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Study on *Bacillus thuringiensis* Indigenous Highland of South Sumatera–Based Bioinsecticide Towards Lepidopteran Insect Pests

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***Abstract*— The objectives of research were 1) to explore the presence of Bacillus thuringiensis from highland soil of South Sumatera; 2) to investigate crystal proteins and their toxicity against diamondback moth Plutella xylostella and armyworm Spodoptera litura; and 3) to produce B. thuringiensis – based product of the most promising B. thuringiensis isolate. Exploration of soil resulted 33 B. thuringiensis isolates in which 21 isolates were toxic against P. xylostella and 15 isolates were toxic towards S.litura. Two isolates, namely SASU and KATB, were very toxic to both insects. Developing of those isolates as bio-insecticide was done in three main growth media i.e. coconut water, soybean soaking water, tofu liquid waste, mixtures of coconut water and soybean soaking water (1:1. v/v), mixtures of coconut water and tofu liquid waste (1:1. v/v), and Nutrient Broth, as control. Total Viable Spore Count (TVSC) showed spore product was ranged from 2.22 x 106 until 8.98 x 108spores/ml resulted in high mortality of P. xylostella and of S. litura, indicating the presence of toxic crystal protein.**

***Keywords*— Bacillus thuringiensis; bio-insecticide; Plutella xylostella; Spodoptera litura.**

I. Introduction

Bacillus thuringiensis(Bt) is a gram-positive, rod-shaped, aerobic and spore-forming bacteria. The presence of inclusions in *B. thuringiensis* have been found and detected in the inclusion parasporal crystal structure containing more than one type of insecticidal crystal proteins (insecticidal crystal protein, ICP) or also called delta endotoxins [1] and it will be produced by Bt during sporulation [2]. This bacterium can be found in soil, various plants, including vegetables, cotton, tobacco, and forest plants. In the environment with good conditions and adequate nutrition, bacterial spores can survive and continue the vegetative growth [3] [4] [5]. Various strains of Bt isolates have been demonstrated to control various plant pests. Some member of these orders Lepidoptera, Coloeptera, Diptera, Hymenoptera, Homoptera, Molophoga, and Acari are target Bt [6]. In Indonesia, important insect pests are armyworm *Spodoptera litura* (Lepidoptera: Noctuidae) and diamondback moth *Plutella xylostella* (Lepidoptera :Plutellidae) because of their characteristic of life.[7].

Bt–based product (known as bio-insecticide) was made from waste material content of carbohydrate, nitrogen, protein and some other minerals. Devi *et al*. [8] used wheat bran based media to produce bioinsecticide toxic to larvae of castor semilooper, *Achaea janata* L. Chilcott and Pillai [9] used coconut wastes for production of *B. thuringiensis* var *israelensis*. High production of Bt based -bioinsecticide was depended on carbon, nitrogen, water content, mineral element and suitable growth condition. The strain of local *B.thuringiensis* also played a role in achievement of manufacturing process. This paper presented earlier observation of *B. thuringiensis* isolated from highland of South Sumatera and their effectiveness to kill insect pests.

II. Material and Method

A. Soil Sample Collection

Samples were collected from soil in location had not been treated by *B. thuringiensis*-bio insecticide. Location was in high land of South Sumatera. Samples were collected by scrapping off material by an sterile spatula and obtaining 50 g soil below 5 cm from surface. Samples were kept on 4°C until use.

B. Isolation of B. thuringiensis

Five g of soil samples is diluted well in 15 ml dH_20 in test tube. Shaked well until perfectly diluted. One ml of upper

part of dilution is taken in eppendorf tube, added by 1 µl Triton X-100, and heated in waterbath 85ºC 15 minutes. With a sterile spatula, the solution was streaked on the medium NaCl Glycine Kim and Goepfert (NGKG) on petridish. Petridish incubated at 30ºC, for 24-72 hours. Colonies of Bt will grow in white colour. After 24-72 hours incubation, proteinaceous parasporal inclusion bodies will presence. Identification of *B. thuringiensis* refers to Thiery and Frachon [10].

C. Insect Test mass-rearing

Groups of eggs of armyworm *S. litura* and diamondback worm *P. xylostella* were obtained from the field and subsequently maintained in the laboratory. Larvae reared in a plastic container maintenance (d = 15 cm and h = 9 cm). Depending on species, food used were the leaves of water spinach (*Ipomoea reptana*) grown without pesticide treatment for mass rearing for *S. litura*, and brassica leaves for *P. xylostella*, as well. Temperature and relative humidity were maintained. Maintenance of container was done by cleaning of residual dirt and food remains to ensure the availability of food and cleanliness. At the bottom of the box was placed maintenance of sterile soil that had been sterilized as a place of *S. litura* to become pupae. If the caterpillar has reached prepupa phase characterized by no activity, meaning caterpillar will enter the pupa stage. Larvae of *S. litura* reared to be a phase of insect pupae, and imago. Insect samples used were second generation (F2).

