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POTENTIAL ENTOMOPATHOGENIC BACTERIA ISOLATED FROM EXTREME CONDITION AREA SIDOARJO MUD, INDONESIA AGAINST *Spodoptera litura* FAB (LEPIDOPTERA: NOCTUIDAE)

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Abstract

Biological control by using entomopathogenic bacteria shows potential to be developed as an alternative technique to control *Spodoptera litura*. Bacteria that can live in extreme environments are reported to have high efficiency and the ability to survive in various environmental conditions, such as in the area of Sidoarjo mud. Related to the issue, this research aimed to find out bacteria that can survive in extreme conditions and are potentially entomopathogenic to control *S. litura*. The study was conducted from January until July 2021 in the Sidoarjo mud area and laboratory of Biological Control, Faculty of Agriculture, University of Brawijaya. The research was conducted using methods including consist of a sampling of Sidoarjo hot mud, isolation, and screening of bacteria that is potential as entomopathogenic bacteria, bioassay of mortality *S. litura*, development of larvae and pupal using Completely Randomized Design with 11 treatments and 4 replications, and molecular identification by 16S rRNA. Data for mortality and development of larvae and pupal were submitted to variance analysis, followed in comparison to the averages of the Duncan test at a 5% level of significance. The results showed that 43 colony bacteria from Sidoarjo hot mud have been successfully isolated and obtained 9 isolates selected as entomopathogenic bacteria against *S. litura* with a percentage of mortality larvae reached 60%. Moreover, bacteria have the potential to inhibit the development of larvae and pupal *S. litura.* Molecular identification showed that potential isolates are *Bacillus subtilis* strain 15A-B92, *Bacillus thuringiensis* strain GTG-29, and *Bacillus anthracis* strain BA1035.

Keywords: Bacillus subtilis; Bacillus thuringiensis; Bacillus anthracis; Entomopathogenic bacteria; Sidoarjo Mud*; Spodoptera litura.*

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Introduction

The Armyworm (*Spodoptera litura* Fab) is an important pest that can attack >120 different host plants (Ahmad et al, 2008). *S. litura* is a highly destructive pest of Agriculture in several countries in Asia and causes great damage of about 54% to 100% (Su et al., 2012; Sang et al., 2016). *S. litura* is generally controlled by applying a chemical insecticide because farmers consider this the easiest and most effective way. In contrast, many research indicates that the use of synthetic pesticides that did not appropriate has some negative impacts, such as killing non-target organisms, causing pathogenic resistance, and adversely affecting the environment (Aktar et al., 2009). Therefore, an effective and environmentally friendly alternative control strategy is needed. Biological control by utilizing entomopathogenic microorganisms is part of an integrated control component that can provide alternative problem-solving due to the use of a synthetic insecticide. The use of entomopathogenic microorganisms in biological control works by producing antibiotics or other compounds that can inhibit the growth and development of insect pests and induce plant resistance (Haouel et al., 2010).

Entomopathogenic bacteria are microorganisms that have the potential to be biocontrol agents in pest management. Previous studies have reported that some bacteria can act against *S. litura* pests Bacillus *thuringiensis* subsp. *aizawai* was able to suppress the development of *S.litura* with mortality rates between 33.3-100% (Bobrowski et al., 2002). In addition, the *Chromobacterium violaceum* bacteria also showed maximum antifeedant activity of 72.46% at 1000 ppm concentration (Baskar and Ignacimuthu, 2012). Another potential bacteria, *Bacillus subtilis* exhibited inhibitory activity capable of controlling *S.litura* by producing various antibiotic compounds and secondary metabolites in the form An extracellular chitinase CS1 and CS2 (Compant et al., 2005).

In Indonesia, the development of entomopathogenic bacteria to control pests *S.litura* is on developing, even in 2008 registered a total of 14 formulations of Bt to control these pests. However, in the field, only a few farmers use Bt to control the *S.litura*. Among the causes of less favored Bt insecticide by farmers is the Bt active ingredients readily decompose when exposed to sunlight. The effectiveness of the application of bacteria as plant pest control agents are influenced by various environmental conditions which can support growth, such as the presence of oxygen, temperature, moisture, salinity, and pH. In general, the bacteria can live optimally at a maximum temperature of 37 ° C, pH between 6.7 to 7.5, and water content of about 80-90%. Therefore, to optimize its role as a controlling agent, it is necessary for bacteria that can play an optimal role in various environmental conditions. Bacteria capable of living in extreme environmental conditions such as high temperature and high salinity are reported to have high efficiency and adapt to various environmental conditions. *Brevibacillus laterosporus* BPM3 bacterial strains isolated from extreme environments natural hot springs in Assam, India, showed inhibitory activity against the growth of corn borer (*Ostrinia nubilalis* and pathogenic fungi (*Fusarium oxysporum, F. semitectum, Magnaporthe grisea, Rhizoctonia oryzae*) and bacterial pathogens (*Staphylococcus aureus*) (Saikia et al., 2011).

