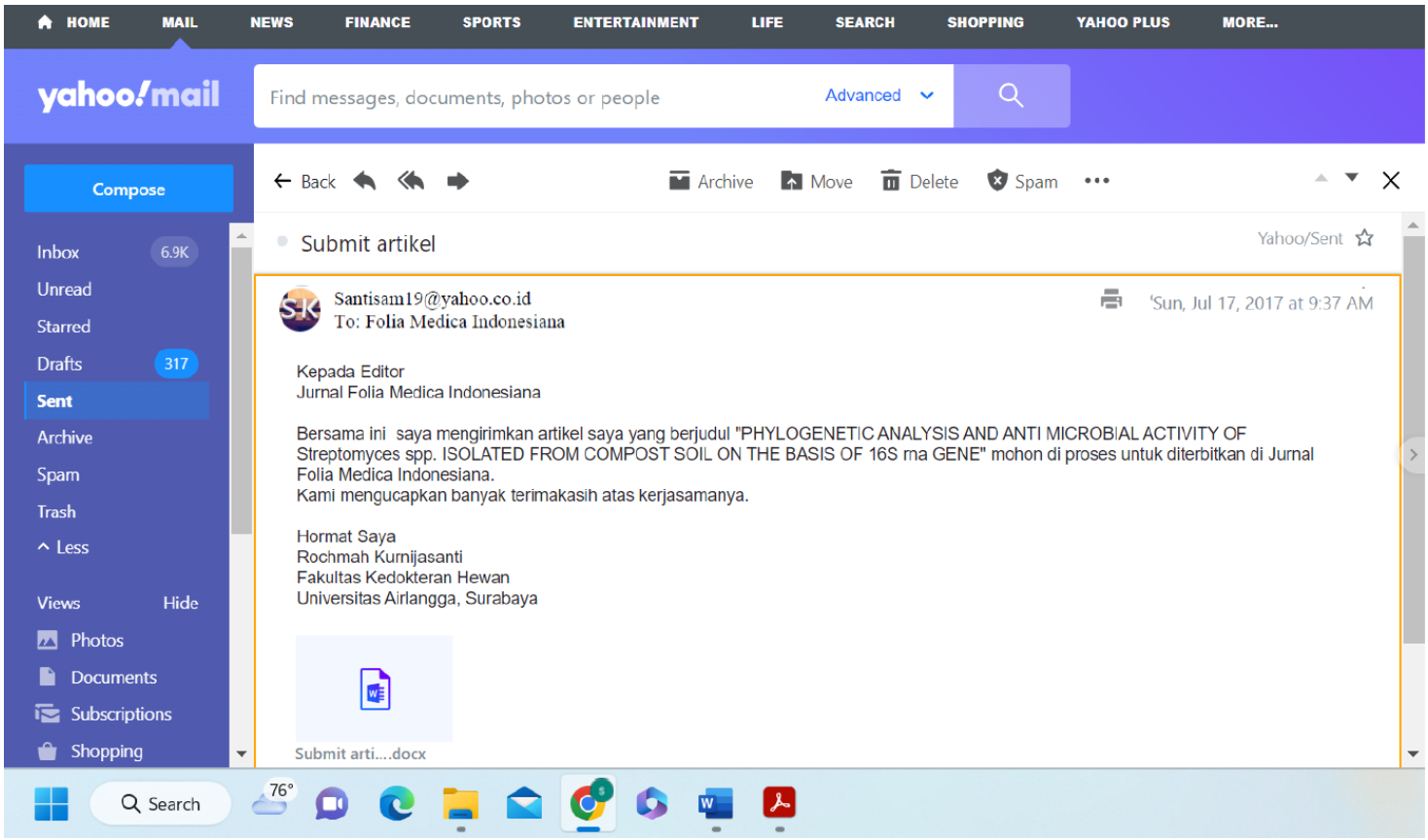
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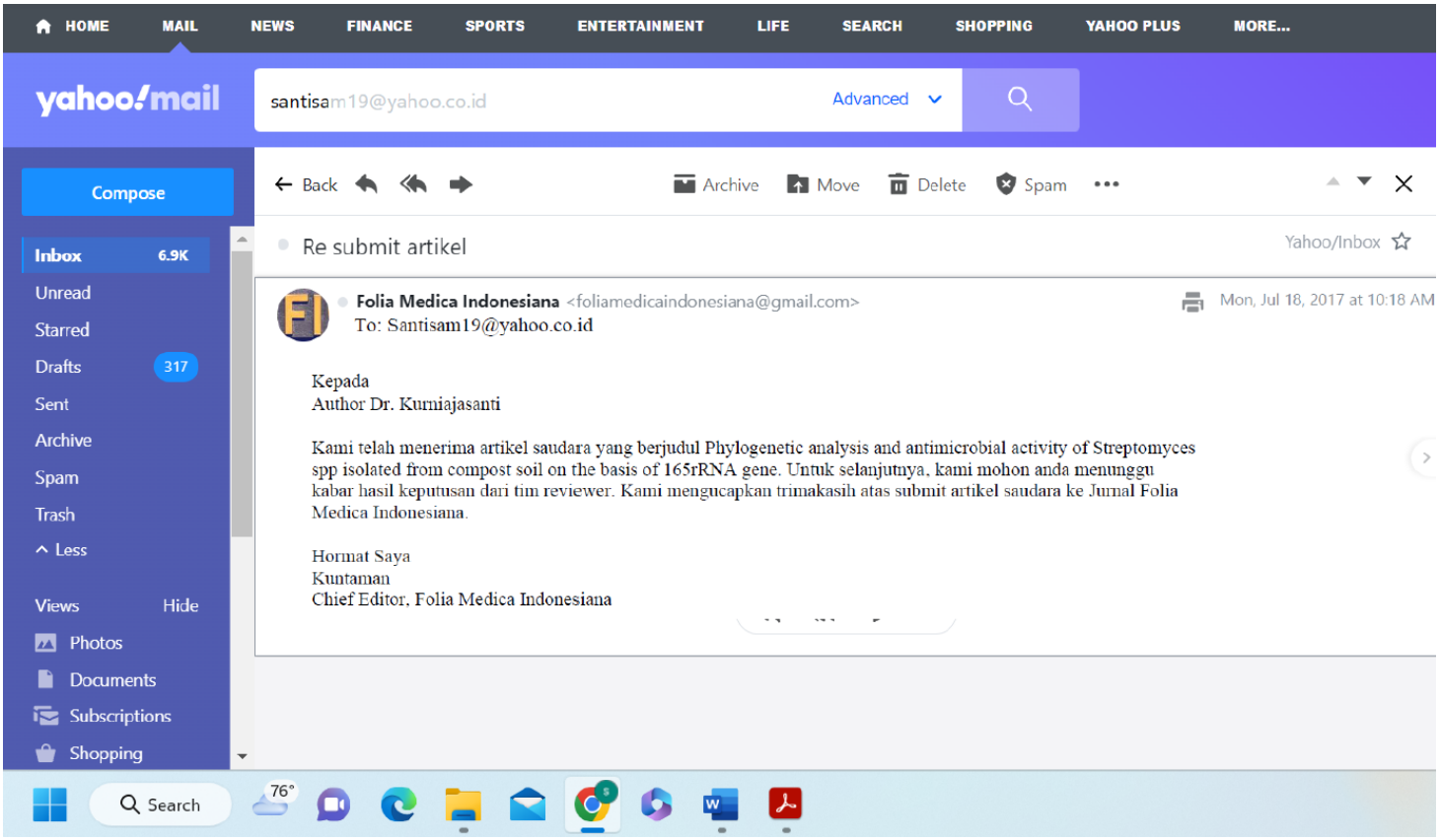
