

PHYLOGENETIC ANALYSIS AND ANTI MICROBIAL ACTIVITY OF *Streptomyces* spp. 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* yogyakartaensis. *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; antimikrobal; streptomises; tanah kompos; 16S rRNA

ABSTRACT

Eight isolates of *Streptomyces* sp. can be isolated from compost soil in Surabaya, Indonesia. The results of biochemical and morphological 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* yogyakartaensis. *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 streptomycetes 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; streptomycetes; compost soil; 16S rRNA

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INTRODUCTION

Streptomyces is largest genus of actinomycetes and constitute 50% of the total population of soil actinomycetes. Moreover, *Streptomyces* spp. provides a larger number and wider variety of new antibiotics than any other actinomycetes genera, suggesting that substantial numbers of *Streptomyces* species or strains with novel

antibiotic productivity exist in nature. Furthermore, over 6000 antibiotics are obtained by different species of *Streptomyces* and many of these compounds are commercially available as anti-infective (antibiotics, antifungal and antiparasitic), anticancer or immunosuppressant agents (Champness 2000, Xiao et al 2002, Barakate et al 2002, Iznaga et al 2004). One species of *Streptomyces* is capable in producing more than 2-3

natural-derived antibiotics. Tens of thousands of such compounds have been isolated and characterized, most of them have been developed into drugs for treatment of widerange of diseases in human, veterinary and agriculture.

Conventional classification methods for the identification of species within the genus *Streptomyces* have mainly relied on the morphological and phenotypic characteristics of the organisms. During the last decade, molecular biological methods such as 16S rRNA gene sequencing and BOX-PCR fingerprinting have had an increasing impact on streptomycete taxonomy (Kim & Goodfellow 2002, Kim et al 2004, Saintpierre et al 2003).

In this study, we determined 16S rRNA gene sequences to classify *Streptomyces* spp. and Antimicrobial Activity isolates from compost soil 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 conducted. 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 antibiotics as alternative drugs.

MATERIALS AND METHODS

Microorganisms and culture conditions

Eight *Streptomyces* spp could be isolated from soil sample collected from compost soil Surabaya, Indonesia. *Streptomyces* were isolated using ISP-4 agar. The plates were incubated for 4 days at 28°C. The isolated *Streptomyces* spp were then screened since they have the potential to generate bioactive compounds. The most potent producer strains was then selected and identified. The cultures were maintained on ISP-4 agar. The inoculated agar medium was incubated for 4 days at 28°C, then maintained at 4°C until further use.

Test microorganisms

Pseudomonas aeruginosa ATCC 27853, *Eschericia coli* ATCC 2593, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231 were used to determine anti-microbial activity of isolated *Streptomyces* strains.

Screening of Actinomycetes for antimicrobial activity

The screening method consisted of steps as primary screening and secondary screening. In primary screening the antimicrobial activity of pure isolates were

determined by perpendicular streak method on nutrient agar (NA). The test organisms were *Pseudomonas aeruginosa* ATCC 27853, *Eschericia coli* ATCC 2593, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231. Secondary screening was done through the agar well method against the standard test organisms *Pseudomonas aeruginosa* ATCC 27853, *Eschericia coli* ATCC 2593, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231 (Dhanasekaran et al 2005).

Primary screening of the antimicrobial activity

The primary antimicrobial activity was done by perpendicular streak method. In this method bacterial colonies were streaked on center of nutrient agar plates as a linear culture and incubated at 28°C for 4 days. After 4 days, the test microorganisms were inoculated perpendicularly to the linear cultures and incubated at 37°C for 48 h. The antimicrobial producer isolates inhibited the growth of test microorganisms and were selected for further experiments (Dhanasekaran et al 2005).

Secondary screening

Secondary screening of potent actinomycetes was done to confirm the results of primary screening. A loopful of the *Streptomyces* spp. from the 4-day culture age was inoculated into 250 ml Erlenmeyer flasks containing 100 ml of liquid ISP-4 medium. The flasks were incubated on a rotary shaker (200 rpm) at 28°C for 4 days. Two-liter of total volume was filtered through Whatman No.1 filter paper, followed by centrifugation at 5000 r.p.m at 10°C for 20 minutes. Culture supernatants was prepared and used for antimicrobial analyses, minimum inhibitory concentration. Secondary screening was performed by the agar well diffusion method against *Pseudomonas aeruginosa* ATCC 27853, *Eschericia coli* ATCC 2593, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923 and *Candida albicans* ATCC 10231.

Molecular assays

In order to identify the strains of the isolated *Streptomyces* and isolation of *Streptomyces* species among them, several molecular and bioinformatical assays were performed.

Genomic DNA extraction

Genomic DNA extraction was conducted according to the protocol described by Corbin method with some modifications. Briefly, a single colony was cultured in 50 ml liquid ISP4 medium for 18 - 24 hours in shaker

incubator at 28°C. Then the culture was centrifuged for 3 minutes at 5000 rpm and supernatant was discarded. The bacterial cells were pulverized in liquid nitrogen, suspended in a solution I containing 10 mM Tris (pH: 7.4), 1 mM EDTA, 0.5% SDS and 0.1 mg/ml of proteinase K, and lysed by incubation at 37°C for 1 hour, then the solution II containing 0.8 M NaCl and 1% CTAB was added to the lysates, and incubated at 65°C for 20 min and extracted with equal volume of chloroform isoamylalcohol (24:1). Nucleic acid was precipitated from the aqueous phase with 0.6 volume of isopropanol and finally purified using ethanol 70% (Corbin et al 2001).

