Silver Nanoparticles Biotransforming Bacteria Isolated from Silver-Craft Waste

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ABSTRACT: The use of silver resistant bacteria in the synthesis of nanoparticles emerges as an eco-friendly approach. In the current study, biotransformation of silver ion becomes silver nanoparticles by silver resistant bacteria BAgBK3 was reported. These transformed silver nanoparticles were observed using UV-Visible spectrophotometery and TEM. The absorption of silver colloids has detected a broad peak at 410 nm corresponding to the plasma resonance of silver nanoparticles. The synthesized nanoparticles were found to be spherical shape with a size in the range of 9 – 13 nm. It was scattered around the edge and around of bacteria cell. Nitrate reductase enzyme involved in the biotransformation of silver ion to silver nanoparticles. Isolate strain BAgBK-3 is a promising bacteria for AgNPs biotransformation.

KEYWORDS: Silver resistant bacteria, Biotransformation, Silver nanoparticles, Silver-craft waste, Nitrate reductase

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INTRODUCTION

Heavy metal such as silver is harmful and toxic to organisms (Rajendran *et al*., 2003). Meanwhile a number of microbes, include bacteria like *Bacillus* sp., *Idiomarina* sp., *Pseudomonas aeroginosa* that able to survive in toxic silver metal have been isolated from different sources (Hidayanti, 2015; Lima de Silva *et al*., 2012; Seshadri *et al*., 2012). Bacteria is silver resistant due to the specific genetic and biochemical mechanism (Singh *et al*., 2012). These silver tolerant bacteria have been explored in recent years for biological metal remediation (Gupta *et al*., 1998; Rajendran *et al*., 2003). However, since the bacteria resistant to silver ion so they are being employed to synthesis nanomaterial (Deepak *et al*.,2011). The synthesis of nanoparticles using bacteria is one of the most interesting fields of research that is easy, ecofriendly, and also enable in controling its size and shape. The first report on AgNPs biosynthesizing bacteria isolated from silver mine was in 1999 by Klauss and co-workers. The bacterium, *Pseudomonas stutzeri* AG259 can accumulate the AgNPs in the cell wall without undergoing cell death. The resistance of the bacteria to ion silver is the requirement for biosynthesize of AgNPs.

Silver nanoparticles (AgNPs) have been defined as a silver mineral with very small size (1-100 nm). Silver nanoparticles can be utilized for broad spectrum application in medical such as for antimicrobial, in textile engineering, in optics, in water treatment or as fungicides for plants (Singh *et al*., 2015). Theoretically colloidal silver nanoparticles, with size range 10- 14 nm, possess optical spectrum (surfaces plasmon resonance) with absorption peak lies ~400 nm (Solomon *et al*., 2007).
Silver as toxic metal imparts two functions one is inducing cell death at higher concentration another is inducing organism to reduce silver ion for bioremediation or biosynthesizing nanoparticles (Deepak *et al*., 2011). On the study of *Bacillus licheniformis*, the increase of silver ion concentration was accompanied by an increase in the catalase and nitrate reductase synthesis. This defense mechanism occurs in the respiratory system of the organism. The three major Sil genes, silE, silP and silS also play significant role for this resistance to silver ion (Singh *et al*., 2015). Silver-craft wastes could be a good source of silver tolerant bacteria. Hence, the present study aims to describe the

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biological transformation of silver ion become silver nanoparticle using silver resistant bacteria from silver-craft waste.

METHODOLOGY

Bacterial Isolates and Selection

The bacterial strain, BAgAK-6, BAgBK-1.1, and BAgBK-3 are silver tolerant bacteria isolated from a slurry of silver-craft waste at Kotagede, Indonesia obtained from the previous research (Hidayanti, 2014). The silver resistant bacteria was grown on Nutrient Broth supplemented with 0.1 mM Silver nitrate ($AgNO_3$) for selecting the potential bacteria. The culture flask was maintained for 48 h in a shaker incubator at 120 rpm, at 37° C. The turbid appearances of the culture flask ensured the growth and were taken for further experimental study.

Bacterial Growth and Biosynthesis of Silver Nanoparticles

The selected bacterial isolate was inoculated in 250-ml Erlenmeyer flask containing 100 ml sterile TYE broth medium (tryptone 1 gr/L, yeast extract 3g/L, pH 7) supplemented with 0,1 mM $AgNO_3$. The control culture were the same except without $AgNO_3$. The cultured flask was incubated for 24 h at 120 rpm, at 37° C. The growth of potential bacteria was periodically monitored spectrometrically at 600 nm. For AgNP synthesis, the stock culture (after 24h) was centrifuged at 12.000 rpm for 10 minutes. The biomass and cell filtrate was separated. The final concentration of 0,1 mM $AgNO_3$ was added in to TYE broth with biomass pellet. The flask were incubated in dark room condition up to 48h, 120 rpm, at 37° C. The control was maintained without addition of $AgNO_3$. The change of culture color from yellow pale to brown was an indication of the synthesized silver nanoparticles.