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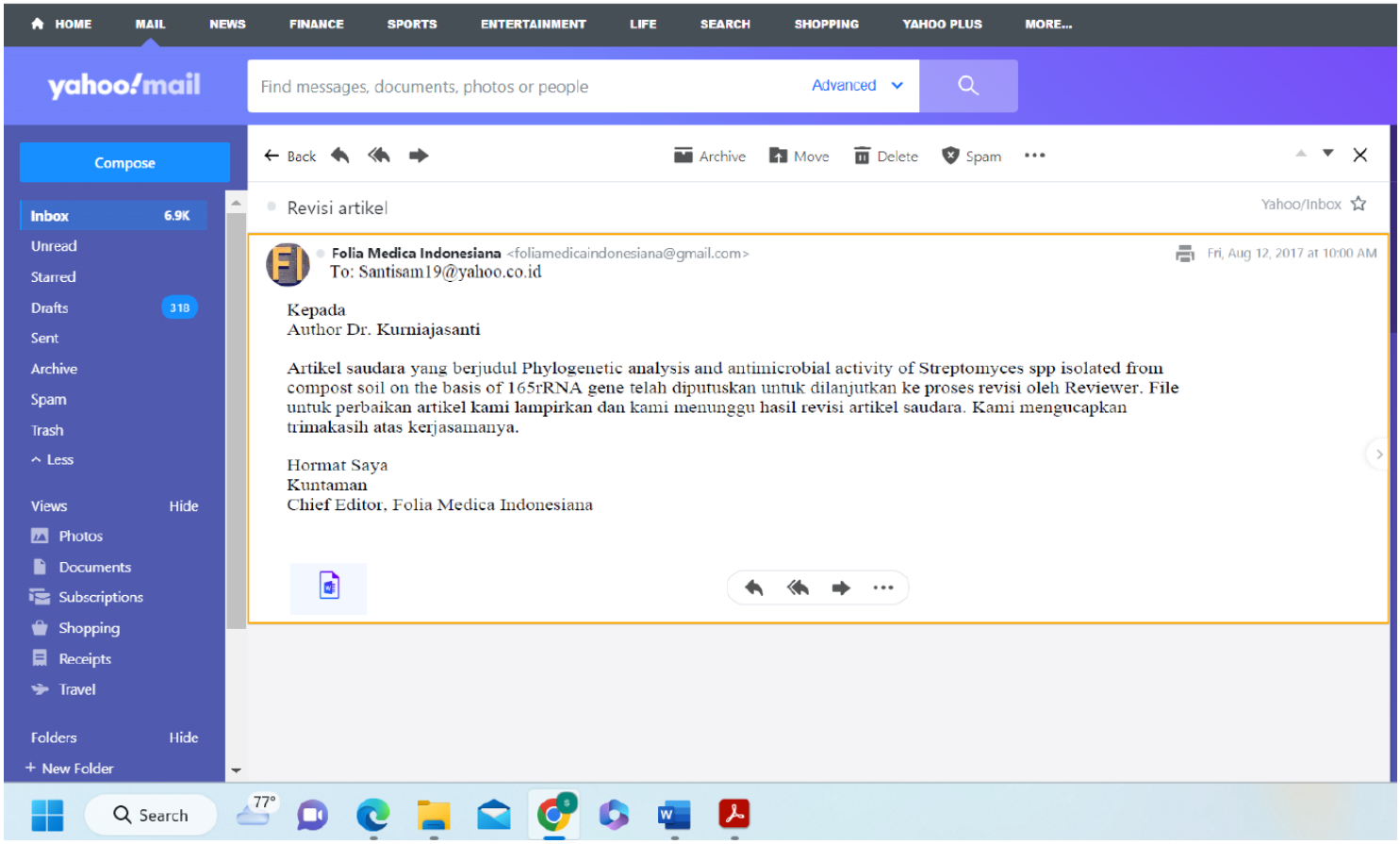

