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Research Article

BACTERIAL CHARACTERIZATION OF SILVER NANOPARTICLES FROM TEMBAGAPURA SOIL SAMPLE ISOLATE, PAPUA, INDONESIA

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ABSTRACT

Characterization of silver nanoparticle-producing bacteria from Tembagapura, Papua, Indonesia soil samples isolates has been investigated. Bacteria characterization was carried out macroscopically, microscopically, biochemically and molecularly. Furthermore, the results of the formed silver nanoparticles were characterized using UV-VIS spectroscopy, Fourier transform infrared (FTIR) and scanning electron microscopy (SEM). The experimental results showed maximum absorbance at 414 nm in TP10-1 isolates in UV-Vis spectroscopy. FTIR spectra of silver nanoparticles samples of TP10-1 isolates showed strong peaks in wave numbers 1637.65 cm^{-1} and 3329.47 cm^{-1}. SEM micrographs reveal the formation of well dispersed silver nanoparticles. Silver nanoparticles of TP10-1 isolates that was measured by the imageJ program had an average particle size of 16,991 nm. Bacterial isolates with TP10-1 sample code which are identical to the Bacillus cereus strain GCF1I2 was able to synthesize silver nanoparticles.

Keywords: silver nanoparticle, characterization, *Bacillus cereus* strain GCF1I2

INTRODUCTION

Nanotechnology development is still developing by the researchers from academic world and from the industrial world. Nano particle synthesis means making particles with sizes less than 100 nm and at the same time changing their properties or functions[1]. One of the most popular nanoparticles is nanosilver particles or nanosilver particles (NSPs). If the particle size is lowered, the surface area with the NSPs volume ratio will increases dramatically, which leads to significant changes in physical, chemical, and biological aspect. NSPs are most commonly used in health care systems for hundreds of years[2].

Biosynthesis (green synthesis) of nanosilver has received widespread attention because of its growing need for environmentally friendly synthesis methods and the use of environmentally friendly reducing and capping agents, such as proteins, peptides, carbohydrates, from various species of bacteria, fungi, yeast and algae and plants[2].

In this paper, we will be used to synthesize nanosilver particles (NSPs) from bacteria isolated from Tembaga Pura, Papua soil and reported the characterization was formed. Bacteria from Tembagapura which produce nanosilver will be identified molecularly.

MATERIAL AND METHOD

Isolation of Silver Nanoparticle-Producing Bacteria

Soil samples obtained from Tembagapura were used as a source for bacterial isolate. A total of 10 g of soil samples were put into 90 ml of NB medium, then incubated for 24 - 48 hours at 37 °C, then it was diluted to $10^{3,4}$. Isolation of metal-resistant bacteria (Ag) was carried out by duplicate plating technique using a nutrient equipped with a 1 mM concentration of sterilized $AgNO_3$ and using NA medium only. Then it was incubated at 37 °C for 48-72 hours, the Petri dish was observed to determine the bacterial growth[5,6]. Cultures of growing isolates were identified as silver resistant bacteria. Separately grown bacterial colonies were then purified using the streak plate method on nutrient agar (NA) medium.

Conventional Identification of Bacteria

Conventional Identification Bacteria that produce silver nanoparticles were further identified by conventional methods. The main identification of bacterial isolates was carried out on the basis of colony, microscopic and biochemical characteristics which refer to the Bergey's Manual of Determinative of Microorganism[3,7].

Molecular Identification of Silver Nanoparticles- Producing Bacteria

Molecular identification of bacterial isolates was done using the 16SrRNA gene. DNA products of bacterial isolates from amplification and purification were sequenced by Macrogen Inc. (South Korea). Sequence data were analyzed using the BLAST program (www.ncbi.nlm.gov.blast) and phylogenetic trees was built[8].

Extracellular Biosynthesis of Silver Nanoparticles

Extracellular synthesis of silver nanoparticles was carried out as described by Shahverdi et al. (2007) with a few modifications as

given below. Isolated colonies were cultured in NB and incubated for 24 hours at 37 °C. Then, NB was centrifuged at 8000 rpm for 10 minutes to collect the supernatant. Silver nitrate solutionas much as 1 mM was prepared with distilled water. As much as 20 mL of 1 mM silver nitrate solution were mixed with 10 mL of the culture supernatant in a 50 mL Erlenmeyer flask. All samples were shaked using shaker tool at 150 rpm and maintained in dark conditions for 36-72 hours. Silver nitrate reduction was monitored with visible color changes from the solution[5,9].

Synthesis Evaluation of Silver Nanoparticle

Synthesis evaluation of silver nanoparticle formed was done using UV-Vis spectrophotometer, Fourier transform infrared(FTIR) and Scanning Electron Microscopy (SEM)[10,11,12].

