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Characteristics of *Bacillus thuringiensis* isolates indigenous soil of South Sumatra (Indonesia) and their pathogenicity against oil palm pests *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract. *Pujiastuti Y, Arsi, Sandi S. 2020. Characteristics of* Bacillus thuringiensis *isolates indigenous soil of South Sumatra (Indonesia) and their pathogenicity against oil palm pests Oryctes rhinoceros (Coleoptera: Scarabaeidae). Biodiversitas 21: 1287-1294. Bacillus thuringiensis* is a gram-positive, entomopathogenic bacterium that could be isolated from soil and be used to control various plant pests. *Oryctes rhinoceros* is an important pest in oil palm. Application of *B. thuringiensis*-based bioinsecticides is an alternative in controlling these pests. The purposes of this study were to isolate and identify *B. thuringiensis* bacteria from the soil of South Sumatra, production of *B. thuringiensis*-based bioinsecticides and to test their toxicity to *O. rhinoceros* larvae. The study was conducted in several cities/districts in the province of South Sumatra. Soil samples were taken from various habitats and *B. thuringiensis* isolates were grown on NGKG agar media. Among 76 soil samples (6 districts and 2 cities) *B. thuringiensis* colonies were obtained leading to 24 isolates of *B. thuringiensis*. Toxicity screening tests for armyworm *Spodoptera litura* were 55.79% (53 isolates) and their mortality to 25.26% *O. rhinoceros* larvae (24 isolates). From these isolates whose effectively killed *O. rhinoceros* larvae, 10 isolates were taken and propagated with Nutrient Broth (NB) and biourine enriched with 5% molasses. Number of spores produced was counted during 24, 48 and 72 hours. Furthermore, a bioassay test was carried out on *O. rhinoceros* larvae for 7 days. Isolate of KJ3P1 caused the highest mortality of *O. rhinoceros* larvae after 7 days of observation. SDS Page resulted in KJ3P1 and KJ3R5 isolates showing several bands whose content of various types of protein molecular weight. Isolation of *B. thuringiensis* in South Sumatra produced 2 isolates potentially to be active ingredients in production of bioinsecticides which were effective in killing *O. rhinoceros* larvae.

Keywords: *Bacillus thuringiensis*, biological control, *Oryctes rhinoceros*, toxicity

INTRODUCTION

Oryctes rhinoceros (Coleoptera: Scarabaeidae) is an important oil palm plant pest both in immature and mature stage of plants (Susanto et al. 2011). The application of zero waste by re-entering empty bunches in oil palm plantations increases *O. rhinoceros* larvae population. Oil palm empty fruit bunches which decompose into organic matter become suitable breeding sites for these larvae (Santi and Sumaryo 2008). The decline in palm oil yields can occur after the attack. Heavy injury to palm oil leaves (≥ 90%) can reduce production by more than 70% in the first year and continue for the subsequent years (Sudharto and Guritno 2003). Control of *O. rhinoceros* by using entomopathogens is an alternative when the use of synthetic chemical insecticides is not able to solve pest problems. For example, *Bacillus thuringiensis* used to pest control has been tried to control *Metisa plana* caterpillars (Khaeruni and Purnamaningrum 2012; Ahmad et al. 2017; Kamarudin et al. 2017), but to controlling *O. rhinoceros* is still not much studied.

Bacillus thuringiensis works like stomach poison. Proteins and spores of *B. thuringiensis* produced during sporulation must be ingested and digested in the midgut of insects (Mizuki et al. 1999; Bravo et al. 2015a). The specificity of host is a unique feature of *B. thuringiensis*. In its grouping, *B. thuringiensis* is divided into groups based on their insecticidal protein content (Jouzani et al. 2017). The discovery of new isolates is a big chance in producing isolates who is toxic to insect pests (Bravo et al. 2015b). After obtaining *B. thuringiensis* isolates, their application for controlling insect pest becomes important. Bioinsecticides need to be made with materials that are easily obtained, inexpensive and abundant. Therefore, waste materials such as bio-urine and molasses can be used as media in the propagation of *B. thuringiensis* (Purnawati et al. 2014; Salazar-Magallon et al. 2015; Pujiastuti et al. 2018). The specificity of the insect pest host of *B. thuringiensis* can also be explained by its protein content. Wiest et al. (2015) and Osman et al. (2015) explained specifically *B. thuringiensis* which active against Lepidoptera content a protein molecular weight of 130 kDa, while those active against Coleoptera own a molecular weight of 14-133 kDa (Fernandez and López-Pazos 2011). Therefore it is necessary to investigate *B. thuringiensis* isolates from soil isolated in South Sumatra province and their toxicity to Oryctes larvae by paying attention to their protein content.

