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The Potential of *Bacillus* sp.'s Isolate of Coffee Beans as Plant Pest Control Candidates

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ABSTRACT

Long-term use of chemical pesticides in high doses might result in chemical residues. Therefore, eco-friendly plant pest control should be getting more attention. To do so, one might consider the utilization of microorganisms that have the enzymatic ability to destroy the pest's body structure. This study aimed to explore the isolates of Bacillus sp. from coffee beans as a plant pest control candidate. Bacillus could be considered due to its ability to produce protein crystals and extracellular enzymes. Three isolates are obtained from isolation from coffee beans: *Bacillus* sp. 1; *Bacillus* sp. 2; and *Bacillus* sp. 3. These isolates have the enzymatic character of protease, chitinase, and lipase. The results of the bioassay test on larvae of *Plutella xylostella* (Lepidoptera order) showed that these isolates were able to cause larvae death within 48 hours.

INTRODUCTION

Plant pests have been a major problem for anyone working in the agricultural business, as they might spread out widely, exacerbating the affected plant's productivity. These pests can reduce plant productivity both qualitatively and quantitatively. A result of pest attacks can reduce the productivity of plantation crops by up to 40% (Anonymous, 2011). According to Van and Muller (2005), the activity of insect larvae in the fruit, such as eating the pulp, causes the puncture marks to expand and the fruit to rot before cooking. The losses caused by these pests reach 30-60%. In general, chemical plant pest control is one of the most popular tools; however, it also causes an adverse effect as it generates chemical residues. Yuantri et al. (2013) show that the use of pesticides by farmers causes poisoning for farmers, pesticide residues settle in the soil, and pesticides sprayed on plants are absorbed through the leaves, stems, and roots of the plants. Therefore, there is a rising demand for eco-friendly pest control, including utilizing microorganisms that have the enzymatic ability to destroy the body structure of pests.

Bacillus is one of the microbes that can be used to control plant pests, as it produces endospores that enable them to survive in harmful environmental conditions (Cavaglieri et al., 2005). *Bacillus* can also yield

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extracellular enzymes to degrade the body structure of plant pests. In addition, *Bacillus* also produces protein crystals that will act as toxins in the insect's metabolism system (Frederici et al., 2006). Three isolates of *Bacillus* sp derived from coffee beans are *Bacillus* sp. 1; *Bacillus* sp. 2; and *Bacillus* sp. 3, which all produce protein crystals (Siallagan et al., 2020). Thus, these isolates could be considered an alternative bioinsecticide. However, further investigation into their effectiveness on that specific matter needs to be tested. This study aimed to explore the isolates of *Bacillus* sp. from coffee beans as a plant pest control candidate.

MATERIALS AND METHOD

This research was conducted from October 2019 - January 2020 at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. Three Isolates of *Bacillus* sp. used in this study also belong to the University of Lampung. The three isolates of *Bacillus* sp 1; *Bacillus* sp 2; and *Bacillus* sp 3, result from isolation from coffee beans (Siallagan, 2020).

Determination of enzymatic characteristics.

Bacillus sp. aged 24 hours were tested for their enzymatic abilities, such as proteolytic, chitinolytic, and lipolytic.

Proteolytic tests

Bacillus sp isolates on slanted Nutrient Agar Media were inoculated with the point method on Nutrient Agar media + 1% skim milk and then incubated for 24 hours at room temperature. Proteolytic ability is characterized by the formation of clear zones around the colony (Firliani et al., 2015)

Chitinolytic ability

The three isolates of *Bacillus* sp were inoculated with the point method on media containing colloidal chitin 0.3%, KH_2PO_4 0.02%, $MgSO_4.7H_2O$ 0.1%, yeast extract 0.1%, and NA 2%. After that, it was incubated at room temperature for 24 hours. The existence of a clear zone around the colony shows the chitinolytic character (Nurdin et al., 2016)

Lipolytic test

Each of the three isolates was inoculated with the point method on the Nutrient Agar (NA) medium added with olive oil and subsequently incubated for 24-48 hours at room temperature. The presence of clear zones around the colony indicates lipolytic ability.

Determination of the enzymatic index

On each enzymatic test result, the area of the clear zone and colony area was measured. The enzymatic index (EI) was determined by the formula:

$$EI = \frac{Area\ of\ clear\ zone - Area\ of\ colony}{Area\ of\ the\ colony}$$

(Agustien, 2010).

Initial bioassay test on Plutella xylostell larvae

Isolate suspension was made for the three isolates with the same turbidity as McFarland 0.5 solution; cell density is equivalent to 105 cells/ml. Mustard leaves as larvae feed, dipped in each *Bacillus* suspension, then placed in a plastic container/box. *Plutella xylostella* larvae were put into the test box, with ten larvae in each container. The observation was carried out for 24 and 48 hours. The number of dead larvae showed the potential of Bacillus isolate. Observation data were analyzed descriptively.

