PHYLOGENETIC ANALYSIS AND ANTI MICROBIAL ACTIVITY OF Streptomyces spp. THAT ISOLATED FROM COMPOST SOIL IN SURABAYA INDONESIA ON THE BASIS OF 16S Rrna GENE

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PHYLOGENETIC ANALYSIS AND ANTI MICROBIAL ACTIVITY OF *Streptomyces* spp. THAT ISOLATED FROM COMPOST SOIL IN SURABAYA INDONESIA ON THE BASIS OF 16S Rrna GENE

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ABSTRAK

Delapan isolat Streptomyces sp. dapat diisolasi dari tanah kompos di Surabaya, Indonesia. Hasil uji biokimia dan morfologi menunjukkan bahwa 8 isolat tersebut merupakan isolat baru. Analisis filogenetik dilakukan berdasarkan sekuen gen 16S rRNA. Sekuen nukleotida dari gen 16S rRNA Streptomyces sp. isolat tanah kompos Surabaya dianalisis dan dibandingkan dengan sekuen gen 16S rRNA dari pustaka. Hasil analisis Streptomyces sp. isolat tanah kompos Surabaya berdasarkan gen 16S rRNA menunjukkan Streptomyces spesies baru. Hasil diagram pohon filogenetik menunjukkan bahwa Streptomyces Sp-D, Sp-Ep, Sp-G dan Sp-I yang ditemukan pada tanah rumah kompos Bratang Surabaya ternyata merupakan isolat baru. Streptomyces Sp-Ep merupakan Streptomyces jenis baru yang masih berkerabat dekat dengan Streptomyces indonesiasis dan Streptomyces nashvillensis. Streptomyces Sp-Ea merupakan Streptomyces olivoreticuli yang masih berkerabat dengan Streptomyces yogyakartensis. Streptomyces Sp-F adalah Streptomyces levis strain NRRL B-24299. Streptomyces Sp-C adalah Streptomyces filamentosus. Streptomyces Sp-D merupakan Streptomyces jenis baru yang masih berkerabat dekat dengan Streptomyces javensis dan Streptomyces roseus. Streptomyces Sp-G merupakan Streptomyces jenis baru yang masih berkerabat dekat dengan Streptomyces roseoviridis strain NBRC 12911 dan Streptomyces thermocarboxydovorans strain AT52. Streptomyces Sp-I merupakan Streptomyces jenis baru yang masih berkerabat dekat dengan Streptomyces cangkringensis dan Streptomyces asiaticus. Streptomyces Sp-A adalah Streptomyces laurentii strain : LMG 19959. ***(FMI 2017;53:204-208)***

Kata kunci*: analisis filogenetik; antimikrobial; streptomises; tanah kompos; 16S rRNA*

ABSTRACT

Eight isolates of Streptomyces sp. can be isolated from compost soil in Surabaya, Indonesia. The results morphological and biochemical tests showed that the 8 isolates were new. Phylogenetic analysis was performed on the sequence of 16S rRNA gene. Nucleotide sequences of 16S rRNA gene Streptomyces sp. the compost soil isolates of Surabaya were analyzed and compared with the 16S rRNA gene sequence from the literature. Streptomyces sp. the compost soil of Surabaya based on the 16S rRNA gene showed the new species of Streptomyces. The result of phylogenetic tree diagram showed that Streptomyces Sp-D, Sp-Ep, Sp-G and Sp-I found in Bratang Surabaya compost house land were new isolates. Streptomyces Sp-Ep was a new type of Streptomyces closely related to Streptomyces indonesiasis and Streptomyces nashvillensis. Streptomyces Sp-Ea was Streptomyces olivoreticuli which was still related to Streptomyces yogyakartensis. Streptomyces Sp-F was Streptomyces levis strain NRRL B-24299. Streptomyces Sp-C was Streptomyces filamentosus. Streptomyces Sp-D was a new type of Streptomyces closely related to Streptomyces javensis and Streptomyces roseus. Streptomyces Sp-G was a new type of Streptomyces closely related to Streptomyces roseoviridis strain NBRC 12911 and Streptomyces thermocarboxydovorans strain AT52. Streptomyces Sp-I was a new streptomyces that was still closely related to Streptomyces cangkringensis and Streptomyces asiaticus. Streptomyces Sp-A was Streptomyces laurentii strain: LMG 19959. ***(FMI 2017;53:204-208)***

Keywords*: Phylogenetic analysis; antimicrobial; streptomyces; compost soil; 16S rRNA*

