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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 *Pyriculariaoryzae* 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%, 10%, 25%, 50%, 75%, and 100%) 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.

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INTRODUCTION

Rice is the primary food crop for societies world wide 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 Vijayabharathi *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 microbial (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; Saifuddin *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 sixwas 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. Mulfinger *et al.* (2007) identified absorption peaks of AgNPs in the range of 370 – 500 nm (Mulfinger *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

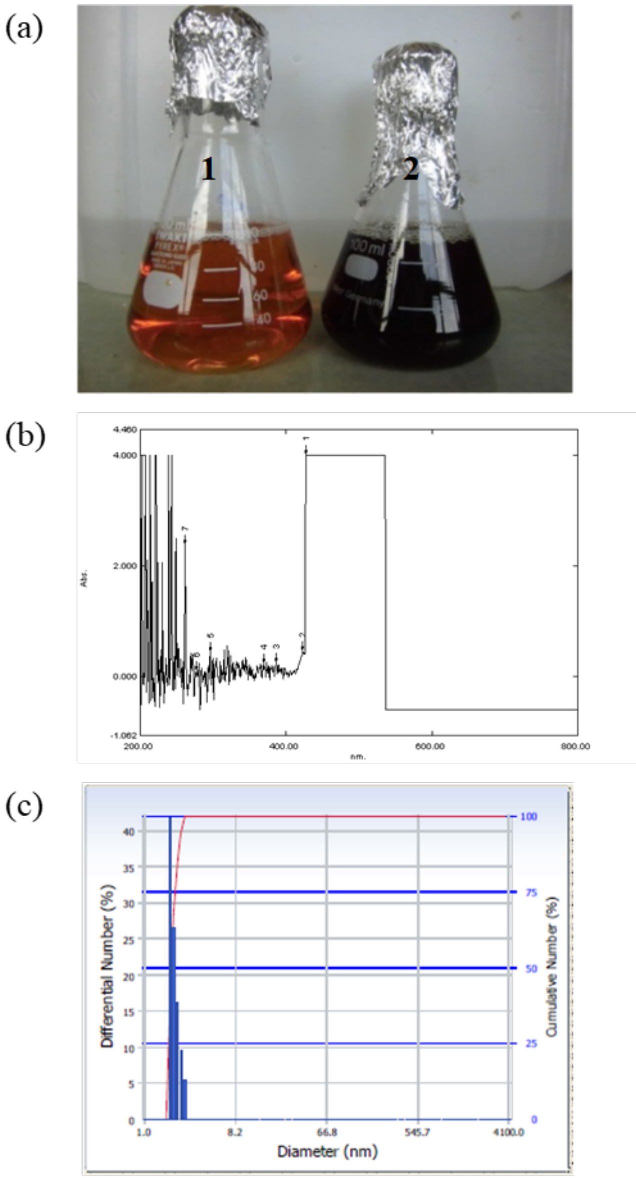

Figure 1: Sample of extracellular metabolite of *L. delbrueckii* (a) withoutAgNO3 (1) and with AgNO3 (2), and characterization of nanoparticle by UV-Vis spectrophotometer (b) and particle size analyzer (c)

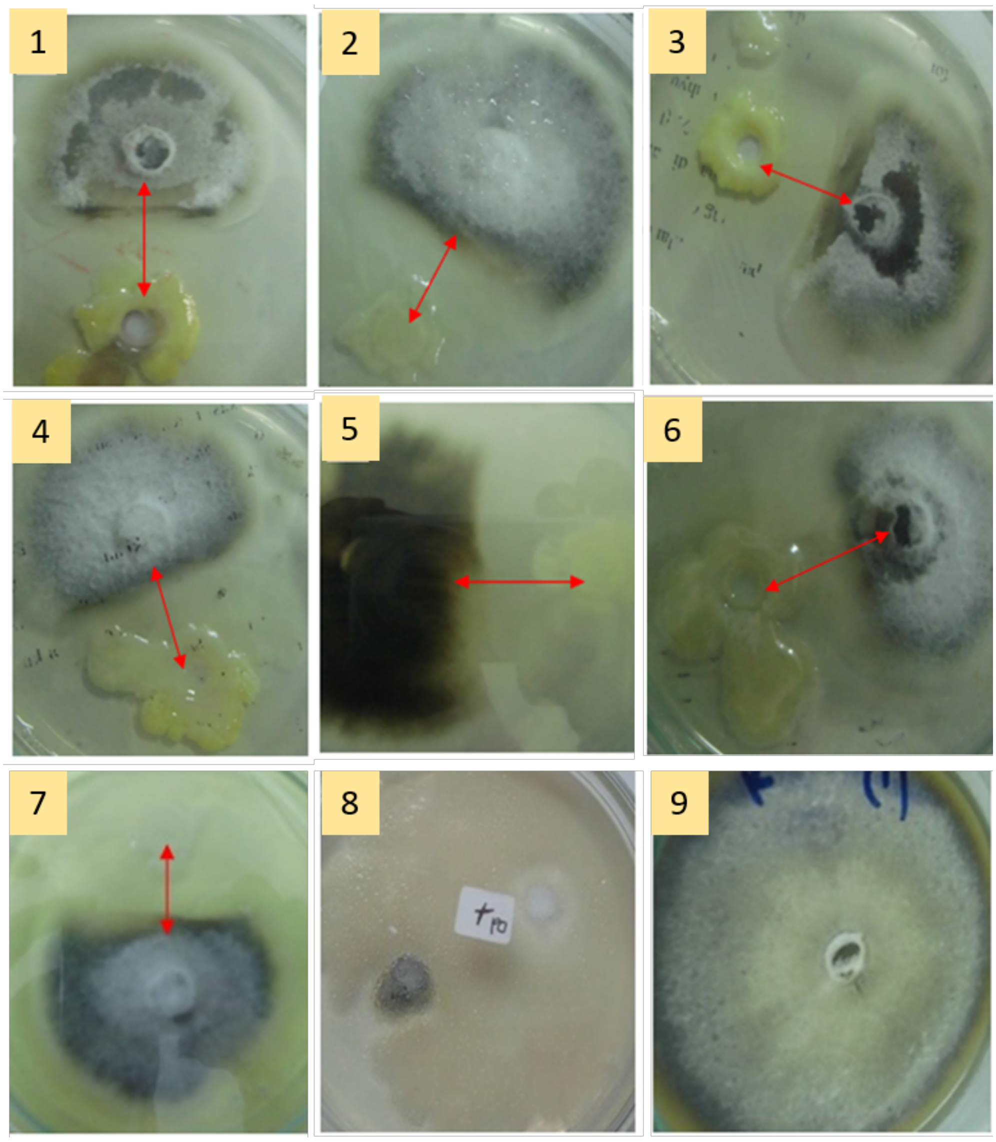

Figure 2: Antifungal activity against *P. oryzae* from AgNPs with concentration of (1) 0.25%; (2) 0.5%; (3) 10%; (4) 25%; (5) 50%; (6) 75%; and (7) 100%; and also (8) nystatin (0.1%) and (9) control. –> = showed of inhibition zone

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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