

## MORPHOLOGICAL IDENTIFICATION OF BACTERIA ISOLATED FROM MUSHROOM BAGLOG AND ITS EFFECT ON SHIITAKE (*Lentinula edodes* (Berk.) Pegler) AND REISHI (*Ganoderma lucidum* (Curtis) P. Karst) MYCELIAL GROWTH

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### ABSTRACT

Reishi (*Ganoderma lucidum* (Curtis) P. Karst) and shiitake (*Lentinula edodes* (Berk.) Pegler) mushrooms have high economic value and contain bioactive metabolites that provide health benefits. Shiitake mushrooms have a delightfully unique taste as an edible mushroom. Mushroom production can be increased by utilizing microbes known as MGPB (Mushroom Growth Promoting Bacteria). This study aimed to determine the presence of bacteria associated with shiitake and reishi mushrooms in the baglog and determine how these bacteria influence the growth of shiitake and reishi mycelium. Bacteria was isolated from the mushroom baglog then were purified and centrifuged to obtain the cell-free extract. Furthermore, the bacterial cell-free extracts were poured on PDA media, then shiitake and reishi mycelium were inoculated to test the effect of bacteria isolates on fungal growth. The results showed that three isolates have the potential as MGPB in reishi mushroom, i.e., isolate S1C, S1D, and S2A. Reishi mycelium grown on isolate S2A achieved the fastest maximum growth on day 6th. In shiitake mushroom, it was seen that all isolates showed a negative effect on mycelium growth. Shiitake mycelium in isolate S1C, S1D, S2A, and S2B were seen to form a clear zone on PDA medium.

*Keywords:* Shiitake, Reishi, bacteria, MGPB

### INTRODUCTION

Shiitake mushroom (*Lentinula edodes* (Berk.) Pegler) has high economic value and delightfully unique taste. Shiitake contains bioactive metabolites with cholesterol-lowering and antitumor properties, such as eritadenine (Duran-Rivera *et al.*, 2020) and polysaccharides lentinan (Ruthes *et al.*, 2016). Moreover, reishi mushroom

(*Ganoderma lucidum* (Curtis) P. Karst) has been known as herbal medicine that has antidiabetic (Vitak *et al.*, 2017) and anticancer properties (Skolastika, 2019).

Based on Badan Pusat Statistik (2019), mushroom yields have decreased from 2016 to 2018. On the other hand, mushroom consumption in Indonesia has increased

every year (Direktorat Jenderal Hortikultura, 2017). Thus it is necessary to increase the yield and production of mushrooms. Fungal growth can be increased by utilizing MGPB (Mushroom Growth Promoting Bacteria). The use of microbes as a substitute for fertilizers can reduce the use of chemicals and can preserve the environment according to the concept of sustainable agriculture.

Several bacteria have been identified as MGPB, as reported by Febriansyah *et al.* (2018), that *B. cereus*, *Acinetobacter pittii*, and *B. aryabhatai* produced IAA and act as MGPB. Apart from stimulating the growth of fungal mycelium, some bacteria are known as pathogens in fungi; for instance, *Pseudomonas tolaasii* cause brown blotch disease on cultivated *Agaricus bisporus* (Abou-Zeid, 2012).

Therefore, the objective of this study was to determine the effect of bacteria associated in the fungal baglog on the growth of shiitake and reishi fungi and determine the morphological form of the isolated bacteria.

## **MATERIALS AND METHODS**

### **Preparation of Culture Media for Bacteria and Fungi**

There were three types of media used in this study, i.e., LB (Lactose Broth), NB (Nutrient Broth), and PDA (Potato Dextrose Agar) instant media with doses of 2.5 g/100 mL, 0.8 g/100 mL, and 3.9 g/100 mL, respectively. The mixture of each medium was homogenized on a magnetic stirrer for 5 minutes, then heated in a microwave for 15 minutes and sterilized in an autoclave at a temperature of 121°C for 15 minutes.