One of the environments with extreme conditions is the area of Sidoarjo mud flow. Sidoarjo mud is a national disaster that occurred on–29 May 2006 due to oil drilling activities conducted by PT. Lapindo Brantas (Davies et al., 2007). The Sidoarjo mudflow zone is reported to have extreme environmental conditions with temperatures between 45-70ºC, alkaline pH conditions

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between 7.5-7.8, as well as a high salinity (salinity) of 30 ppt or 30,000 ppm (Dagdag et al., 2015). Sidoarjo mudflow disaster directly affects the various aspects of the environment and creates a unique new ecosystem formation, one of which is the emergence of a simple life of bacteria characterized by the presence of various signs of microorganism activity such as changes in mud color in some overflow locations. A total amount of about 5.1x104 cfu /g (Santosa et al., 2014). Previous studies have reported that the bacteria of *Orchobacterium intermedium* isolated from the Sidoarjo mud (Muhidin, 2016) may act as biological agents against the *Leptinotarsa decemlineata*, Additionally, the thermophilic bacteria producing the xilinase enzyme known in the *Bacillus lichenformis* species have also been isolated from the Sidoarjo mud (Habibie et al, 2014). Bacteria *B. lichenformis* N1 was reported to be able to suppress the development of pest infestation in chili plants caused by *Bemicia tabacii* pest (Kim, et al., 2007).

The existence of entomopathogenic bacterial life in the extreme environment of Sidoarjo mud gives the potential to be developed as the controlling agent against other plant pests. Therefore, this research aimed to collect bacteria that can survive in the extreme condition (high salinity & thermal), find the potential entomopathogenic bacteria that can be used to control *S.litura* and identify the selected entomopathogenic bacteria to *S.litura* by molecular analysis.

Materials And Methods

The research was conducted from January until July 2021 in the Biological Control Laboratory, Department of Pest and Disease of the Faculty of Agriculture, University of Brawijaya, Malang. There are several steps of this research among others: 1). Determination of research location; 2). Exploration of bacteria that can survive in extreme conditions; 3). Bioassay of potential entomopathogenic bacteria to mortality larvae of *S.litura* and larvae and pupal period, and 4). Molecular identification of potential entomopathogenic bacteria *S.litura* by 16S rRNA gene sequencing.

Determination of research location

Samples were taken at 10 points around the area representative in the mud using the purposive sampling method. The sampling criteria used is an area that has a high temperature, the content of salt, and the area that changes color that indicates the activity of microorganisms (Fig. 1). There were 4 of the selected area 25, 40 which is closest to the affected area residential area, the central area of the mudflow and gryphon area which is formed by a small volcano. The sampling in areas with high temperatures is expected to obtain thermotolerant bacterial properties, while in the area is expected to obtain a salt content of bacteria halotolerant. Meanwhile, the area of sampling is recording the coordinates and altitude-specific locations using GPS (Global Positioning System) as well as temperature measurement using an infrared thermometer. The result of recording the coordinates, altitude, and temperature measurements is presented in Table 1. Sample the mud that has been collected and then take it to the laboratory to isolate bacteria.

Exploration of bacteria that can survive in extreme conditions.

Exploration of halotolerant bacteria was done based on Rahman et al., (2017), 10 g of each the mud samples was taken to prepare a suspension. Afterward serially diluted (10^{-1}, 10^{-3}, 10^{-5} ,and 10^{7}), each dilution took 100 µL and incubated on artificial medium Nutrient Agar for 24-48 hours at 37^{0}C. The emerging colonies were then purified by taking a single colony and breeding on the same medium. The bacteria are then stored in 20% glycerol or skim milk and stored at -30^{0}C. Each bacteria is characterized based on morphologically (color, shape, edges, elevation, mucoid).