MATERIALS AND METHODS

Materials

Soil sampling was carried out in 6 districts and 2 cities in the province of South Sumatra, Indonesia as shown in Figure 1. Altitude varies from lowland (8 m) to highlands (850 m). The habitat for sampling also varies according to local conditions including lowland peat soils, tidal swamp soils and annual plantations (Table 3).

Procedures

Soil sampling

Soil samples were taken from various habitats. The surface of soil was cleaned from litter and then measured a distance of about 30 cm from the plant stem with a depth of 5 cm. The soil was taken as much as ± 500 g in each place. The soil samples were then put into a plastic bag, tightly bound, labeled with location and date of collection, then taken to the laboratory and stored in the refrigerator until further analyses.

Isolation method

Isolation of *B. thuringiensis* was performed in accordance with the procedures of Rusmana and Hadinata (1994). One gram of soil samples was diluted well in 15 mL H_2O in test tube by shaking well until perfectly diluted. One mL of upper part of dilution was taken in eppendorf tube, added by 1 µL Triton X-100, and heated in water bath 85º C for 15 minutes. With a sterile spatula, the solution was streaked on the NaCl Glycine Kim and Goepfert (NGKG) medium on Petri dish. Petri dish was incubated at 30ºC for 24-72 hours. Colonies of *B. thuringiensis* grow in white color. After 24-72 hours incubation, proteinaceous parasporal inclusion bodies produced. Identification of *B. thuringiensis* was done by observing microscopic cells test and gram staining test.

Screening test

Bacillus thuringiensis isolates that have been identified were grown on Nutrient Broth media. Their toxicity was tested using armyworm 3rd instar larvae of *S.litura* and 1st instar of *O. rhinoceros*. Every isolate required 20 individuals of each species. Mortality was used as a reference for subsequent testing.

Propagation of Bacillus thuringiensis

Propagation of *B. thuringiensis* as bioinsecticide was carried out using cow bio-urine enriched with 5% molasses. For production of bioinsecticides, preculture or seed culture was first made (Valicente et al. 2010). A total of 10 mL NB in 200 mL-Erlenmeyer was given one pre-determined isolate ose needle. The Erlenmeyer was then put in the fermenter for 12 hours 200 rpm at room temperature. From the Erlenmeyer, it was taken 5 mL of seed culture and added with 10 mL of NB and once again fermented for 12 hours. Seed culture was ready to be used as a basis for making Bt-based bioinsecticides. A 100 mL of cow biourine enriched with 5% molasses were sterilized with autoclave for 20 min at 121 °C and 1 atm pressure. Under aseptic conditions, as much as 10 mL of seed culture was put into an Erlenmeyer containing 100 mL of cow biourine enriched with 5% molasses. It was fermented at room temperature at 200 rpm for 72 hours. Numbers of bacterial/ spore cells were observed at 24, 48, and 72 hours. Ten isolates that showed the highest number of spores were chosen to test their toxicity against *O. rhinoceros* larvae.

