LARVICIDAL ACTIVITY OF *Streptomyces* sp. LIQUID CULTURES AGAINST *Aedes aegypti* LARVAE

Ni Wayan Desi Bintari[*], Putu Ayu Parwati[**]

[*]*STIKES Wira Medika Bali, Study Program of Medical Laboratory Technology (Diploma III), Denpasar, Indonesia, desibintari@gmail.com*

[**] *STIKES Wira Medika Bali, Study Program of Medical Laboratory Technology (Diploma IV), Denpasar, Indonesia, parwatiputuayu@gmail.com*

Email Correspondence : desibintari@gmail.com

Received : November 26, 2021 Accepted : November 21, 2022 Published : December 31, 2022

Abstract: The tropics have significant future challenges in controlling the vectors of dengue hemorrhagic fever. The occurrence of resistance to chemical control encourages the development of strategies based on biological control. This study aimed to test the larvicidal activity of *Streptomyces* sp. liquid culture toward *A. aegypti* larvae. The selection of *Streptomyces* as a bio-larvicide was carried out by a chitinase test. Chitinase-producing bacteria were cultivated in biomass culture. The study was conducted using a completely randomized design. The results of this study can be isolated 4 *Streptomyces* isolates from muddy soil. Among the isolates, *Streptomyces* sp.4 showed chitinolytic activity on in vitro tests; therefore, it was used for larvicidal activity. Based on the Duncan test result, *Streptomyces* sp.4 culture showed a significant effect on larval mortality compared to the negative control ($p<0.05$). The highest rate of larval mortality was found in the A6B1 treatment (34.35%). The results of the Probit test showed that the LD_{50} value of the *Streptomyces* sp.4 culture was 24.6±5.4 mL. Based on the in vivo test, showed that *Streptomyces* sp.4 liquid culture affected the mortality rate of *A. aegypti* larvae and was significantly different from the negative control ($p<0.05$). *Streptomyces* sp.4 is known to have potential benefits as a biological larvicidal agent.

Keywords: chitinolytic bacteria; dengue hemorrhagic fever; *integrated vector management*

Abstrak: Daerah tropis memiliki tantangan besar kedepannya dalam pengendalian vektor demam berdarah dengue (DBD). Adanya kejadian resistensi pengendalian dengan zat kimiawi mendorong strategi pengembangan berbasis pengendalian biologis. Penelitian ini bertujuan untuk melakukan pengujian aktivitas kultur *Streptomyces* sp. sebagai larvasida *A. aegypti*. Seleksi *Streptomyces* sebagai biolarvasida dilakukan dengan uji aktivitas kitinase. Bakteri penghasil kitinase dilakukan kultivasi kultur biomassa untuk pengujian *in vivo*. Rancangan uji menggunakan Rancangan Acak Lengkap. Hasil penelitian dapat diisolasi 4 isolat *Streptomyces* yang diisolasi dari tanah berlumpur. Diantara keempat isolat, satu isolat yaitu *Streptomyces* sp.4 menunjukkan aktivitas kitinolitik sehingga digunakan untuk uji aktivitas larvasida secara *in vivo*. Hasil uji larvasida menunjukkan perlakuan kultur *Streptomyces* sp.4 berpengaruh nyata terhadap persentase kematian larva dibandingkan kontrol negatif ($p<0.05$) berdasarkan hasil uji Duncan. Persentase kematian tertinggi didapatkan pada perlakuan A6B1 yaitu sebesar 34.35%. Hasil uji Probit menunjukkan nilai LD_{50} dari kultur *Streptomyces* sp.4

adalah 24.6 ± 5.4 mL. Berdasarkan hasil uji *in vivo* diketahui bahwa perlakuan kultur cair *Streptomyces* sp.4 berpengaruh terhadap tingkat kematian larva *A. aegypti* dan berbeda nyata dengan kontrol negative ($p<0.05$). *Streptomyces* sp. 4 diketahui memiliki potensi sebagai salah satu agen larvasida biologis.