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Bioassay of potential entomopathogenic bacteria to mortality larvae of *S. litura* and larvae and pupal period Rearing of *S. litura*

The Insects' test that was used was 2nd instar larvae of *S. litura* which were obtained from the Laboratory of Pest, Department of Pest and Disease Plant, University of Brawijaya. *S.litura* reared on cabbage leaves in plastic containers (Dimensions: 18 cm height and 15 cm diameter) covered with a screened window with stencil paper. Larvae were fed with fresh cabbage leaves daily until full maturity. The armyworm *S.litura* took 16.70 ± 0.54 days to complete the larval period. And prepare sterilized soil for pupation. A pupal period was found to be 12.70 ± 0.56 days. After emergence, the adult moths were kept inside a plastic container (11 cm diameter and 10 cm high) for mating and provided with a sheet of paper for oviposition. The Cotton swab soaked in 10% sucrose solution was provided as a source of food for adult moths. Adults lived for 8.10 ± 0.28 days and the oviposition period was found to be 4.50 ± 0.17 days. Egg masses laid on the papers were collected daily and placed in clean containers (12 cm diameter x 6.5 cm high). Eggs were incubated at a temperature of 25 ± 1 °C and relative humidity of 70–80%. The incubation period was observed to be 4.60 ± 0.16 days. After hatching, neonate larvae were released on the tender cabbage leaves and reared to maturity as explained above. The larvae from the second and subsequent generations were used for the experiments (Kaleeswaran et al., 2018).

Preparation of bacterial suspension

Bacterial isolates from stock cultures were transferred to fresh NA plates to obtain single colonies for each isolate. The obtained single colonies were inoculated into Nutrient Broth (NB) and incubated at 30 ^{0}C for 48 h. After incubation, the bacterial density was measured at an optical density (OD600) and adjusted to 1.89 (approximately 1.8 x10^9 CFU mL^{-1}) (Moar et al, 1995). Five milliliters of each culture were centrifuged at 3000 rpm for 10 min. After that, the pellet was resuspended in 5 ml of sterile phosphate buffer solution (PBS) and used in bioassays.

Selection of bacteria that are potentially entomopathogenic to *S. litura*

The selection process refers to the method Zimmer et al. (2013). The newly second instar larvae of *S. litura* (n= 30) were placed in each vial with organic cabbage leaves (approximately 10 cm^2) were surface sterilized with 5% (v/v) NaOCl followed by washing with distilled water. These leaves were treated by dipping in 5 ml bacterial suspension (10^{-9} CFU mL^{-1}) of each isolate prepared as described first. The vials were kept at 27 ± 2 ^{0}C temperature and 65 ± 5% relative humidity condition. After every 48 h, the larvae were provided with organic cabbage leaves treated with bacterial suspension till pupation. The observation was done with a counted number of dead larvae after 48 hours.

The bioassay of selected potential entomopathogenic bacteria at the mortality of larvae of *S. litura*, larvae period, and pupal period

Bioassay was performed on selected Sidoarjo mud bacterial isolates that had entomopathogenic potential against *S.litura*. The tests were performed using the same method as in the selection process of entomopathogenic bacteria (Zimmer et al., 2013), for control negative larvae we added *aquadest* instead of bacteria suspension. And control positive use bioinsecticide commercial that contains *B. thuringiensis* (Thurex), The vials were placed inside larger containers with sawdust to obtain the 3rd instar larvae post-feeding, then covered with cheesecloth tissue for pupation, and later adult emergence. There are 4 treatments (LS 4.4, LS 5.1, LS 9.2, control) and 5 replicate. The experiment was kept at 27 ± 2 ^{0}C temperature and 65 ± 5% relative humidity condition. The individual

surviving insects were observed until emergence as an adult and the leaves changed every 2 days. The parameters observed were mortality of larvae, larvae period, and pupae period. The calculated percentage of mortality was calculated using the proposed formula by Finney (1971). Percentage of mortality larvae = Number of dead larvae/ Number of observing larvae. The larvae period counted from the time of application (2nd instar) till became pupae. Furthermore, the pupae period calculated began to form pupae until it becomes imago. Data for mortality of larvae, larvae period, and pupae period were evaluated by analysis of variance, when differences were detected, the Duncan test at a 5% significance level was applied to classify the means.

Molecular identification of potential entomopathogenic bacteria againts *S. litura* by 16S rRNA gene sequencing

Identification of selected bacterial isolates possessing the ability of entomopathogenic bacteria was done molecularly based on the results of the 16S rRNA gene sequencing. 16S rRNA gene sequencing consists of a DNA extraction stage, amplification of 16S rRNA gene with PCR (Polymerase Chain Reaction), PCR electrophoresis, and sequencing (Boukedi et al., 2016).

The stages in DNA extraction refer to the PrestoTM Mini gDNA Bacteria Kit protocol. Pure bacterial isolates were cultured on NB media and in the shoots at shakers at 150 rpm for 48 hours. The process of DNA extraction consists of the following steps.