### **Isolation and Purification of Bacteria**

Bacteria were isolated from reishi mushroom baglog originating from mushroom cultivation in Sumedang, West Java, and shiitake mushroom baglog from Sanggar Tani Media Agro Merapi, Yogyakarta Special Region. From each type of fungus, two baglogs were used as the population. The isolation method used was serial dilution ( $10^{-4}$  dilution factor). Bacteria isolated from the fungal baglog were then inoculated in LB agar medium by the pour plate method, each colony which had a different macroscopic morphology purified by the streak plate method to obtain a single colony.

### **Isolation of Shiitake and Reishi Fungi**

Fresh shiitake and reishi fruit bodies are used as a source of mycelium. Mushroom fruit bodies were sterilized with 70% alcohol, then split and cut into small pieces and inoculated on PDA medium for 7 days.

### **Morphological Identification of Bacteria**

Observation of bacterial morphology was carried out macroscopically and microscopically, including the shape, color, margin, and elevation of the colony, as well as gram staining and observation of the bacterial cell shape. Observation on a microscope using 1000x magnification with the aid of oil immersion to achieve greater clarity.

### **Preparation of Bacterial Cell-Free Extract**

The bacteria isolates were inoculated on NB medium and shaken for 24 hours, then it was centrifuged for 30 minutes at 8000 rpm at 4°C to obtain the bacterial cell-free extract.

### **Test on Effect of Bacterial Cell-Free Extract on Shiitake and Reishi Mycelial Growth**

The bacterial cell-free extract was tested toward the growth of fungal mycelium. 2 mL of bacterial cell-free extract

was poured into 18 mL of PDA medium, then shiitake and reishi mycelium were inoculated on the medium. The diameter of the growing mycelium was measured on day 3 until the mycelium in the control treatment fully cover the medium in petri dish.