Kata kunci: bakteri kitinolitik; demam berdarah dengue; manajemen vektor terintegrasi

Recommended APA Citation :

Bintari, N. W. D., & Parwati P. A. (2023). Larvicidal Activity of *Streptomyces* sp. Liquid Cultures Against *Aedes aegypti* Larvae. *Elkawnie*, *8*(2), 209-220. https://doi.org/10.22373/ekw.v8i2.11436

Introduction

Dengue hemorrhagic fever (DHF) is a mosquito-borne disease transmitted by Aedes mosquitoes, primarily *Aedes aegypti* infected with the dengue virus (Haryanto, 2018). DHF infection in Indonesia was first reported in 1968. However, until 2019 as many as 80% of provinces were infected and could be treated by DHF (Harapan, 2019). As a dengue-endemic area, Indonesia has significant challenges in controlling the infection in the future. The increase in rainfall and extreme earth surface temperature predictions for 2038 will correlate with the growth of the DHF vector. As a tropical area, Indonesia is expected can constantly to handle and control DHF (Haryanto, 2018).

Vector control of DHF generally uses synthetic insecticides and larvicides such as abatization and fogging (Atikasari and Sulistyorini, 2018). However, several studies have evaluated a decrease in effectiveness and an increase in larvae resistance to both techniques. Synthetic control increases the potential of polluting the environment, threatens with toxic to non-target animals, and leaves residues in the environment (Zhou et al., 2020). In the future, dengue vector control is expected to be developed through an Integrated Vector Management (IVM) strategy that focuses on the use of microorganisms (Braks et al., 2019).

Microbes as biocontrol agents are very suitable because of their ability to produce toxic metabolites that target animals. In addition, its activity is reported to be more selective, permanent, and does not cause resistance (Benelli et al., 2016). The development of bio-larvicidal agents can be focused on the group of chitin-degrading microorganisms. Chitin is the insect exoskeleton component, chitin degradation by bacteria can cause damage and death (Veliz et al., 2017). Various studies have proven that chitinolytic bacteria are effective biological controllers against insects (Jabeen et al., 2018; Al-qwabah et al., 2018). Therefore, these bacteria may be used as biological larvicides against mosquitoes, especially *A. aegypti*.

The chitinolytic bacteria explored are Actinomyces, especially the genus *Streptomyces* (Lacombe-Harvey et al., 2018). Almost all members of the genus *Streptomyces* can produce chitinase. It is designated as one of the best microorganisms to study chitinase's production and biochemical aspects through

various conditions and environments (Jha et al., 2016; Poorna and Pradeep, 2016). The presence of chitinolytic activity by *Streptomyces* such as *S. rubiginosus* (Jha et al., 2016) and *S. albus* FS12 (Santhi, 2016) encourages the need to explore this species, which can be developed as a bio-larvicide. Research related to the larvicidal activity of *Streptomyces* sp. against *A. aegypti* larvae has been carried out previously (Yotopranoto et al., 2017). However, the selection of *Streptomyces* bacteria that produce chitinase enzymes as candidates for bio larvicides has not been widely explored. This study aims to isolate and perform laboratory tests on the *Streptomyces* sp. liquid culture as larvicides of *A. aegypti* larvae. In this study, the larvicidal activity test was carried out using a liquid culture of *Streptomyces* which was able to produce the chitinase enzyme on in vitro test.

Research methods

Instruments

The instruments used in this research are a laminar flow cabinet, microscope (Olympus™), autoclave (Hirayama™), micropipette (Eppendorf™), rotary shaker (Ratex Instrument Australia™), incubator (Memmert™), vortex mixer (Ohaus™), Erlenmeyer (Pyrex™), microscope slide, wire loop, petri dish, cork borer, bunsen burner, plastic container (500 mL).

Materials

The material used in this research is *A. aegypti* larvae (stage III), Yeast Extract Malt Agar (Merck™), Yeast Extract Malt Broth (Merck™), Chitin Agar Medium, Gram stain, acid-fast stain, distilled water, immersion oil.

Research Design

This research was experimental. The test design used is a 1-factor design with eight levels using a completely randomized design. In this design, all experimental materials have an equal chance to receive treatment. The research was conducted at the Bacteriology Laboratory of STIKES Wira Medika Bali in January-September 2019.

