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The Characterization of *Bacillus thuringiensis* from soil habitat of Auky Island, Padaido District in Biak Numfor Regency and Its Toxicity against Mosquito Larva of *Anopheles sp*

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Abstract: *Bacillus thuringiensis* (Bt) is bacteria that produce protein crystals as an insecticidal against various vector diseases in plants and animals including humans. The purpose of this research is to discover the local isolate toxicity of *B. thuringiensis* from Auky Island Padaido District in Biak Numfor Regency against mosquito larva *Anopheles sp*., as well as to discover the local isolate of *B. thuringiensis* with toxicity ≥85 of laboratory scale. The method used in the research is toxicity detection method where 1ose colony inserted into a container containing 200 mL sterile soil water and 20 larvae of Anopheles instar 3 for 24 h. The percentage of larval deaths is calculated by the number of dead larvae divided by the total number of larvae multiplied by 100%. The result of the research shows three isolates (ABNP 8, ABNP P and ABNP 11) of isolate local *B. thuringiensis* which the toxicity is ≥ 85%. The difference in toxicity may becaused by the difference of strain, the ability of the enzymes in larvae stomach to dissolve δ endotoxin, in which when dissolved in insect gut, it will turn into shorter insecticidal peptides (27-149 kd) and subsequently interact with epithelium cells in the larval midgut and cause pores formation (very small holes) inside the channel digestion membrane and disrupt the osmotic balance, the cell swells and eventually ruptures causing the death of larvae. The difference in toxicity can also be caused by the difference of molecular weight of Gen Cry, in this study the probability of isolates whose toxicity ≥85 has the gen Cry IV b and IV c. Therefore, this study should be continued to determine the genotype of toxic causes of Anopheles larvae.

Keywords: Toxicity, Local Isolate *B. thuringiensis*, Auky Island, Anopheles Instar Larvae 3

Introduction

Bacillus thuringiensis could be found in a variety of habitats and can be isolated from various soil habitats. These bacteria are entomopathogenic microorganisms that are being developed as a biological control of insects from the Ordo of Lepidoptera, Coleoptera and Diptera, as the vectors of various infectious diseases of plants, animals and humans (Blondine, 2010; Lantang and Runtuboi, 2012). Blondine *et al*. (2000), isolated *B. thuringiensis* in the soil habitat in Salatiga and found 12 isolates, in which one of the isolates had a high toxicity to the *Aedes aegypti* mosquito larvae. Similarly, Lantang (2005) isolated 43 isolates of *B. thuringiensis* from various soil habitats in Papua, in which two of 43 isolates have a very high toxicity to Anopheles farauti Laveran as a major malaria vector in Papua. One of the two isolates remains unidentified serologically based on isolate antigen H password 18 Skow Mabo (Lantang, 2010).

Lantang and Runtuboi (2012) characterized 290 *Bacillus thuringiensis* isolates from various species of protected forest trees in Cenderawasih University

Jayapura and found the average of toxicity ranging from 55% to 80% against Anopheles mosquito larvae. Lidwina *et al*. (2013) reported that although *Bacillus thuringiensis* are dominantly found in the lowlands, it can be isolated also in highlands area as found in isolated areas in East Java highlands. Moreover, its toxicity is quite effective against the death of Aedesagypty instar III mosquito larvae, which is 24 h 100% and 72 h 62%. Lantang and Tanjung (2015) reported the pathogenesis of *Bacillus thruringiensis* code 18 local isolates Skow Mabo on a semi-field scale, showing prenatal deaths of Anopheles mosquito larvae on day one, 72.8%, day two 49,1% and day three 21.8%.

Despite there has been much research on bacterial characterization from various habitats in some areas, isolation from various soil habitats to obtain potential indigenous isolates as bio insecticides are needed. Thus, this research chooses Auky Island as an area of research for indigenous isolate as well as its uniqueness which had been affected by Tsunami in 1996. By characterizing *Bacillus thuringiensis* phenotypically, isolate indigenous will be obtained and furthermore to address its toxicity towards Anopheles mosquito larvae as the main malaria vector in Papua and surrounding islands.