PHYLOGENETIC ANALYSIS AND ANTI MICROBIAL ACTIVITY OF Streptomyces spp. ISOLATED FROM COMPOST SOIL ON THE BASIS OF 16S rna GENE

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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 yang masih berkerabat dekat dengan Streptomyces roseoviridis strain NBRC 12911 dan Streptomyces thermocarboxydovorans strain AT52. Streptomyces Sp-I merupakan Streptomyces jenis yang masih berkerabat dekat dengan Streptomyces cangkringensis dan Streptomyces asiaticus. Streptomyces Sp-A adalah Streptomyces laurentii strain : LMG 19959.

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***Kata kunci**: anclisis filogenetik; antimikrobial; 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 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.

***Keywords**: Phylogenetic analysis; streptomyces; 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 spesies 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. 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 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 the 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 aeroginosa ATCC 27853, *Eschericia coli* ATCC 2593, *Bacillus subtillis* 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 aeroginosa* ATCC 27853, *Eschericia coli* ATCC 2593, *Bacillus subtillis* 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 aeroginosa* ATCC 27853, *Eschericia coli* ATCC 2593, *Bacillus subtillis* 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 alinear 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 throughWhatman No.1 filter paper, followed by centrifugationat 5000 r.p.m at 10°C for 20 minutes. Culture super- natants was prepared and used for antimicrobial analyses, minimum inhibitory concentration. Secondary screening was performed by the agar well diffusion method against *Pseudomonas aeroginosa* ATCC 27853,*Eschericia coli* ATCC 2593, *Bacillus subtillis* 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 with somemodifications. 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.- AGAGTTTGATCCTGKGTCAG -3.and Strep R; 5.AAGGAGGTGATCCAKKGKGA -3. 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 wasachieved at 94°C for 1 minute as primary denaturing temperature, then 94°C for 1 minute as denaturing temp- erature, 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).