Soil Sample Collection

Streptomyces sp. bacteria isolated from muddy soil samples in the Subak ecotourism area in Badung Regency, Bali. Approximately 20 grams of soil was collected using a sterile spatula with a depth of roughly 10 cm. Soil samples were stored in sterile pots with closed containers and stored at room temperature during transport.

Streptomyces sp. Isolation and Identification

Isolation is carried out using the serial dilution method and inoculated by pour plate method on Yeast Extract Malt Agar/ YEMA media (International Standard Project 4/ISP4). Samples were incubated for 5-7 days at a temperature of 30 ± 2^0C aerobically. The growth colonies were observed macroscopically and

microscopically. Macroscopic observations of colony characteristics included color, shape and the presence or absence of aerial hyphae growth. Microscopic observations included observations on the structure of hyphae and conidia, Gram stain test, and acid-fast staining test.

Selection of Chitinase-Producing *Streptomyces*

Streptomyces cultures at five days of incubation were taken using a cork borer (colony diameter ± 5 mm). The bacterial culture was placed on the surface of the chitin agar medium and incubated at 30 ± 2^0C aerobically for 24 hours. Chitinase activity was indicated by the formation of a clear zone around the colony. The magnitude of the ability of the chitinase enzyme activity is carried out by measuring the clear zone formed (Shrivastava et al., 2017).

Cultivation of *Streptomyces* sp. Biomass Culture

Streptomyces which are capable of producing chitinase enzymes undergoes biomass cultivation for larvicidal activity testing. Isolates were inoculated in 100 mL of Yeast Extract Malt Broth (YEMB) media. The culture was incubated for five days on a rotary shaker at 80 rpm at a temperature of 30 ± 2^0C (Soeka, 2015).

Larvicidal Activity Test

Larvae used for testing in vivo are larvae of *A. aegypti* stage III with an average length of 4-5 mm. The larvae are the result of spawning from the Entomology Section of the East Java Provincial Health Office. Based on the test design used, the number of experimental units in this test is 32. In each container, 25 larvae were stocked. Before the treatment was done, the larvae were acclimatized for 24 hours, and every day the larvae were fed ad libitum. *Streptomyces* sp. culture was divided into 8 treatment levels. Each treatment level was carried out using a culture density of 1x10^8 CFU/mL (Table 1). The culture treatment was given once, and the larva mortality was calculated after 48 hours of treatment to determine the percentage of larvae mortality and LD_{50} value based on Vinodhkumar *et al*. (2015).

Table 1. Treatment of *Streptomyces* sp. culture

Treatment	*Streptomyces* sp. culture (mL)	Sterile Aquadest (mL)
Negative control	0	100
Positive control	0 + temephos	100
A1B1	5	100
A2B1	10	100
A3B1	15	100
A4B1	20	100
A5B1	25	100
A6B1	30	100

Data Analysis

Data were analyzed using Analysis of Variance (ANOVA) through the F test at the 5% level. If the results of the F test show a significant difference, then continue with the Duncan test (DMRT) at the 5% level. The determination of LD_{50} is done by observing the mortality of larvae during 48 hours of treatment. LD_{50} value data was calculated using Probit analysis. The analysis is conducted using Minitab 20.0 software.

Result

Isolation and Characterization of *Streptomyces* sp. Bacteria

Streptomyces sp. bacteria isolated from a sample of muddy ricefield soil in the ecotourism area of Subak, Badung Regency, Bali. It is located at coordinates 8°34'03.8" S latitude and 115°04'41.3" E. The isolation results on YEMA media can be characterized by four species of *Streptomyces* bacteria, namely *Streptomyces* sp.1, *Streptomyces* sp.2, *Streptomyces* sp. 3, and *Streptomyces* sp. 4 (Figure 1). The four *Streptomyces* species were characterized by colony shape, aerial hyphae color, conidia shape and size, Gram and acid-fast characteristics, and catalase test results (Table 2).

