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Research Article

CHARACTERIZATION AND IDENTIFICATION OF A SOIL *NOCARDIA sp.* TP-1 ISOLATED FROM INDONESIAN VOLCANIC SOIL

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ABSTRACT

Tangkuban Perahu mountain in West Java was one of volcanic mountain in Indonesia. Volcanic soil contained some unique minerals that microorganism need for producing their secondary metabolite. TP-1 strain is a soil microbe, isolated from soil taken from the area in Tangkuban Perahu mountain Since the secondary metabolite of *Nocardia sp.* can combat pathogen resistant bacteria, fungi and cell line cancer so it can be applied in pharmaceutical industries. The objective of this research is identified and characterized actinomycetes that have potency to produce secondary metabolite for further utilization in pharmacy. The methods are by analyze of its morphological, physiological, biochemical and molecular characteristics of TP-1 strain. The results of analysis of its morphological, physiological, and biochemical characteristics strongly suggested that TP-1 strain belonged to the *Nocardia sp*. Analysis of the nucleotide sequence of the 16S rRNA gene of *Nocardia sp*. TP-1 strain showed a strong similarity (90%) with the 16S rRNA gene of *Nocardia sp*. YIM 65630. Conidia , usually round, chalk, and white light grey. It is a new information that *Nocardia sp.* can produce some secondary metabolites like antibacterial, antifungal and anticancer activity.

Keywords: Morphological, Physiological, Biochemical and molecular identification**,** Homology, *Nocardia sp*. YIM 65630 TP-1

INTRODUCTION

Actinomycetes group has been known as a producer of secondary metabolites that are beneficial to the pharmaceutical industry. Microorganisms that grow in extreme environments such as volcanic area have way of self-defense in order to survive its life. *Nocardia sp.* is one genus of this group which can find in volcanic soil will appear to be the most promising source of the future antibiotics. Ways in which microorganisms among others, by holding entry toxic inorganic compounds such as selenium or the mechanism of resistance in cell microorganisms by changing the compound toxic to non-toxic. The bacteria that live in extreme environment have a mechanism of resistance toxic heavy metals such as selenium that only specific bacteria can survive on environment containing selenium. The mechanism of resistance is due presence of genes that encode proteins so that it can bind to the compound selenium and converted into complex selenium-proteins are not toxic. Selenium-protein complexes can be used as a component of the active site of the enzyme glutathione peroxidase. This enzyme plays as antioxidant enzymes in protecting cells from free radicals. In laboratory tests, selenium inhibits tumor growth and regulates the natural life of the cells[1,2].

The genus Nocardia become one of the most important organisms in genetic research since the genera was reported a new anticancer Nocardicyclins A and B production , which have been isolated from *Nocardia pseudobrasiliensis* IFM0624 (JCM9894). Nocardicyclin A and B has antibacterial activity against gram-positive bacteria including *Mycobacterium spp* and cytotoxic activity against leukemic cells L1210 and P388[3]. Anthracycline antibiotic 3'-O-demethyl mutactimycin and 4-O, 3'-Odidemethyl new mutactimycin been isolated from *Nocardia transvalensis* have antimicrobial activity against gram-positive bacteria and cytotoxic activity against cancer cells P388, L1210 and HeLa[4].

Methichillin resistant *Staphylococcus aureus* (MRSA), a multidrug resistant variation of common *S. aureus* has become a considerable public health issue during the past decade, due to a significant increase in the incidence of MRSA isolated from patients with complicated infection[5]. Since resistance was not because of the antibiotic destruction by enzyme β-lactamase, the resistance was termed as intrinsic. Increased outbreaks had subsequently been reported from many countries after the emergence of MRSA, as nosocomial pathogen in the early 1960s. There were reports of life-threatening sepsis, endocarditis, and osteomyelitis caused by this organism[6].

On this study, identification of *Nocardia sp*. TP-1 strain which was isolated from Tangkuban Perahu mountain in West Java was carried out based on morphological, physiological, biochemical and genetic analysis partially on 16S ribosomal RNA.