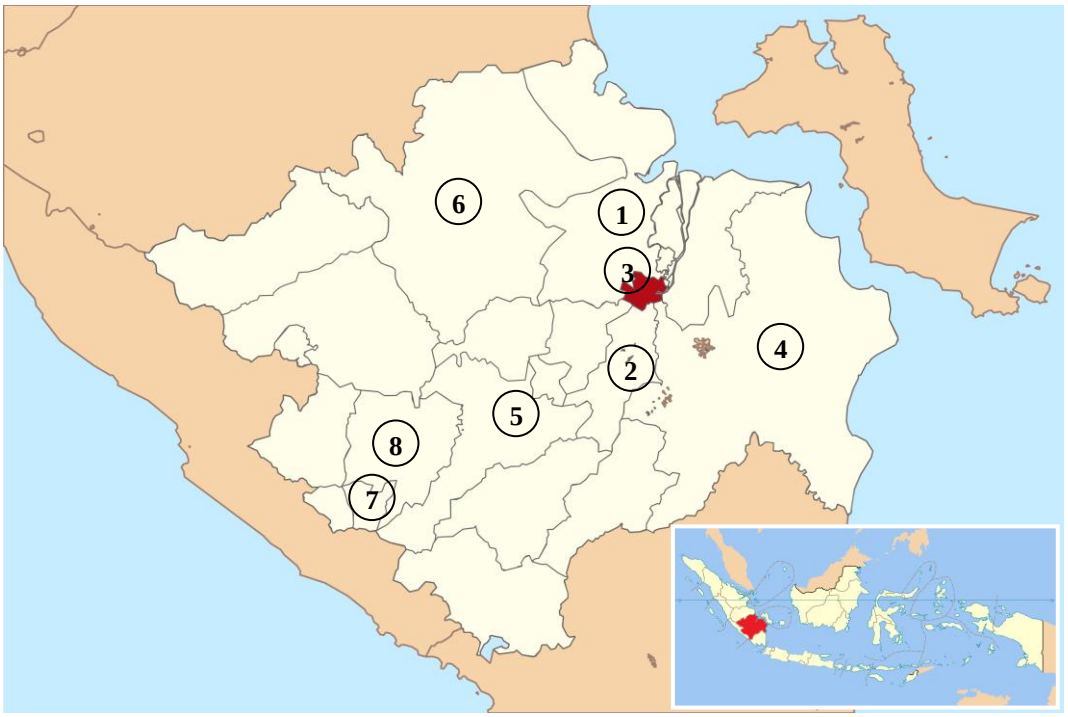


Figure 1. Location of soil sampling in 6 districts and 2 cities in South Sumatra Province, Indonesia, i.e. 1. Banyuasin, 2. Ogan Ilir, 3. Palembang, 4. Ogan Komering Ilir, 5. Muara Enim, 6. Musi Banyuasin, 7. Pagar Alam, 8. Lahat

Tabel 1. *Bacillus thuringiensis* isolates original from soil in some cities and districts of South Sumatra, Indonesia

No.	Cities/dustricts (m asl)	No. of soil samples	No.of colony (BC)	No. of *B. thuringiensis* colonies (Bt)	% isolat Bt (Bt index = (Bt/BC)x100%)
1	Banyuasin (63)	10	56	19	33,9
2	Ogan Ilir (18)	18	73	15	20,5
3	Palembang (8)	13	26	8	30,8
4	Ogan Komering Ilir (18)	6	30	4	13,3
5	Muara Enim (45)	8	57	15	26,3
6	Musi Banyuasin (15)	10	36	10	27,8
7	Pagar Alam (780)	6	25	18	72,0
8	Lahat (500)	5	18	6	33,3
Total/average of % Bt isolates		76	321	95	29,6

Bioassay test

Bioassay test was conducted by using 1st instar larvae of *O. rhinoceros*. For each *Bt* isolate-based bioinsecticide treatment, there was be 5 replications, with 10 individuals/replication. A total of 50 g of decomposed-oil palm bunches were put into 10 × 10 × 5 cm plastic containers. The soil was sprayed singly with 5 mL of bioinsecticide and air-dried. *Oryctes rhinoceros* were then put into the container and allowed to eat. The mortality was observed every day until 7th day.

Isolation of Bacillus thuringiensis *protein*

One colony of *B. thuringiensis* from slant agar was transferred using inoculation loope of into 1 mL NB medium and shook at 25ºC overnight. This culture was then transferred into10 mL of NB medium and shook at 25ºC for 2 days. The cells were harvested after centrifugation at 13 krpm for 10 min, washed in distilled water twice and collected white pellets. The pellet was suspended in distilled water. Precipitation was collected by centrifugation at 13 krpm for 10 min. It was re-suspended in water and added 3 ml of 0.5 M NaCL. For collecting precipitation it was centrifuged at 10 krpm for 15 min. This step (NaCl wash) was done at least two times and continued with distilled water wash two times. The cells were re-suspended in 5 mL of 10 mM Tris HCl pH 8 containing 10 mM EDTA and disrupted by a sonicator. This solution was kept on ice for 30 min after addition of 100 µL of mercapto-ethanol and adjustment of pH 10.5. To collect supernatant contained protein, it was centrifuged down at 10 krpm for 10 min. The last step was adjusting to pH 4.4 and kept the crystal protein on 4ºC for long storage.