## **RESULTS AND DISCUSSION**

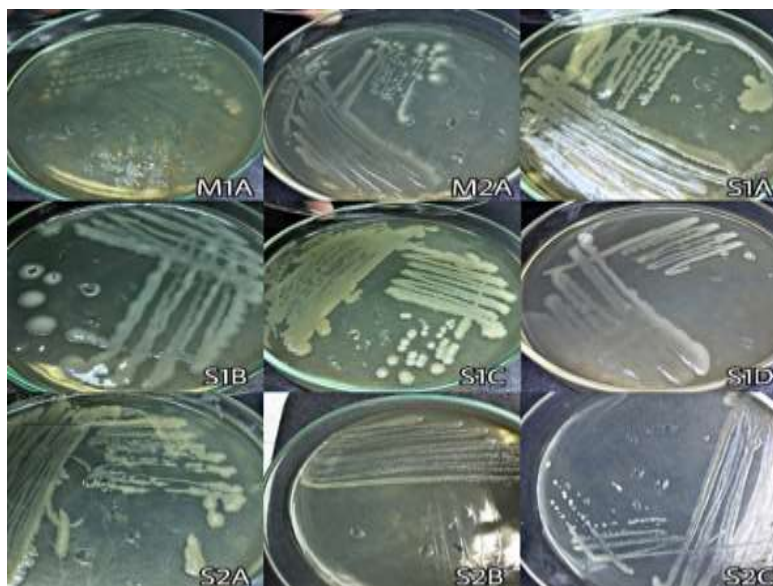
### **Result**

#### **Isolation of Bacteria from Shiitake and Reishi Baglog**

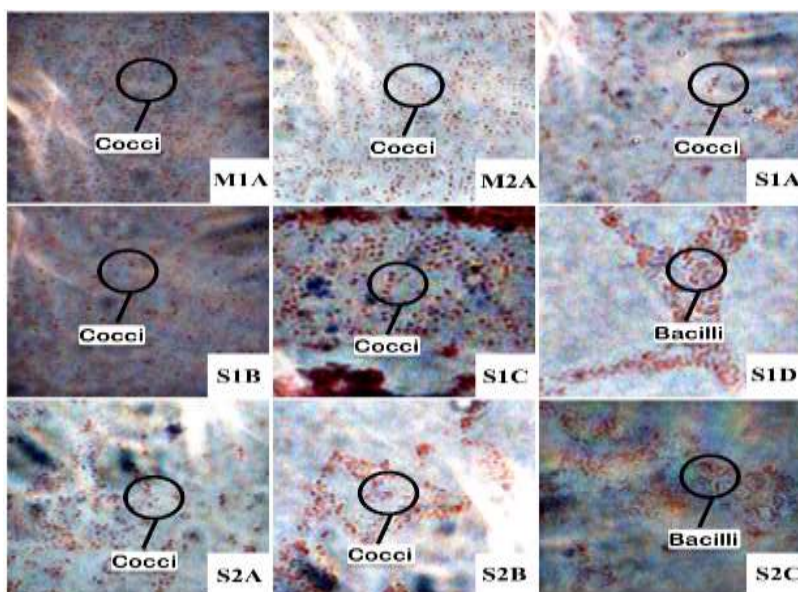
The total bacteria isolated in this study were nine isolates, i.e, isolates M1A, M2A, S1A, S1B, S1C, S1D, S2A, S2B, and S2C. The letter M shows the bacteria isolated from baglog originating from Merapi, while the letter S is the bacteria that isolated from baglog originating from Sumedang.

#### **Isolation of Shiitake and Reishi Mushrooms**

The results of mushroom isolation showed a variation in the growth rate of shiitake and reishi mycelium. Shiitake mycelium on PDA medium (without bacterial treatment) fully covered the medium on day 13, while reishi mycelium on PDA medium (without bacterial treatment) fully covered the medium on day 9.



**Fig. 1.** Colony morphology of bacteria isolated form shiitake and reishi baglog



**Fig. 2.** Gram stained bacteria under microscope

### Morphological Identification of Bacteria

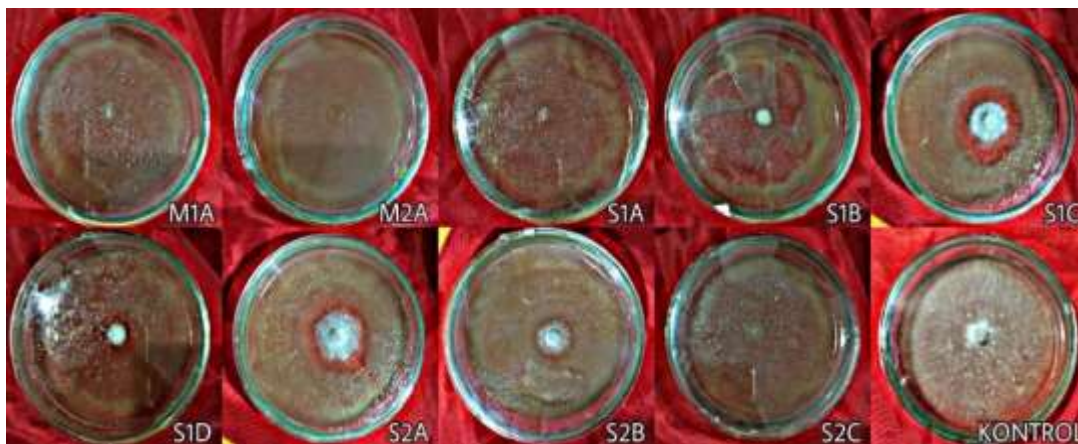
Figure 1. shows the various macroscopic morphological characteristics in terms of colony shape, color, margin, and colony elevation.

Figure 2. shows all bacteria exhibit red color after gram staining. Seven bacteria isolates were cocci (round-shaped cells), and two bacteria were bacilli (rod-shaped cells).

**Test on Effect of Bacterial Cell-Free Extract on Shiitake and Reishi Mycelial Growth**

Figure 3. shows that on the day 13 of observation, only the mycelium in the control fully covered the medium (on 9 cm diameter petri dish). It can be seen that there is a clear zone around the shiitake mycelium

grown on isolate S1C, S1D, S2A, and S2B. Compared to the control treatment, there were no isolates potentially promote the growth of shiitake mycelium (Table 1). Shiitake mycelium on isolate M1A, M2A, S1A, S1B, and S2C not growing from day 3 until the last day of observation (day 13).