Table 2. Characterization of *Streptomyces* spp. isolates

Isolate Code	Characteristics
Streptomyces sp.1	Round colonies, entire margin, septate hyphae, gray aerial hyphae, and conidia are round and in chains (0.08-0.10µm), Gram-positive, catalase-positive, non-acid-fast bacteria.
Streptomyces sp.2	Irregular colonies, entire margin, septate hyphae, white to pink aerial hyphae, and conidia are round in chains (0.06-0.08µm), Gram-positive, catalase-positive, non-acid-fast bacteria.
Streptomyces sp.3	Irregular colonies, wavy edges, septate hyphae, gray aerial hyphae, and conidia are round and in chains (0.06-0.12µm), Gram-positive, catalase-positive, non-acid-fast bacteria.
Streptomyces sp.4	Irregular colonies, wavy margins, septate hyphae, gray aerial hyphae, and conidia are round and in chains (0.07-0.10µm), Gram-positive, catalase-positive, non-acid-fast bacteria.

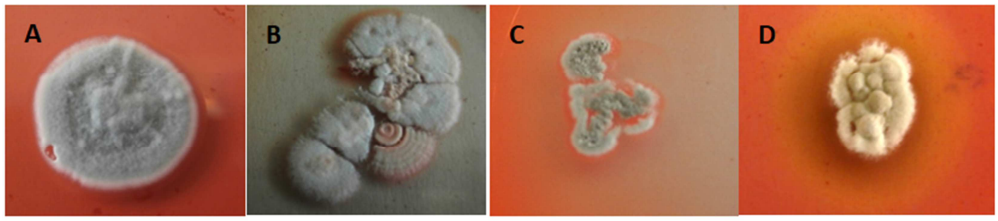

Figure 1. *Streptomyces* colonies on YEMA. (A) *Streptomyces* sp.1, (B) *Streptomyces* sp.2, (C) *Streptomyces* sp.3, (D) *Streptomyces* sp.4

Chitinase Enzyme Activity Test

A chitinase enzyme activity test was carried out to select *Streptomyces* isolates which are capable of producing chitinase enzymes. The test results showed that the isolate producing the chitinase enzyme was *Streptomyces* sp.4, which was indicated by forming a clear zone in the in vitro test. The diameter of the clear zone created by *Streptomyces* sp. 4 is 6.2 ± 0.4 mm (Table 3, Figure 2).

Table 3. chitinase enzyme production activity test result

Isolate Code	Clear zone diameter (mm)
Streptomyces sp.1	0.0±0.00
Streptomyces sp.2	0.0±0.00
Streptomyces sp. 3	0.0±0.00
Streptomyces sp.4	6.2±0.40

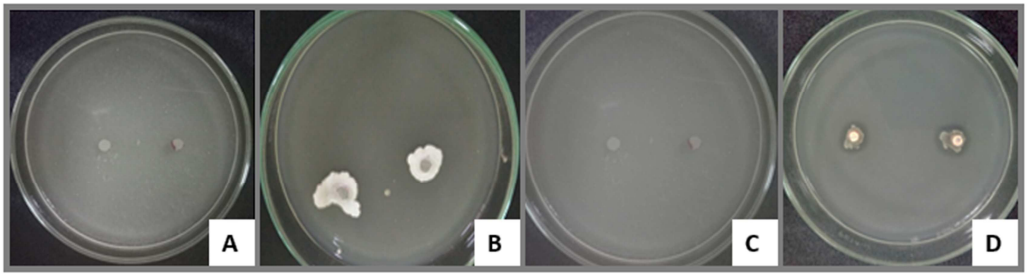

Figure 2. Chitinase activity test. (A) *Streptomyces* sp.1, (B) *Streptomyces* sp.2, (C) *Streptomyces* sp.3, (D) *Streptomyces* sp.4

Larvicidal Activity Test

The larvicidal testing activity was carried out by applying *Streptomyces* sp.4 cultures. The test results showed that *Streptomyces* sp.4 culture treatment had a significant effect on the percentage of larval mortality compared to negative controls ($p<0.05$). The highest death percentage after 48 hours was shown by the A6B1 treatment which can cause 34.25 % larval mortality. However, the positive control treatment (temephos) showed the greatest larvicidal effectiveness with an average mortality percentage of 47.00%.