Genomic DNA extraction

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

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. The 16S rRNA gene sequences with high similarities to those determined in the study were retrieved and used in the construction of phylogenetic tree.

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Table 1. Characteristics of *Streptomyces*.

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

Commented [ws8]: Harap dirubah *Streptomyces spp*

Table 2. Antimicrobial activity of strains

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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

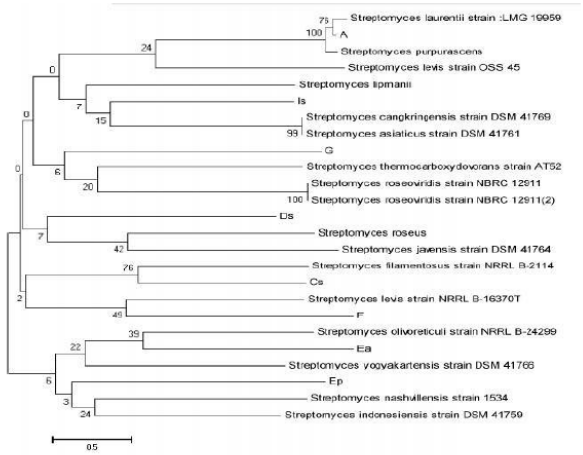

Fig. 1. phylogenetic tree based on 16S rRNA gene sequences

Phylogenetic analysis of Sp-A, Sp-C, Sp-D, Sp-Ea, Sp-Ep, Sp-F, Sp-G, Sp-I strains 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. cangkringensis* 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. yogyakartensis* DSM 41766, *S. nashwillensis* 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 yogyakartensis*. *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 cangkringensis* 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 of Surabaya based on the 16S rRNA gene revealed new Streptomyces species. Phylogenetic tree diagram show- ed that *Streptomyces* Sp-D, Sp-Ep, Sp-G and Sp-I foundin the soil from Bratang compost house Surabaya were new isolates.

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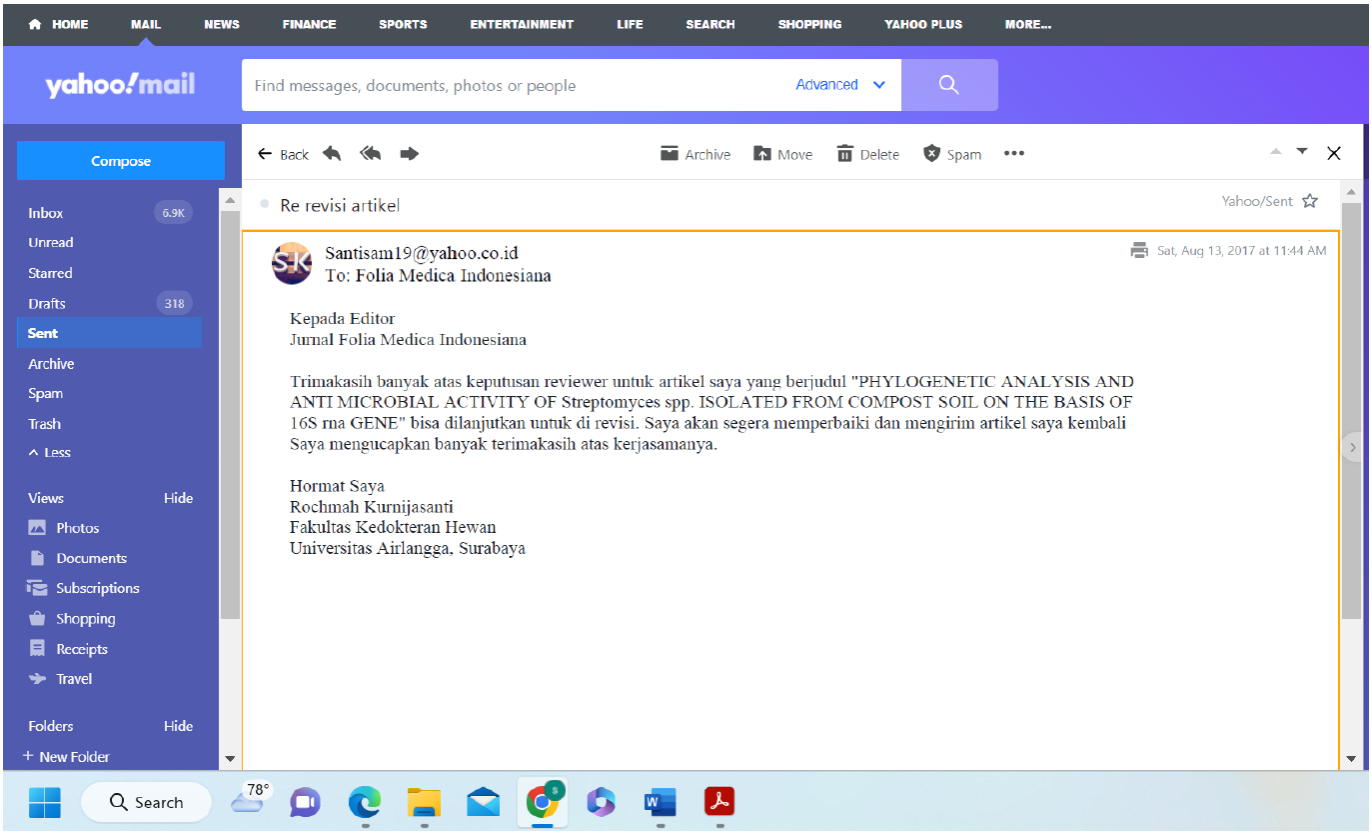