RESULTS AND DISCUSSION

From the enzymatic ability test, the three isolates of *Bacillus* sp from coffee beans have the potential to produce proteases, chitinases, and lipases. From observation, these isolates produced protease enzymes, as it is proved by the presence of clear zones around the colony, as shown in Figure 1. According to Gupta et al. (2002), the species of *Bacillus* sp. is one of the bacteria that has the potential to produce protease enzymes. The clear zone around the bacterial colony shows the degradation activity of the protein substrate in the milk medium into its monomers, namely amino acids.

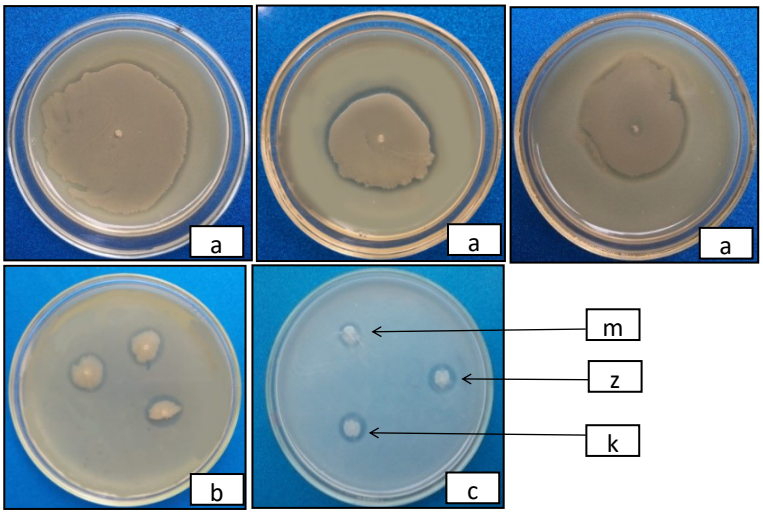

Figure 1. Protease test results,
(a) isolate B1; (b) isolate B2; (c) isolate B3; m = protease selective media; z = clear zone; k = a colony of *Bacillus* sp.

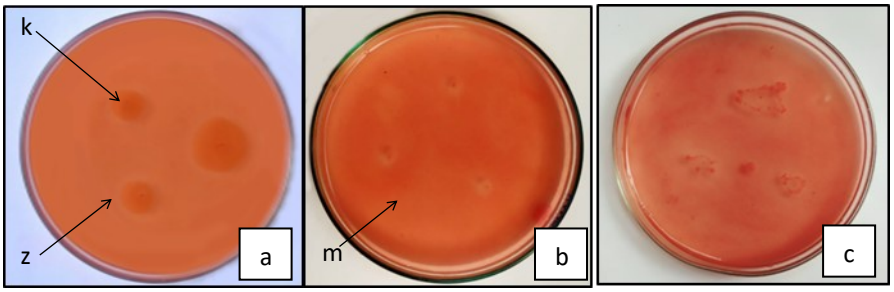

Figure 2. Chitinase test results, (a) *Bacillus* sp. B1 isolates; (b) *Bacillus* sp. B2 isolates; (c) *Bacillus* sp B3 isolates; m = chitinase selective medium; z

The three isolates also produced the enzyme chitinase, indicated by a clear zone around the colony after adding congo red. In Figure 2, a clearly visible spot suggests that the substrate in the chitinase selective media is hydrolyzed by the enzyme chitinase produced by *Bacillus* sp. The number of monomers produced could influence the difference in the size of the clear zone.

Besides producing protease and chitinase, the three isolates also had lipase enzymes. This is indicated by the presence of a clear yellowish zone around the colony of *Bacillus* sp., as seen in Figure 4. The yellowish color of the media indicates pH is becoming more acidic. Conditions around acidic colonies are caused by the breakdown of lipids into fatty acids and glycerol due to lipase activity (Bestari and Suharjono, 2015). The ability of the three isolates to distribute lipids in different media can be seen in the significant differences in the zones produced.

Based on the determination of the enzymatic index, the three isolates showed different enzymatic activity. The enzymatic index data is presented in Figure 4.

Bacillus sp. B2 isolates show higher proteolytic and chitinolytic index than the other two isolates, while *Bacillus* sp. B3 isolates have the highest lipolytic index compared to the other two. It is plausibly caused by the optimum environmental condition for *Bacillus* sp. B2 isolates to synthesize proteases and chitinases, as well as being very suitable for *Bacillus* sp. B3 isolates to produce lipase enzymes. The difference in the ability of *Bacillus* sp in enzyme synthesis is influenced by the needs of the environmental factors of each isolate; hence the amount of enzymes produced differs from each isolate.