Weighing molecular mass of crystal protein by SDS-PAGE analysis

Crystal proteins isolated from *B. thuringiensis* strains were analyzed by sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was carried out by the method of He (2011) using 10% running and 4% stacking gels. The crystal proteins were prepared as follows: 10 µl of crystal protein kept at pH 4.4 was centrifugated down at 10 k rpm for 1 min to collect precipitation. It was suspended in the equal volume of TE. After addition of SDS sample buffer (1: 4, v/v), protein sample was boiled for 3 min. The centrifugation at 7 krpm for 1 min was done to purify the protein solution and electrophoresed.

Data analyses

Data on spores density of *B. thuringiensis* and mortality of *O. rhinoceros* were analyzed using analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) Test was employed to test for significant differences among the treatments (isolates) at P = 0.05. All data were analyzed using software of SAS University Edition 2.7 9.4 M5.

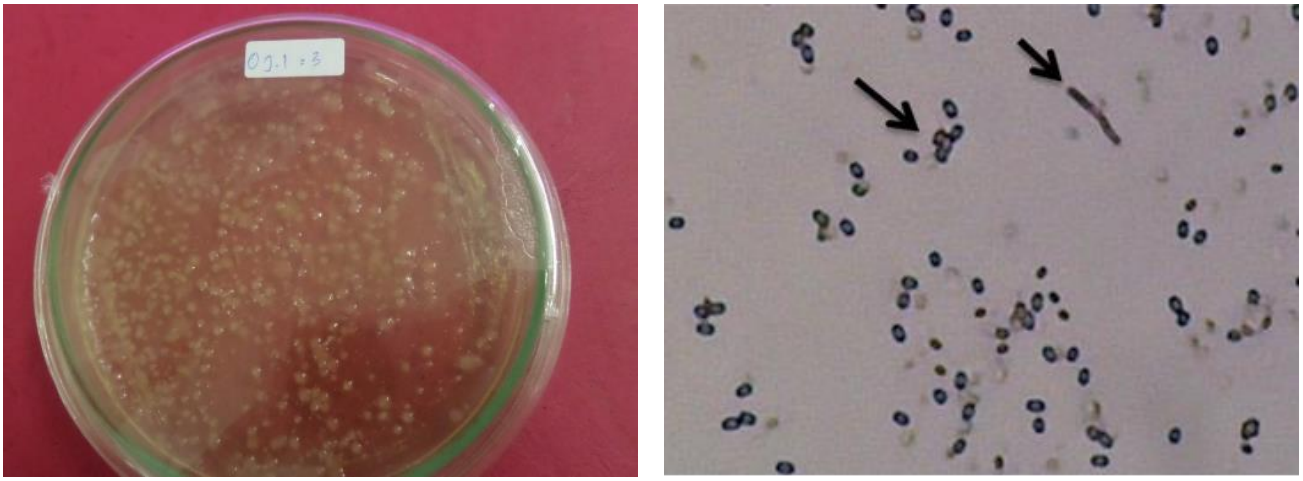
RESULTS AND DISCUSSION

Soil sampling

Soil sampling was carried out in 6 districts and 2 cities in the province of South Sumatra. Soil samples were taken from a variety of habitats, especially in free polluted habitats by chemicals and generally annual plantation or secondary forests. Among the number of soil samples, the average *B. thuringiensis* isolates obtained was 29.6% (Table 1). Bacterial colonies grow after incubation on the first day were very small and grow almost flat with the media. On the second day, the shape of the bacteria was enlarged and rather wide however they did not collide with each other. On the third day enlargement of bacterial elevation was clearly seen on the solid media (Figure 2.A). Of cell observation under a microscope, it appeared in the form of bacilli and some obtained formed spores (Figure 2.B).

Screening test

Screening tests were carried out on third-instar *Spodoptera litura* larvae and first-instar *Rhinoceros oryctes* larvae. From the screening test results obtained that not all isolates of *B. thuringiensis* were found to be able to kill *S. litura* or *O. rhinoceros*. The screening test resulted in 55.79% of these isolates were able to kill *S. litura* larva while 25.06% of *B. thuringiensis* isolates were active against *O. rhinoceros* larvae (Table 2).



A **B**

Figure 2. Colonies of *B. thuringiensis* (A) dan bacterial cel of *B. thuringiensis* (magnification 400 x) (B)

Among 24 isolates of *B. thuringiensis* which actively killed *O. rhinoceros* larvae, 10 isolates were chosen (Table 3). They were grown on NGKG media and propagated with NB media to determine spore density (Figure 2). In NGKG agar medium, *B. thuringiensis* isolates grew well to form colonies. In NB media, it was proved that 72 hours after propagation, the number of cells/spores increased. Each isolate grown on NB media was also propagated on Biourine media enriched with 5% molasses. Furthermore, spore densities were calculated at 24, 48, and 72 hours (Table 3).