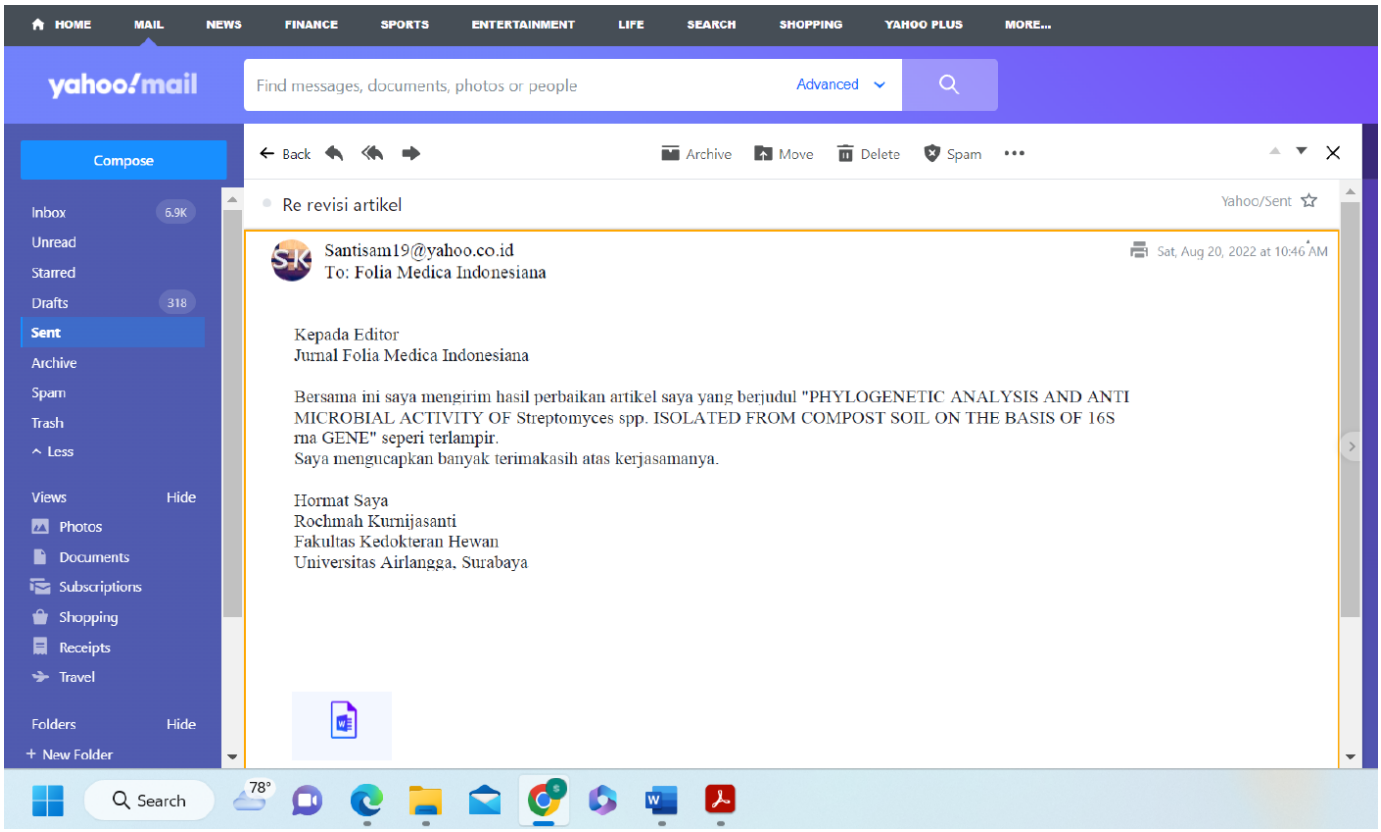