RESULTS AND DISCUSSION

Isolation of Silver Nanoparticles-Producing Bacteria

Pure colonies grown on nutrient agar (NA) medium which had been added with 1mM $AgNO_3$ solution were taken one of them and given isolate code TP10-1. These colonies are considered as resistant bacteria to silver solution, then characterization of the bacteria and the products of silver nanoparticles formedwas done. Characterization of bacterial isolates obtained was done by comparing the isolate with standard literature. The result showed that the bacteria was Gram positive and has bacillary form (Fig. 1). Endospora tests was carried out on these isolates and showed that these bacteria can form endospores. Furthermore, biochemical tests were carried out, one of which was catalase test. This test result showed that the bacterial isolates were positively catalase, characterized by the formation of bubbles. These results indicate that the isolate is likely to be Bacillus.

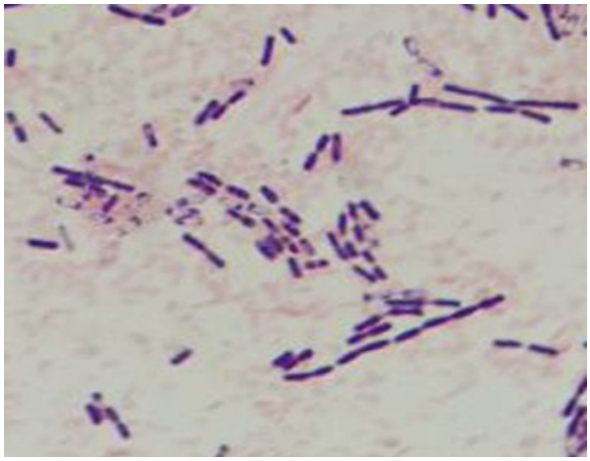

Figure 1: The results of Gram staining isolation of TP10-1 bacteria with 10x100 magnification

Molecular identification of silver nanoparticles-producing bacteria

The sequencing results of TP10-1 bacterial isolates were compared with GeneBank data using the BLAST program which was conducted online on the NCBI website.

>10-1
AGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTTCCGG
GAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGA
TGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCAACCTGAGAGGGTGAT
CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTC
TGACGGAGCAACGCCCCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTG
AATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTG
GCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAA
CCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGT
AGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCA
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGATG
AAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACA
AGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGA
TAGGGCTTCTCCTTCGGGAGAAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGT
CCCGCAACGAGCGCAACCCTTGATTTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACCGCCGGTGACAAACCGG
AGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGA
GCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTACATGAA
GCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACC
ACGAGTAGTTTGTAACACCCGGAAGTCGGTGGGGTAACCTTTTGGAGCCAGCTATCAA

Figure 2: Nucleotide sequencing results of TP10-1 bacterial isolates

TP10-1 bacterial isolates have an 99% similarity with Bacillus cereus strain GCF1I2. In the observation of phylogenetic trees, the distance between bacterial isolates of TP10-1 and Bacillus cereus strain GCF1I2 was very close (Fig. 3). This indicates that the bacterial isolate TP10-1 was found to be identical to the Bacillus cereus strain GCF1I2. This is in accordance with Hagström, Pinhassi and Zweifel (2000) whom stating that the isolates with similar sequences of more than 97% can represent the same species. While the sequence similarity between 93% - 97% can represent the identity of bacteria at the genus level but from different species[13]. This is also consistent with Tamisier et al. (2015) who stated that the recommended threshold is 95% (for genus) and 98.7% (for species) to classify bacterial isolates[14].

