# Potential of Biodiversity in Microbial Indigenous *Aedes Aegypti* Mosquito Control in Indonesia: Surface Water Protection Efforts

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**Abstract:** An outbreak of dengue hemorrhagic fever (DHF) has attacking several areas in Indonesia, both rural and urban areas. DHF is a dangerous virological disease because it can cause the death of the sufferer. The spread of DHF disease was aided by the vector of *Aedes aegypti* mosquito and is transmitted through their bites. *A. aegypti* mosquitoes breed in shelters and around human habitation. Mosquito control is often done using synthetic chemical insecticides. The use of chemicals to control *A. aegypti* mosquitoes continuously can cause environmental pollution and improving insect resistance against chemical insecticides. One way that can be done to control mosquitoes and safe for the environment is the use of biological insecticides (microbes). As a country with a very large biodiversity, second to the world after Brazil, Indonesia provides a wealth of invaluable natural resources. Searching for indigenous bacterial isolates and the type of organisms with the potential to control *A. aegypti* mosquito's vector should continue to be done. Several researchers have searched for local isolates of *Bacillus thuringiensis* and other potential locals isolate of bacterial for mosquito control. They also conduct researches for local alternative media as multiplication media to isolates bacteria with cheap and easily obtained, without reducing the level of pathogenesis against mosquitoes.

Keywords:

## 1. Introduction

*A. aegypti* is the primary vector of dengue hemorrhagic fever (DHF) in the tropics. In Indonesia, DHF is known as an endemic disease and is one of the major public health problems. The number of DHF patients in Indonesia is tend to increase and widespread. According to WHO (World Health Organization), starting from the year 1968 until the year 2009, Indonesia is the country with the highest dengue cases in Southeast Asia (Ditjen PP & PL, 2009). In 2011 the number of dengue fever patients in Surabaya has reached 921 people and five people died (Republika 2011). South Sumatra recorded 1721 cases of dengue fever with a mortality rate of 19 people (Kompas 2012). In Bogor has recorded 608 dengue cases in 2011, while 2010 to 1600 patients. Five years ago, Bogor regency has set an extraordinary event of DHF in January 2007 where there were 427 dengue cases and 14 deaths (Republika 2012).

DHF diseases are caused by a virus of the genus Flavivirus, family Flaviviridae. DHF is transmitted to humans through the bite of an infected mosquito vector of dengue virus. Vaccines for the prevention of viral infections and cures for diseases DB / DBD does not exist and is still in the research process, so the control is primarily intended to break the chain of transmission, with the control vector (Sukowati 1996). Prevention efforts that have been routinely carried out are such as environmental control and chemical control. Environmental control is done by burying items that can collect rain water and keep the water storage of the larval of *A. aegypti* mosquito (Soedarmo 1998). Chemically control can be done such as placing an abate powder SG 1% in stagnant water and fogging with malathion or fenitrothion (Morley, 1999).

The use of chemical insecticides in the control of dengue vector of *A. aegypti* does give effective results and optimal, but many negative impacts for both the living organisms and the environment. According to WHO, approximately 20,000 people die per year due to pesticide poisoning, but it also cause fatal effects, such as cancer, disability, and infertility. Another negative impact is the death of natural enemies from nuisance organism, the death of beneficial organisms that interfere with the quality and balance of the environment due to the residue and the emergence of resistance in the target animal (Novizan 2002).

Number of negative impacts of the use of chemical insecticides led to new research in disease vector control using the biological insecticide that is defined as a biological insecticide basic material derived from living things that contain chemicals (bioactive) that are toxic to insects but easily decompose (biodegradable) in nature, so it does not pollute the environment and are relatively safe for humans (Kardinan 1999). One of the biological insecticides developed is derived from microbes.

### Several potential of Indigenous Microbes in Controlling Mosquitoes Based on Biological Insecticide of B. thuringiensis.