Research Method

Soil sampling was obtained from various location in Auky Island, Padaido District, Biak Numfor Regency in February 2018. The soil sample was collected based on the method used by Blondine (2000) and Lantang (2010). Microbiological characterization was done in FMIPA Microbiology Laboratory, Biology Department, Cenderawasih University in Papua. Soil samples obtained from each location weighed 1 gram, then put into a 10 mL sterile water reaction tube, later stirred for 1 min and put into a water heater at 75°C for 20 min.

Following this, the soil samples were streached on Nutrien Agar and incubated at 37°C for 24 h. Colonies indicated by *B. thuringiensis* are white, cream, uneven, rounded colon, protruding. These suspected colonies later observed microscopically with endospore staining and protein crystals using black naphthalene under contrast phase microscope with maximum magnification 1000x, to ensure the isolate was *B. thuringiensis*. (Blondine, 2000; Ayomi *et al*., 2014).

The mosquito's larvae used in this research are the *Anopheles sp* instar 3 taken from the Nimbokrang District and West Sentani District. The larvae then brought to the laboratory to be reared (maintained) prior to testing. Bioassay or toxicity test conducted according to Ayomi *et al*. (2014) by putting 1ml isolate of *B. thuringiensis* into a plastic bowl containing sterile groundwater and 20 larvae *Anopheles sp* Instar 3. Next, in 24 h after the first step of toxicity test, observation is needed to identify numbers of death larvae to the toxicity of *B. thuringiensis*. Dead larvae are brownish black, also identified with swelling of the body, fragile and easily damaged when removed.

Data Analysis

The data obtained in this research are types of local isolates of *B. thuringiensis* from Auky Island and its toxicity towards Anopheles larvae instar 3 laboratory scale. The data analyzed descriptively with an in-depth explanation.

Result

The result of this research obtained 66 soil samples from 19 locations in Auky Island, Padaido District. The macroscopic observation on Nutrien medium agar 87indicated *B. thuringiensis* colony in white color, rough, smooth, round and protruding. These colonies are similar as reported by Khaeruni *et al*. (2012) and Lantang (2010).

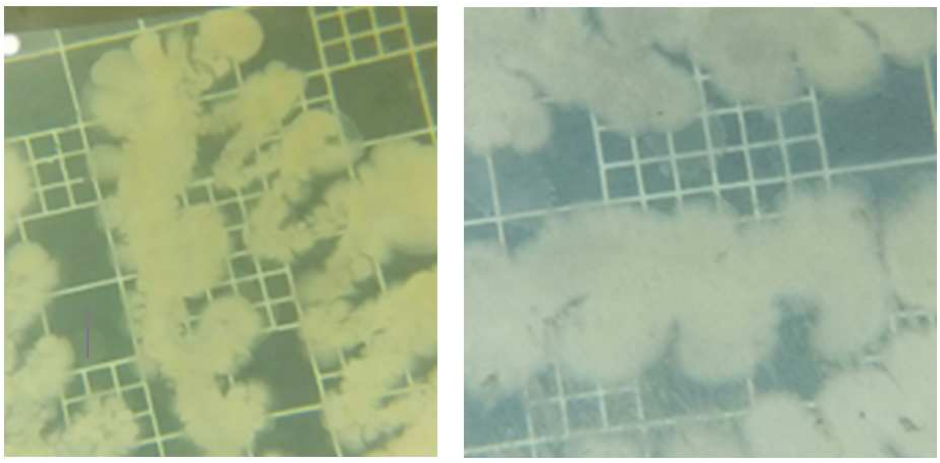
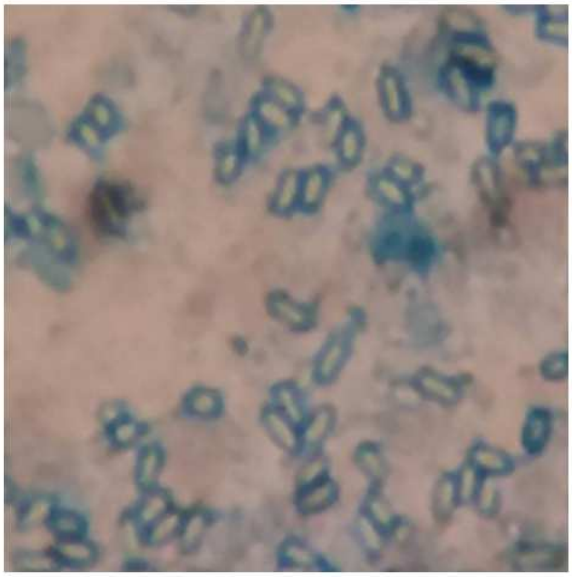


