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"Perspectives on Innovative Sciences"

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BOGOR AGRICULTURAL UNIVERSITY
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FOREWORD

The International Seminar on Sciences 2013, which had the main theme "Perspectives on Innovative Sciences", was organized on November 15th -17th, 2013 by the Faculty of Mathematics and Natural Sciences, Bogor Agricultural University. This event aimed at sharing knowledge and expertise, as well as building network and collaborations among scientists from various institutions at national and international level.

Scientific presentations in this seminar consisted of a keynote speech, some invited speeches, and about 120 contributions of oral and poster presentations. Among the contributions, 66 full papers have been submitted and reviewed to be published in this proceeding. These papers were clustered in four groups according to our themes:

A. Sustainability and Science Based Agriculture

B. Science of Complexity

C. Mathematics, Statistics and Computer Science

D. Biosciences and Bioresources

In this occasion, we would like to express our thanks and gratitude to our distinguished keynote and invited speakers: Minister of Science and Technology, Prof. Manabu D. Yamanaka (Kobe University, Japan), Prof. Kanaya (Nara Institute of Science and Technology, NAIST, Japan), Prof. Ken Tanaka (Toyama University, Japan), Emmanuel Paradis, PhD. (Institut de Recherche pour le Développement, IRD, France), Prof. Dr. Ir. Rizaldi Boer, MS (Bogor Agricultural University), and Prof. Dr. Ir. Antonius Suwanto, M.Sc. (Bogor Agricultural University).

We would like also to extend our thanks and appreciation to all participants and referees for the wonderful cooperation, the great coordination, and the fascinating efforts. Appreciation and special thanks are addressed to our colleagues and staffs who help in editing process. Finally, we acknowledge and express our thanks to all friends, colleagues, and staffs of the Faculty of Mathematics and Natural Sciences IPB for their help and support.

Bogor, March 2014

The Organizing Committee

International Seminar on Sciences 2013

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SYNTHESIS OF SILVER NANOPARTICLES BY USING EXTRACELLULAR METABOLITES OF *Lactobacillus delbrueckii* subsp. *bulgaricus*

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Abstract

The silver nanoparticles were used for many applications such as antifungal, anti bacterial, nanosensors, and food wrappers. Synthesis of silver nanoparticles using microbes as well as metabolites have been developed with consideration of the hazardous waste free environment. The aim of this research was to synthesize silver nanoparticles by using extracellular metabolites of L. delbrueckii subsp. bulgaricus. The synthesis silver nanoparticles was carried out by reacting the extracellular metabolites of L. delbrueckii subsp. bulgaricus with silver nitrate ($AgNO_3$) solution. Reduction of Ag^+ ions occurred by interaction of the extracellular enzyme that produced by L. delbrueckii subsp. bulgaricus. The silver nanoparticles was detected by UV-Vis spectrophotometer based on specific absorption spectrum at the 400 nm wavelength. The result of Fourier Transform Infrared (FTIR) analysis showed that organic component played an important role in the silver nanoparticles formation. The enzyme was estimated as the organic component in the extracellular metabolites of L. delbrueckii subsp. bulgaricus and had a role in the formation of silver nanoparticles. Furthermore, particles size analysis conformed that average size of formed silver nanoparticles was 2.7 nm and considered as silver nanoparticles.

Keywords: *$AgNO_3$, extracellular metabolites, Lactobacillus delbrueckii subsp. bulgaricus, synthesis of silver nanoparticle*

I. INTRODUCTION

Silver nanoparticles have been used in various fields such as antifungal (Vivek *et al* 2011), antibacterial (Prasad *et al* 2011), nanosensor, pesticides, cleaners for water and soil, food wrappers (Bouwmeester, 2007), also can reduce infections after surgery (Kalishwaralal *et al* 2009).

Silver nanoparticles can be synthesized by physically, chemically, and biologically (Kumar 2011). Synthesis of silver nanoparticles by physically and chemically have disadvantages for its stability and aggregation (Kaliswaralal *et al* 2010) and pollute the environment. Synthesis of silver nanoparticles biologically is one of the alternative to produce silver nanoparticles that more safe to the environment. Silver nanoparticles can be obtained from reduction of silver ions by microorganisms such as bacteria, yeast, or fungi (Tolaymat *et al* 2010). Synthesis of nanoparticles using microorganisms are less expensive, non-toxic, high productivity, and easily adapted to the ambient temperature and pressure (Reyes 2009). Therefore, silver nanoparticles have great potential to be developed in the future.