The result of the initial bioassay test on *Plutella xylostell* larvae is provided in Table 1. The data shows that more than half of the *Plutella xylostella* larvae tested died after being treated for 24 hours. The number of death was even boosted in 48 hours of testing. *Bacillus* sp. B2 isolates have a higher killing power, shown by the death of ten larvae within 48 hours, while none of those larvae died for the other two isolates. The distinct killing

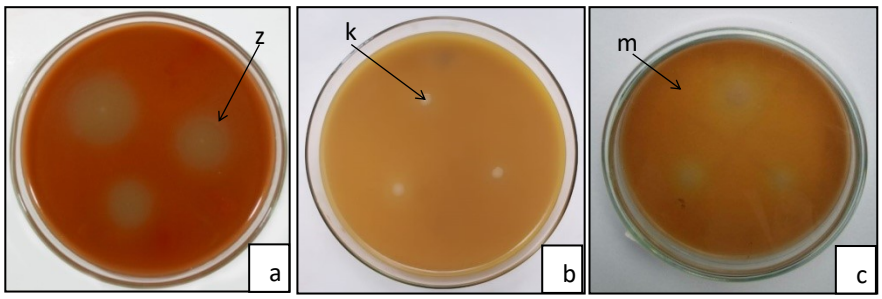

Figure 3. Lipase test results, (a) *Bacillus* sp. B1 isolate; (b) *Bacillus* sp. B2 isolate; (c) *Bacillus* sp. B3 isolate; m = the lipase selective media; z = clear zone; k = colony of *Bacillus* sp.

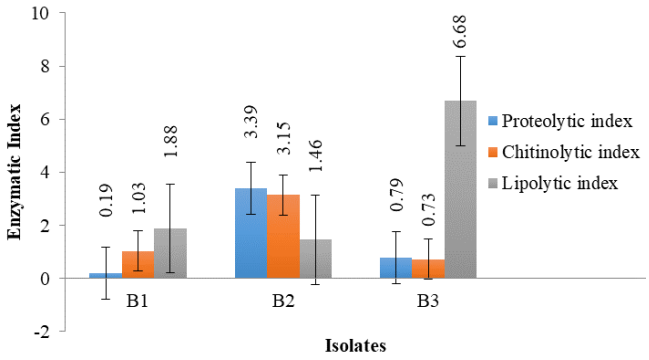

Figure 4. Enzymatic index of *Bacillus* sp.

power of *Bacillus* sp. B2 isolates were supported by the greater proteolytic and chitinolytic ability, with a high amount of

Table 1. The Results of Initial Bioassay Observations on the Number of Larval Deaths

No	Isolate	The Result of Observation			
		24 hours		48 hours	
1	B1	3	7	2	8
2	B2	1	9	0	10
3	B3	4	6	1	9

Note: the number of larval test models for each treatment is 10 larvae

protease and chitinase enzymes, *Bacillus* sp. B2 isolates can penetrate the larvae's body quickly, causing the faster death of larvae. In Figure 6, we can see the difference in the body color of the larvae infected by *Bacillus* sp., which tends to be yellowish, while larvae still alive are green but move slower.

Protein crystals cause larval death in *Bacillus*, which enter the mouth from the larva to the insect's digestive tract. Hadi et al. (2009) confirmed that if sensitive insects eat the spores and protein crystals, paralysis will occur, resulting in the host's death. In the digestive apparatus, protein crystals will be active due to the activity of proteolytic enzymes, which convert protoxin into toxins for target larvae. These toxins can disrupt the larvae's digestive system and even cause larvae death (Bahagiawati, 2002). The characteristics of insects exposed to the toxin from Bt include changes in appetite, slow movement, and changes in body color in insects (Lee et al., 2003).

Previous studies suggest that the three isolates of *Bacillus* sp. produce protein crystals (Siallagan et al., 2020). Therefore, the three isolates of *Bacillus* sp have the killing power of plant-insect pests, especially against *Plutella xylostella*. According to Frederici et al. (2006), protein crystals entering insects' digestive tract will actively become toxins in the alkaline atmosphere. This causes osmotic changes so that the toxin molecule spreads throughout the body through the blood, causing insect deaths.

CONCLUSION

The three isolates of *Bacillus* sp. from coffee beans have the ability of proteolytic, chitinolytic, and lipolytic, as well as being able to kill *Plutella xylostella* larvae. However, *Bacillus* sp. B2 isolates were able to cause the death of *Plutella xylostella* larvae more than *Bacillus* sp. B1 isolates and *Bacillus* sp. B3 isolates during 48 hours of incubation. Thus, the three isolates of *Bacillus* sp. could be considered alternative bioinsecticides.

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