Biosynthesis of silver nanoparticles using *Lactobacillus delbrueckii* subsp. *bulgaricus* cultured for anti-fungal activity against *Pyricularia oryzae*

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ABSTRACT

The rice is the main food crop worldwide. The fungi *Pyricularia oryzae* infected the rice and caused a decrease in rice production. The silver nanoparticle (AgNPs) are popular in several research areas such as cosmetics, agriculture, and medicine due to their biological activities and environmentally friendly. Interestingly, the biosynthesis using the microbe is a preferred alternative route for AgNPs production because it does not involve a supplementary reducing agent. The objective of this work was to biosynthesize the AgNPs using *Lactobacillus delbrueckii* subsp. *bulgaricus* and then also evaluate for antifungal activity against *P. oryzae*. Biosynthesis of AgNPs was used *Lactobacillus delbrueckii* subsp. *bulgaricus* cultured with AgNO3 in Man, Rogosa, and Sharpe medium. AgNPs characterized using UV-Vis spectrophotometer and particle size analyzer (PSA). The AgNPs sample at different concentrations (0.1%, 0.25%, 0.5%, 1%, 2.5%, 5%, 7.5%, and 10%) were evaluated for antifungal properties against *P. oryzae* using the agar diffusion method. Results confirmed the absorbance maximum of AgNPs at 423 nm. The size of the AgNPs was 2.0 nm. AgNPs showed antifungal activity against *P. oryzae* and the highest activity was observed at 0.75% concentration. The results showed the AgNPs could be used as a complementary to bio-fungicide against *P. oryzae* in the blast disease treatment of rice.

INTRODUCTION

Rice is the primary food crop for societies worldwide as well as in Indonesia (Panuju et al., 1961; Ma et al., 2020). Based on global data in 2017, Indonesia (81.4 million tons) are the third-largest producer after that China (427.1 million tons) and India (168.5 million tons) (FAOSTAT, 2020). One of the problems in rice production is rice blast disease, which caused by fungus of *Pyricularia oryzae* (Xiao et al., 2018). Globally, rice blast causes a decline in rice production estimated at around 30% (Nalley et al., 2016). Therefore, the search for antifungal of *P. oryzae* is essential needed for improving rice production in agricultural management.

Recently, silver nanoparticles (AgNPs) have been significantly used as an antimicrobial agent in several area application such as cosmetics (Kokura et al., 2010), agricultural Vijayabharchathi et al. (2018); Khaleghi et al. (2019); Torrent et al. (2019), food storage (Bocate et al., 2019), and medicine (Khorrami et al., 2018). The AgNPs can be
produced by the chemical and physical method and also the green synthesis routes, which uses microbrial (yeast, algae, bacteria, and fungi) and plant extracts (de Souza et al., 2019; Khodashenas and Ghorbani, 2019). For hazardous and toxic chemical reasons from the chemical and physical methods, the microbe is a crucial source of a biological system for the AgNPs biosynthesis. Several reports have investigated different microbe which produced AgNPs such as Lactobacillus (Gomma, 2016), Bacillus (Kalishwaralal et al., 2008; Saihudin et al., 2009), Enterobacter (Shahverdi et al., 2007), Escherichia (Maharani et al., 2016), Klebsiella (Müller et al., 2016), Saccharomyces cerevisiae (Korbekandi et al., 2016), and Caulerpa racemosa (Edison et al., 2016).

In this work, the biosynthesis AgNPs was studied using Lactobacillus delbrueckii subsp. bulgaricus culture. Furthermore, antimicrobial activity of AgNPs against P. oryzae was investigated.

MATERIALS AND METHODS

Biosynthesis of AgNPs

The strain L. delbrueckii was purchased from the IPB culture collection, IPB University, Indonesia. The AgNPs was produced according to Saravanan et al. (2011) method with some modification. Briefly, L. delbrueckii was newly inoculated with Man, Rogosa, and Sharpe medium. After incubated in 24 h for 150 rpm at 37°C, the culture filtrates were obtained using centrifugation at 8000 rpm for 10 min. The culture supernatant (100 ml) at pH six was mixed with 0.017 g AgNO3. Finally, the mixture was incubated in a dark room for 30 min. AgNPs characterized by changed color solutions from yellow to brown. The control was used without AgNO3 in solution.