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INTRODUCTION

Streptomyces is the biggest genus of actinomycetes that consist of 50% of the total population of actinomycetes in soil. In addition, Streptomyces spp.contains a larger number and also a greater number of variations of its new antibiotics than other genus of actinomycetes, it shows that compounds of streptomyces species number or strains can produce new antibiotics in nature actinomycetes genus. Therefore, there are more than 6000 antibiotics have been found in various species of Streptomyces where many of these substances are used commercially as anticancers, anti-infection (antibiotics, antifungals, and antiparasites), and immuno suppressant agents (Champness 2000, Xiao et al 2002, Barakate et al.2002, Iznaga et al 2004., Iznaga et al 2004) new antibiotics in nature. One spesies of Streptomyces is capable in producing more than 2-3

natural-derived antibiotics. Thousands of many substances of Streptomyces have been characterized and isolated, which most of them have been utilized in the pharmacy industry as drugs for any kind of diseases in humans, veterinary, and agriculture.

Common classification methods that used for the characterization of species from the genus Streptomyces mainly use the phenotypic and morphological to characteristic of the organism. It has been reported from many researchers that did molecular biological method, such as 16S rRNA gene sequencing and BOX-PCR fingerprinting that has had an increase in streptomyces taxonomy (Kim & Goodfellow 2002, Kim et al 2004, Saintpierre et al 2003).

In this research, we determined 16S rRNA gene sequences to classify *Streptomyces* spp. and Antimicrobial Activity isolates from compost soil that was in Surabaya, Indonesia. Therefore, a study on 16S rRNA gene sequence of antibiotic-producing *Streptomyces* spp. isolated from compost soil in Surabaya, Indonesia should be conduct-ed. The objective of this study was to find new types of Streptomyces from compost soil in Surabaya, Indonesia which are immune, specific, and able to produce anti-biotics as alternative drugs.

MATERIALS AND METHODS

Microorganisms and culture conditions

Eight Streptomyces spp have been isolated from compost soil obtained from Surabaya, Indonesia. Streptomyces were isolated using ISP-4 agar, and then the plates those were used, they were incubated at 28°C for 4 days. The isolated Streptomyces spp that have the potential to generate bioactive substances are screened. The most potent strain of its producer then it would be selected and identified. The cultures were maintained on ISP-4 agar. The strain inoculated in the agar medium was incubated at 28°C for 4 days and saved at 4°C until further use.

Test microorganisms

Pseudomonas aeroginosa ATCC 27853, Candida albicans ATCC 1023, Eschericia coli ATCC 2593,, Staphylococcus aureus ATCC 25923, and Bacillus subtillis ATCC 6633 were utilized to identify activity of anti-microbial of isolated strains of Streptomyces.

Screening of Actinomycetes for antimicrobial activity

The screening method consist of measures, namely primary screening and secondary screening. In primary screening, the pure isolates for the antimicrobial activity were conducted by method of perpendicular streak on nutrient agar (NA). The organisms to test namely using Pseudomonas aeroginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Eschericia coli ATCC 2593, Candida albicans ATCC 1023, and Bacillus subtillis ATCC 6633. In secondary screening was conducted which utilized the method of agar well against the standard test organisms Pseudomonas aeroginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Eschericia coli ATCC 2593, Candida albicans ATCC 1023, and Bacillus subtillis ATCC 6633 (Dhanasekaran et al 2005).

Primary screening in antimicrobial activity

The primary screening in antimicrobial activity was conducted by perpendicular streak method. In this method, the colonies of bacterial were streaked on center of nutrient agar plates as a linear culture and it's incubated for 4 days at 28°C. and then after 4 days of the microorganisms test that were inoculated in perpendicullarly with the linear culture and incubated for 48 h at 37°C. So, it's obtained isolates of antimicrobial manufacturers that inhibit the growth of microorganisms and these to be selected for experiment (Dhanasekaran et al 2005)

Secondary screening

Confirming the results of the main screening was very potent carried out secondary screening actinomycetes. It can be done in a loopful of Streptomyces spp. from the cultural age of 4-days inoculated into 250 ml of Erlenmeyer pumpkin, it contains 100 ml of isp-4 media liquid. The thermos were incubated on a rotary shaker (200 rpm) for 4 days at 28 °C, then, Two liters of total volume are filtered through Whatman No.1 filter paper, and followed by centrifugation at 50.00 r.p.m for 20 minutes at 10°C. Cultural supernatants are prepared and used for antimicrobial analysis in minimum inhibition concentrations. Secondary screening is carried out by method so that well diffusion against Pseudomonas aeroginose ATCC 27853, Staphylococcus aureus ATCC 25923, Eschericia ATCC 2593, Candida albicans ATCC 1023, and Bacillus subtillis ATCC 6633.