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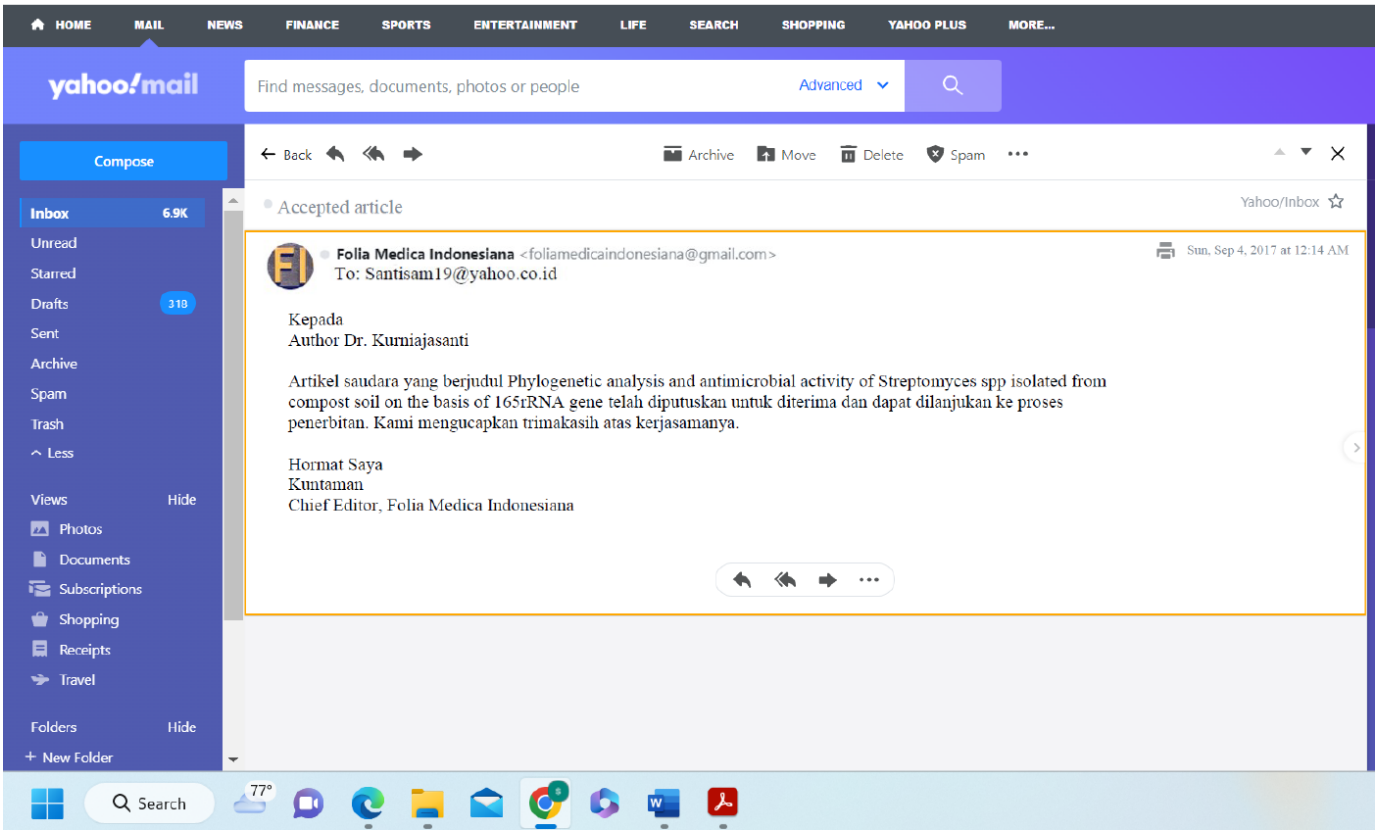