Synthesis of silver nanoparticles using the fungus has also been reported by Sadowski *et al* (2008) from *Aspergillus niger* as an agent biosynthesis. Kumar & Mamidyala (2011) states that the metabolites of *Pseudomonas aeruginosa* can be used for extracellular biosynthesis of silver nanoparticles. *L. delbrueckii* is a Gram positive, facultative anaerobic, homofermentative, rod-shaped, not sporulating, Synthesis of nanoparticles of this bacterium needs to be studied.

This aim of this research was to synthesis of silver nanoparticles by using extracellular metabolites of *L. delbrueckii* subsp.*bulgaricus*. The result of this research can provide scientific information on the biosynthesis of silver nanoparticles by reaction of extracellular metabolites that safe for the

environment and can be applied in the field of medical and industries.

II. Methodology

a. Determination of Growth Curve

Lactobacillus delbrueckii subsp. *bulgaricus was refreshed* on MRS medium and incubated at 42°C for 16 hours at shaker incubator (120 rpm). The growth curve was determined by calculating the optical density (OD) of *Lactobacillus delbrueckii* subsp. *bulgaricus* after incubated at 0, 4, 8, 16, 32, and 64 hours and measured the absorbance at 620 nm wavelength.

b. Synthesis of Silver Nanoparticles (Saravanan *et al* 2011)

The bacteria were incubated at 42 ° C with agitation speed 150 rpm for 24 hours. Cells biomass and medium were separated by centrifugation (16000 g, 10 min). The 100 mL of supernatant culture were mixed with 0.017 grams $AgNO_3$ and incubated in dark chamber at room temperature. Mixture was allowed to stand for 30 minutes until the color changed from clear to brown as an indicator of the reaction between the supernatant and $AgNO_3$

c. Analysis of Silver Nanoparticles With UV-Vis Spectrophotometer (Saravanan *et al* 2011)

Supernatant (reacted with $AgNO_3$) was taken 2 mL for analysis by UV-Vis spectrophotometer and scanning at 200-800 nm wavelength. The formed peak will be analyzed for indication of silver nanoparticles.

d. FTIR Analysis for Silver Nanoparticles (Kumar & Mamidyala, 2011)

Potassium bromide/KBr powder (200 mg) was made into tablets. The supernatant was dripped into potassium bromide tablet and then measured at wavelength 400-4000 cm^{-1}. The results of frequency spectrum measurement will further analyzed using correlation to determine the chemical bonds in organic compounds contained in the sample.

e. Analysis of of Silver nanoparticles Size by Particles Size Analysis/PSA (Kim *et al.* 2006)

The refractive index and viscosity of solution that contained silver nanoparticles were analyzed first and then PSA. The average size of the silver nanoparticles were known by compared with size distribution of silver nanoparticles.

III. RESULTS AND DISCUSSIONS

Growth curves of *Lactobacillus delbrueckii* subsp. *bulgaricus* and $AgNO_3$ Treatment results

Biosynthesis of silver nanoparticles were started with rejuvenation of *Lactobacillus delbrueckii* subsp. *bulgaricus* in order to maintain the growth of bacteria under optimum conditions. *L. delbruecki* subsp. *bulgaricus* grown at 40-43°C as optimum temperature. The culture of *L. delbrueckii* subsp. *bulgaricus* were grown on MRS medium which indicated the changes of medium from clear to cloudy that caused by accumulation of biomass cell.

The aim of growth curves making is to determine optimum growth conditions of *L. delbrueckii* subsp. *bulgaricus*. Observation was done for 64 hours at the 2, 4, 8, 16, 32, 48, 64 hours after incubation (Figure 1). The *L. delbrueckii* subsp. *bulgaricus* was still clear after 2 hours incubation as considered lag phase i.e the phase of bacterial adaptation to the growth medium. Cell biomass increased only slightly during this phase that caused slightly changed culture density. The log phase (exponential) started after 8 hours up to 16 hours incubation which indicated the optical density or turbidity value increased and reached 1,754 (Figure 1). In this phase *L. delbrueckii* subsp. *bulgaricus* have been able to adapt to the medium and population growth increased exponentially (Black 2008). Furthermore, the stages of growth deceleration occurs after 16 hours incubation time. In this phase, changes of nutrition and environmental caused limitation of bacterial growth.