Spore density of *Bacillus thuringiensis* isolates

Ten of *B. thuringiensis* isolates active to *O. rhinoceros* larvae grown on NB and Biourine enriched with 5% molasses produced a fairly high spore density and varied from each isolate. Statistically, the amount of spore density was significantly different at each time of observation. Spore growth was gradually increased starting from 24 hours in number until 72 hours of observation. In propagation with NB media after 72 hours of fermentation, the highest spore density in *Bt*-isolates was obtained on KJ3R5 code (9.27 x 10^{12} spores mL^{-1}), whereas in biourine media enriched with 5% molasses the highest density was in KJ3P1 (0.71x10^{12} spores-ml^{-1}). Spores densities of *B. thuringiensis* were showed in Table 4.

Mortality of insect test

In the bioassay test, death occurred starting on the third day after application, ranging between 1.3-8%. On the fifth day, the deaths increased to 4-40%. Some isolates began to show effectiveness in causing death on the fifth day. From the statistical tests, there were significant differences between isolates from controls (without treatment). The process of death symptoms may begin around 24 hours after application, however insect mortality started on the third day ranging from 1.3 - 8.0% 4.0-40% and 6.67-81.33% on the third day, fifth day and seventh day, respectively. On the seventh day, there were 6 isolates showed a mortality rate above 50%. Mortality data of *O. rhinoceros* larvae were presented in Table 4.

Molecular weight of *Bacillus thuringiensis* protein

Of the results of calculating molecular weights of protein using SDS-Page, several bands had been produced in various isolates. In isolate 1 (SMR) it was estimated to have a molecular weight of about 90 kDa and 45 kDa, while in isolate 2 (MSP) was around 90 kDa. , Isolate 3 (KJ3R5) was predicted to possess a molecular weight of 130 kDa, 90 kDa, and 45 kDa, while isolate 4 (KJ3P1) was 50-60 kDa. Isolate 5 (SASU) was suspected a molecular weight of <45 kDa and isolate 6 (MSKS) was around 90 kDa (Figure 4).

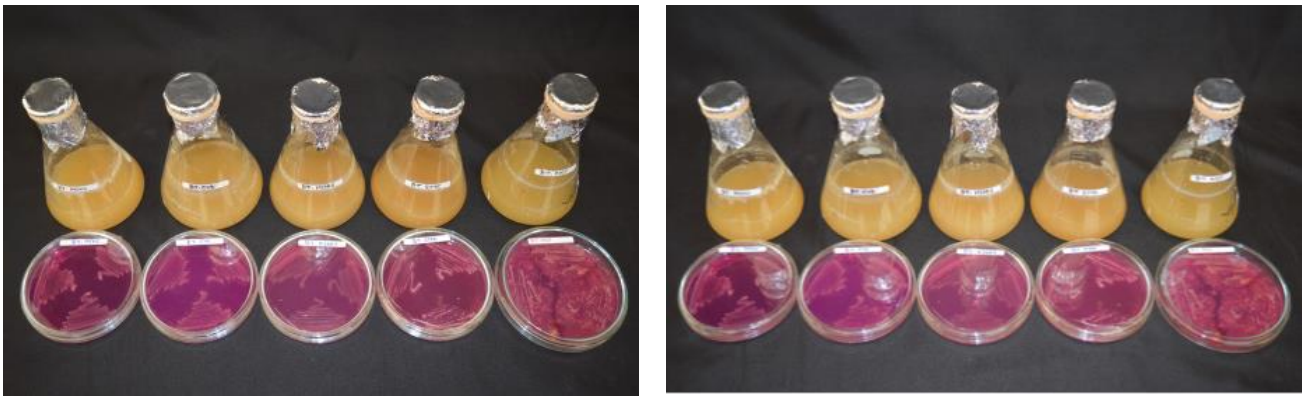
Mortality symptom of larva *Oryctes*

Mortality of *O. rhinoceros* larvae was characterized by changes in color, body texture and body shape. Firstly, healthy Oryctes larvae were white, however, 3 days after application, the symptoms of *B. thuringiensis* infection were marked by discoloration. On the seventh day, larvae died with a black body-color (Figure 3).