MATERIALS AND METHODS

Isolation of strain TP-1 by total plate count (TPC) method

The strain TP-1 was isolated from Tangkuban Perahu mountain soil, West Java, Indonesia by total plate count (TPC) method. 1 gram of soil sample from Tangkuban Perahu mountain area was diluted in 100 ml aqua sterile. The suspension of soil was mixed and diluted to 10^{-6} dilution serial. One ml of a diluted sample was placed in each of sterile petri dish, and then the liquid of potato dextrose agar (PDA) was added and mixed slowly. After the medium was solidified, petri dishes were incubated at 30°C for one to three weeks. The separated colony was removed to PDA slant and incubated at 30°C until the rough and white light grey spores was appeared. The isolation process was repeated to obtain the pure isolate.

Morphology, physiology and biochemical characteristics

After cultivating TP-1 at 30°C for 3-5 days, the morphological characteristics of the substrate mycelium, aerial mycelia, sporophore and the spore shape of TP-1 were observed by both the light and scanning electron microscope (SEM) as well as physiological and biochemical characteristics[7,8]. Strain TP-1 was selected for molecular identification.

Molecular identification of *Nocardia sp.* TP-1

TP-1 isolate was obtained from the soil of Tangkuban Perahu mountain of West Java Indonesia. Molecular identification of *Nocardia sp.* TP-1 was carried out based on genetic analysis partially to that of 16S ribosomal DNA of bacteria. Isolation of DNA was carried out by inoculated the strain TP-1 in medium Yeast Starch Agar (YSA) and incubated for 48 hours. Biomass of mycelia was then harvested for DNA extraction process. Extraction of genomic DNA strain TP-1 as a template for polymerase chain reaction (PCR) was carried out by using GES method and then 16S rRNA gene was amplified by PCR. The primer of PCR amplification on 16S rRNA was used 9F (5'GAGTTTGATCCTGGCTCAG –3') dan 1541 R (5-

AAGGAGGTGATCCAACC –3')[9]. PCR product purification was carried out by PEG precipitation method and continued by sequencing cycle. The purification of this product was repeated by ethanol purification method. The analysis of nitrogen base sequence was read using the automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems). Raw data's from the sequencing was then trimmed by MEGA 4 program and assembling by BioEdit program, then converted in FASTA format. DNA sequencing data's in FASTA format was continued using BLAST program to identified the homology by on line method in data base center on NCBI (http://www.ncbi.nlm.nlh.gov/) or DDBJ (http://www.ddbj.nig.ac.jp). The last step of identification was analysis the phylogenetic tree using Clustal X[10] and NJ plot program[11,12].

RESULTS AND DISCUSSION

The morphological characteristics of the substrate mycelium, aerial mycelia,sphorophore, and the spores of the strain TP-1 could be clearly observed. In an observation using the light and scanning electron microscope, the sporophore appeared to be the round-shaped spores (Table 1 and Fig. 1). The morphological characteristics indicated that the strain may be actinomycete. The results of physiological and biochemical characteristics of TP-1 were shown in Table 1 and Fig. 2. The TP-1 strain was able to hydrolyze starch and casein, but could not produce hydrogen sulfide. It was able to utilize glucose, sorbitol, arabinose and xylose as a carbon source.

Nocardia sp. TP-1 isolate was selected for molecular identification because of its antibacterial, antifungal and anticancer activities. DNA sequencing data's in FASTA format was continued using BLAST program that shown on Fig. 4. 16S rRNA or 16S ribosomal RNA is a component of the 30S small subunit of prokaryotic ribosomes. It is 1542 kb (or 1542 nucleotides) in length (Fig. 3). The genes coding for it are referred to as 16S rDNA and are used in reconstructing phylogenies. Results of homology analysis to *Nocardia sp*. TP-1 to that of ribosome DNA of bacteria using 16S rRNA are shown on Table 2.

Family and phylogenetic trees of *Nocardia sp.* TP-1 were analyzed using Clustal X and NJ plot program. To construct the phylogenetic trees, the homology sequence from BLAST and FASTA format results was calculated the data for three construction by Clustal X, and then conversion of calculated data into trees by NJ plot. Neighbors-joining (NJ) method is the simple program to construct the phylogenetic tree from evaluationary distance data by finding the pairs of operational taxonomic units (neighbors) that minimize the total branch length at each stage of clustering of neighbors starting with a star-like tree. The phylogenetic trees of 16S rRNA were shown on Fig. 5. Based on phylogenetic analysis using 16S rRNA methods, *Nocardia sp.* TP-1 has a close family (90%) with *Nocardia sp.* YIM 65630.