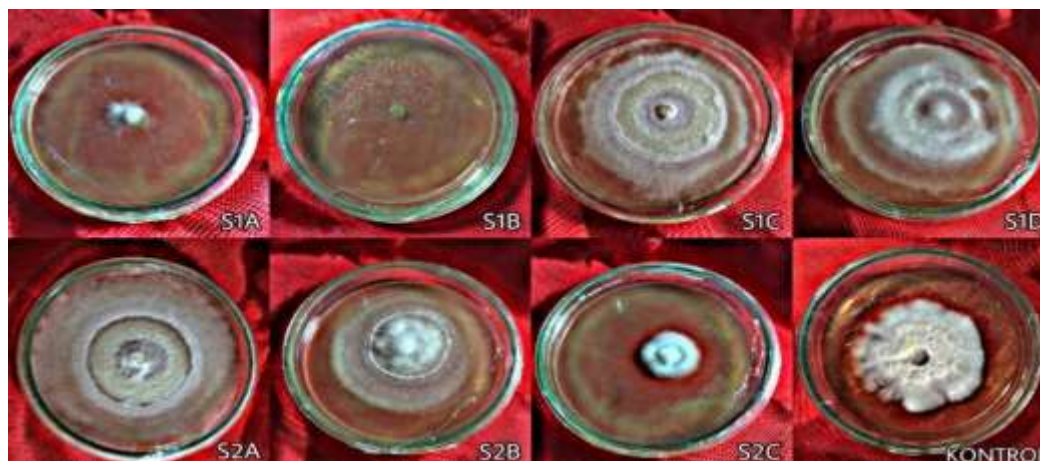


**Fig. 3.** Shiitake mycelium on PDA media contain bacterial cell-free extract on day 13 observation

Table 1. The diameter of shiitake mycelium from day 3 to day 13 observation

Day of observation	M1A	M2A	S1A	S1B	S1C	S1D	S2A	S2B	S2C	kontrol
3	0	0	0	0	1,2	1	1	1,3	0	1,4
4	0	0	0	0	1,6	1,2	1,4	1,8	0	1,9
5	0	0	0	0	1,9	1,2	1,6	2	0	2,7
6	0	0	0	0	2,2	1,2	2	2,1	0	3,6
7	0	0	0	0	2,6	1,2	2,3	2,1	0	4,4
8	0	0	0	0	2,7	1,2	2,5	2,1	0	5,2
9	0	0	0	0	2,7	1,2	2,8	2,1	0	6,2
10	0	0	0	0	2,9	1,2	3,1	2,1	0	7,1
11	0	0	0	0	2,9	1,2	3,2	2,1	0	7,9
12	0	0	0	0	2,9	1,2	3,2	2,1	0	8,5
13	0	0	0	0	2,9	1,2	3,2	2,1	0	9





**Fig. 4.** Reishi mycelium on PDA media contain bacterial cell-free extract on day 6 observation

Table 2. The diameter of reishi mycelium from day 3 to day 9 observation

Day of observation	S1A	S1B	S1C	S1D	S2A	S2B	S2C	kontrol
3	0	0	6,2	5,1	6,1	5,4	1,2	2,8
4	1	0	6,8	5,8	6,8	6,5	1,5	3,9
5	1,3	0	7	6,5	7,3	6,8	2,1	5
6	2,0	0	7,8	8,2	9	7	2,2	6
7	3,2	0	9	9	9	8	3,3	7
8	4,5	0	9	9	9	8,5	3,7	8,3
9	5,7	0	9	9	9	8,7	4	9

Figure 4. shows reishi mycelium on isolate S2A treatment had fully covered the medium on the day 6 of observation. Table 2 shows that the mycelium treated with isolate S1C, S1D, and S2A grew faster than mycelium in control treatment. Those isolates fully covered the media on day 7th. Compared to the control treatment and other isolates, mycelium that treated with isolate

S1A, S1B, and S2C cell-free extract had much slower growth rate.

### Discussion

The result of this study indicates that the number of bacteria isolates found in the reishi baglog is more than in the shiitake baglog. It is assumed that low bacteria count in shiitake baglog is caused by antimicrobial component in shiitake mushrooms, which

suppresses bacterial growth. Kupcová *et al.* (2018) found that Lenthionine is a component of shiitake mushrooms that exhibits antibacterial and antifungal activity.