Table 2. Screening tests for *Bacillus thuringiensis* isolates against *Spodoptera litura* and *Oryctes rhinoceros*

Cities/districts	Number of *B. thuringiensis* isolates	Active against *S. litura* (%)	Active against *O. rhinoceros* (%)
Banyuasin	19	10 (52.63)	5 (26.32)
Ogan Ilir	15	8 (53.33)	2 (13.33)
Palembang	8	6 (75.00)	3 (37.50)
Ogan Komering Ilir	4	4 (100)	0 (0.00)
Muara Enim	15	9 (60)	5 (33.33)
Musi Banyuasin	10	5 (50)	4 (40.00)
Pagar Alam	18	7 (38.89)	3 (16.67)
Lahat	6	4 (66.67)	2 (33.33)
Total	95	53 (55.79)	24 (25.26)

Notes: Larvae of Spodoptera (n=20 individuals); Larvae of Oryctes (n=20 individuals)



A B

Figure 3. *B. thuringiensis* isolates and their propagation on NB media. Isolate codes: MSKS, CI4, KJ3R5, SMR, MSP. Isolate codes: KJ3P1, KJ3R3, LK, SASU, TPP

Table 3. The origin of isolated soil habitats

Cities/districts	Elevation (m asl)	*B. thuringiensis* active to *O. rhinoceros*	Number of *B. thuringiensis* chosen	Habitat	Isolates codes
Banyuasin	63	5	1	Peat soil	TPP
Ogan Ilir	18	3	1	Jackfruits tree	CI4
Palembang	8	2	1	Sawo tree	SASU
Muara Enim	45	5	2	Mangoes tree	MSP, MSKS
Musi Banyuasin	15	4	3	Rambutan tree	KJ3P1, KJ3R5, KJ3R3
Pagar Alam	780	3	1	Coffee tree	SMR
Lahat	500	2	1	Pepper tree	LK
Total		24	10		

Table 4. Spores density of *Bacillus thuringiensis* active against *Oryctes rhinoceros* larvae and propagated on NB media and biourine enriched with 5% molasses

Isolates codes	Spores density (x 10^{12} spores-mL^{-1})					
	24 h		48 h		72 h	
	Biourine + 5% Molase	NB	Biourine + 5% Molase	NB	Biourine + 5% Molase	NB
SMR	3.25 ± 0.13 ab	3.73 ± 0.20 a	5.8 ± 0.52 b	5.42 ± 0.20 ab	8.07 ± 0.52 ab	7.82 ± 0.24 abc
KJ3P1	6.73 ± 0.47 c	5.99 ± 0.36 b	8.31 ± 0.28 b	8.33 ± 0.26 d	9.71 ± 0.28 b	8.59 ± 0.24 c
KJ3R5	5.26 ± 0.55 bc	4.55 ± 0.29 ab	6.13 ± 0.43 b	6.74 ± 0.24 bcd	8.19 ± 0.43 ab	9.27 ± 0.29 c
MSP	4.21 ± 0.30 bc	4.47 ± 0.35ab	6.14 ± 0.26 b	7.93 ± 0.12 cd	8.11 ± 0.26 ab	9.21 ± 0.14 c
SASU	2.65 ± 0.37 a	3.85 ± 0.17 a	4.04 ± 0.61 a	6.43 ± 0.28 abcd	8.09 ± 0.61 ab	8.07 ± 0.29
MSKS	5.70 ± 0.60 bc	4.78 ± 0.06 ab	6.91 ± 0.47 a	6.23 ± 0.47 abcd	7.98 ± 0.47 ab	7.70 ± 0.40 bc
KJ3R3	5.11 ± 0.63 bc	5.14 ± 0.23 ab	6.37 ± 0.60 a	6.78 ± 0.36 bcd	7.40 ± 0.60 ab	7.39 ± 0.22 abc
LK	6.39 ± 0.40 c	4.73 ± 0.09 ab	7.23 ± 0.26 a	5.97 ± 0.20 abc	7.36 ± 0.26 ab	7.46 ± 0.39 abc
TPP	5.51 ± 0.51 bc	4.18 ± 0.29 ab	6.77 ± 0.50 a	5.09 ± 0.40 ab	7.72 ± 0.50 ab	6.38 ± 0.46 ab
CI4	4.32 ± 0.44 bc	3.87 ± 0.50 a	5.5 ± 0.47 a	4.78 ± 0.27 a	6.95 ± 0.47 a	6.26 ± 0.33 a
F value	6.49	3.47	4.12	8.92	2.51	6.54
F Table	2.39	2.39	2.39	2.39	2.39	2.39
P-Value	0.00	0.01	0.00	0.00	0.04	0.00
Tukeys HSD tests	0.25	0.17	0.21	0.13	0.12	0.11