Table 4. Percentage of cumulative mortality of *A. aegypti* larvae by treating the *Streptomyces* sp.4 culture

Treatment	Percentage of Larval Mortality After 48 hours (%)
Negative control	0.00 $_{g}$
Positive control	47.00 $_{a}$
A1B1	9.00 $_{f}$
A2B1	14.25 $_{e}$
A3B1	20.00 $_{d}$
A4B1	26.75 $_{c}$
A5B1	22.00 $_{cd}$
A6B1	34.25 $_{b}$

Description: The values in the table are the average of 4 repetitions. Based on Duncan's test, different letters (a,b,c,d,e,f) in the same column showed significantly different results ($p<0.05$).

Based on the percentage of cumulative mortality of larvae after 48 hours, Probit test analysis was carried out to determine the LD_{50} value of the *Streptomyces* sp.4 culture. The results of the Probit test showed that the LD_{50} value of the *Streptomyces* sp.4 culture was 24.6 ± 5.4 mL. These results indicate a dose of 24.6 mL of *Streptomyces* sp.4 culture in 100 mL of distilled water can kill as much as 50% of the number of larvae in the treatment container.

Discussion

Isolation of *Streptomyces* bacteria in this study was carried out on muddy soil in the rice fields (subak ecotourism) in Badung Bali. The isolation results obtained four types of bacteria: *Streptomyces* sp.1; *Streptomyces* sp.2; *Streptomyces* sp.3; and *Streptomyces* sp.4 (Table 2). This finding shows that muddy soil can be used as a source of new locations to explore *Streptomyces* bacteria, especially for discovering species-producing metabolites. These results support the research by Kurniawari et al. (2015), Law et al. (2017), and Basik et al. (2020), which can also isolate *Streptomyces* from rice fields.

Streptomyces bacteria are a Gram-positive group that has a structure resembling a filamentous fungus. The filamentous structure resembling the hyphae in its development will differentiate into spore chains (Chater, 2016). In this study, the general characteristics of the isolated *Streptomyces* were Gram-positive, non-acid-fast bacteria, and catalase-positive (Table 2). Colonies generally grow slowly on YEMA media and adhere tightly to the media. It is also covered by aerial hyphae and produces powdery spores. Microscopically it has a filamentous structure resembling hyphae. Then, it has conidia of round to oval shape with various sizes. The genus *Streptomyces is* also widely known to produce active metabolites (Bintari et al., 2017; Fatmawati et al., 2019). One of the active metabolites that can be produced by the genus Streptomyces is the

chitinase enzyme. Chitinase-producing Streptomyces bacteria were reported as potential bio larvicide candidates (Jha et al., 2016; Harir et al., 2018).

In this study, the larvicidal activity of the liquid culture of *Streptomyces* bacteria was tested against *A. aegypti* larvae. The activity test was carried out using *Streptomyces* bacteria which is capable of producing chitinase enzymes based on in vitro chitinase activity tests. The results of the chitinase activity test showed that from 4 *Streptomyces* isolates, there was one isolate, namely *Streptomyces* sp.4 capable of producing chitinase enzymes. These isolates formed a clear zone with 6.2 ± 0.4 mm (Table 3, Figure 2.) using in vitro testing. The clear zone formed in the chitin medium is thought to be caused by the contained chitin compounds and decomposed by the chitinase enzyme produced by *Streptomyces* sp.4.

This study supports the results of previous studies which stated that *Streptomyces* is very potential as a chitinase enzyme-producing agent. Based on the previous study, several species reported to produce chitinase enzymes include *S. rubiginosus* (Jha et al., 2016), *S. albus* FS12 (Santhi, 2016), *S. maltophilia* (Salas-Ovilla et al., 2019), *S. macrospores* M1 (Sukalkar et al., 2018) and *S. philanthi* RM-1-1-38 (Boukaew et al., 2016). The ability of these various *Streptomyces* species to decompose chitin, according to Veliz et al. (2017), is due to the ability of hyphae to penetrate the substrate followed by the release of extracellular chitinase enzymes.