The chromosomal DNA of the isolated bacterial sample was centrifuged at 13,000 rpm for 5 min, then ~~it~~ used as a template in the amplification of the 16S rRNA gene. The 16S rRNA gene was amplified by PCR (Polymerase Chain Reaction) technique using Mycycler Thermal Cycler System (Bio-Rad, USA). Primers used for the amplification of the 16S rRNA gene are primers uinF (5'-AGAGTTTGATCATGGGTCAG 3 ') and uinR (5'-TACGGCTACCTTGTTACGA-3') which is a universal primer for various bacterial strains. The PCR stage begins with making Master Mix which consists of Master Mix Go Tag Green (Promega) 10 µl, Universal uinF 1 µl and uinR 1 µl universal, DNA template 2 µl, and ddH2O 11 µl.

PCR product results can be viewed using agarose gel electrophoresis. Gel agarose was made by dissolving agarose gel powder as much as 1.9% then 100 mg of TBE (tris boric EDTA) solution was added and heated in the microwave until homogeneous. When still in a warm condition, SYBR is added as much as 10 µl and mixed until homogeneous, then poured into molds that have been installed comb well maker. After the gel agarose solid, added 1 TB solution until submerged. The next step is to insert a marker (1kb DNA ladder) and PCR product results into the wells contained in the gel, then electrophoresis with a voltage of 100 volts for + 45 minutes. Visualization of electrophoresis result was done by using Gel-Doc XR 1000 (Bio-Rad, USA).

The sequencing of PCR products is performed using the 1st BASE service which is channeled by PT. Genetics Science Indonesia, Jakarta. The sequencing results were processed using BioEdit software, then analyzed using the BLAST program to search for a homologous 16S rRNA sequence on the DNA database (GenBank) from the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov/.

Results And Discussions

Exploration and selection of bacteria that are potentially entomopathogenic to *S. litura.*

The populations of bacteria were calculated in mud samples collected from four areas in Sidoarjo Mud, Indonesia (Table 1). A total of 43 bacteria were isolated from areas 25 (15), 40 (13), Mudflow Center (4) and Gryphon (11). Each isolate bacteria has different characteristic morphology and biochemical.

Morphological observations based on shape, color, elevation, edge and mucoid. Moreover, biochemical in terms of gram hypensitive tests. 34 isolates were gram-negative while the other 8 isolates were gram-positive, 39 isolates showed hypersensitive negative and 3 isolates were hypersensitive positive. The existence of different morphology and biochemical character from each isolate indicated that the isolates have different genera

The Sidoarjo mudflow zone is reported to have extreme environmental conditions with temperatures between 45-70°C, alkaline pH conditions between 7.5-7.8, as well as a high salinity (salinity) of 30 ppt or 30,000 ppm (Dagdag et al., 2015). Four mud location samples were collected from Sidoarjo Mud. The isolated bacteria from the mud samples had successfully survived in a limited range of salinities and thermo-tolerant bacteria. Isolated bacteria from mud samples are determined by their composition. Mud composition broadly reflects the normal distribution of species of bacteria in the environment (Heijs, et al., 2006). However, isolates obtained from isolation using the serial dilution method cannot be said to have represented much bacterial diversity in the environment (Schoenborn et al., 2004).

Bioassay of selected potential entomopathogenic bacteria at the mortality of larvae of *S. litura*, larvae period, and pupal period

The 43 isolates bacteria from the mud were selected to determine the inhibitory potency against *S. litura* pests was done by dipping test bacteria in the feed. The selection of 43 isolates of the mud showed that nine isolates BLS10, BLS11, BLS12, BLS14, BLS15, BLS16, BLS18, BLS22, and BLS33 were capable of caused mortality in *S. litura* larvae. Mortality of *S. litura* treated with bacterias from Sidoarjo Mud in of 9 days observation are illustrated in Fig 2. The highest mortality was controlled positive (commercial bioinsecticide *B. thuringiensis*) at 66.67%. However, it is not significantly different from BLS10 which aused mortality to reach 60%. Meanwhile, it is interesting there are 2 treatments BLS 11 and BLS 12 that causing almost a similar mortality percentage of around 40%. Followed by BLS 33, BLS 15, BLS16, and BLS 14 with caused mortality of around 20%. Moreover, BLS22 and BLS 18 subsequently caused only 10% mortality at the end of an experiment.