*B. thuringiensis* is one of the insect pathogenic bacteria. Bacterial strains showed a wide range of specificity in various insect orders (Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, and Mallophaga) and Acari (Bravo *et al.* 1998 in Suryanto, 2009). According to Agaisse

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and Lereclus (1995) in Suryanto (2009), all sub-types of *B. thuringiensis* is known to produce large amounts of protein crystals in the body of segregating insecticide paraspora (d-endotoxins) during sporulation. Glycoprotein toxin is actually still a protoksin in the cells of *B. thuringiensis* that does not have any toxic properties. Insect-sensitive bacteria are having an alkaline digestive tract, producing minerals and enzymes that can hydrolyze crystalline protoksin to become a toxin. Damage mainly occurs in the middle of insect gut (Abdel-Mohammed *et al*. 1991 in Gama *et al.* 2010).

Some researchers have conducted a study of *B. thuringiensis* var. israelensis for mosquito control, but the bacteria are expensive because it needs to import from abroad. Therefore, it needs to continue to do research using *B. thuringiensis* local isolates potential for mosquito control.

**B. thuringiensis H-14 strains Local Salatiga**

Center for Research and Disease Reservoir (B2P2VRP) has bred *B. thuringiensis* H-14 strains land local isolated in Salatiga, and high toxicity against larvae of *A. aegypti*, and Anopheles aconitus. Bacteria of *B. thuringiensis* H-14 local strains can be grown in coconut water media in the range of pH 7 to 8.5 with the optimum pH 7 (Blondine and Susanti 2010).

In addition to local strains of *B. thuringiensis* exploration Indonesia as a potential mosquito control is also continuing research into the use of local media for breeding of *B. thuringiensis*. Utilization of local isolates of *B. thuringiensis* on a wide scale is still not economical. This is because it is difficult to obtain and the expensive standard medium for propagation, it is necessary to look for alternative media that are cheap and easy to get by not reducing the level of toxicity. Utilization of local media such as coconut water, soybeans soaking water, rice water and molasses as propagation media for *B. thuringiensis* can be an alternative solution to this problem. Local media are readily available, relatively inexpensive and can be prepared within time. Coconut water is a waste of manufacturing Virgin coconut oil (VCO), rice water is household waste, molasses is a sugar mill waste and waste water soaking soy is untapped tofu and tempeh. Coconut milk and soybean soaking water can be used as an alternative medium for propagation of bacterium *B. thuringiensis* mass (Putrina and Fardedi 2007).

The results of Blondine *et al.* (2005) showed that breeding of *B. thuringiensis* H-14 using local media coconut (coconut water and endosperm) has good growth and toxicity. Blondine (2003), states that the use of media in the form of coconut milk powder (powder) have a level of toxicity that is higher than IPS medium (standard medium of Pasteur). Then Blondine (2008), states that C4 rice water can be used as a medium for the growth and development of *B. thuringiensis* H-14 strains and effectively shut down local mosquito larvae of *A. aegypti* and A. aconitus. Similarly, soy infusion media can be used as a medium for the growth of *B. thuringiensis* H-14 strains that can control local mosquito larvae of A. maculates with effectiveness from 6.75 to 12.58 days (Blondine and Widiarti 2008).

In the management of microbial insecticides, as an alternative measure of the selectivity of the reduction and the use of synthetic chemical insecticides, it is necessary to engage the community to participate in order to be more effective for mosquito control. After doing counseling and guidance to the public, there was 83.33% of respondents were willing to breed *B. thuringiensis* H-14 local strains in coconut fruits, storing and applying these bacteria in ponds breeding mosquito larvae (Blondine *et al*., 2004).