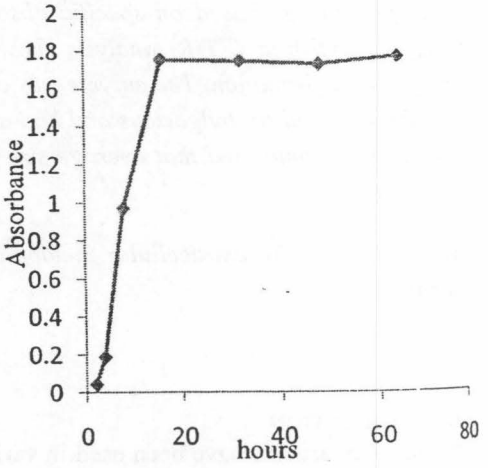
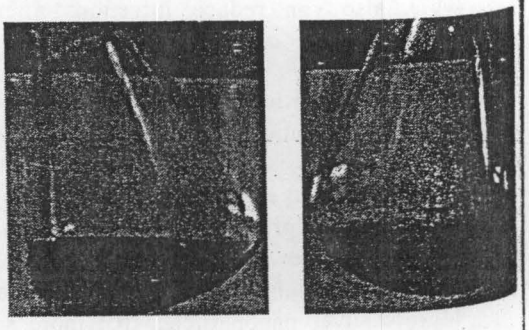


Figure 1. The turbidity growth curve of *Lactobacillus delbrueckii* subsp. *bulgaricus*



before after

Figure 2. Reaction with $AgNO_3$ before addition of $AgNO_3$ and addition of $AgNO_3$ after 30 minutes incubation

Harvesting of *L. delbrueckii* subsp. *bulgaricus* for biosynthesis of silver nanoparticles were carried out after 24 hours incubation time when reached statisioner phase. At the statisioner phase *L. delbrueckii* subsp. *bulgaricus* produced organic compounds such as primary metabolites and secondary metabolites more than at the exponential phase (Kunaepah, 2008). The next stage was separation of cell biomass of *L. delbrueckii* subsp. bulgaricus with growth medium containing compounds the metabolites. Supernatant that containing metabolites of *L. delbrueckii* subsp. *bulgaricus* then added with $AgNO_3$ (0.017 g/100 mL). There was color changes from clear to dark brown and became turbid after incubation for 30 minutes (Figure 2). Reaction of reduction directly observed by color changes of the solution (Kumar 2011). The color changes occurred due to the reaction between organic compounds that accumulate in the bacterial growth medium contained $AgNO_3$ that indicated bioreduction. Then, UV – vis analysis was conducted to confirm whether the compound formed silver nanoparticles.

Biosynthesis of Silver Nanoparticles by *Lactobacillus delbrueckii* subsp. *bulgaricus*

UV-Vis Spectrophotometer Analysis Result

The objectives for confirmation bioreduction of $AgNO_3$ compound into silver nanoparticles was to ensure that the color changes was biochemical reaction that indicates the formation of silver nanoparticles. Indication of nanoparticles formation was known from peak absorption at 370-500 nm wavelength (Solomon *et al.*, 2007). In this research, the formation of absorption peak was at 400 nm wavelength (Figure 3).

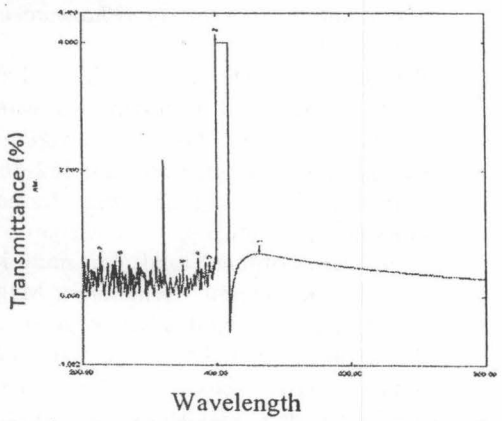


Figure 3. Absorption spectrum of silver nanoparticles by *L. delbrueckii* subsp. *bulgaricus*

The absorption peak at 400 nm wavelength indicated that there had been formation of silver nanoparticles using extracellular metabolites *L. delbrueckii* subsp. *bulgaricus*. According to Solomon *et al.* (2007), absorption of silver formed at range 370-500 nm wavelength. Analysis of UV - Vis spectrophotometer (Figure 3) provided information the amount of nanoparticles formed. The higher absorbance values can be assumed that the number of nanoparticles formed. Absorption peaks were very high that reached maximum absorbance value was 4 that indicated formation of silver nanoparticles in the solution.

Absorption spectrum was very important indicator to determine the formation of silver nanoparticles in the bioreduction of silver (Ag^+) ions. Visual observation of the color changes was not enough to prove whether or not the silver nanoparticles formation.