Molecular assays

There are many molekuer and bioinformatics tests conducted to identify isolated strains of Streptomyces as well as the isolation of streptomyces species that are among them.

Genomic DNA extraction

Genomic DNA extraction was done according to the protocol that was described by Corbin method with modification. Briefly, one colony was cultured in 50 ml liquid medium of ISP4 in shaker for 18 - 24 hours

and has been incubated at 28°C. Furthermore, the culture was centrifuged at 5000 rpm for 3 minutes and supernatant was removed. In the liquid nitrogen, the cell of bacterial were pulverized, those were suspended in a solution I, those were containing 10 mM Tris (pH: 7.4), 0.5% SDS, 1 mM EDTA & 0.1 mg/ml of protein-ase K, and lysed by incubation for 1 hour at 37°C , and then the solutions II contains 0.8 M NaCl& 1% CTAB that was added by the lysates, and incubated for 20 min at 65°C & it was extracted with equal volume of chlorroform isoamyllalcohol (24:1). In the aqueous phase with 0.6 volume of isopropanol, Nucleic acid was precipitated that purified with ethanol 70% (Corbin et al 2001).

Amplification and sequencing of 16S rRNA gene

PCR amplification using two primers used to conduct the 16S rRNA gene of the local Streptomyces strain, StrepF; 5.- AGAGTTTGATCCTGKGTCAG -3.and Strep R; 5.AAGGAGGTGATCCAKKGKGA -3, (Davelos et al 2004). In PCR there are several mixtures consisting of 30 pmol in each primer, 100 ng of chromosomal DNA, 200 µM of dNTPs and 2.5 unit of Taq polymermeracy, in a 50 µl polymerase buffer. PCR amplification is carried out for 1 minute at a temperature of 94 °C which is as the primary denaturation temperature, and then for 1 minute 94ºC, this is referred to as the denaturation temperature, whereas at a temperature of 57ºC for 60 seconds referred to as an anil temperature, while the temperature is 72 °C for 60 seconds as an extension time, it is carried out in 35 cycles, and 72ºC for 5 minutes as the last extension time. (Fermentas Co.) as a size marker. The QIA quick PCR purification reagents was used to purifi remaining mixture (Qiagen, USA). The gene of 16S rRNA was sequenced on both strands through method of the dideoxy chain termination. The PCR product for gene of 16S rRNA (1.5 kb) sequencing was obtained by using a Terminator Cycle Sequencing kit (ABI Prism 310 Genetic Analyzer, Applied Biosystem,USA).

Sequence similarities and phylogenetic analysis

Blast program (http://ncbi.nlm.nih.gov/blst) is used to measure DNA similarity levels, then MEGA 5.0 software, it is used to evaluate multiple sequences of molecular alignment and phylogeny (Tamura et al 2011). While TREE VIEW is used to display phylogenetic trees.

Identification of Streptomyces isolate

The determination was done according to the recommendation of international Key's viz and Numeric al taxonomy of Streptomyces species program. On the basis of the 16 S rRNA.

RESULTS

The data results showed that differences of morphological from eight streptomyces which were isolated from compost soil in Surabaya, Indonesia. The results of morphological observations are shown in Table 1.

DISCUSSION

In knowing, the basic local alignment search tool (BLAST) on the NCBI (http://ncbi.nlm.nih.gov) website (Shayne et al 2003) is used to determine the simillarity of the rRNA 16S gene sequence compared to the GenBank database sequence in a public database. Where was in this study the high-like rRNA sequence 16S gene would be identified in the study, where it would be taken and used in the construction of phylogenetic trees.

Table 1. Characteristics of *Streptomyces* spp.