Characterization

The characterization of AgNPs was performed using UV-vis spectrophotometer and particle size analyzer.

Antifungal activity of AgNPs

The biosynthesized Ag nanoparticles were evaluated against P. oryzae by agar well diffusion method, according to Shanthi et al. (2016) with slight modification. P. oryzae was purchased from the IPB Collection Culture, IPB University, Indonesia. P. oryzae was sub-cultured newly in PDA (potato dextrose agar) plates. In the PDA plate, the well with a size of 8.00 mm was created using gel puncture. The sample AgNPs (0.1%, 0.25%, 0.5%, 10%, 25%, 50%, 75%, and 100%) of 20 ml loaded on to the wells. Nystatin and sterile water (control) were also investigated as controls treatment. After P. oryzae incubated at 25°C for five days, the diameter of the inhibition zone was measured, and the data presented in mm.

RESULTS AND DISCUSSION

Biosynthesis and characterization of silver nanoparticle (AgNPs) from L. delbrueckii is shown in Figure 1. Generally, the formation of AgNPs is characterized by the change color from yellow to dark brown (Figure 1 a) in solution AgNPs with L. delbrueckii (Dakhil, 2017). Up to date, the mechanism of AgNPs synthesis by microorganisms is still unclear. Still, the mechanism through microorganisms’ enzymes such as nitrate reductase possible can contribute to reducing silver ions in AgNPs formation (El-Batal et al., 2013; Wang et al., 2018). The nanoparticle of AgNPs was characterized by UV-Vis and PSA, data presented in Figure 1 (b and c). The maximum peak of the UV-Vis spectrophotometer was shown at 423 nm. Mullinger et al. (2007) identified absorption peaks of AgNPs in the range of 370 – 500 nm (Mullinger et al., 2007). This result indicated the colloid formation of AgNPs (Khaleghi et al., 2019). PSA results presented that the average size of AgNPs was 2 nm. Several reports demonstrated different particle sizes of AgNPs from different strain of Bacillus with range of 4 (Babu and Gunasekaran, 2009) to 94 nm (Das et al., 2014; Pourali and Yahyaei, 2016).

The antifungal effect of AgNPs was evaluated in P. oryzae. The inhibition zone diameter of the AgNPs on P. oryzae has been provided in Table 1 and Figure 2. The data clearly presented that AgNPs inhibited P. oryzae growth at different concentrations. The 0.75% AgNPs showed the highest for inhibiting P. oryzae. P. oryzae infected in rice which most destructive effect such as reduces of rice yield and the characteristic of rice (Xiao et al., 2018). This result indicated that potency of the AgNPs for bio-fungicide in agriculture area especially in rice blast management. Similar reports exhibited that AgNPs had antimicrobial activity on fungal pathogens (Vijayabharathi et al., 2018; Bocate et al., 2019; Gulbagca et al., 2019). Several researchers suggested mechanism antimicrobial of AgNPs through disrupting in DNA replications, damage cell membrane, disorderly respiration role, and creating reactive oxygen species (Shanthi et al., 2016; Singh et al., 2016; Khorrami et al., 2018).
Table 1: Antifungal activity of AgNPs from *L. delbrueckii* against *P. oryzae*

| Sample concentration | Inhibition zone (mm) |
|----------------------|----------------------|
| Ag NPs (0.25%)       | 14.67                |
| Ag NPs (0.50%)       | 15.67                |
| Ag NPs (10%)         | 16.33                |
| Ag NPs (25%)         | 18.67                |
| Ag NPs (50%)         | 22.67                |
| Ag NPs (75%)         | 24.33                |
| Ag NPs (100%)        | 15.33                |
| Nystatin (0.1%)      | 29.00                |
| Control              | 0.00                 |

Figure 1: Sample of extracellular metabolite of *L. delbrueckii* (a) without AgNO3 (1) and with AgNO3 (2), and characterization of nanoparticle by UV-Vis spectrophotometer (b) and particle size analyzer (c)
CONCLUSIONS

The present work reports characterization silver nanoparticles (AgNPs) from *Lactobacillus delbrueckii* subsp. *bulgaricus*. Also, the potential of the AgNPs in the application antifungal activity against *Pyricularia oryzae* has been evaluated. Based on the results, the AgNPs characterized by wavelength of absorbance and the average size of 423 nm and 2 nm, respectively. The antifungal activity of AgNPs was shown with highest activity at 0.75% concentration.