Streptomyces sp.4 bacteria in this study were then tested for larvicidal activity against *A. aegypti* larvae using in vivo study. Cultures of *Streptomyces* sp.4 with a population density of $1x10^8$ CFU/mL with volume variations (Table 1) were inoculated in a test container containing 25 larvae of *A. agypti* (stage III). The test results showed that the treatment of *Streptomyces* sp.4 culture affected larval mortality. Based on Table 4, the higher the volume of culture used for the test is directly proportional to the percentage of larval mortality. The A6B1 culture treatment was known to have the highest mortality percentage, which was 34.25%, and significantly different from the negative control ($p<0.05$). However, compared to positive controls, the administration of chemical larvicide temephos had a higher mortality percentage, namely 47.00%, and significantly different from the culture treatment and negative control. Based on the Probit analysis, the LD_{50} value of the *Streptomyces* sp.4 culture was 24.6 ± 5.4 mL. These results indicate a dose of 24.6 mL of *Streptomyces* sp.4 culture in 100 mL of distilled water can kill as much as 50% of the number of larvae in the treatment container.

Based on the in vivo test results, it was found that the application of liquid culture of *Streptomyces* sp.4 isolate had an effect on larval mortality and was significantly different from the negative control treatment ($p<0.05$). The results of this study support Widiastuti and Marbawati (2016) who stated that chitinase-producing bacteria are potential for mosquito bio larvicide candidates. The chitinase enzyme produced by bacteria is reported to have active activity in

destroying the exoskeleton structure of mosquito larvae which is composed of chitin material. The damage to the exoskeleton structure of mosquito larvae can trigger such as impaired growth and death. The results of observations of the test group with *Streptomyces* sp.4 culture treatment also showed that most larvae can't develop into pupae. It is different from the control treatment, where the surviving larvae then turn into pupae. These results are in line with the research of Aggarwal et al. (2015), who obtained the effects that the chitinolytic activity of bacteria affects the process of turnover from the pupa stage.

Conclusion

Liquid cultures of *Streptomyces* sp.4 based on in vivo test is known to have larvicidal activity against *A. aegypti* larvae. The test result showed that the treatment of *Streptomyces* sp.4 liquid culture had a significant effect on larval mortality compared to the negative control ($p<0.005$). Based on Probit analysis showed that the LD_{50} value of the *Streptomyces* sp.4 liquid culture was 24.6±5.4 mL.

Acknowledgement

The authors would like to thank for Institution of Research and Community Service of STIKES Wira Medika Bali and the Entomology Section of the East Java Provincial Health Office for support in this research grant under contract 035/PP/LPPM/III/2019.

References

Aggarwal, C., Paul, S., Tripathi, V., Paul, B., & Khan, M. A. (2015). Chitinolytic activity in *Serratia marcescens* (strain SEN) and potency against different larval instars of *Spodoptera litura* with effect of sublethal doses on insect development. *BioControl*, *60*(5), 631–640. https://doi.org/10.1007/s10526-015-9674-3

Al-Qwabah, A. A., Al-Limoun, M. O., Al-Mustafa, A. H., & Al-Zereini, W. A. (2018). *Bacillus atrophaeus* A7 crude chitinase: characterization and potential role against *Drosophila melanogaster* larvae. *Jordan Journal of Biological Sciences*, *11*(4), 451–459. https://jjbs.hu.edu.jo/files/v11n4/Paper%20Number%2015.pdf

Atikasari, E., & Sulistyorini, L. (2018). Pengendalian vektor nyamuk *Aedes aegypti* di Rumah Sakit Kota Surabaya. *The Indonesian Journal Public Health*, *13*(1), 71–82. https://doi.org/10.20473/ijph.vl13il.2018.71-82

Basik, A. A., Juboi, H., Shamsul, S. S. G., Sanglier, J. J., & Yeo, T. C. (2020). Actinomycetes isolated from wetland and hill paddy during the warm and cool seasons in Sarawak, east Malaysia. *Journal of Microbiology, Biotechnology and Food Sciences*, *9*(4), 774–780. https://doi.org/10.15414/jmbfs.2020.9.4.774-780

Benelli, G., Jeffries, C. L., & Walker, T. (2016). Biological control of mosquito vectors: Past, present, and future. *Insects*, *7*(4), 1–18. https://doi.org/10.3390/insects7040052