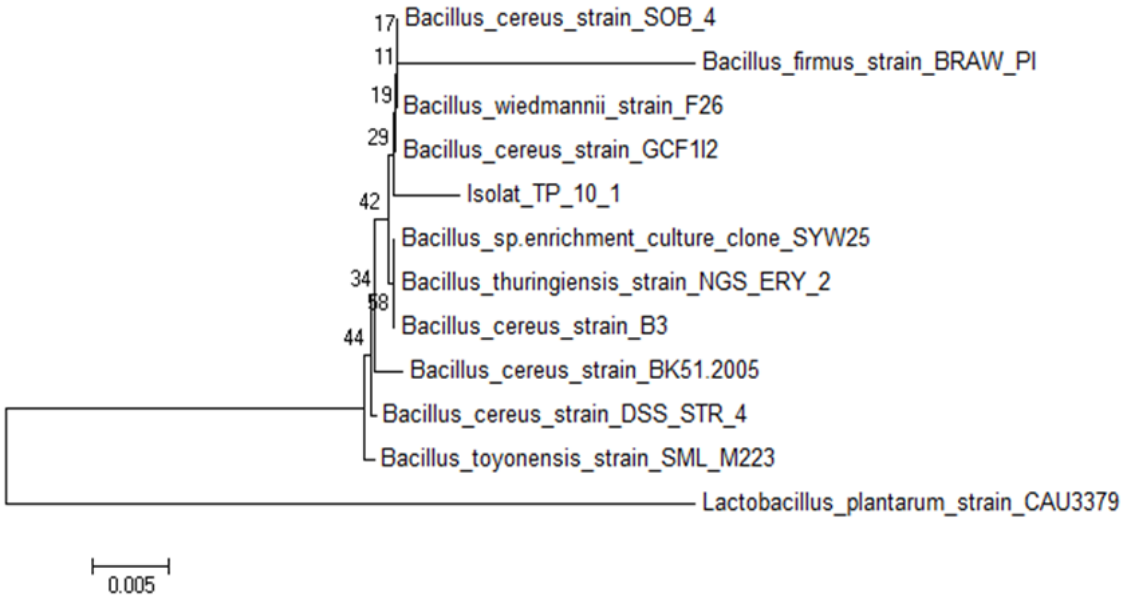

Figure 3: Phylogenetic tree of TP10-1 bacterial isolate

Extracellular Biosynthesis of Silver Nanoparticles

On visual observation, after the fifth day there was a change in the solution color from being initially yellow to ground brown. The supernatant culture without silver nitrate and 1 mM $AgNO_3$ solution also observed for color changes and used as controls (Fig. 4). The appearance of brownish color in the solution which added with silver nitrate was identified as the formation of silver nanoparticles. This is in accordance with Agrawal and Kulkarni (2017) opinion that the color of the supernatant solution added with 1 mM sterile $AgNO_3$ will turned brown after incubation[6]. Kalishwaral et al. (2008) also stated that supernatant culture that incubated with silver nitrate showed color changes from yellow to brown while supernatant culture without silver nitrate and silver nitrate solution (as a control) were observed to have no color changes[15].

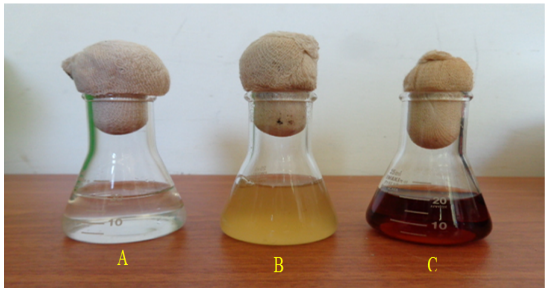

Figure 4: Color change test on bacteria isolates after incubated for 5 days on 37 °C in a dark atmosphere; A. 1 mM $AgNO_3$ solution, B. Bacterial supernatant C. Bacterial supernatant + 1 mM $AgNO_3$solution with color changes in TP 10-1 bacterial isolate.

Evaluation of the synthesis of silver nanoparticles

The result of UV-Vis spectrophotometer observation was indicated by the formation of the maximum absorption peak of isolate TP10-1 was 414 nm (Fig. 5). This is consistent with the statement of Sileikaite et al. (2006) stated that silver nanoparticle colloids have typical absorption peaks in visible light with a range of 350 - 530 nm in spectrophotometer analysis[16].

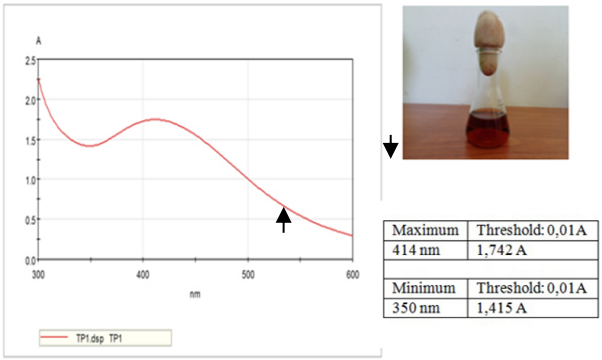

Figure 5: The maximum absorption peak of TP10-1 at 414 nm wavelength

Fourier Transform Infra Red (FTIR) was used to prove the interaction of TP 10-1 bacterial isolate protein during the process of formation of silver nanoparticles (Fig. 6). FTIR spectra of silver nanoparticles from TP10-1 isolates between wave numbers 400-4000 cm^{-1} showed strong peaks in wave numbers 1637.65 cm^{-1} and 3329.47 cm^{-1}. This is consistent with the opinion of Kong and Yu (2007) whom states that the spectrum region most sensitive to the secondary structural component of protein is the amide I band (1700-1600 cm^{-1}) which is almost entirely caused by vibration of C = O stretching connected to the peptide (around 80%). The frequency of amide component I was found to correlate closely with each of the secondary structural elements of the protein[17]. Banker (1992) also states that other amide vibrational bands are very complex depending on the details of the force field, properties of the side chain and hydrogen bonds. Therefore, this method is more practical to used in protein conformation studies[18].