FTIR Analysis Result for the detection of Functional Groups in Formation of Silver Nanoparticles

FTIR analysis results indicated that there were several strong absorption peaks, i.e at wavelengths of 3432, 1642.1401, 1124 cm^{-1}. The peak indicated the bond contained in organic compounds in solution of silver nanoparticles (Figure 4). Organic compounds released by *L. delbrueckii* subsp. *bulgaricus* had an important role in the bioreduction of silver nitrate compounds. Spectrum with a wavelength of 3432 cm^{-1} showed the content of organic compounds with the N-H bond of amines and amides compounds with moderate intensity. Shaligram *et al.* (2009) stated that presence of amine group indicated presence of proteins in biosynthesis of silver nanoparticles, because this compound was a peptide bond that linked amino acids to form proteins. FTIR analysis results indicated that there was an organic compound that played an important role during formation of silver nanoparticles

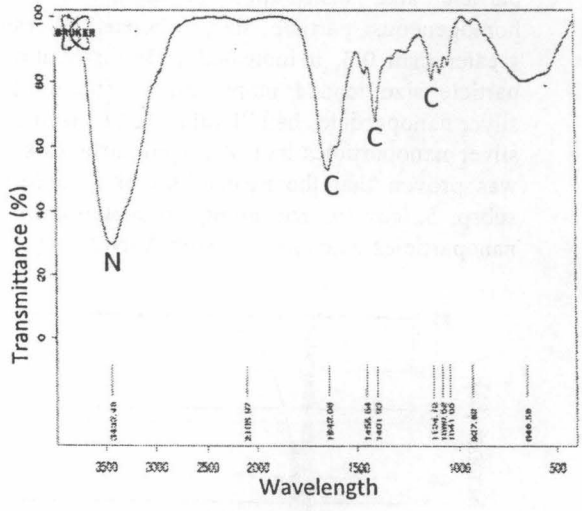


Figure 4. Spectrum of FTIR in silver nano particles solution

These compounds had role in reducing and wrap silver nanoparticles to form stable and homogen sizes of nanoparticles. Protein suspected as one of organic component that had role in the formation of silver nanoparticles. Further analysis is needed to determine specific protein or other organic

compounds that play a role in formation of silver nanoparticles. Reactions that occurred in formation of silver nanoparticles are oxidation and reduction that there was electron transfer between oxidation and reduction agent. The silver (Ag^+) ions was positively charged ions if reacted with organic compounds that produced by metabolic processes of bacteria will release electrons. This condition will change silver (Ag^+) ions turn into Ag^0. The enzyme binded to the nitrate compounds from silver nitrate ($AgNO_3$) and using nitrate as substrate. The enzyme was nitrate reductase that played a role in the reduction of nitrate became nitrite.

The Size of Silver Nanoparticles by PSA

Analysis of the nanoparticles size was performed by using PSA (Particle Size Analysis). PSA was considered more accurate than Scanning Electron Microscope (SEM). Transmission Electron Microscope (TEM) and Scanning Force Microscope (SFM), especially for samples in the order of nanometer and submicron which usually have a high agglomeration tendency (Lidiniyah 2011). PSA measurement result in the form of distribution can be used to determine the overall particle size. Measurements were performed at 25°C using refractive index 1.3390 and viscosity samples were 2.1700 cp. Value of refractive index and viscosity will improve the accuracy of PSA measurement.

Based on the PSA analysis, it was known that average size of silver nanoparticles was 2.7 nm and PI (Polydispersity Index) was 0.351. PI value describes of the width of the size distribution of particles. If PI value less than 0.3, it indicated that particle size distribution is narrow and more homogeneous particle size, whereas PI values greater than 0.3, it indicated wide distribution and particle size tended more varied (Figure 5). The silver nanoparticles had PI value 0.351 that indicated silver nanoparticles had homogen particles size. It was proven that the metabolites of *L. delbrueckii* subsp. *bulgaricus* had ability to synthesis of silver nanoparticles when reacted with $AgNO_3$.

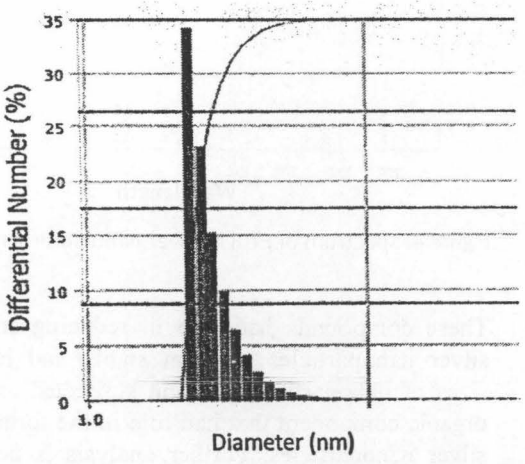


Figure 5. Distribution of silver nanoparticles size

IV. CONCLUSIONS

Metabolites of *Lactobacillus delbrueckii* subsp. *bulgaricus* had ability to synthesize of silver nanoparticles when reacted with $AgNO_3$. The absorption peak of silver nanoparticles occurred at 400 nm wavelength. The organic molecules had a role in formation of silver nanoparticles by reducing $AgNO_3$ into silver nanoparticles. The size of silver nanoparticles was 2.7 nm.

ACKNOWLEDGEMENT

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