D. Preliminary Test of Bt isolate (Screening test)

Leaves of spinach and leaves of brassica were prepared for screening test. Bt isolates were prepared in single dose of 10^6 spores/ml. Leaves were dipped in Bt about 3 minutes, dried-air and transferred into petri dish. Second-instar larvae of *S.litura* were placed in petri dish with Bt treated spinach leaves, and third instar larvae of *P. xylostella* were place in treated leaves of brassica. Each isolates was tested by 20 larvae. Mortality of larvae was observed and counted.

E. Mass Production of Bt spores in Various Media

Two isolates will be chosen for mass-production of Bt spores with criteria they showed the highest mortality towards both insect pests. Media used for mass production was 1). Coconut water, 2). Soybean soaking water, 3) Tofu liquid waste, 4). Mixture of coconut water and soybean soaking water (1:1. v/v), 5). Mixture of coconut water and tofu liquid waste (1:1. v/v), and 6). Nutrient Broth (Control). The media were individually added by 0.3 g/l MgSO4.7H2O, 0.02 g/l FeSO4.7H2O, 0.02 g/l MnSO4.7H2O, 0.02 g/l ZnSO4.7H2O and 0.01 g/l CaCO3 following the method of Dulmage and Rhodes [11] Those media were shaken 300 rpm for 72 days. Total Viable Spore Count (TVSC) was observed. Two isolates chosen were checked their protein shape by SEM (Scanning Electron Microscope)

F. Bioassay of Bt-product towards S.litura and P. xylostella

TVSC of Bt product was used as treatment for bioassay towards *S. litura* and *P. xylostella*. Experiment was done by Completely Randomized Design (CRD) with 6 treatments and 5 replications. Leaves were dipped in Bt about 3 minutes, dried-air and transferred into petri dish. Second-instar larvae of *S.litura* were placed in petri dish with Bt treated spinach leaves, and third instar larvae of *P. xylostella* were place in treated leaves of brassica. Each replication was tested by 10 larvae. Mortality was observed until 5 days.

III. Result and Discussion

A. Isolation of Bacillus thuringiensis

Soil sampling was conducted in highland of South Sumatera consisted of 4 districts namely Pagaralam district (985m asl), Lahat district (925 m asl), OKU Selatan district (950 m asl) and Muara Enim district (915 m asl). Exploration of soil resulted 33 *B. thuringiensis* isolates in which 21 isolates were toxic against *P. xylostella* and 15 isolates were toxic towards *S.litura*. Data was shown in Table 1.

TABLE I
SCREENING TEST OF BT ISOLATED FROM HIGHLAND OF SOUTH SUMATERA AGAINST *SPODOPTERA LITURA* AND *PLUTELLA XYLOSTELLA*

No.	Isolate code	Location	Mortality (%)	
			S.litura	*P. xylostella*
1	BAK	Pagaralam (985m asl)	35	40
2	BAC		65	0
3	PWP		35	70
4	KDu		40	45
5	KRa		0	0
6	PKa		0	0
7	PKe		30	45
8	PKo		0	40
9	PCe	Lahat (925 m asl)	0	0
10	DMS		40	0
11	DMA		30	50
12	DMP		0	65
13	DMK		0	0
14	SRK		0	0
15	SRA		0	0
16	SRJ		0	55
17	SKD		0	50
18	PGK	OKU Selatan (950 m asl)	0	40
19	APLB		55	0
20	CELB		0	60
21	PILB		70	0
22	DRPD		0	0
23	DUPD		60	45
24	MAPD		55	50
25	JABD	Muara Enim (985 m asl)	0	0
26	KHBD		0	65
27	SEBD		35	40
28	KATB		90	95
29	PITB		0	40
30	MATB		45	45
31	PELM		0	45
32	SASU		95	95
33	RASU		50	40

Two isolates toxic to both *S.litura* and *P. xylostella* were chosen among 33 isolates, i.e. SASU and KATB. Their toxicity towards *S.litura* was 90 and 95 % (SASU-product) and 95 and 95 % on *P. xylostella*. There was any possibility that these isolates contained of *cry 1* gene and specific shape of crystal proteins. Asano *et al* [12] showed that *B. thuringiensis* toxic to *S. litura* belongs to *cry 1 gene* group. These two isolates will be used as material to mass-production of Bt. The shape of two proteins was observed by Scanning Electron Microscope (SEM). The photographs of these proteins were shown in Figure 1.