Nitrate Reductase Assay

The prepared bacterial culture was considered for the assay of nitrate reductase according to the method of Harley (2005) with a slight of modification. The substrate used were 25 mM Phosphate Buffer with 10 mM KNO3 and 0.05 mM EDTA pH 7.3. For assaying the enzyme sample, the crude extract of supernatant solution of bacterial strain was mixed with the substrate. 1% (w/v) Sulphanilamide solution in 3M HCl and 0.02 % (w/v) NEED (N-(1-naphtyl) ethylene diamine dihydrochloride) was added in each of the sample. After 10 minutes of incubation the intensity of the developed color was estimated in a UV-Vis spectrophotometer (Thermo Genesys 10) at 540 nm.

Characterization of silver nanoparticles

The visual observation of silver nanoparticle colloid was using laser beam which exposed to bacterial culture, red line indicates positive result. The biosynthesized silver nanoparticles were analyzed using a UV-visible spectrophotometer (Thermo Genesys 10) at 300-500 nm. The size and shape were characterized using a Transmission Electron Microscope (JEOL-JEM 1400), at an accelerating voltage of 120 kV.

RESULT AND DISCUSSION

Silver resistant bacteria used ini this research were BAgAK-6, BAgBK-1.1, and BAgBK-3. In the current study, isolate BAgBK-3 able to grow in liquid medium containing 0.1 mM AgNO3 (Table 1).

Table 1. The growth bacterial strain on NB supplemented with $AgNO_3$

Concentration of $AgNO_3$ mM	Bacterial strain		
	BAgAK6	BAgBK1.1	BAgBK3
0 mM	+	+	+
0,1 mM	-	-	+

Note: + = turbid medium the isolates strains capable to grow;
- = clear medium no grow of the isolates strains

Generally silver ion is often toxic for microbial. The ionic silver inactivates thiol groups of vital enzymes and direct binding the DNA lead to disruption of replication and cell death (Pandian *et al*., 2010). Because of the special ability the silver resistant bacteria could survive by efflux pump and enzymatic mechanism or by secrete inorganic metabolic products such as sulfide, carbonate or phosphate ion from their respiratory system to precipitate or detoxify of harmful metal (Rajendran *et al*.,2003; Pandian *et al*., 2010). But, each bacterial strain has a different maximum threshold of silver tolerance, when the concentration over the threshold can lead cell death of the organism. That condition makes it useless in higher concentration (Deepak *et al*., 2011).

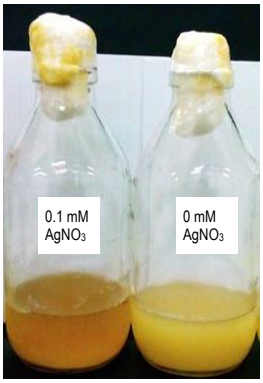

Figure 1. Color change from yellow pale to brown of 48h BAgBK-3 culture medium containing 0.1 mM $AgNO_3$

Figure 2. AgNp colloids synthesized by BAgBK-3. Red line appeared (arrowed) after exposed to laser beam

Figure 3. Purple color (right) on BAgBK-3 culture indicate presence of nitrite

Isolate BAgBK-3 is selected as a potential silver resistant bacteria for further studies because of the ability to grow in higher silver concentration. The silver resistant properties of bacteria emerge them to reduce or accumulate silver ion comes to silver nanoparticles (Klauss *et al*., 1999; Kalimuthu *et al*., 2008). Fig. 2 shows red line appearances in culture BAgBK-3 using a laser beam, indicate as colloidal silver nanoparticles. The red line was caused Tyndall effect, by scattering light of colloidal particle exposed to laser beam (Soetarto *et al*. 2012). Reduction of silver ion into AgNP also exhibit brown dark color in culture medium (Fig. 1). The distinctive color of colloidal silver is due to a phenomenon known as plasmon absorbance. Incident light creates an oscillation in conduction electrons on the surface of the nanoparticles and electromagnetic radiation is absorbed (Solomon *et al*., 2007). Similar observation was previously reported for the culture of *Idiomarina* sp. PR58-8, where pale yellow culture change to brown color was formed due to reduction of silver ion (Seshadri *et al*., 2012). This was further confirmed using UV-Visible spectroscopy which measures the absorption spectra of AgNP formed due to the collective excitation of a conduction electron in the metal. The absorption spectrum broad peak is located at 410 nm (Fig 4). TEM analysis reveals biosynthesized AgNP by BAgBK-3 is spherical in shape with size range of 9 – 13 nm (Fig 5).