Table 1: Morphology, Physiology and Biochemistry Characterization of *Nocardia sp*. TP-1

Character of Strain TP-1	Result of assay
Macroscopic colony and microscopic of cell in Potato Dextrose Agar (PDA) medium and light microscope	Circular (round), entire, umbonate, moderate colonies, chalk & rough, pigmented, white, dark brown-black reverse, slow growth, the color of young culture is white and the old culture is white light grey, spore chains are spiral, gram positive.
Motility	Motile
Utilize of carbon source:	
Inositol	Negative
Glucose	Positive
Mannose	Negative
Sorbitol	Positive
Fructose	Negative
Lactose	Negative
Maltose	Negative
Galactose	Negative
Xylose	Positive
Sucrose	Negative
Arabinose	Positive
Growth in NaCl 10%	Negative
Starch hydrolysis	Positive
Fat hydrolysis	Negative
Casein hydrolysis	Positive
Liquefaction of gelatin	Negative
Methyl red	Negative
Voges proskauer	Negative
Nitrate reduction	Negative
Growth in 1% of Tripton broth	Pellicle
Degradation of urea	Negative
Citrate reduction	Negative
Production of H_2S	Negative
I-Indol	Negative

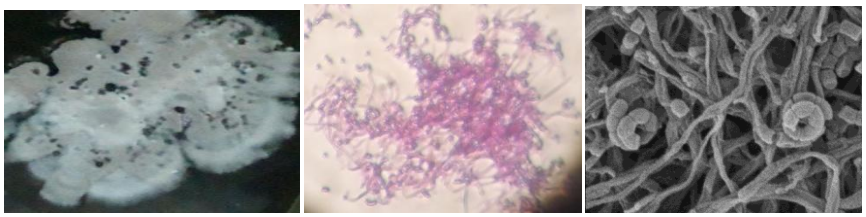

Fig. 1: Morphology of *Nocardia sp*. TP-1 in PDA medium, Light & Scanning electron microscope