Table 1. Conditions of sampling the Sidoarjo Mud and results of exploration bacteria

Area Sampling	Point	Code	Coordinate		Elevation	Temperature	Number of bacteria
			South	East			
Point area 25	1	LS1	7031'57,24 "	112042'27,61 "	20	28	8
	2	LS2	7032'02,01 "	112042'28,59 "	21	29	7
Point area 40	1	LS3	7032'19,61 "	112043'01,61 "	24	31	7
	2	LS4	7032'19,00 "	112042'59,83 "	23	30	6
Mudflow Center	1	LS5	7031'55,15 "	112042'29,13 "	25	30	1
	2	LS6	7052'85,70 "	112070'90,80 "	26	59	2
	3	LS7	7031'84,40 "	112042'55,60 "	25	38	1
Gryphon	1	LS8	07°31.910 "	112°42.587 "	23	36	2
	2	LS9	07°31.418 "	112°42.389 "	25	40	4
	3	LS10	07°31.898 "	112°42.606 "	24	33	5

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Significant differences were recorded in the percentage of larvae period and pupal period compared to control negative (Table 2). Generally, treatment of bacteria could prolong the larvae period. Larvae periode in treatment was prolonged than other treatments until 3 days. Larvae period count commences with infestation larvae (2nd instar) until becoming pupae. The results indicated that Control positive, BLS 10, BLS11 and BLS 12 could prolong the larvae period by approximately 2-3 days. Mostly, in their treatment larvae were mortality and did not develop become pupae. On the other that, the pupal period was extended from 3 days until 5 days after treatment with bacterial suspension. The pupal development period was significantly influenced by treatment, with the prolonged pupal period in Control positive, BLS 10, BLS11, and BLS 12.

In the present result, a significant difference in the larvae and pupal development period of *S. litura* was recorded after treatment with bacteria. Overall, treatment of Sidoarjo Mud bacteria was delayed on diet containing bacteria and the larvae and pupal period was also significantly prolonged. Metabolites active compounds produced by *B. subtilisis* species were also reported as exhibiting activities against the larva and pupal stage of mosquitoes (Manonmani et al., 2011) and another lepidopteran (Salam et al, 2011; Ghibri et al 2011; Mnif et al, 2012). To develop become pupae, larvae need more energy to build some organs. Some Bacillus species could reduce Lactase dehydrogenase (LDH) which play a key role in carbohydrate metabolism and has been used as an indicator of chemical stress, on the other that LDH is relevant in the production of energy (Malaikozhundan and Vinodhini, 2018). It is suspected that treatment with Bacillus species can reduce LDH which affects the availability of energy needed by *S. litura* to be developed in the larvae and pupal stages.species able to reduce LDH which affect the availability of energy needed by *S. litura* to be developed in larvae and pupal stage.

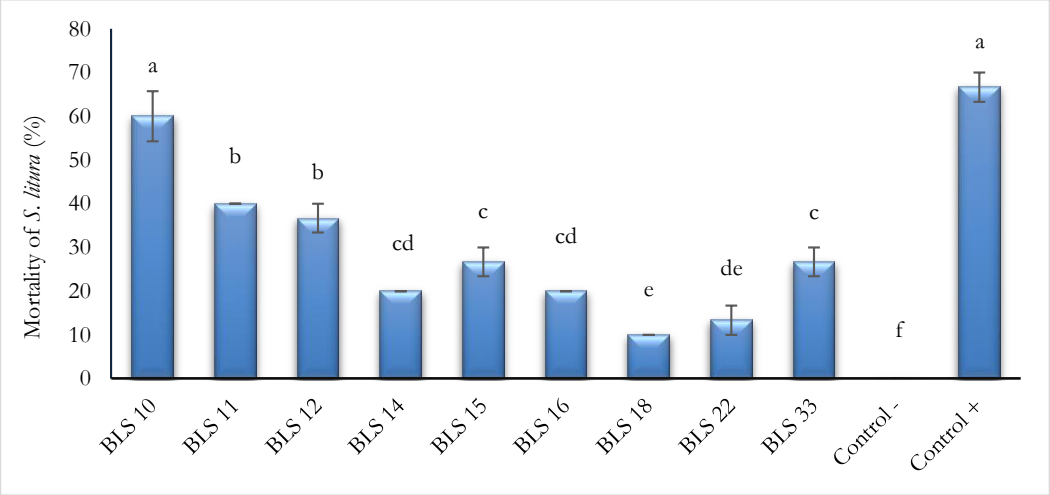

Figure 2. Percentage mortality of *S. litura* that treated entomopathogenic bacteria from Sidoarjo Mud under laboratory conditions in 9 Day After Treatment (DAT). Differences among treatment bacteria were established by Duncan post hoc tests; significant differences ($p<0,05$) are represented by different letters among treatments. Error bars correspond to standard errors. BLS; Bacteria from Sidoarjo Mud.

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Table 2. Means of Larvae and pupal development of *S. litura,* when treated with suspensions of entomopathogens bacteria from Sidoarjo Mud at concentrations of 10^{-9} CFU ml^{-1} under laboratory conditions.

