Activities of Bio-Larvacides from Symbiont Bacterial of Soft Coral Sarcophyton sp. SCRTG4P4 Against Aedes aegypti

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RESEARCH ARTICLE

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Activities of Bio-Larvacides from Symbiont Bacterial of Soft Coral *Sarcophyton* sp. SCRTG4P4 Against *Aedes aegypti*

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Background: Synthetic insecticides are known as the substances that can lead to an destabilization of ecosystem and enhance a resistance to insecticides. Bio larvacides is an eco-friendly insecticide that can be an alternative vector control for *Aedes aegypti*. The objective of the research was to determine the activity of biolarvacides of symbiont bacterial soft coral *Sarcophyton* sp. SCRTG4P4 against *Ae. aegypti* and molecular identification of Symbiont bacterial of soft coral *Sarcophyton* sp. *Method*: This research was done by an experimental method with post-test only control group design. The variation concentrations of symbiont bacterial extract, soft coral SCRTG4P4 (0.15; 0.20; 0.25; 0.30; 0.35; 0.40; 0.45%) and control, were applied to the III–IV instar of *Ae. aegypti* larva. Data analysis carried out with one-way ANOVA followed by Probit Analysis. Moleculer identification used PCR amplification and DNA sequencing. *Results*: The result showed that there were significant differences in various concentration of extract soft coral *Sarcophyton* sp. SCRTG4P4 ($p = 0.000$) and LC_{50} values of 0.275% and LC_{90} values of 0.40%. Based on the result of ANOVA analysis, there were significant differences of various concentration extract as biolarvacides with significance $p = 0.000$. Molecular identification showed that SCRTG4P4 was similar with *Bacillus subtilis*. *Conclusion*: SCRTG4P4 as symbiont bacteria of soft coral *Sarcophyton* sp. had activities of biolarvacide and it was similar to *Bacillus subtilis*.

Keywords: Bio Larvacides, Symbiont Bacteria, Softcoral.

1. BACKGROUND

Mosquitoes serve as vector of several diseases, causing public health problems. Vector controls commonly use synthetic insecticides, like organochlorine, organophosphorus, carbamates, pyrethrins and pyrethroids. The synthetic insecticides can lead to the destabilization of ecosystems and enhance a resistance to insecticides. Bio larvacide is an agent of insect control from biological agents that is easy to use, not harmful to natural enemies and other beneficial insects. It is one of eco-friendly alternative insect controls.

Ali et al.,[1] reported the seaweeds extract, *Caulerpa racemose* showed its toxicity against 4th instar larvae of *Aedes aegypti*, *Culex quinquefasciatus, Anopheles stephensi* with equivalent LC50 value (0.0556 ± 0.0103) μg/mL, (0.0675 ± 0.1360) μg/mL and (0.0661 ± 0.0076) μg/mL. Another research by Poonguzhali and Nisha[2] reported that Algae extract of *Grateloupia lithophila* was effective against the *Culex* larvae.

A limited supply of marine animal and plant including marine invertebrate becomes a problem. A low content of active compound caused many researchers focused on marine microorganism as sustainable resources. Indonesia has biodiversity of marine organism. One of the archipelagos in Indonesia, Karimunjawa archipelago in Jepara, is famous of their beautiful corals.[3]

SCRTG4P4 is a symbiont bacteria from soft coral *Sarcophyton* sp. The objective of this research was to determine the activity of biolarvacides from symbiont bacterial of soft coral *Sarcophyton* sp. SCRTG4P4 against *Ae. aegypti* and molecular identification of SCRTG4P4.

2. METHOD

This research was done by an experimental method with post test only control group design. The sample was soft corals from Tanjung Gelam Islands Karimunjawa, Center of Java, using Scuba Diving. The isolation of symbiont bacteria of soft coral *Sarcophyton* sp. used streak method. Molecular identifications of symbiont bacterial used PCR, Sequencing, and BLAST. PCR amplification was carried out according to the method of Radjasa et al.[4] Universal primers described by Weisburg et al.[5] was used for PCR amplification. The larvicidal bioassay followed the World Health Organization (WHO) standard protocols. It was used to determine the susceptibility or resistance of mosquito larvae

