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Bacteria as Bioreductor in Synthesis of Silver Nanoparticles

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Abstract:
Background: *Nanotechnology is the most active research field in modern science today. Nanotechnology is thought to influence science, economics and everyday life in the 21st century. The silver nanoparticles have important applications in the pharmaceutical industry. Currently, to develop an environmentally friendly synthesis of nanoparticles, a biological synthesis is carried out using bacteria. In this review, we will explain about bacteria as bioreductors in the manufacture of silver nanoparticles which contains the mechanism of synthesis of silver nanoparticles from bacteria and any bacteria that can be utilized in the synthesis of silver nanoparticles and what are the advantages of synthesizing silver nanoparticles using bacterial bioreductors. In addition, we also discuss the methods for characterizing these silver nanoparticles.*
Materials and Methods: *In compiling this review article, a literature study was carried out with sources in the form of official books and international journals in the last 10 years (2010-2020). In addition, in making this review article, data search was carried out using online media with the keywords being bacteria as bioreductors in synthesis of silver nanoparticles..*
Results: *From this review, the most active bacteria in the synthesis of nanoparticles is Bacillus subtilis, which is capable of producing nanoparticles with a size of 5-94 nm. The characterization of the resulting nanoparticles can use UV-Vis spectrophotometry, FTIR, XRD, and SEM. The advantages of biosynthesis using these bacteria are fast, stable, easy and cost-effective on a large scale, as well as easier control of the shape, size and distribution of the silver nanoparticles produced.*
Key Words: *nanotechnology, silver nanoparticles, bacteria, bioreductors.*

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I. Introduction

Nanotechnology is the most active research field in modern science today. Nanotechnology impacts science, economics and everyday life in the 21st century[1]. Nanotechnology means any technology at the nanoscale that has real-world applications. Nanotechnology encompasses the production and application of physical, chemical and biological sciences at scales from atomic or molecular to submicron dimensions[2]. Nanoparticles are particles in the nanometer size which is about 1-100 nm[3]. Metallic nanoparticles such as silver and gold are most in demand because of their applications in areas such as medicine, electronics, cosmetics, coatings, packaging and biotechnology[1]. The synthesis of metal nanoparticles is an important branch of research in nanotechnology.

Silver is the most commonly used nanomaterial technique in all consumer products[4]. Silver has an important application in the pharmaceutical industry as an anti-bacterial agent such as in the manufacture of topical ointments to prevent infection of burns and open wounds. Currently, to develop environmentally friendly nanoparticle synthesis, biological synthesis is utilized using microorganisms, which has been recognized as a promising source for the manufacture of nanoparticles[5]. The organisms that play the most role in biotechnology are bacteria. Many bacterial cultures are used for various types of nanoparticles, especially silver nanoparticles (SNPs)[1]. The bacteria play the most role in the synthesis of nanoparticles.

Biosynthesis (green synthesis) from nanosilver has received wide attention due to the growing need for environmentally friendly synthesis methods using environmentally friendly reducers using various bacterial species capable of synthesizing NSPs to 60-150 nm sizes using silver-binding peptides[6]. The advantage of biological synthesis over other methods is that green synthesis avoids organic solvents and toxic reagents. Synthetic method for AgNP with biological method seems simple, fast, reliable, and a green approach that can produce good size and morphology in conditions that be optimized for the research[6,7,8].

According to Mudock (2008) in Zhang (2016) that the characteristics feature of nanomaterials are categorized into size, shape, size distribution, surface area, shape, solubility, aggregation, etc.[8]. The shapes of the particles are round, cylindrical, tubular, conical, hollow core, spiral, flat, irregular, etc. The sizes vary from 1 nm to 100 nm in size. The surfaces can be uniform or irregular depending on surface variations. Some forms of nanoparticles are crystalline or amorphous with single or multiple crystalline solids[9].

Characterization of AgNP is important in order to determine the functional aspects of the particles being synthesized. As for the evaluation of synthesized nanomaterials, many analytical techniques have been used, including UV-Vis spectroscopy, x-ray diffractometry (XRD), fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), scanning electron microscope (SEM), transmission electron microscope (TEM), atomic force microscope (AFM), etc.[8,10].