Isolate	Characteristics		
	Colony color	Colony form	Colony surface
Sp-A	white	round	Smooth, flat
Sp-C	creamy	Round, small	Smooth, convex
Sp-D	gray	round	Convex
Sp-Ea	gray	round	Flat
Sp-Ep	white	round	convex, shiny
Sp-F	pink	Round like a flower	uneven
Sp-G	pink	round	flat
Sp-I	gray	round	Smooth, shiny

Table 2. Antimicrobial activity of strains against test organisms

Isolate	Inhibition zone (mm)				
	E. coli ATCC 2593	*P. aeroginosa* ATCC 27853	*B. subtillis* ATCC 6633	*S. aureus* ATCC 25923	*C. albicans* ATCC 10231
Sp-A	13.7	13.7	12.7	12.7	14.7
Sp-C	-	-	-	6.1	0
Sp-D	-	7.1	-	-	7.9
Sp-Ea	-	9.1	-	-	7.8
Sp-Ep	-	-	-	-	5.5
Sp-F	13.4	14.8	12.9	11.6	14.7
Sp-G	13.9	12	11.1	11.6	14.8
Sp-I	-	5.9	6.9	5.7	9.3

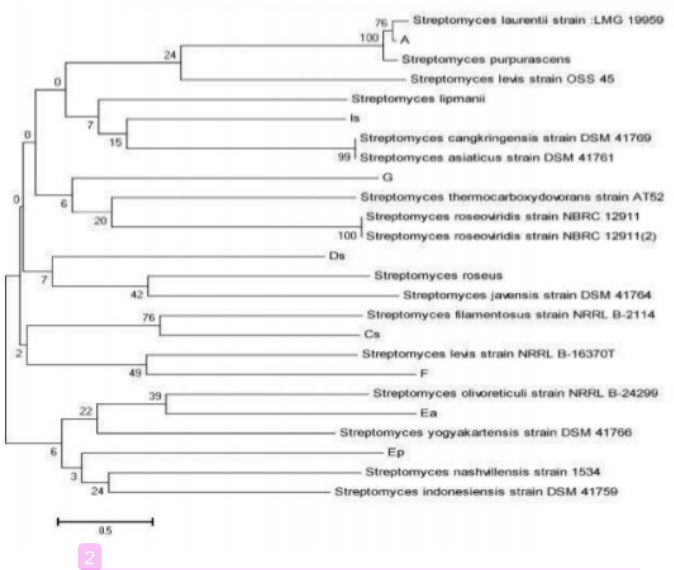

Fig. 1. phylogenetic tree based on 16S rRNA gene sequences

Phylogenetic analysis of Sp-A, Sp-C, Sp-D, Sp-Ea, Sp-Ep, Sp-F, Sp-G, Sp-I strains showed that they were belonged to the Streptomyces genus comparing with several of the type strains validly described and local isolate of Indonesian was selected as an outgroup (Figs. 1). They were closed to the strains of *S. laurentii* LMG 19959, *S. purpurascens*, *S. lewis* OSS 45, *S. lipmanii*, *S. cangkringensis* DSM 41761, *S. thermocarboxydovorans* AT 52, *S. roseoviridis* NBRC 12911, *S. Roseus*, *S. javensis* DSM 41764, *S. filamentosus* NRRLB-2114, *S. lewis* NRRL B-16370T, *S. olivoreticuli* NRRL B-24299, *S. yogyakartensis* DSM 41766, *S.nashwillensis* 1555534 and *S. indonesiensis* DSM41759.

The results showed that Streptomyces Sp-Ep clustered closely with *Streptomyces indonesiasis*and *Streptomyces nashvillensis* which was a new type. *Streptomyces* sp-Ea should be identified as *Streptomyces olivoreticuli* that was related to the *Streptomyces yogyakartensis*. *Streptomyces* sp-F should be identified *Streptomyces levis* strain NRRL B-24 299. *Streptomyces* sp-C shouldbe identified *Streptomyces filamentosus*. *Streptomyces* sp-D was a new type that was closely related to *Strepto-myces javensis* and *Streptomyces roseus*. *Streptomyces* sp-G was a new type that was closely related to *Streptomyces roseoviridis* strain NBRC12911 and *Streptomyces thermocarboxydovorans* strain AT52. *Streptomyces* sp-I was a new type that was still closelyrelated to *Streptomyces cangkringensis* and *Streptomy-*

ces asiaticus. *Streptomyces* sp-A should be identified *Streptomyces laurentii* strain LMG 19959. Basically, allthe samples are the same genus Streptomyces with different types. Phylogenetic tree *Streptomyces* spp. can be seen in Figure 1.

CONCLUSION

The analysis of *Streptomyces* spp from compost soil taken in Surabaya based on the 16S rRNA gene can be revealed the existence of a new species of *Streptomyces*. where is in the phylogenetic tree diagram, it is shown that Streptomyces Sp-D, Sp-Ep, Sp-G and Sp-I found in the soil of Bratang Surabaya compost house is a new isolate.

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