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THE PROBIOTIC PROPERTIES OF BACILLUS SP. IN THE INHIBITION OF FUSARIUM SOLANI CAUSING STEM ROT IN CASSAVA

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ABSTRACT:
The purpose of this research was to study the probiotic properties and to evaluate the probiotic potential of *Bacillus* sp. by Morphology and biochemical test with a gram stain kit to study the cell appearance and shape. Next, tested the efficiency of *Bacillus* sp. sporulation to see the growth of bacteria under inappropriate conditions. Then, *Bacillus* sp. was used to inhibit the plant pathogenic fungi *Fusarium solani* by dual culture method to see the efficacy of Bacillus sp.

Keywords :- Probiotic, Bacillus sp., *Fusarium solani*, plant pathogenic fungi.

INTRODUCTION :

Nowadays, in Thailand, there are many economic vegetables affected by the infection that causes damage to the agricultural industry. Which fungi can cause the most plant diseases out of all the factors that cause the diseases. Most of the plant diseases such as wilt, root rot, leaf spot and leaf blight etc. In order to inhibit fungi or pests, most farmers choose to use chemical methods to control fungi, but those methods can only control fungi for a short period of time. When chemicals are used in large quantities and for a long time, the bacteria will become resistant to these chemicals. Causing the farmers to increase the amount of chemicals and create a high risk of chemical residues in plants. It's also has a dangerous effect on farmers and consumers as well. Most of the fungi that occur in economic crops are *Fusarium* sp., causing wilting, root rot, and stem rot. It's very prevalent in beans, cassava, corn, many kinds of melons and orchids, etc.

Bacillus sp. is a rod-shaped bacteria in the family Bacillaceae with a length of 4-10 micrometers with Gram positive and move with flagella. It's commonly found in soil and water, has probiotic properties and also can produce antibiotics that used to control plant diseases such as bacillomycin, iturin, mycosuptilin and fenjimycin etc. In addition to the previous research has indicated that *Bacillus* sp. has the ability to inhibit many pathogenic fungi such as *Collectrotrichum* sp., *Phythium aphanidermatum*, *Sclerotium rolfsii*, which causes disease in grapes (Ponphan, 2007), etc. Therefore, this research aims to study the efficacy of *Bacillus* sp. in inhibiting plant pathogenic fungi. However this will be a guideline for physical studies on bioactivity to inhibit *Fusarium* sp.

OBJECTIVE :

To study the potential as a probiotic of *Bacilus* sp. against *Fusarium solani*

MATERIAL AND METHOD :

Step 1: morphology and biochemical test

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The probiotic properties were tested by Morphology with Gram staining and biochemical test to evaluate the probiotic potential of *Bacillus* sp. Bacteria that cultured on Nutrient Agar (NA) medium was used for Gram staining to determine cell shape using Gram staining kit. *Bacillus* sp. are gram-positive bacteria and rod-shaped. Biochemical tests were performed, including Catalase test, Oxidase test, Starch hydrolysis Motility test and Indole test.

Step 2: The sporulation efficiency of *Bacillus* sp. test

Bacillus sp. were streak on NA, incubated at 37 °C for 24 h, then cultured in LB medium and incubated at 37 °C in a shaking incubator at a speed of 150 rpm for 24 h, then transfuse on DSM medium, incubated at 30 °C for 72 h, then scraped off the spores from the surface medium and washed with distilled water. Then bacterial spores were heated at 80°C for 10 min and non-heated. Then diluted at a concentration of 10^{-4}–10^{-6} was spread on NA medium. Three replicates of each concentration were incubated at 37 °C for 24 h. The number of colonies formed in each group was counted to compare sporulation efficiency. Spore formation was examined (Ghelardi et al., 2013).

Step 3: Inhibition test of *Bacillus* sp. against plant pathogenic fungi with the dual culture method

Plant pathogenic fungi *Fusarium solani* were cultured on PDA medium, incubated at 30 °C for 24 h. Then streak the *Bacillus* sp. at a distance of 2 cm from the fungi. Fungal growth and inhibition were measured daily for 7 days, and results were reported as inhibition zones in cm as the distance from the fungal colony edge to the *Bacillus* sp. colony edge (Ashwini and Srividya, 2012).

RESULT & DISCUSSION :

morphology and biochemical test

Morphology and biochemical test, the result showed that bacteria was gram-positive and bacilli-shaped it has color, such as blue and purple, as shown in Figure 1. The research of Lakshmi S.S. et al, (2020) showed that *Bacillus* sp. biochemical tested have positive results for catalase test, starch hydrolysis, motility test and negative for oxidase test and indole test. The properties were corresponding with *Bacillus* sp. The results in this research are consistent with the research report as shown in Table 1 and Figure 1.

Note : Symbol + means positive biochemical test result, symbol - means negative biochemical test result. positive results for catalase test, starch hydrolysis, motility test and negative for oxidase test and indole test.

The sporulation efficiency of *Bacillus* sp. test

The sporulation performance of *Bacillus* sp. in DSM food was tested. *Bacillus* sp. was incubated at 30° C for 72 hours. It was found that the spore production efficiency was high at 87.41%. which has an efficiency of sporulation higher than 80% will be used in the next experiment.

Inhibition test of *Bacillus* sp. against plant pathogenic fungi with the dual culturemethod

From the test results of *Bacillus* sp. in inhibiting the plant pathogenic *Fusarium solani* by Dual culture method, the results showed that *Bacillus* sp. was effective in inhibiting *Fusarium solani* with an inhibition area at 76.98% as shown in Figure 2

CONCLUSION :

From the results of morphology and biochemical tests, it's estimated that bacteria belong to the *Bacillus* group and have probiotic properties. Which had a good sporulation efficiency at 87.41%. Then tested for antifungal efficacy by dual culture method, it was found that *Fusarium solani* was effectively inhibited with 76.98% inhibition when tested in the laboratory. Therefore, this bacterium may be

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developed and applied in agricultural fields as an alternative for farmers to avoid using chemicals to inhibit the plant pathogen *Fusarium solani*.

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Table 1 Morphology and biochemical test of *Bacillus* sp.

Gramstain	Shape	Catalase	Biochemical test			
			Motility	Starch hydrolysis	Oxidase	Indole
+	Rod	+	+	+	-	-

Figure 1 : Morphology of Bacillus species under microscope

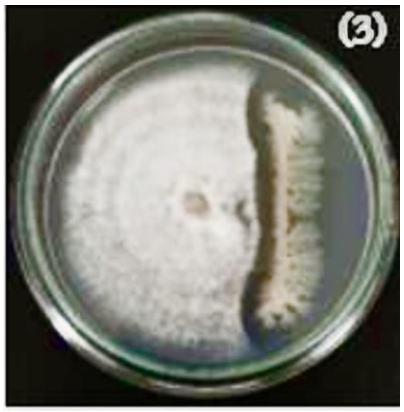

Figure 2 : The efficacy of Bacillus sp. (right) against pathogenic fungi Fusarium solani (left) by dual culture method in Day 7

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