II. Material and Methods

In compiling this review article, a literature study was carried out with sources in the form of official books and international journals for the last 10 years (2010-2020). In addition, in the preparation of this review article, data search was carried out using online media with the keywords being bacteria as bioreductors in synthesis of silver nanoparticles.

III. Results

Potential organisms such as bacteria can be used in the synthesis of nanoparticles where this biosynthetic technique develops an environmentally friendly and economical process, which does not use toxic chemicals in the synthesis process. The types of bacteria that can be used as bioreductors in the process of making silver nanoparticles according to scientific articles that meet international standards.

Table 1. Several types of bacteria play a role in the synthesis of silver nanoparticles as bioreductors using various methods and the size of the particles formed.

No.	Bacterial Species	Type and Concentration of Silver	Instrument Engineering	Particle Size	Reference
1.	*Bacillus cereus* (2011)	$AgNO_3$ with different concentrations of 50,100,500,1000,1500 and 20000 ppm	- UV-Vis spectroscopy (between 430 - 440 nm) -X-ray diffraction (crystalline and metallic)	15 nm (TEM)	11
2.	*Pseudomonas aeruginosa* (2014)	$AgNO_3$1mM	- UV-Vis (420 nm) spectroscopy	50-100 nm (SEM)	12
3.	*Bacillus sp. CS 11* (2014)	$AgNO_3$1mM	UV-Vis spectroscopy (450 nm)	42–94 nm (TEM)	13
4.	Bacillus subtilis T-1 (2015)	$AgNO_3$1mM	- UV-Vis spectroscopy (450-500 nm)	average 13–19 nm (TEM)	14
5.	*Bacillus sp.* (*Brevibacillus borstelensis* MTCC10642) *(2016)*	$AgNO_3$ with various concentrations of 500, 1000, 1200, 1500, 2000, 2500, 3000, 4000, and 5000 ppm	- UV-Vis spectroscopy (430 nm) -FTIR (1345, 1651, 3072, 3626 / cm^{-1}) - X-ray diffraction (crystalline) - SEM (cuboid)	5- 15 nm (SEM)	15
6.	*Escherichia coli (2016)*	$AgNO_3$ 1mM	-Conventional (the change of color from yellow to brown indicates the formation of nanoparticles)	-	2
7.	*Bacillus amyloliquefaciens* - *Bacillus subtilis* (2017)	$AgNO_3$ 3 mM	- UV-Vis spectroscopy (405 nm) - FTIR (3384.91 cm^{-1}) - X-ray diffraction (crystalline) - UV-Vis spectroscopy (420 nm) - FTIR (3386.75 cm^{-1}) - X-ray diffraction (crystalline)	15.9 - 80 nm (SEM)	16
8.	*Pseudomonas sp.* (2017)	$AgNO_3$ 1 mM	- UV-Vis spectroscopy (420 nm) - FTIR (1000-1600 cm^{-1})	-	17
9.	*Bacillus cereus* GCF1I2 (2018)	$AgNO_3$ 1 mM	- UV-Vis spectroscopy (414 nm) - FTIR (1000-1600 cm^{-1})	16,991 nm (SEM)	18
10.	*Bacillus subtilis* T (DA-12) (2018)	$AgNO_3$ 1 mM	- FTIR (absorption occurs at wavelengths 458, 1335, 1338, 1407, 1456, 1506, 1558, 1635, 1653, 1670 and 3442 cm^{-1}) - Optimum formation at 37 ° C within 24 hours - Optimum pH 7	-	19
11.	*Bacillus endophyticus* (2018)	$AgNO_3$ 2 mM	- UV-Vis spectroscopy (420 nm) - FTIR (3400, 2969, 1650, 1560, 1453, 1401, 1227 and 1083 cm^{-1}) -XRD (111, 200, 220 and 311)	5.1 nm (TEM)	20
12.	*Lactobacillus* sp. (2020)	$AgNO_3$ 1 mM	- UV-Vis spectroscopy (420 nm)	3 - 35 nm (TEM)	21

13	Isolate BES-01 - BES-10 (Highest concentration of BES-04) (2020)	$AgNO_3$ 3 mM	- UV-Vis spectroscopy (300 - 800 nm). The result is the BES-04 isolate had the highest AgNP concentration with an absorbance of 2,381	-	22