**B. thuringiensis Madura isolates**

The bacteria of *B. thuringiensis* var. Israelensis has been widely studied for mosquito control, whether in the form of commercial products and pure isolates, but local isolate was rarely used as a natural enemy of mosquitoes. Gama *et al.* (1998), comparing the pathogenicity of *B. thuringiensis* serotype H-14 var. Israelensis and *B. thuringiensis* Madura isolates against mosquito larvae. The results showed that the power of killing from *B. thuringiensis* Madura isolates greater than the power of killing from *B. thuringiensis* var. Israelensis. Furthermore Gama *et al.* (2010), stating that the toxicity of *B. thuringiensis* Madura isolates is large enough that it can kill mosquito larvae of *A. aegypti* at instar I until 88.89%. Similarly, the influence of the crystal (toxin) of *B. thuringiensis* Madura isolates on the structure of epithelium and intestinal tissues is very real, because after treatment with *B. thuringiensis* Madura isolates, the epithelium structure and bowel tissue has perforated, crushed and neatly arranged.

**B. thuringiensis North Sumatra isolates**

Suryanto *et al.* (2007a) in Suryanto (2009), isolating *B. thuringiensis* originates from North Sumatra and found 9 isolates were relatively similar in morphology and biochemistry. TU1 isolates and isolates of commercial bioinsecticide, has relatively the same morphological and biochemical properties. Possibility is that both of isolates are genetically similar. Variation that is not too big from morphological and biochemical properties among these isolates showed morphological and biochemical diversity relatively not large from *B. thruringiensis* origin of North Sumatra isolate.

Furthermore (Suryanto *et al*. 2007b in Suryanto 2009) states that the spectrum of bioassay isolates against larvae of some insects showed a different spectrum. The results showed that the TU1 isolates can kill larvae, with variations in the ability to kill. Although in general it must be admitted that the tested of commercial bioinsecticide showed better performance in controlling insect larvae, but it seems the ability of TU1 isolates has a similar tendency to the commercial bioinsecticide. This similarity may be due to the presence of the gene encoding the protein crystals similar in the both isolates. This similarity can be seen if both isolates were analyzed from genomes (Suryanto 2009).

**Chitinolytic Bacteria**

The use of microorganisms to control plant diseases one of which is by using chitinolytic bacterial isolates. Chitinolytic bacteria are often used as a biological control agent because of its ability to hydrolyze chitin to become chitin derivatives (Ohno *et al.* 1996). Biological control of fungal pathogens in plants using chitinolytic microbes based on the ability of chitinolytic microbes producing chitinases and ß-1,3-glucanase can degraded yeast cells (El-Katatny *et al.* 2000 in Suryanto 2009).

Chitinolytic bacteria besides can degraded yeast cells, it can also degrade mosquitoes exoskeletons composed of chitin material. Pujiyanto (2008) tested the ability of 10 chitinolytic bacterial isolates of water samples obtained from several areas in Central Java and West Java in controlling *A. aegypti* mosquito larvae. After the observation, based on the properties of LMB1-5 chitinolytic bacterial isolates possessed a high ability to control *A. aegypti* mosquito larvae, easy to reproduce and have a high viability. Chitinolytic bacteria can cause larval mortality of 86.7% within 7 days. This is based on components of the mosquito exoskeletons which composed of chitin material that logically can be degraded by chitinase enzyme produced by the endophytic chitinolytic bacteria. Damage to the structure of the exoskeletons of mosquito larvae can result in stunted growth and death. Therefore, LMB1-5 isolates is a high potential to be studied and developed a strain of mosquito control of *A. aegypti* so that it can be used as an alternative to prevent outbreaks of dengue fever.

**Endophytic Chitinolytic Bacterial Batu East Java Isolates**

One source of bioactive compounds from endophytic microbes is bacteria. Endophytic microbes (bacteria and fungi) are microorganisms present (living) in the plant organs or in part during its life cycle, can colonize the plant tissues without causing disease on host plants (Petrini 1991). Bacteria used as the source of a biological product will simplify the process and reduce the cost of production, which in turn produce the product at a cheaper price (Tan and Zhou 2001). Secondary metabolites produced by endophytic microbes may include antimicrobial compounds, anti-cancer, anti-nematode, decomposer enzymes, and plant growth regulators. Secondary metabolites may be useful in the field of industry, agriculture, and pharmaceuticals (Strobel *et al*. 2005).