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Conflict of Interest

None.

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REFERENCES

Babu, M. M. G., Gunasekaran, P. 2009. Production and structural characterization of crystalline silver nanoparticles from Bacillus cereus isolate. Colloids and Surfaces B: Biointerfaces, 74(1):191–195.

Bocate, K. P., Reis, G. F., de Souza, P. C., Junior, A. G. O., Durán, N., Nakazato, G., Furlaneto, M. C., de Almeida, R. S., Panagio, L. A. 2019. Antifungal activity of silver nanoparticles and simvastatin against toxigenic species of Aspergillus. *International Journal of Food Microbiology*, 291:79–86.
Dakhil, A. S. 2017. Biosynthesis of silver nanoparticles (AgNPs) using Lactobacillus and their effects on oxidative stress biomarkers in rats. Journal of King Saud University - Science, 29(4):462-467.

Das, V. L., Thomas, R., Varghese, R. T., Soniya, E. V., Mathew, J., Radhakrishnan, E. K. 2014. Extracellular synthesis of silver nanoparticles by the Bacillus strain CS11 isolated from industrialized area. 3 Biotech, 4(2):121-126.

de Souza, T. A. J., Souza, L. R. R., Franchi, L. P. 2019. Silver nanoparticles: An integrated view of green synthesis methods, transformation in the environment, and toxicity. Ecotoxicology and Environmental Safety, 171:691-700.

Edison, T. N. J. I., Atchudan, R., Kamal, C., Lee, Y. R. 2016. Caulerpa racemosa: a marine green alga for eco-friendly synthesis of silver nanoparticles and its catalytic degradation of methylene blue. Bioprocess and Biosystems Engineering, 39(9):1401-1408.

El-Batal, A. I., Amin, M. A., Shehata, M. M. K., Hallol, M. M. A. 2013. Synthesis of silver nanoparticles by Bacillus stearothermophilus using gamma radiation and their antimicrobial activity. World Appl Sci J, 22(1):1-16.

FAOSTAT 2020. Crops: rice, paddy. [Accessed On 2020, January 24].

Gomma, E. Z. 2016. Exopolysaccharide-mediated silver nanoparticles produced by Lactobacillus brevis NM101-1 as antibiotic adjuvant. Microbiology, 85(2):207-219.

Gulbagca, F., Ozdemir, S., Gulcan, M., Sen, F. 2019. Synthesis and characterization of Rosa canina-mediated biogenic silver nanoparticles for antioxidant, antibacterial, antifungal, and DNA cleavage activities. Heliyon, 5(12):e02980-e02980.

Kalishwaralal, K., Deepak, V., Ramkumarpandian, S., Nellaiah, H., Sangiliyandi, G. 2008. Extracellular biosynthesis of silver nanoparticles by the culture supernatant of Bacillus licheniformis. Materials Letters, 62(29):4411-4413.

Khaleghi, M., Khorrami, S., Ravan, H. 2019. Identification of Bacillus thuringiensis bacterial strain isolated from the mine soil as a robust agent in the biosynthesis of silver nanoparticles with strong antibacterial and anti-biofilm activities. Biocatalysis and Agricultural Biotechnology, 18:101047-101047.

Khorashehs, B., Ghorbani, H. R. 2019. Synthesis of silver nanoparticles with different shapes. Arabian Journal of Chemistry, 12(8):1823-1838.

Khorrami, S., Zarrabi, A., Khaleghi, M., Danaei, M., Mozafari, M. R. 2018. Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. International Journal of Nanomedicine, Volume 13:8013-8024.

Kokura, S., Handa, O., Takagi, T., Ishikawa, T., Naito, Y., Yoshikawa, T. 2010. Silver nanoparticles as a safe preservative for use in cosmetics. Nanomedicine: Nanotechnology, Biology and Medicine, 6(4):570-574.

Korbekandi, H., Mohseni, S., Jouneghani, R. M., Pourhossein, M., Iravani, S. 2016. Biosynthesis of silver nanoparticles using Saccharomyces cerevisiae. Nanomedicine, and Biotechnology, 44(1):235-239.

Ma, Z., Wang, Y., Cheng, H., Zhang, G., Lyu, W. 2020. Biochemical composition distribution in different grain layers is associated with the edible quality of rice cultivars. Food Chemistry, 311.