IV. Discussion

Nanoparticles are particles in the nanometer size which is about 1-100 nm[3]. The silver nanoparticle method is the most commonly used nanomaterial technique in all consumer product[4]. Silver has an important application in the pharmaceutical industry as an anti-bacterial agent such as in the manufacture of topical ointments to prevent infection of burns and open wounds[5]. The technique of making silver nanoparticles using a biosynthetic process (green synthesis) has received widespread attention using various bacterial species[6]. The use of bacteria in the synthesis process of silver nanoparticles acts as a bioreductor. According to Duran *et al*. (2005) the exact mechanism of nanoparticle synthesis using microbes has not known certainly yet, but it is thought that enzymes such as nitrate reductase secreted by microbes help in the bioreduction of metal ions into metal nanoparticles[23]. This was also reported in *Bacillus licheniformis* where nitrate reductase secreted by bacteria was found to be responsible for the reduction of Ag^+ to silver nanoparticles[24].

The formation of silver nanoparticles through the reduction of Ag^+ ions by bacteria can be influenced by culture media when this biosynthesis process has been reported by Baltazar *et al.* (2019) where the production of Ag-NPs with *E. coli* top 10 (Ec-Ts) cultures with different media where the media Luria-Bertani Lennox medium (LB) appeared to be more efficient for the production of nanoparticles of uniform diameter than the other two media tested. This explains that the composition of the media affects the reduction of Ag^+ ions by bacteria where the more nutrients the media has, the more it can cause metabolic changes in bacteria which can cause the appearance of substances that affect the process of reducing Ag^+ ions to AgNPs[25]. Report by Bajracharya (2018) revealed that the silver nanoparticle synthesis test with bacteria *Bacillus subtilis* with a concentration of 1mM silver nitrate ($AgNO_3$), it was found to have the ability to form silver nanoparticles at 37^0C within 24 hours of incubation at pH 7[19]. This means that the optimum conditions for bacteria also need to be controlled in the synthesis of silver nanoparticles using bacterial bioreductors.

Characterization of Silver Nanoparticle Synthesis

Characterization of silver nanoparticles was carried out to determine the functional aspects of the synthesized particles can be carried out by the UV-Vis spectroscopy method. UV-Vis spectroscopy is used to see the maximum absorption peak in the synthesis of nanoparticles by bacteria. Figure 1 shows the maximum absorption peak for the formation of nanoparticles with the help of bacteria as a bioreductor which the use of *Bacillus sp.* CS 11 culture supernatant forms the absorption spectrum for the formation of silver nanoparticles at a strong peak of 450 nm.

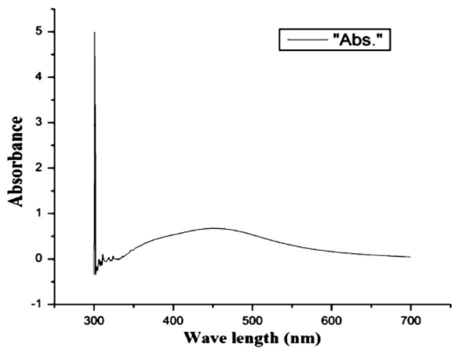

Fig. 1. UV-Vis absorption spectrum of silver nanoparticles synthesized by supernatant *Bacillus sp* CS 11[23].

This method is often used because it is fast, easy, simple, sensitive, selective for various types of nanoparticles, short time for measurement, and calibration is also not required for particle characterization of colloidal suspensions.

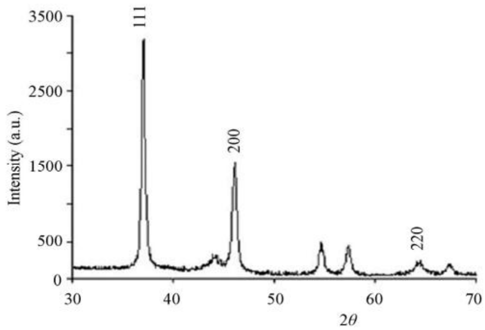

Fig. 2. X-ray diffraction pattern of silver nanoparticles synthesized from *Bacillus cereus* bacteria[11].