Fig. 2. Hydrolisis of Starch and Protein by *Nocardia sp*. TP-1

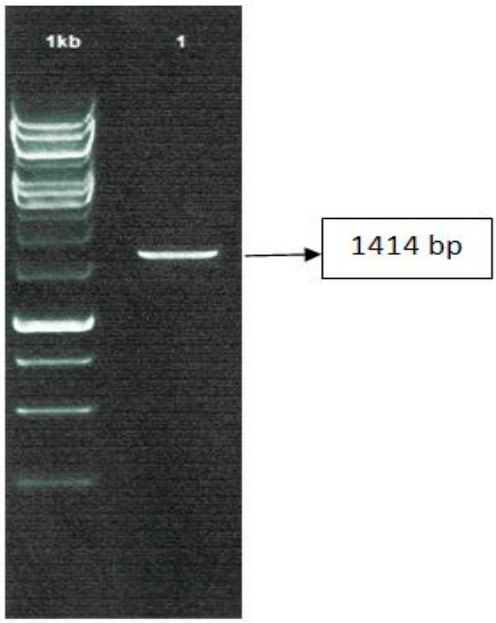

Fig. 3: **Electrophoresis of PCR product of *Nocardia sp*. TP-1**

Table 2: Result of Molecular Identification of *Nocardia sp.* TP-1

Region	*Nocardia sp.*	Homology (%)
16S rRNA	*Nocardia sp.* YIM 65630	90

16S ribosomal RNA of Nocardia sp. YIM 65630, length 1414

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   1 tgcaagtcga gcggtaaggc ccttcggggt acacgagcgg cgaacgggtg agtaacacgt
  61 gggtgatctg ccctgtactt cgggataagc ctgggaaact gggtctaata ccggatatga
 121 cccaaggttg catgactttg ggtggaaaga tttatcggta caggatgggc ccgcggccta
 181 tcagcttgtt ggtggggtaa cggcctacca aggcgacgac gggtagccga cctgagaggg
 241 tgaccggcca cactgggact gagacacggc ccagactcct acgggaggca gcagtgggga
 301 atattgcaca atgggcgaaa gcctgatgca gcgacgccgc gtgggggatg acggccttcg
 361 ggttgtaaac ccctttcgac agggacgaag cgcaagtgac ggtacctgta gaagaagcac
 421 cggccaacta cgtgccagca gccgcggtaa tacgtagggt gcgagcgttg tccggaatta
 481 ctgggcgtaa agagcttgta ggcggtctgt cgcgtcttct gtgaaaactt ggggctcaac
 541 cttaagcttg caggggatac gggcagacta gagtacttca ggggagactg gaattcctgg
 601 tgtagcggtg aaatgcgcag atatcaggag gaacaccggt ggcgaaggcg ggtctctggg
 661 aagtaactga cgctgagaag cgaaagcatg ggtagcaaac aggattagat accctggtag
 721 tccatgccgt aaacggtggg tactaggtgt gggtttcctt ccacgggatc cgtgccgtag
 781 ctaacgcatt aagtaccccg cctggggagt acggccgcaa ggctaaaact caaaggaatt
 841 gacgggggcc cgcacaagcg gcggagcatg tggattaatt cgatgcaacg cgaagaacct
 901 tacctgggtt tgacatacac cggaaacctg cagagatgta ggccccttg tggtcggtgt
 961 acaggtggtg catggctgtc gtcagctcgt gtcgtgagat gttgggttaa gtcccgcaac
1021 gagcgcaacc cttatcttat gttgccagcg cgtaatggcg gggactcgtg agagactgcc
1081 ggggtcaact cggaggaagg tggggacgac gtcaagtcat catgcccctt atgtccaggg
1141 cttcacacat gctacaatgg ccggtacaga gggctgcgat accgcgaggt ggagcgaatc
1201 ccttaaagcc ggtctcagtt cggatcgggg tctgcaactc gaccccgtga agttggagtc
1261 gctagtaatc gcagatcagc aacgctgcgg tgaatacgtt cccgggcctt gtacacaccg
1321 cccgtcacgt catgaaagtc ggtaacaccc gaagccggtg gcctaaccct tgtggaggga
1381 gccgtcgaag gtgggatcgg cgattggacg aagt
```

Fig. 4: BLAST results of 16S rRNA gene PCR product

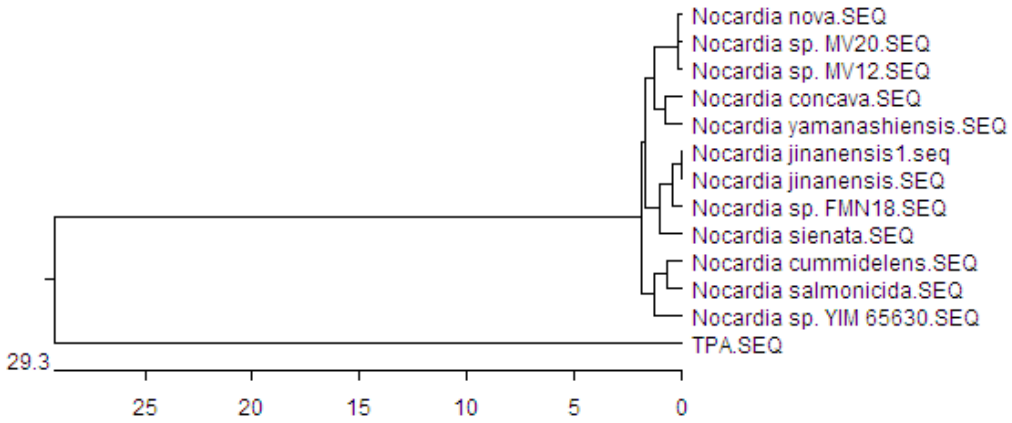

Fig. 5: Phylogenetic Tree of 16S rRNA of *Nocardia sp.* TP-1

CONCLUSION

Homology analysis to *Nocardia sp* TP-1 to that of ribosome RNA of bacteria using 16S rRNA sub unit, the isolate is homology to *Nocardia sp*. YIM 65630. Based on phylogenetic analysis using 16S rRNA, *Nocardia sp*. TP-1 has 90% close with *Nocardia sp.* YIM 65630.

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