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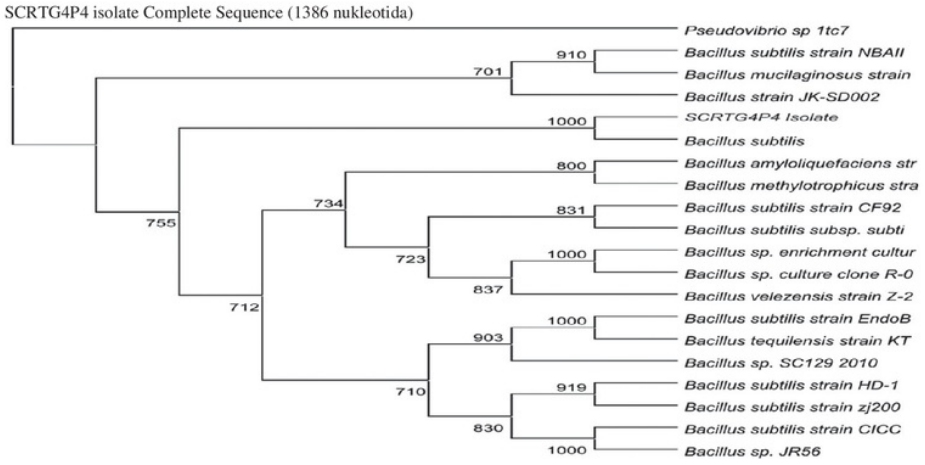

Fig. 1. Phylogenetic tree of SCRTG4P4.

against insecticides.[6] The extraction was carried out according to the method described by Kanjana et al.[7] The various concentrations of symbiont bacterial extract, soft coral SCRTG4P4, (0.15%, 0.20%, 0.25%, 0.30%, 0.35%, 0.40% dan 0.45% and control) were applied to against the III–IV instar of *Ae. aegypti* larva. Data analysis carried out with one-way.

3. RESULTS

There were significant differences of various concentration extract of SCRTG4P4 as bio-larvacides to the mortality of larva *Ae. agypti*. The Probit analysis showed the bio-larvacides activity of symbiont bacterial soft coral *Sarcophyton* sp. SCRTG4P4 had LC_{50} value = 0.275% and LC_{90} value = 0.40%.

Using the 16S rRNA gene for sequencing, SCRTG4P4 isolate had 1386 nucleotide. Molecular identification of SCRTG4P4 isolate was *Bacillus subtilis*. Phylogenetic tree of SCRTG4P4 described in Figure 1.

4. DISCUSSION

SCRTG4P4 is a symbiont bacterial of soft coral *Sarcophyton* sp. Several marine organisms have adapted themselves through symbiotic association. The microorganisms living in their invertebrate hosts could be the actual producers of these secondary metabolites.[8]

Table I. The mortality of larva *Ae. aegypti*.

Concentration (%)	Total mortality	Mean of mortality	% total
Control	0	0	0
0.15	6	2	10
0.20	14	4.67	23.35
0.25	27	9	45
0.30	36	12	60
0.35	40	13.33	66.65
0.40	56	18.67	93.33
0.45	59	19.67	98.35

Molecular identification result showed that SCRTG4P4 is *Bacillus subtilis*. *Bacillus* is the gram-positive, aerobic, rod-shaped endospore-forming bacteria.[9] Genus *Bacillus* are the most widely represented organisms in the soil and aquatic environment. *B. cereus* and *B. subtilis*, *B. pumilus,* are considered as a major component of marine bacterial communities.[10] *Bacillus subtilis* strains can act as bio fungicides for benefiting agricultural crops and antibacterial agents. *Bacillus subtilis* is used as a fungicide, fortunately, and does not affect humans. Some strains related to *Bacillus subtilis* are capable to produce toxins for insects. *Bacillus thuringiensis*, as example, is another bacterium at the same genus that is used for insect control.[11]

Based on the phytochemical test, symbiont bacterial SCRTG4P4 contained alkaloids, triterpenoids, and saponin.[12] Marine organism has been found to produce secondary metabolites such as alkaloid, terpenoid, peptides, sulfated polysaccharides, sesterterpene.[13] Alkaloids have functioned as a toxin toinsects as a digestive toxin that can reduce the tense of tractus digestivus mucosal larva. Alkaloids can also work as a growth inhibition, especially to inhibit brain hormone, ediction hormone and a juvenile hormone that make a failure of methamorphosis.[14]

5. CONCLUSION

SCRT4P4 had an activity as bio larvacides against *Ae. aegypti*. SCRTG4P4 isolate is similar to *Bacillus subtilis*.

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