Note: significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at $P < 0.05$ according to Tukey's HSD test. Original data were transformed using Log transformation prior to statistical analysis

Table 5. Bioassay tests on *Oryctes* larvae during 7 days of observation

Isolates codes	Average of mortality days of ..... (n=75 ind.)		
	3	5	7
SMR	8.00 ± 2.31 b	28.00 ± 4.00 d	58.67 ± 4.35 b
KJ3P1	6.67 ± 1.33 b	25.33 ± 4.81 cd	81.33 ± 7.62 b
KJ3R5	8.00 ± 2.31 b	30.67 ± 3.53 d	86.00 ± 1.88 b
MSP	6.67 ± 1.33 b	40.00 ± 2.31 d	66.67 ± 2.88 b
SASU	8.00 ± 2.31 b	33.33 ± 4.81 d	58.67 ± 2.18 b
MSKS	5.33 ± 1.33 b	32.00 ± 2.31 d	58.67 ± 6.06 b
KJ3R3	1.33 ± 1.33 ab	8.00 ± 2.31 bc	18.67 ± 2.18 a
LK	1.33 ± 1.33 ab	4.00 ± 0.00 ab	10.67 ± 2.88 a
TPP	0.00 ± 0.00 a	5.33 ± 1.33 ab	13.33 ± 1.09 a
CI4	1.33 ± 1.33 ab	4.00 ± 4.00 ab	6.67 ± 2.18 a
Control	0.00 ± 0.00 a	0.00 ± 0.00 a	4.00 ± 0.00 a
F value	7.19	22.74	26.84
F Tab	2.30	2.30	2.30
P-Value	0.00	0.00	0.00
Tukeys HSD tests	12.40	14.08	19.80

Note: significantly different; values within a column (the data of each isolate) followed by the same letters were not significantly different at P < 0.05 according to Tukey's HSD test. Original data were transformed using Arcsin transformation prior to statistical analysis

A B C

Figure 4. Symptoms of death of *Oryctes rhinoceros*. A. Healthy larvae, B. Larvae with symptoms of Bt infection, C. dead larvae

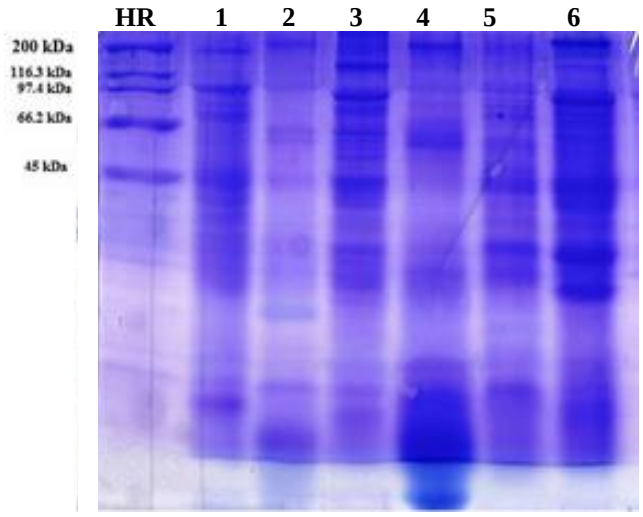


Figure 5. Molecular weight of *B. thuringiensis* isolates as a result of isolation from the soil South Sumatra. Note: 1. SMR, 2. MSP, 3. KJ3R5, 4. KJ3P1,5, SASU, 6. MSKS

Discussion

Sampling sites were varied in microclimate and altitude. The city of Pagar Alam located at an altitude of 780 m is a plateau area (Table 1). From overall isolation, soil samples taken from the city of Pagar Alam were found to be the highest of *B. thuringiensis* isolates (72%). In observations, it may approve that sampling in natural habitats and not widely applied to chemicals (pesticides and fertilizers). Fernandes and López-Pazos (2011) reported *B. thuringiensis* composition seems to be influenced by several factors including soil humidity, organic matter, temperature, structure and pH, macro/micro-nutrients, richness, and local insect distribution.