Fig. 1: *Bacillus thuringiensis* colony found in Auky Island, Padaido District, Biak Numfor Regency



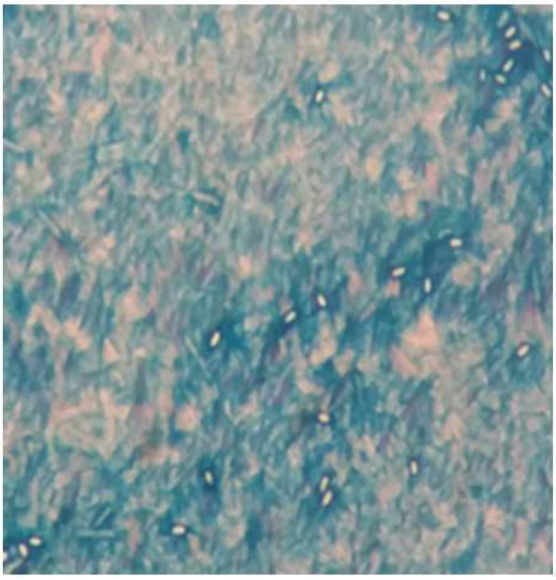


Fig. 2: Protein crystal and endospore

Table 1: 19 local isolates with endospore and protein crystals with rod-shaped cells and positive grams found in Auky Island, Padaido District, Biak Numfor

IsolateAuky Biak Numfor Papua (ABNP)	Repetition 1	2	3	Total of dead larvae	Average	Mortality percentage
Isolate ABNP 1	4	8	8	20	0.33	33.33
Isolate ABNP 2	8	10	8	26	0.43	43.33
Isolate ABNP 3	3	7	4	14	0.23	23.33
Isolate ABNP 4	4	2	5	11	0.18	18.33
Isolate ABNP 5	10	8	14	32	0.53	53.33
Isolate ABNP 6	4	4	6	14	0.23	23.33
Isolate ABNP 7	16	18	12	46	0.77	76.67
Isolate ABNP 8	18	17	18	53	0.88	88.33
Isolate ABNP 9	17	19	16	52	0.87	86.67
Isolate ABNP 10	1	2	1	4	0.07	6.67
Isolate ABNP 11	18	16	18	52	0.87	86.67
Isolate ABNP 12	14	16	14	44	0.73	73.33
Isolate ABNP 13	1	4	1	6	0.10	10.00
Isolate ABNP 14	1	0	4	5	0.08	8.33
Isolate ABNP 15	3	3	2	8	0.13	13.33
Isolat ABNP 16	6	3	4	13	0.22	21.67
Isolate ABNP 17	18	14	12	44	0.73	73.33
Isolate ABNP 18	6	8	6	20	0.33	33.33
Isolate ABNP 19	8	10	14	32	0.53	53.33

Discussion

Microscopic observation of spores and protein crystal towards 87 indicated isolate of *B. thuringiensis* obtained 19 isolates with endospore and protein crystals with rod-shaped cells and positive grams as shown in Fig. 2. These results are similar as reported by Bahagiawati (2002), Khaeruni *et al*. (2012), Suwarno *et al*. (2015).

Toxicity detection test against Anopheles Instar III mosquito as shown in Table 1.