Code of Isolate Bacteria	Larvae periode (days) ± SD[a]	n	Pupal periode (days) ± SD[b]
BLS 10	10.77 ±1.15 a	12	5.5 ± 0.21 a
BLS 11	10.47 ± 0.17 b	18	5.59 ± 0.08 a
BLS 12	10.50 ± 0.12 b	19	5.4 ± 0.13 ef
BLS 14	8.07 ± 0.12 e	24	4.42 ± 0.11 cde
BLS 15	8.50 ± 0.10 c	22	3.92 ± 0.11 e
BLS 16	8 .00± 0.10 e	24	4.03 ± 0.13 de
BLS 18	8.13 ± 0.15 de	27	4.68 ± 0.28 bcd
BLS 22	8.07 ± 0.12 e	26	3.95 ± 0.10 e
BLS 33	8.37 ± 0.06 cd	22	4.24 ± 0.45 cde
Control -	8.13 ± 0.29 de	30	4.9 ± 0.61 abc
Control +	10.6 ± 0.10 ab	10	5.6 ± 0.61 a

[n] Number of surviving pupae from 30 treated larvae

[a] Day from instar 2 larvae to pupation

[b] Day from pupation to adult emergence.

[c] Means followed by different letters are significantly different (GLM followed by Duncan test: $P < 0.05$).

Molecular identification of potential entomopathogenic bacteria against *S. litura* by 16S rRNA gene sequencing

Based on the selection results were known, there are 3 bacteria with the potential to cause mortality in *S. litura* larvae. The result of molecular identification 16S rRNAs of 3 selected entomopathogenic bacterial isolates from Sidoarjo Mud against *S. litura* showed that BLS 10 isolates had 99% similarity with 16S rRNA gene sequences *Bacillus subtilis* strain 15A-B92, isolate BLS 11 has a homology value of 98% with a 16S rRNA isolate gene isolate *Bacillus thuringiensis* strain GTG-29, and BLS 12 isolates have similarities with *Bacillus anthracis* strain BA1035 with levels likeness of 96% (Table 3 and Fig 3). Interesting mortality results are BLS 10 isolate which after being identified as *Bacillus subtilis* strain 15A-B92 afford the same effect as commercial bioinsecticide that contains *B. thuringiensis*. Recently, Salam et al (2018) reported that *B. subtilis* subsp. *subtilis* BTN7A strain has chitinase gen CHI-NRC-4 and T-CHI-NRC-6, which could inhibit egg hatching *Meloidogyne javanica* until 96%. Chitinase play role in the degradation of wall insect and nematode (Husain et al, 2017). Furthermore, *B. subtilis* produces lipopeptide biosurfactants that cause mortality of the second instar of *Papilio demoleus* (Linn) (Osouli and Afsharmanesh, 2016). Other results, bacteria that have been detected cause mortality of *S. litura* are *B. thuringiensis* strain GTG-29 (BLS11), and *Bacillus anthracis* strain BA1035 (BLS12). *Bacillus* species are gainful bacteria that can be developed as bioinsecticides (Garci'a *et al*., 2011). Crystal toxins (Cry) from *B. thuringiensis* are eminent as efficacious in controlling insects pest. Several reports explain that Cry toxin is effective to lepidopteran spesies (Gahan et al, 2010; Stevens et al, 2017; Liu et al, 2018). The uniqueness is *B. anthracis* strain BA1035 which causes mortality too. It is a gram-positive causing the acute mammalian disease anthrax (Hadjinicolaou et al, 2009), some report *B. anthracis* also mainly affects herbivores (Hugh-Jones and Blackburne, 2009).

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Table 3. Result of molecular identification of bacteria that have potential as entomopathogens bacteria againts *S. litura* from Sidoarjo Mud

Code of Isolate Bacteria	Accession number	Length	Gene	Linkages (%)
BLS 10	MF062627.1	1310	*Bacillus subtilis* strain 15A-B92	99%
BLS 11	KJ769222.1	1418	*Bacillus thuringiensis* strain GTG-29	98%
BLS 12	CP009700.1	1407	*Bacillus anthracis* strain BA1035	96%