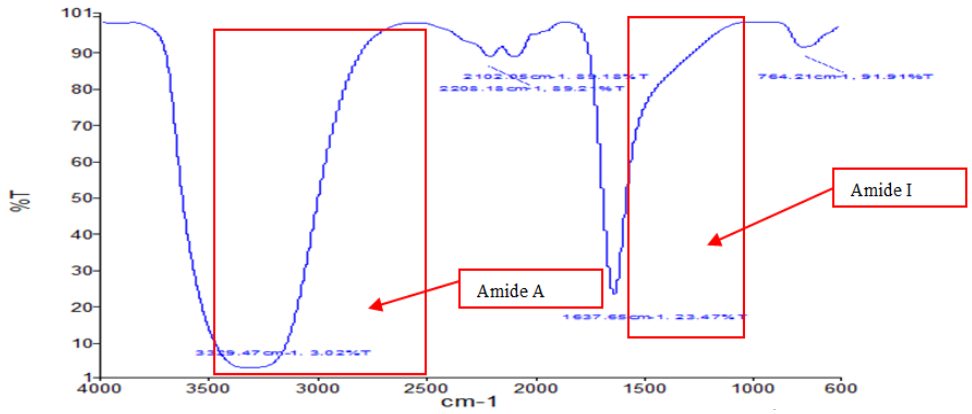

Figure 6: FTIR result of TP10-1 isolate showed the formation of amide peak

The particle size observation of silver nanoparticles was carried out using the imageJ program. This is consistent with Mazolli and Favoni (2012) that stated to evaluate particle size and size distribution, Scanning Electron Microscopy (SEM) and ImageJ processing programs were used (Fig. 7). The number of analyzed nanoparticles was 5813 of the total 98765 with the average size of the nanoparticles formed was 16.991 nm[19].

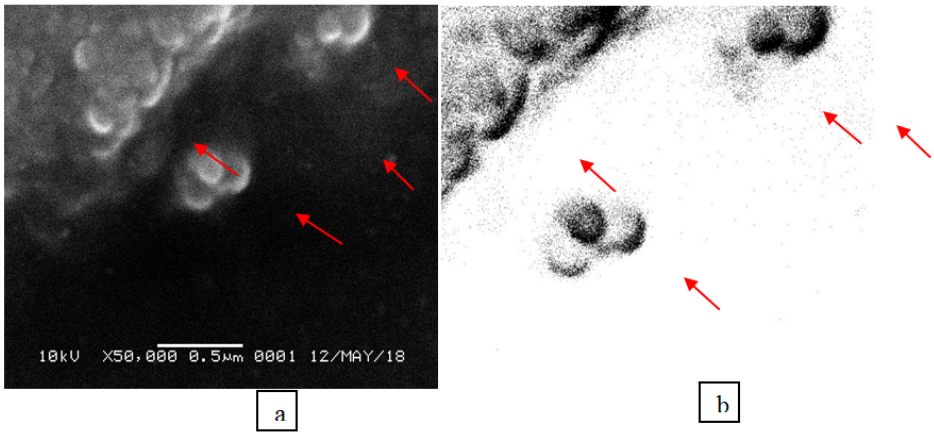

Figure7: SEM result of TP10-1 bacterial isolate, a. SEM results showed the formation of silver nanoparticles; b. The results of the particle size scanner for silver nanoparticles using ImageJ

The results showed that the TP10-1 bacterial isolate was able to synthesize $AgNO_3$ solution into silver nanoparticles. This is in accordance with Pugazhenthiran *et al*. (2009) who using Bacillus sp. to synthesize silver into silver nanoparticles and obtained a nanoparticle with size of 5-15 nm. Banu et al. (2014) was synthesized the nanoparticle from Bacillus thuringiensis and obtained nanoparticle with size of 43.52–142.97 nm. El-Shanshoury *et al.* (2011) also synthesized the nanoparticle with *Streptococcus thermophilus* and obtained nanoparticle with size of 28–122 nm[20]. This is in accordance with Srikar *et al.* (2016) which states that nanoparticle synthesis means making particles with sizes less than 100 nm and at the same time changing their properties or functions[1].

CONCLUSION

This study showed the characterization of TP10-1 bacterial isolates that was tested molecularly had 99% similarity with Bacillus cereus strain GCF1I2. This results was confirmed using phylogenetic trees. Bacillus cereus strain GCF1I2 has the potential to synthesize silver nanoparticles. The average particle size formed was 16.991 nm. UV-Vis spectrophotometer observation results were characterized by the formation of a maximum absorption peak at 414 nm which is a typical absorption peak for silver nanoparticles. The results of the Fourier Transform Infra Red (FTIR) proved the interaction of TP 10-1 bacterial isolate protein during the process of forming silver nanoparticles.

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