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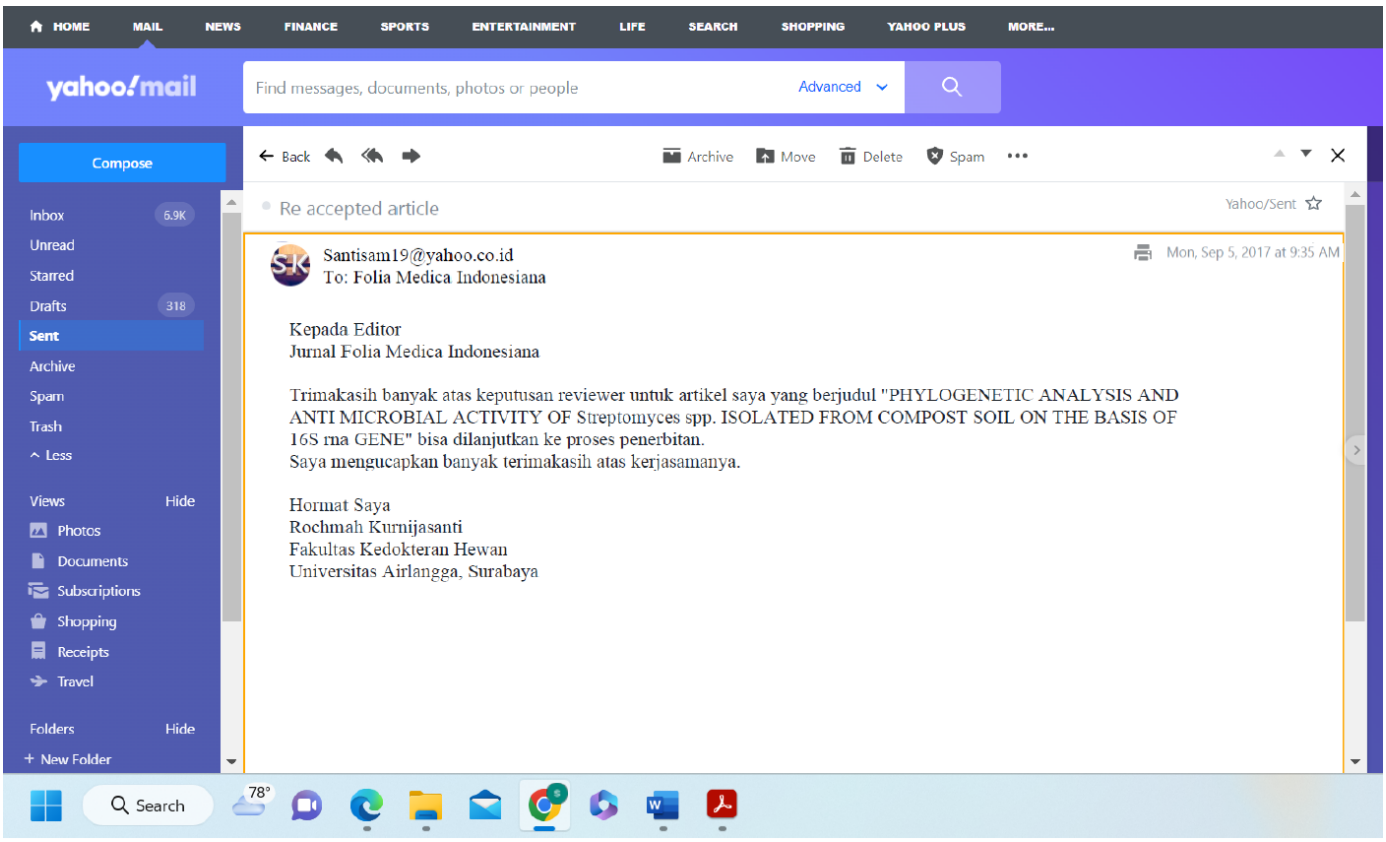
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