Wardhani (2010), managed to isolate and identify endophytic bacteria from the roots of potato plants from Batu areas in East Java, namely Bacillus mycoides, Pseudomonas pseudomallei and Klebsiella ozaenae. The three endophytic bacteria are either single or consortia of bacteria treatment has the potential to produce the chitinase, protease and cellulase enzyme (Faticha 2011). Subsequent research by Fitroh and Utami (2012), which examine the potential of three endophytic bacteria such as *A. aegypti* mosquito larvae biolarvacide. The results showed that all three endophytic bacteria such as potential biolarvacide against *A. aegypti* mosquito, the concentration of the filtrate 1.5 ml of Pseudomonas pseudomallei bacteria combined with Klebsiella ozaenae percentage mortality of larvae and pupae of *A. aegypti* mosquitoes highest at 97.5%. Mortality is the effect of the damage on the larval exoskeleton structure that resulted in the disruption of the process of growth and metabolic processes of the body. Damage to these structures can be observed in larvae that die from treatment of chitinolytic endophytic bacteria with a macro microscope NIKON SMZ645 (Fig. 1)

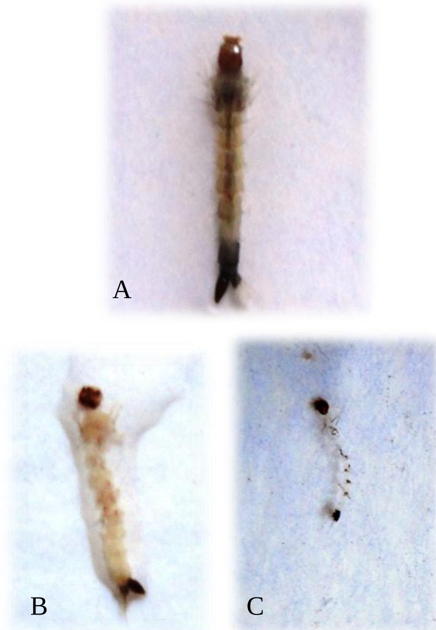


Fig. 1. (A) alive larva, (B) dead larvae on control and (C) damage to the structure of the exoskeletons of the dead larva due to chitinolytic endophytic bacteria treatment.

**Future Expectation**

From some of the researches that has been done can be seen that the potential diversity of indigenous microbes in controlling *A. aegypti* mosquitoes in Indonesia is huge and needs to be explored and developed among others by:

1. Mechanical preparation of raw materials (isolate local bacterial and local media) that is easy and inexpensive, so that biological insecticides (microbial) can be provided by the community.
2. Increasing public understanding of the biological insecticide (microbes) that does not rely on chemical insecticides (synthetic) and realize that there is an alternative control, by the use of biological insecticides (microbial).
3. Form of guidance counseling to the people and willing to breed local bacterial isolates mass in the local media as a biological insecticide. Then do the application in places around the house and water storage in ponds breeding with *A. aegypti* mosquito larvae, as one of the protection of surface water pollution synthetic chemical insecticides.
4. Research and development to overcome the disadvantages of biological insecticides (microbes) in addition to obtain new indigenous isolates.
5. Development and measurement of sustainability indicators, among others, can be viewed from: a) the safety and benefit of society, b) reduction in the use of synthetic chemical insecticides, c) low synthetic chemical insecticides residue on the environment, land and water; d) public acceptance of biological insecticides.
6. The studies that have been done are expected to provide information about the potential (bioprospecting) of biological resources in Indonesia. This stored potential can be raised for the purpose of development of domestic

industry in an environmentally friendly, though still requiring research stages which are quite long.

**5. Conclusion**

1. Several indigenous bacterial isolates have potential in controlling *A. aegypti* mosquito in Indonesia to support the eradication of dengue vectors.
2. Local media can be used as a medium for the growth and proliferation of indigenous bacterial isolates and effective enough to kill mosquitoes.

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