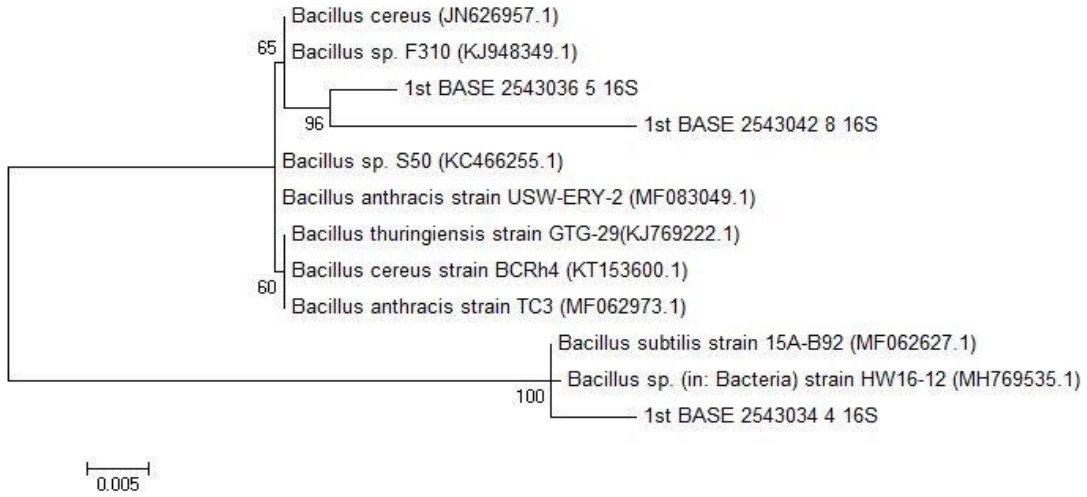

Figure 3. Phylogenetic tree derived from BLS 10, BLS 11, and BLS 12

Conclusion

Retrieved 9 bacteria that could potentially cause mortality in *S.litura* larvae. And 3 isolates causing the high mortality in the larval *S.litura* are BLS 10, BLS 11, and BLS 12 with a mortality rate of 60%. Morphological characters of three bacteria that could potentially cause mortality in *S.litura* larvae are BLS 10, BLS 11, and BLS 12 have different morphological characters, Molecular characterization based on the sequence partial of 16S rRNA showed that three isolates of entomopathogenic bacteria (BLS 10, BLS 11, and BLS 12,) identified as *Bacillus subtilis* strain 15A-B92, *Bacillus thuringiensis* strain GTG-29 and *Bacillus anthracis* strain BA1035.

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References

Amad M, Sayyed AH, Saleem AM, Ahmad M, 2008. Evidence for field evolved resistance to newer insecticides in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. Crop Prot. 27: 1367-1372.

Aktar MS, Sengupta D, Chowdhury A, 2009. Impact of pesticides use in agriculture: their benefits and hazards.

Interdisciplinary Toxicology: 1-12.

Baskar K, Ignacimuthu S, 2005. Bioefficacy of violacein against Asian armyworm *Spodoptera litura* Fab.(Lepidoptera: Noctuidae). J of the Saudi Society of Agr Scie. 73-77.

Bobrowski VL, Pasquali G, Bodanese-Zanettini, MH, Pinto LM, Fiuza LM, 2002. Characterization of two Bacillus thuringiensis isolate from South Brazil and their toxicity against *Anticarsia gemmitalis* (Lepidoptera: Noctuidae). J. Biological Control. 20: 129-135.

Boukedi H, Sellami S, Ktari S, Belguith N-Ben H, Sellami T, Touris BS, Mesrati LA, 2016. Isolation and characterization of a new *Bacillus thuringiensis* strain with a promising toxicity against Lepidopteran pests. Microb Research.186-187: 9-15.

Compant S, Duffy B, Nowak J, Clement C, Barka EA . 2005. Use of plant growth-promoting bacteria for biocontrol of plant disease: principles, mechanisms of action, and future prospects. Appl and Environ Microb. 491-4959.

Dagdag, E., Sukoso, & Asthervina, W. (2015). Isolation and characterization of isolates thermophilic bacteria from water and solid sediment of Lapindo mud. Resources and Environment. Resourche and Envi: 66-71.

Davies RJ, Swarbrick RE, Evans RJ, Huuse M, 2007. Birth of a mud volcano: East Java, 29 May 2006. Environment. J of Gen Eng and Bio. 15: 77–85.

Finney. DJ, 1971. Probit Analysis, 3 Ed. application of regression. Cambridge. Cambridge University Press.

Ghribi D, Elleuch M , Abdelkefi L, Chaabouni SE, 2018. Evaluation of larvicidal potency of *Bacillus subtilis* SPB1 biosurfactant against *Ephestia kuehniella* (Lepidoptera: Pyralidae) larvae and influence of abiotic factors on its insecticidal activity. J of Stor Prod Res. 48: 68-72.

Habibie FM, Wardani AK, Nurcholis M, 2014. Isolation and molecular identification of thermophilic microorganisms xilinase producer of hot mudflow. Journal of Food and Agro-industry. J of Food and Agro-industry: 231-238.