Amplification and sequencing of 16S rRNA gene

PCR amplification of the 16S rRNA gene of the local Streptomyces strain was conducted using two primers, StrepF; 5.- AGAGTTTGATCCTGKGCAG -3.and Strep R; 5.AAGGAGGTGATCCA KKGKGA -3, (Davelos et al 2004). The PCR mixture consisted of 30 pmol of each primer, 100 ng of chromosomal DNA, 200 µM dNTPs and 2.5 units of Taq polymerase, in 50 µl of polymerase buffer. The PCR amplification was achieved at 94°C for 1 minute as primary denaturing temperature, then 94°C for 1 minute as denaturing temperature, 57°C for 60 seconds as annealing temperature, 72°C for 60 seconds as extension time, in 35 cycles, and 72°C for 5 minutes as final extension time. The PCR, reaction mixture was then analyzed via agarose gel electrophoresis and marked using 1 kb DNA ladder (Fermentas Co.) as the size marker. The remaining mixture was purified using QIA quick PCR purification reagents (Qiagen, USA). The 16S rRNA gene was sequenced on both strands via the dideoxy chain termination method. The 16S rRNA gene (1.5 kb) sequence of the PCR product was acquired using a Terminator Cycle Sequencing kit (ABI Prism 310 Genetic Analyzer, Applied Biosystems, USA).

Sequence similarities and phylogenetic analysis

The BLAST program (www.ncbi.nlm.nih.gov/blst) was used in order to assess the degree of DNA similarity. Multiple sequence alignment and molecular phylogeny were evaluated using MEGA 5.0 software (Tamura et al 2011). The phylogenetic tree was displayed using TREE VIEW program.

Identification of Streptomyces isolate

The identification was performed basically according to the recommended international Key’s viz. and Numerical taxonomy of Streptomyces species program. On the basis of the 16 S rRNA.

RESULTS

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

DISCUSSION

The 16S rRNA gene sequences were compared to sequences in the public database using basic local alignment search tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov/) in order to determine similarity between sequences in the GenBank database (Shayne et al 2003). The 16S rRNA gene sequences with high similarities to those determined in the study were retrieved and used in the construction of phylogenetic tree.

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)				
	<i>E. coli</i> ATCC 2593	<i>P. aeruginosa</i> ATCC 27853	<i>B. subtilis</i> ATCC 6633	<i>S. aureus</i> ATCC 25923	<i>C. albicans</i> 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

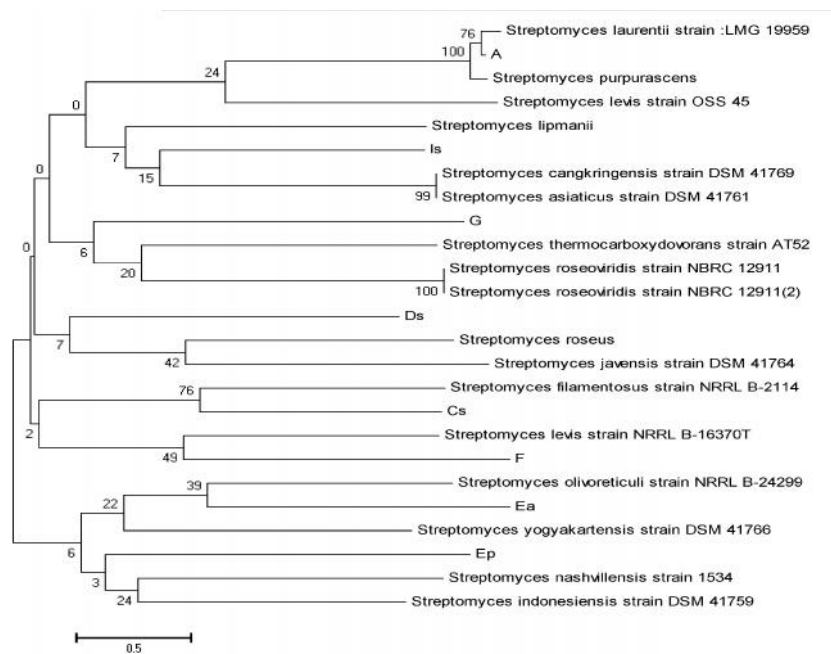


Fig. 1. phylogenetic tree based on 16S rRNA gene sequences

Phylogenetic analysis of Sp-A, Sp-C, Sp-D, Sp-Ea, Sp-Ep, Sp-F, Sp-G, Sp-I strains revealed that they were belonged to the genus *Streptomyces* comparing with some of the type strains validly described and Indonesian local isolates 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. kangkringensis* DSM 41761, *S. thermocarboxydovorans* AT 52, *S. roseoviridis* NBRC 12911, *S. Roseus*, *S. javensis* DSM 41764, *S. filamentosus* NRRL B-2114, *S. lewis* NRRL B-16370T, *S. olivoreticuli* NRRL B-24299, *S. yogyakartaensis* DSM 41766, *S. nashvillensis* 1555534 and *S. indonesiensis* DSM 41759.

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 yogyakartaensis*. *Streptomyces* sp-F should be identified *Streptomyces levis* strain NRRL B-24 299. *Streptomyces* sp-C should be identified *Streptomyces filamentosus*. *Streptomyces* sp-D was a new type that was closely related to *Streptomyces 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 closely related to *Streptomyces kangkringensis* and *Streptomy-*

ces asiaticus. *Streptomyces* sp-A should be identified *Streptomyces laurentii* strain LMG 19959. Basically, all the 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 of Surabaya based on the 16S rRNA gene revealed new *Streptomyces* species. Phylogenetic tree diagram showed that *Streptomyces* Sp-D, Sp-Ep, Sp-G and Sp-I found in the soil from Bratang compost house Surabaya were new isolates.

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