Bacillus thuringiensis As Local Biological Agent

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Abstract

The chemical insecticides are still contributing to human life enormously, but they have been distributed in ecological system of organisms including human beings because of their low specific toxicity to any organism and their low specific toxicity to any organism and their slight decomposition in nature. Therefore, many biological control of insects have been investigated. Currently, researches on the use pathogenic microorganisms to control insect pests are increasing. Microbial pest control is practiced in different parts of the world though utilization of pathogen like fungi, bacteria, viruses and nematodes. Bacterial research causing disease in insects began in the late nineteenth century. It was a study of flacherie of the silkworm, *bombx mor*i in this report on the discovery of *sotto bacillus*, reffered briefly to occurrence of sotto bacillus-like organism, which causes the disease to silkworm larvae. Ten-fold serial dilutions of the heated suspension in sterile distilled water were placed on nutrient agar (NA-pH 7.5). After two days of incubation at 28^{o}C, Bacillus colonies were recorded. After 2 to 3 days incubation, crystalliferous sporeforming bacteria were determined in phase contrast microscop. Isolation from five soil samples yielded about 35 isolates, only one was identified as *B. thuringiensis*.

Key Word: *B. thuringiensis*. Biological Agent

Introduction

Cabbage moth, *C. binotalis* Zell. (Lepidoptera: Pyraustidae) is considered the most important limiting factor for a successful production of cruciferous vegetable not only in the Indonesia but in other country in the world. The larva feeds on folage from seedling to harvest cousing 100% yield loss if not control (Rejesus and Sayaboc, 1990).

Numerous chemical insecticides have been used in order to control pests, which damage for agriculture. While chemical insecticides have knock down effect to the insect pests, they are too expensive in the developing countries and harmful to both human and the environment. In addition, target insect pests rapidly develop biological resistance especially at higher rates of application. The chemical insecticides are still contributing to human life enormously, but they have been distributed in ecological system of organisms including human beings because of their low specific toxicity to any organism and their low specific toxicity to any organism and their slight decomposition in nature (Shorey and Hall, 1962). Therefore, many biological control of insects have been investigated. Currently, researches on the use pathogenic microorganisms to control insect pests are increasing. Microbial pest control is practiced in differen parts of the world though utilization of pathogen like fungi, bacteria, viruses and nematodes. Bacterial research causing disease in insects began in the late nineteenth century. It was a study of flacherie of the silkworm, *bombx mori* (Burges and Hussey, 1971; Burges, 1981). Ishiwata (1901) in this report on the discovery of *sotto bacillus*, reffered briefly to occurrence of sotto bacillus-like organism, which causes the disease to silkworm larvae .

Berliner (1911) proposed the name of *B. thuringiensis* for a species of bacillus which was isolated from the diseased larvae of the Mediterranean flour moth *Anagasta (Ephestia) kuhniella* Zell. Later, Berliner (1915) noted infection of the larvae after the ingestion of the bacillus or its spore, described and named it *Bacillus thuringiensis*. Mattes (1927) isolated the same bacillus from the same insect host, which Berliner had found earlier.

B. thuringiensis is a gram-positive soil bacterium, and produce a crystalline inclusion body during sporulation (Bulla et all., 1980). This parasporal body is composed of proteins termed “delta-endotoxin”, and specifically toxic to insects. In addition, *B. thuringiensis* produce another toxins namely: alpha-toxin, beta-exotoxin, and gamma-exotoxin. All of the toxic substance may not present in the bacterium (Heimpel, 1967). In another hand, Krieg (1961) has defined various toxic substance produced *B. thuringiensis* as follow: (a) thermolabile endotoxic; (b) thermostable exotoxin; (c) bacillogenic antibiotic; (d) lecithinase; (e) proteinase.

Most strains of *B. thuringiensis* produce delta-endotoxin crystals toxic to lepidopteran insects such as moth (Dulmage et al., 1970). Recently, however several researches have shown that *B. thuringiensis* is also widely distributed in natural soils of various area. Delucca et al., (1982) reported that *B. thuringiensis* made up less than 0.5% of more than 46,000 bacterial isolates recovered from various soils in the United States.