Some *B. thuringiensis* isolates were toxic to *S. litoralis* larva and some were toxic to *O. rhinoceros* larvae (Table 2). It has been expected that *B. thuringiensis* isolates which actively control *S. litoralis* larvae contained a special protein. Likewise, isolates that kill *O. rhinoceros* larvae contained special genes and proteins. This was consistent with the opinion of Fernandes and López-Pazos (2011) who reported biodiversity of active proteins against insects. Proteins were composed of several polypeptides bound together and the polypeptides had molecular masses ranging from 27 kDa to 140 kDa. *B. thuringiensis* active against *S. litoralis* larvae (Lepidopteran order) may be inactive against *O. rhinoceros* larvae (Coleopteran order) (van Frankenhuyzen 2009).

The growth of various isolates in NB media and Biuorine media enriched with 5% molasses showed similar tendency. Spore production at 24-hour observations was seen to be lowest followed by 48-hour and 72-hour observations. The increasing number of spores may be caused by the process of sporulation in which the less the content of materials needed to grow, the bacterial cells were forced to form spores and proteins. This occurrence was also reported by Valicente et al. (2010), Marzban (2012) and Sarrafzadeh (2012) which stated a certain C/N ratio was required to produce a number of spores and proteins toxic to insects. In addition, it was suspected that utilization of biourine enriched with 5% molasses can be substitute for expensive chemical manufacturing materials such as NB. The use of agricultural and livestock waste materials (in this case biourine cattle) and molasses (sugar factory waste) was very important. Aside from utilizing abundant waste, this method was easily imitated and used by farmers. Some researchers use agricultural or livestock waste materials and apparently showed high toxicity to target insects (Prabakaran et al. 2008; Valicente et al. 2010; Salazar-Magallon et al. 2015)

Mortality of insects was caused by the presence of spores and bacterial proteins ingested in the midgut. Death of an insect usually took time. The process of mortality started from changing protein into a toxin (in the midgut) and then spreads throughout the hemolymph. Insects usually experienced septicemia and eventually die (Bravo et al. 2015c; Soberon et al. 2018). Until recently, it was known that few isolates were able to actively kill *O. rhinoceros* larvae. With a hard body texture and large size, it was necessary to isolate *B. thuringiensis* which contained

a special protein with a molecular weight of around 14-133 kDa (Jouzani et al. 2017). In addition, it is also necessary to match the midgut conditions with alkaline pH in order to break down proteins into toxins (Soberon et al. 2018). According to Khaerani et al. (2012), the toxicity difference of *B. thuringiensis* local isolate might be caused by the difference of strain, size, and type of crystal protein produced.

Protein of *B. thuringiensis* infected larvae through midgut epithelial cells resulting in death of the host by septicemia and production of toxins. The body becomes soft as reported by Frederici et al. (2010). In some target insects, Cry proteins merely were sufficient to intoxicate larvae by destroying enough midgut epithelial cells. As a result, it allowed the alkaline midgut juices to flow into the hemolymph and raise the blood pH induced paralysis and cessation of feeding.

In relationship with mortality rate of *O. rhinoceros* where KJ3P1 and KJ3R5 isolates were 81.33% and 86% (Table 4), it was seen both isolates contained proteins that potent to kill *O. rhinoceros* larvae (70-80 kDa). Therefore they caused high mortality against *O. rhinoceros* larvae. Interestingly, a single strain of *B. thuringiensis* may contain more than one crystal protein gene (Letowski et al. 2005; Pedro and Ibarra 2010). As reported by Fernandes and López-Pazos (2011) Coleopteran specificity Cry proteins had molecular weights Cry1Ia (81.2 kDa), Cry1Ba (139.6 kDa), Cry3 (72-75 kDa), Cry6Aa1 (54 kDa), Cry8 (130-133 kDa), Cry34 (14kDa) and Cry35 (44 kDa). Jouzani et al. (2017) also reported that cry gene cry3, cry7, cry8, cry14, cry18, cry23, cry26, cry28, cry34, cry35, cry366, cry366, cry37, cry38, cry43, and cry55 had effectiveness in killing Coleopteran insect.

In general, *B. thuringiensis* bacteria isolated and identified from soil of South Sumatra possessed a high potential in controlling *O. rhinoceros* even though mortality rate of the insect was not optimal yet. *B. thuringiensis* with molecular weight characteristics of 14-133 kDa has the opportunity to control pests of the order Coleoptera because of the alleged suitability of acidity in the midgut larvae of *O. rhinoceros*. Application of agricultural and livestock waste as propagation media was one alternative in production of *B. thuringiensis*- based bioinsecticides that are cheap and safe for the environment.

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