and identification of *Streptomycetes* is based on morphological and physiological test, thus molecular methods (16S rDNA gene sequence analysis) could represent an improvement (Rintala *et al*., 2001). Nowdays, the detection and clasification of *Streptomyces* are now most commonly performed by molecular approaches based on selective PCR amplification (Locatelli *et al*., 2002). Many approaches have been tried to aid in the classification of *Streptomyces* isolates to the genus, species, and strain levels. Genetic methods are more rapid and convenient than classification methods based on phenotypic characteristics (Park *et al*., 2006).

PCR Amplification

Experimental analysis of the PCR amplification performance the forward primer 9F in conjunction with the reverse primer 1541R was conducted under the reaction conditions described. The primer pairs 9F/1541R amplified a fragment of the expected size from DNA isolated from positive control strain *Streptomyces griseus* ATCC 10971. The specificity of the primers was further examined by PCR amplification using DNA isolated from *Streptomyces* sp-MWS1. The specificity of the PCR is affected by multiple factors, such as the primers, the properties of the gene regions flanking the target site, the annealing temperature in the PCR reaction and the reaction conditions.

Phylogenetic Taxonomy of Streptomyces

The alignment of the nucleotide sequences (1430 bp) of *Streptomyces* sp.MWS-1 was done through matching with the 16S rDNA reported genes sequences in the gene bank. The database of NCBI Blast available at (www.nvbi.nlm.nig.gov) was used to compare the isolated strains with sequence of the reference species of *Streptomyces* contained in genomic database bank. The results exhibited similarity level 98% with 17 strains from the uncultured known *Streptomyces* species. The 16S rDNA nucleotide sequence of *Streptomyce* sp-MWS1 consisted of 1430 bp and the GC-content was 70%. These results were in accordance to many authors who mentioned that, the GC-content of *Streptomyces* DNA is 60–78%. (Anderson ad Wellington. 2001).

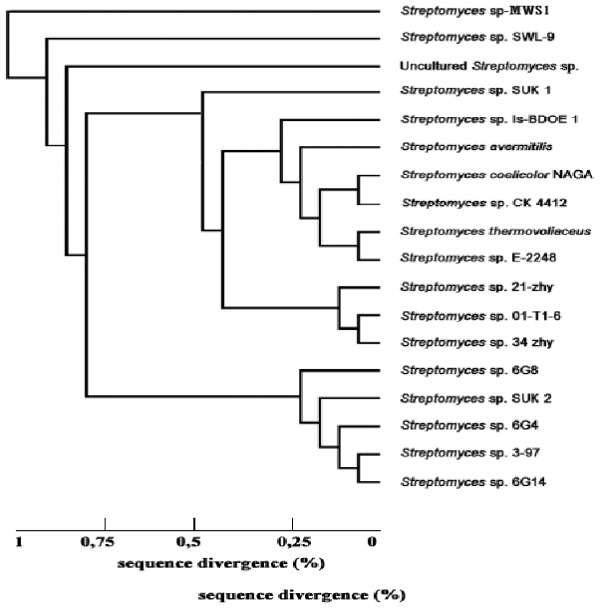

Figure 2. The phylogenetic position of *Streptomyces* sp-MWS1 among neighboring method showing 16S rDNA tree of the phylogenic similarity (%) comparing with the sequences of other known *Streptomyces* species

Modern Streptomyces identification systems are based on the 16S rDNA sequence data, which have provided invaluable information about Streptomyces systematic and then have been used to identify several newly isolated Streptomyces (Hyo *et al*., 2006). For data analysis, the phylogenetic tree in Fig. 2 was derived from distance matrices using neighbor-joining method (Saitou and Nei, 1987). The majority of sequences clustered into groups in the phylogenetic analysis. However, the results indicate the presence of several different type of Streptomyces 16SrDNA sequences in buildings, suggesting higher diversity with several species. These strains together with

sequences from uncultured known Streptomyces species which were differed for them in the some morphological characters; carbon utilization and active secondary metabolites production, where the different detection limits of the methods make presence/absence comparisons difficult (Suutari *et al*., 2002).

CONCLUSION

Streptomyces sp-MWS1 producing antibacterial compounds isolated from the soil of *mangrove* ecosystem in the eastern coast of Surabaya indicated similarity binary 98% with *Sreptomyces* species.

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