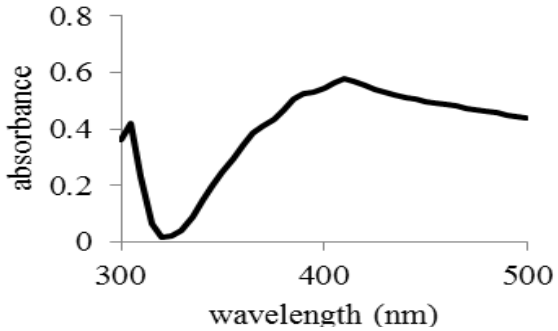

Figure 4. Absorption spectrum of colloidal AgNP synthesized by BAgBK-3

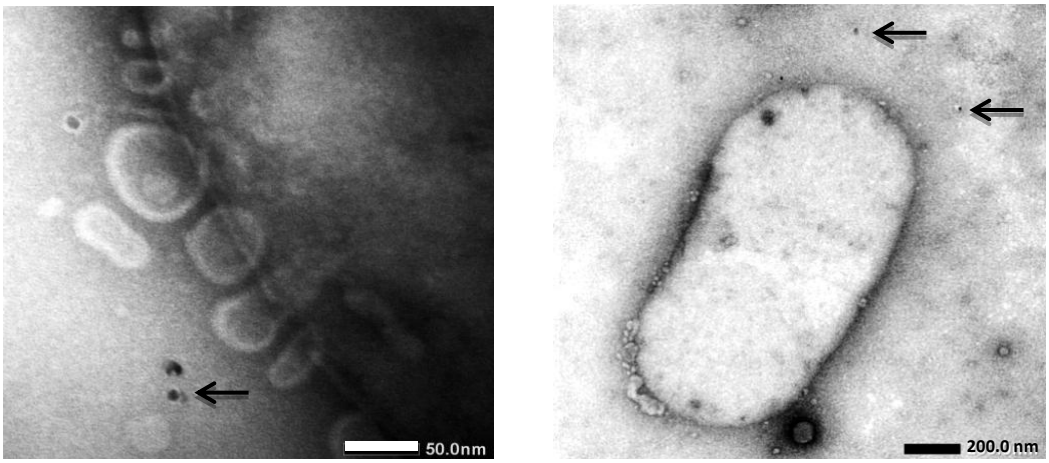

Figure 5. TEM micrograph of AgNP (arrowed) synthesized by BAgBK-3

The bacterial strain BAgBK3 was inoculated in TYEB medium with and without 0.1 mM $AgNO_3$. Both of the cultures showed different growth kinetics form. The culture with silver ion takes same lag phase with control, indicates that *sil* operon is activated during the existence of silver ion. Sil genes play an important roles conduct to responses of silver ion in silver resistance bacteria. The *Salmonella* sp. plasmid pMG100 isolated from hospital involves nine *Sil* genes in three transcription units. The *sil*E operons codes for the periplasmic silver binding proteins, whereas *sil*P and *sil*CBA codes for two efflux pumps (Silver, 2003). Figure 6 exhibits two exponential growth phases namely the diauxic growth curve that caused by catabolite repression. The first exponential phase occur at 10^{th} till 16^{th} hour, which bacteria using carbon source from TYEB medium to synthesize metabolite in order to reduce silver ion. At the second exponential phase (18^{th}–22^{nd} h) bacteria start to use NO_3 inducing nitrate reductase activity.

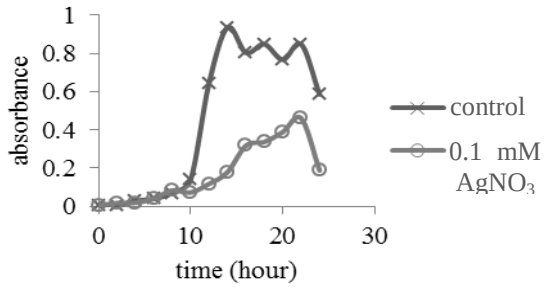

Figure 6. Growth kinetics of strain BAgBK-3 with and without $AgNO_3$

Nitrate reductase is an extracellular enzyme in the nitrogen cycle responsible for the conversion of nitrate to nitrite (Duran *et al.*, 2005). Appearances of nitrate reductase activity were noticed from the purple color change of biomass culture due to the coupling of sulphanilamide with

nitrite forming azo compound (Fig 3). It is confirmed that NADH-dependent nitrate reductase might be responsible in the reduction of silver ions to silver nanoparticles in BAgBK-3 strain culture. This has been demonstrated in *Bacillus licheniformis* (Kalimuthu *et al.*, 2008). The enzyme gains electron from NADH and oxidize it to NAD^+ then undergoes oxidation to reduce the silver ions comes to AgNPs and act as capping and stabilizing agent for AgNPs (Singh *et al.*, 2015). It explains the presence of AgNP in outer cell of bacteria (Fig. 5) correspond to extracellular silver biotransforming by nitrate reductase. Further studies are required on extracting and purifying the biosynthesized AgNPs from bacterial culture.

CONCLUSION

Silver resistant bacteria, BAgBK-3 can transform the silver, $AgNO_3$ as precursor, by reducing Ag^+ ion being colloidal silver nanoparticle at 48 h. UV-Visible absorbance scan of the culture revealed a broad peak at 410 nm, a characteristic of silver nanoparticles. Biosynthesized silver nanoparticles were spherical shape, ranging 9 – 13 nm. The AgNP biotransformation was mediated by NADH-dependent enzyme, nitrate reductase as the stress response of silver ion.

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