Maharani, V., Sundaramanickam, A., Balasubramanian, T. 2016. In vitro anticancer activity of silver nanoparticle synthesized by Escherichia coli VM1 isolated from marine sediments of Ennore south-east coast of India. Enzyme and Microbial Technology, 95:146–154.

Mulflinger, L., Solomon, S. D., Bahadory, M., Jayarajasingam, A. V., Rutkowsky, S. A., Boritz, C. 2007. Synthesis and Study of Silver Nanoparticles. Journal of Chemical Education, 84(2):322-322.

Müller, A., Behsnilian, D., Walz, E., Gräf, V., Hogekamp, L., Greiner, R. 2016. Effect of culture medium on the extracellular synthesis of silver nanoparticles using Klebsiella pneumoniae, Escherichia coli and Pseudomonas Jessinii. Biocatalysis and Agricultural Biotechnology, 6:107–115.

Nalley, L., Tsiboe, F., Durand-Morat, A., Shew, A., Thoma, G. 2016. Economic and Environmental Impact of Rice Blast Pathogen (Magnaporthe oryzae) Alleviation in the United States. PLOS ONE, 11(12):e0167295–e0167295.

Panuju, D. R., Mizuno, K., Trisasongko, B. H. 1961. The dynamics of rice production in Indonesia. Journal of the Saudi Society of Agricultural Sciences, 12(1):27–37.

Pourali, P., Yahyaei, B. 2016. Biological production of silver nanoparticles by soil isolated bacteria and preliminary study of their cytotoxicity and cutaneous wound healing efficiency in rat. Journal of Trace Elements in Medicine and Biology, 34:22–31.

Saifuddin, N., Wong, C. W., Yasumira, A. A. N. 2009. Rapid Biosynthesis of Silver Nanoparticles Using Culture Supernatant of Bacteria with Microwave Irradiation. E-Journal of Chemistry, 6(1):61–70.
Saravanan, M., Nanda, A., Kingsley, S. J. 2011. Lactobacillus delbrueckii mediated synthesis of silver nanoparticles and their evaluation of antibacterial efficacy against MDR clinical pathogens. *International Conference on Nanoscience, Engineering and Technology.*

Shahverdi, A. R., Minaeian, S., Shahverdi, H. R., Jamalifar, H., Nohi, A.-A. 2007. Rapid synthesis of silver nanoparticles using culture supernatants of Enterobacteria: A novel biological approach. *Process Biochemistry, 42*(5):919–923.

Shanthi, S., Jayaseelan, B. D., Velusamy, P., Vijayakumar, S., Chih, C. T., Vaseeharan, B. 2016. Biosynthesis of silver nanoparticles using a probiotic Bacillus licheniformis Dahb1 and their antibiofilm activity and toxicity effects in Ceriodaphnia cornuta. *Microbial Pathogenesis, 93*:70–77.

Singh, P., Singh, H., Kim, Y. J., Mathiyalagan, R., Wang, C., Yang, D. C. 2016. Extracellular synthesis of silver and gold nanoparticles by Sporosarcina koreensis DC4 and their biological applications. *Enzyme and Microbial Technology, 86*:75–83.

Torrent, L., Marguí, E., Queralt, I., Hidalgo, M., Iglesias, M. 2019. Interaction of silver nanoparticles with mediterranean agricultural soils: Lab-controlled adsorption and desorption studies. *Journal of Environmental Sciences, 83*:205–216.

Vijayabharathi, R., Sathy, A., Gopalakrishnan, S. 2018. Extracellular biosynthesis of silver nanoparticles using Streptomyces griseoplanus SAI-25 and its antifungal activity against Macrophomina phaseolina, the charcoal rot pathogen of sorghum. *Biocatalysis and Agricultural Biotechnology, 14*:166–171.

Wang, L., Zhang, H., Rehman, M. U., Mehmood, K., Jiang, X., Iqbal, M., Tong, X., Gao, X. 2018. Antibacterial activity of Lactobacillus plantarum isolated from Tibetan yaks. *Microbial pathogenesis, 115*:293–298.

Xiao, M., Ma, Y., Feng, Z., Deng, Z., Hou, S., Shu, L., Lu, Z. 2018. Rice blast recognition based on principal component analysis and neural network. *Computers and Electronics in Agriculture, 154.*