Table 1 shows that from 19 local isolates of *B. thuringiensis* identified in Auky Island has a different toxicity against larvae of *Anopheles sp*. Isolate ABNP 7, ABNP 12 and ABNP 17 toxic against mosquito larvae Anopheles 76% and 73%, meanwhile isolate ABNP 8, ABNP 9, ABNP 11 toxic against mosquito larvae Anopheles ≥ 86%.

Fig. 3: Dead Larvae of Anopheles Mosquito caused by toxin of *Bacillus thuringiensis*

The result of this research shows that the laboratory scale of local isolate of *B. thuringiensis* in Auky Island (isolate 8, isolate 9 and isolate 12) are higher compared to local isolate of *B. thuringiensis* in forest learning center FMIPA Uncen Jayapura and *B. thuringiensis* local isolate code 18 and 9 in Skow Mabo against CulexSp mosquito larvae and *Anopheles sp*. ≥86%. The differences in toxicity may be caused by the difference of strain and type of protein crystal, size and shape produced during sporulation.

It may also be caused by the ability of strain enzyme to dissolve protein crystal that is ingested by the larvae then eventually enter into insect intestine with an alkaline pH atmosphere. According to Blondine (2010) and Ayomi *et al*. (2014), protein crystal is toxic against the vector when it is soluble in the intestine of larvae by the condition of alkaline pH, which affects metabolic activity of the larvae due to toxin that spread throughout larvae body and leads to the formation of small holes or pores on larvae stomach that causing the inability of larvae to eat and eventually die (Fig. 3).

Hofte and Whiteley (1989) describe that δ endotoxin is a protoxin which when dissolved inside insect gut will turn into a shorter polypeptide (27-149kd). This insecticidal polypeptide further interacts with epithelium cells in larvae gut and causes the formation of pores (very small homes) inside gastrointestinal cell membrane that will disturb the osmotic balance, causes cell swells and eventually breaks out and causes the death of larvae.

According to Khaerani (2012) the toxicity difference of *B. thuringiensis* local isolate could be caused by the difference of strain, size of a protein crystal and type of protein crystal which produced. In addition to this, Bt endotoxin crystals are grouped into 8 main classes: Cry 1A to Cry X based on the sequence homology of its N-terminal aminodic acid, its molecular weight and its insecticidal activity against plant pests and disease vectors in humans.

The result of *B. thuringiensis* protein crystal classification and its specification to insects shows that 1Aa, Cry 1Ab, Cry 1Ac, Cry 1Cb, Cry 1F, CryIIA, Cry IIB and Cry IIC toxic to Lepidoptera group, while Cry IIIA, Cry IIIB and Cry IIIC toxic against Koleoptera and Cry IVB and Cry IVC toxic against the Diptera ordo, Bahagiawati (2002). The difference of *B. thuringiensis* local isolate toxicity could also be caused by the difference in the molecular weight of isolate protein crystal. In this research, the researchers did not analyze the weight of protein crystal and its insecticidal impact towards larvae but Hermanto *et al*. (2013) reported in their findings that the molecular weight hugely determines the toxicity of the larvae. Hermanto also reported that Protein Cry 23, 25 and 35 has a toxicity against Diptera ordo and protein Cry 25 istoxicagainst Coleoptera ordo.

Conclusion

The result of this research found 19 local isolates of *B. thuringiensis* in Auky Island and obtained 3 local isolates with toxicity more than 85 percent against anopheles instar III mosquito larvae in laboratory scale that is ABNP 8, ABNP 9 and isolate ABNP 11 with death rate of Anopheles mosquito larvae respectively 88.33%, 86, 67% and 86,67%.

Recommendation

Further research on the characteristic of *B. thuringiensis* local isolate in Auky Island on genotype sequencing method is needed as well as research on PCR against isolate ABNP 8, 9 and ABNP 11 to obtain information about the toxic gene.

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Authors Contributions

Lantang Daniel and Rampa Ester: Participated in all experiments, coordinated the data-analysis and contributed to the writing of the manuscript.

Lunga Nelly: Participated in data-analysis and contributed to the writing of the manuscript.

Ethics

This study was approved by the *Pusat Lembaga Penelitian dan Pengabdian Masyarakat Universitas Cenderawasih* with ethical clearance.

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