Fungal mycelium isolated from the fruiting body and grown on PDA media without bacterial cell-free extract treatment showed that shiitake mycelial growth was slower than reishi. On day 9 of observation, the reishi mycelium had fully covered the medium (9 cm), whereas shiitake mycelium reached 9 cm in diameter on day 13. These results match those observed in the earlier study by Nguyen *et al.* (2019) and Quaicoe *et al.* (2014), who investigated the growth of reishi and shiitake mushroom, respectively.

Figure 1 shows the different macroscopic morphology of bacteria colonies. A bacteria colony is a visible mass formed by large numbers of bacteria. Each species of bacteria has a different colony morphology. Figure 2 shows that all bacteria isolates are gram negative as indicated by the appearance of a red color after the gram staining process. These results are consistent with those of Tejedor-Calvo *et al.* (2020), who found that the majority of the isolates, which isolated from the shiitake growing media, were gram negative. Isolate S1D and S2C are bacteria that have rod-

shaped cells. Based on the macroscopic and microscopic morphology, it is assumed that isolate S1D and S2C have similarities with *Pseudomonas* (Public Health England, 2013) and *Ewingella* genus (Bukhari *et al.*, 2008).

As shown in Table 1, there were no isolate that promote shiitake mycelial growth. It is suspected that the nine isolates are antagonistic bacteria that produce toxins that inhibit mycelial growth. Lo Cantore *et al.* (2015) found that *P. tolaasii* is a pathogen that produce volatile compounds such as methanethiol, dimethyl disulfide, and 1-undecene, which inhibit growth and cause browning of mycelium in various fungi. There were clear zone around the shiitake mycelium that was grown on isolate S1C, S1D, S2A, and S2B (Figure 3). It is assumed that the clear zone formed due to extracellular enzymes produced by shiitake mycelium. Those enzymes can hydrolyze the free extract of bacterial cells containing organic compounds. Several types of extracellular enzymes produced by shiitake are cellulase, laminarinase, and xylanase (Mata *et al.*, 2016).

It can be seen from the data in Table 2 that three isolates can be utilized as MGPB. The rapid growth of reishi mycelium in cell-

free extracts of isolate S1C, S1D, and S2A is thought to be caused by the secretion of compounds that act as hormones or nutrients for fungal mycelium growth. Young *et al.* (2013) found that some MGPB are able to fix nitrogen, dissolve phosphate, and have cellulase activity associated with *Agaricus blazei*. On the other hand, the reishi mycelium in isolate S1A, S2C, and S1B bacteria did not grow well. It is assumed that those three isolates produce compounds that are toxic to reishi mycelium. Irma *et al.* (2018) found that *P. aeruginosa* bacteria inhibited the growth of the *Ganoderma boninense* fungus, where glucanase is a fungal cell wall degrading compound produced by *P. aeruginosa* (Suryadi *et al.*, 2014).

Taken together, the results of this study indicated that the bacteria that potentially act as MGPB on reishi mushrooms have the opposite effect on shiitake by suppressing mycelium growth. Tejedor-Calvo *et al.* (2020) found that *Ewingella americana* is a bacterium that produces toxins and causes the degradation of shiitake mycelium. In contrast, the study by Zarenejad *et al.* (2012) discovered that fluorescent *Pseudomonas* spp. gave positive impact on the cultivation of *Agaricus*

*bisporus*. Moreover, Oh *et al.* (2018) has indicated that *E. americana* can increase 194% the growth of *Tricholoma matsutake* mycelium. Therefore, it seems that the interactions between bacteria and fungi are complex and specific. Within one genus or species of bacteria, it can cause different effects on fungi, stimulating growth and development, as well as damaging and being pathogenic to fungi.

## CONCLUSION

This study has shown that two bacteria (isolate S1D and S2C) isolated from mushroom baglog had similarities to *Pseudomonas* and *Ewingella* genus. It was also shown that there were no bacteria that could stimulate the growth of shiitake mycelium; however, there are three isolate (S1C, S1D, and S2A) that have the potential as MGPB to reishi mushroom.

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