The objective of the studies to survey, collect and determine the distribution of *B. thuringiensis* in selected diverse crop-growing area.

Materials and Methods

Isolation of *B. thuringiensis*

Soil samples were collected in areas planted to vegetables, rice, citrus, peanut and corn in South Kalimantan (Indonesia) following multistage random sampling. Soil samples were collected at random in a 1- hectare area for a total of 5 kg. The soil sample were taken from the top 1 cm of the soil layer. The 5-kg soil samples were mixed thoroughly and composite sample of 1 kg was taken from which isolation were made for as long as one month. The samples were labeled denoting date, place of collection and crops planted.

Five 1-g soil samples were separately suspended to 9 ml of distilled water. After allowing the suspension to stand for 5 minute, 3-4 ml of the the suspension were taken. One half of the suspension was transferred to a test tube and heated in a waterbath of 80^{o}C for 15 minutes, so that all microorganisms were killed except Bacillus and other sporeforming bacteria, then allowed to cool at room temperature. Ten-fold serial dilutions of the heated suspension in sterile distilled water were placed on nutrient agar (NA-pH 7.5). After two days of incubation at 28^{o}C, Bacillus colonies were recorded. After 2 to 3 days incubation, crystalliferous sporeforming bacteria were determined in phase contrast microscop.

RESULTS AND DISCUSSION

Isolation and distribution of *B. thuringiensis* in different plants growing areas.

Fifty *Bacillus sp*. were isolated from 5 soil samples collected from diverse crop growing areas in the South Kalimantan (Indonesia) (Table 1). The different isolates were obtained from the same area planted with diverse crop. Two soil samples great were found in this area namely: typic calciborrols with pH 6.5 was planted to rice and typic tropudults pH ranging from 4.8 – 5.7 planted to vegetable, corn, citrus, peanut and cabbage. Out of the 35 *Bacillus sp*. isolates, only one (2%) was identified as *B. thuringiensis* based on phase contrast microscope examination for the presence of parasporal inclusion bodies. Only the soil sample from citrus yielded *B. thuringiensis*.. The possible reasons for the low incidence of *B. thuringiensis* isolated from the samples taken in the areas surveyed are the small number of samples size from which the isolation were made, the area where sampling was done and also the difference in the physic-chemical characteristic of the soil where samples were taken. The low incidence of *B. thuringiensis* was also reported in Japan by Ohba and Aizawa (1966) in soil sample from non-agricultural areas. Out of 6910 isolates only 189 (2.7%) isolates were identified as *B. thuringiensis*

Table 1. South Kalimantan Isolates with different plants areas source

ISOLATES	SOIL SOURCE		
	Crop Planted	Great Soil	pH
AA	Watermelon	Typic calciborolls	6.5
AA 1.1			
AA 1.2			
AA 2.1 (1)			
AA 2.2			
AA 2.3			
AA 4.1			
AA 2.5			
AA 2.5 (1)			
AA 2.5 (2)			
BB	Cucumber	Typic tropudults	4.8
BB 1.1			
BB 1.2			
BB 2.3			
BB 1.4			
BB 3.1			
CC	Corn	,,	5.2
CC 1.2			
CC 2.1			
CC 2.3			

CC 2.4			
CC 3.2			
CC 5.1			
CC 5.1 (1)			
DD	Citrus	,,	5.7
DD 1.2 (1)			
DD 1.3			
EE	Peanut	,,	5.1
EE 1			
EE 2			
EE 1.3			
EE 1.4			
EE 3.1			
EE 4.3			

Conclusion

Six soil samples were collected from different crop growing areas in South Kalimantan (Indonesia). Isolation from five soil samples yielded about 35 isolates, only one was identified as *B. thuringiensis*. This result suggest that *B. thuringiensis* is rare and not widespread in the places where sampling was done and isolates of *B. thuringiensis* will screen to the cabbage worm (*Crocidolomia binotalis*).

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