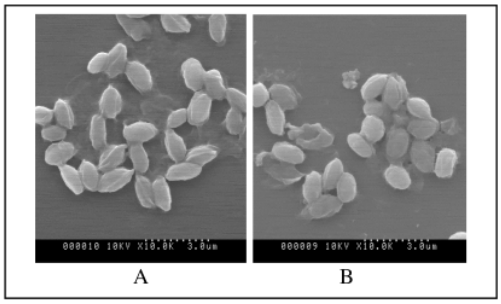

Fig 1.Crystal protein of SASU Bt isolate (A) and KATB Bt isolate (B)

B. Number of Total Viable Spores Count (TVSC) SASU dan KATB isolate-based product

TVSC of SASU-based product was in the range of 5.18 x 10^4 - 8.98 x 10^8 spores/ml and KATB-based product was 5.29 x 10^5 - 7.34 x 10^8 spores/ml. Content of growth media for culturing *B. thuringiensis* was very important. It can be seen in the media of mixture coconut water and soybean soaking water, in SASU and KATB isolates, produced the highest spores. Compare with standard growth medium (nutrient broth), spores produced was similar. It indicated that crystal protein content was high, as well. Data was shown in Table 2.

TABLE II
TOTAL VIABLE SPORES COUNT (TVSC) OF SASU AND KATB ISOLATE-BASED PRODUCT

Treatment	TVSC (spore/ml)	
	Bt - SASU	Bt - KATB
A. coconut water	2.22 x 10^6	3.02 x 10^6
B. soybean soaking water	3.14 x 10^6	3.40 x 10^7
C. tofu liquid waste	5.18 x 10^4	5.29 x 10^5
D. mixture A and B (1:1,v/v)	8.98 x 10^8	7.34 x 10^8
E. mixture A and C (1:1 , v/v)	3.67 x 10^6	4.18 x 10^6
F. nutrient broth	3.56 x 10^8	5.09 x 10^8

C. Toxicity of SASU dan KATB isolate-based product

Mortality of insect pest (*S.litura* and *P. xylostella*) was the highest on media coconut water and soybean soaking water. Carbon and nutrient content of this media could be factor affect the growth of spores. The more number of spores consumed by larvae, the more number larvae will die, since Bt played a role as stomach poisons. Prabakaran *et al* [13] also showed coconut waste media could produce high number of spores, similar with production of spores in NYS medium.

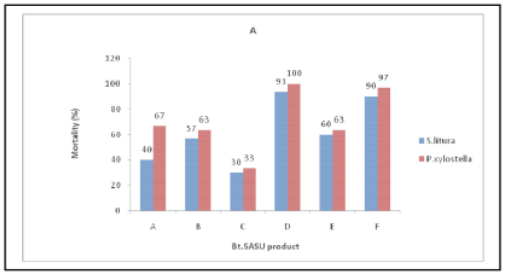

Fig. 2. Mortality of *Spodoptera litura* and *Plutella xylostella* on various media growth of Bt-SASU-based product .

Note:
A. coconut water
B. soybean soaking water
C. tofu liquid waste
D. mixture A and B (1:1,v/v)
E. mixture A and C (1:1 , v/v)
F. nutrient broth

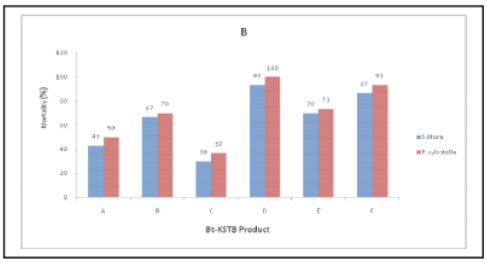

Fig. 3. Mortality of Spodoptera litura and Plutella xylostella on various media growth of Bt- KATB-based product

Note:
A. coconut water
B. soybean soaking water
C. tofu liquid waste
D. mixture A and B (1:1,v/v)
E. mixture A and C (1:1 , v/v)
F. nutrient broth

IV. CONCLUSIONS

Exploration of soil resulted 33 *B. thuringiensis* isolates in which 21 isolates were toxic against *P. xylostella* and 15 isolates were toxic towards *S.litura*. The highest mortality of both S. litura and P. xylostella was occurred on treatment of mixture of coconut water and soybean soaking water. The highest spores produced was Mixture of coconut water and soybean soaking treatment indicated as the best media for producing bio insecticide.

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