Hadjinicolaou AV, Victoria L. Demetriou A, Johana H, Wolfgang B, Ted LH, Leondios GK, 2009. Use of molecular beacons and multi-allelic real-time PCR for detection of and discrimination between virulent *Bacillus anthracis* and other Bacillus isolates. J of Microb Methods. 78: 45–53.

Haouel, S, Mediouni-Ben J, Khouja ML, 2010. Postharvest control of the date moth *Ectomyelois ceratoniae* using eucalyptus essential oil fumigation.Tunisian J. Plant Protect. 5: 201-212.

Heijs SK, Aloisi G, Bouloubassi I, Pancost RD, Piere C, Damste JS, Forney LJ, 2006. Microbial community structure in three deep-sea carbonate crusts. Microb Eco: 451-462.

Hugh-Jones and Blackburne, 2009. The ecology of *Bacillus anthracis*. Mol. Aspects Med. 30: 356–367.

Hussain AA, Abdel-Salam MS, Hoda H, Abo-Ghalia A, Wafaa KH, Safa SH, 2017. Optimization and molecular identification of novel cellulose degrading bacteria isolated from Egyptian GSA Today, 4-9.

Kaleeswaran G, Firake DM, Sanjukta R, Behere GT, Ngachan SV, 2018. Bamboo-Leaf Prickly Ash extract: A potential bio-pesticide against oriental leaf worm, *Spodoptera litura* (Fabricius) Lepidoptera: Noctuidae). J of Env Manage: 46-55.

Kim JH, Lee SH, Kim CS, Lim EK, Choi KH, Kong HG, Moon BJ, 2007. Biological control of strawberry gray mold caused by Botrytis cinerea using *Bacillus licheniformis* N1 formulation. J of Microb and Biotech: 438-444.

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Manonmani AM, Geetha I, Bhuvaneswari S, 2011. Enhanced production of mosquitocidal cyclic lipopeptide from *Bacillus subtilis* subsp. *subtilis*. *Ind*. J. Med.Res. 134: 476-482.

Mnif I, Elleuch M, Chaabouni SE, Ghribi D, 2013. *Bacillus subtilis* SPB1 biosurfactant: Production optimization and insecticidal activity against the carob moth Ectomyelois ceratoniae. Crop Prot. 50. 66-72.

Moar WJ, Al E, 1995. Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae). Appl Env Microb: 2086-2092.

Muhidin A, 2015. Development of biological technology antagonistic bacteria from Sidoarjo Mud to control pathogenic bacteria *Erwinia carotovora* caused , translate from [pengembangan teknologi hayati bakteri antagonis dari lumpur sidoarjo untuk mengendalikan bakteri patogen *Erwinia carotovora* penyebab penyakit busuk lunak]. [Thesis]. Univeristy of Brawijaya.

Osouli S, Hamideh A, 2016. To evaluate the effects of secondary metabolites produced by *Bacillus subtilis* mutant M419 against *Papilio demoleus* L. and *Aspergillus flavus*. Acta Ecologica Sinica. 36 .492–496.

Saikia R, Gogoi D, Mazumder S, Yadav A, Sarma R, Bora T, Gogoi B, 2011. *Brevibacillus laterosporus* BPM3 strain, a potential biocontrol agent isolated from a natural hot water spring of Assam, India. Microb Res: 216-225.

Salam MS, Hoda H, Ameen B, Abdallah SM, Kassab C, Ahmed EA. Mahgoob C, Usama S. Elkelany B. Enhancement of nematicidal potential through cloning and expression of chitinase gene from *Bacillus subtilis* subsp. *subtilis* BTN7A strain. J. of Gen Eng and Biotech.

Sang S, Shu B,Yi X, Liu J, Hu M, Zhong G, 2016. Cross-resistance and baseline susceptibility of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) to cyantraniliprole in the south of China. Pest Manag. Sci. 72: 922-928.

Santosa S, Kusuma Z, Prasetya B, Yanuwiadi B, 2014. The effect of Sidoarjo's mud soil with organic materials toward the growth of Albizian (Samanea saman Merr). Natural and Appl Scie: 57-65.

Schoenborn L, Yates PS, Grinton BE, Hugenholtz P, Janssen PH, 2004. Liquid serial dilution is inferior to solid media for isolation of cultures pepresentative of the phylum-level diversity of soil bacteria. Appl Env Microb: 4363-4366.

Su J, Lai T , Li Ja, 2012. Susceptibility of field populations of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes. Crop Prot. 42: 217-222.

Zimmer M, Kautz G, Topp W, 2003. Leaf litter-colonizing microbiota: Supplementary food source or indicator of food quality for *Porcellio scaber* (Isopoda: Oniscidea). Europe J of Soil Bio: 209-216.

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