To determine the crystalline properties of silver nanoparticles synthesized from bacteria, it can be examined using X-ray diffraction (XRD) techniques. In fig. 2 is an X-Ray Diffraction Pattern (XRD) showing a strong Bragg reflection indexed based on the silver face-centered cubic structure where the XRD patterns that be obtained clearly show the fields [111], [200], [220], [222] and [311] showed that the AgNPs synthesized by *Bacillus cereus* is crystalline in nature[1].

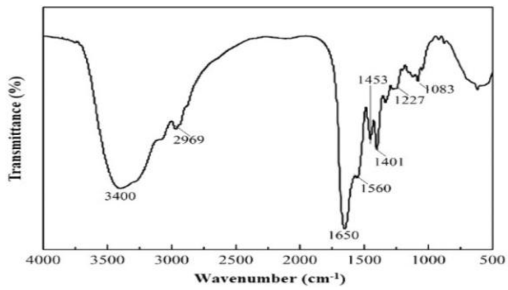

Fig. 3. FTIR spectrum of AgNPs synthesized from bacteria *Bacillus endophyticus* SCU-L[20].

Further analysis was carried out using FTIR analysis. FTIR is a device for identifying the types of chemical bonds in molecules present in AgNPs. Figure 3 shows the FTIR spectrum of AgNPs synthesized from bacteria *Bacillus endophyticus* SCU-L showed several absorption bands around 3400, 2969, 1650, 1560, 1453.1401, 1227 and 1083 cm^{-1}. The peak of 3400 cm^{-1} shows the O - H functional group. At the peak of 2969 cm^{-1} shows the asymmetric CH_2 functional group. The peak at 1453 cm^{-1} shows symmetrical COO functional groups while the absorption peaks at 1650, 1560, 1227, and 1083 cm^{-1} show the functional groups –NH, C = C, C = O and C – O[20].

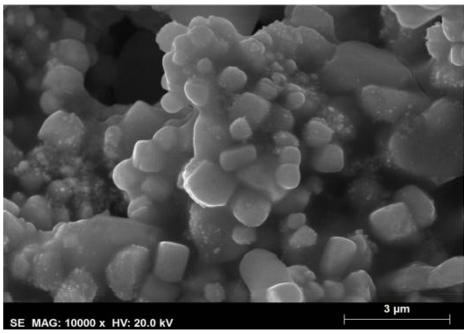

Fig. 4. SEM micrograph of silver nanoparticles synthesized from *Brevibacillus borstelensis* MTCC10642[15] .

Furthermore, the SEM analysis can be carried out where this method aims to determine the size and shape of the synthesized nanoparticles. Figure 4 shows SEM of silver nanoparticles synthesized from *Brevibacillus borstelensis* MTCC10642 confirming that the AgNP obtained is cuboid with a diameter ranging from 5 nm to 15 nm[26]. Apart from the SEM method, you can also use other methods such as the TEM method for characterizing silver nanoparticles synthesized from various types of bacteria.

Finally, the biosynthesis of nanoparticles using bacteria shows a lot of hope that syntheses by this method are more stable than those produced chemically, and can remain stable over a long period of time. In addition, biosynthesis using bacteria is also fast, easy and cost-effective methodology for a large scale production, biological synthesis makes it possible to produce NSPs below the toxic concentration of silver nitrate because the microbial cells can continue to multiply. The biological methods allow easier in control of the shape, size, and distribution of the resulting nanoparticles than the chemical methods. However the disadvantage of biosynthesis with these bacteria is that the purification process can cause pathogenic bacteria and potential bacteria to cause contamination, which should be reason for serious concern in medical applications [6,8,27,28].

V. Conclusion

This review describes bacteria as bioreductors in the manufacture of silver nanoparticles, which contains the synthesis of silver nanoparticles from bacteria and the advantages of synthesizing silver nanoparticles using bacterial bioreductors. From this review, the most active bacteria in the synthesis of nanoparticles is *Bacillus subtilis*, which is capable of producing nanoparticles with a size of 5-94 nm. The characterization of the resulting nanoparticles can use UV-Vis spectrophotometry, FTIR, XRD, and SEM. The advantages of biosynthesis using these bacteria are fast, stable, easy and cost-effective on a large scale, as well as easier control of the shape, size and distribution of the silver nanoparticles which are produced.

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