Bintari, N. W. D., Kawuri, R., & Dalem, A. A. G. R. (2015). *Streptomyces* sp. as a biocontrol of vibriosis on larvae of Macrobrachium rosenbergii (de Man) Prawns. *Journal of Biological and Chemical Research*, *34*(1), 238–248. www.sasjournals.com

Boukaew, S., Petlamul, W., Suyotha, W., & Prasertsan, P. (2016). Simultaneous fermentative chitinase and β-1,3 glucanase production from *Streptomyces philanthi* RM-1-1-38 and their antifungal activity against rice sheath blight disease. *Biotechnologia*, *97*(4), 271–284. https://doi.org/10.5114/bta.2016.64544

Braks, M., Giglio, G., Tomassone, L., Sprong, H., & Leslie, T. E. (2019). Making vector-borne disease surveillance work: new opportunities from the SDG perspectives. In *Frontiers in Veterinary Science* (Vol. 6, Issue 232, pp. 1–9). Frontiers Media S.A. https://doi.org/10.3389/fvets.2019.00232

Chater, K. F. (2016). Recent advances in understanding *Streptomyces F1000Research*, *5*, 1–16. https://doi.org/10.12688/f1000research.9534.1

Fatmawati, U., Meryandini, A., Nawangsih, A. A., & Wahyudi, A. T. (2019). Screening and characterization of actinomycetes isolated from soybean rhizosphere for promoting plant growth. *Biodiversitas*, *20*(10), 2970–2977. https://doi.org/10.13057/biodiv/d201027

Harapan, H., Michie, A., Mudatsir, M., Sasmono, R. T., & Imrie, A. (2019). Epidemiology of dengue hemorrhagic fever in Indonesia: Analysis of five decades data from the National Disease Surveillance. *BMC Research Notes*, *12*(1), 2–6. https://doi.org/10.1186/s13104-019-4379-9

Harir, M., Bendif, H., Bellahcene, M., Frotas, Z., & Pogni, R. (2018). Streptomyces secondary metabolites. In *Basic Biology and Applications of Actinobacteria*. IntechOpen. https://doi.org/10.5772/intechopen.79890

Haryanto, B. (2018). Indonesia Dengue Fever: Status, Vulnerability, and Challenges. In *Current Topics in Tropical Emerging Diseases and Travel Medicine*. IntechOpen. https://doi.org/10.5772/intechopen.82290

Jabeen, F., Hussain, A., Manzoor, M., Younis, T., Rasul, A., & Qazi, J. I. (2018). Potential of bacterial chitinolytic, *Stenotrophomonas maltophilia*, in biological control of termites. *Egyptian Journal of Biological Pest Control*, *28*(1), 1–10. https://doi.org/10.1186/s41938-018-0092-6

Jha, S., Modi, H. A., & Jha, C. K. (2016). Characterization of extracellular chitinase produced from *Streptomyces rubiginosus* isolated from rhizosphere of Gossypium sp. *Cogent Food and Agriculture*, *2*(1), 1–12. https://doi.org/10.1080/23311932.2016.1198225

Kurniawati, S., Mutaqin, K. H., & Giyanto. (2015). Eksplorasi dan uji senyawa bioaktif bakteri agensia hayati untuk pengendalian penyakit kresek pada

padi. *J. HPT Tropika*, *15*(2), 170–179. http://jhpttropika.fp.unila.ac.id/index.php/jhpttropika/article/view/27

Lacombe-Harvey, M. È., Brzezinski, R., & Beaulieu, C. (2018). Chitinolytic functions in actinobacteria: ecology, enzymes, and evolution. In *Applied Microbiology and Biotechnology* (Vol. 102, Issue 17, pp. 7219–7230). Springer Verlag. https://doi.org/10.1007/s00253-018-9149-4

Law, J. W. F., Ser, H. L., Khan, T. M., Chuah, L. H., Pusparajah, P., Chan, K. G., Goh, B. H., & Lee, L. H. (2017). The potential of Streptomyces as biocontrol agents against the rice blast fungus, Magnaporthe oryzae (Pyricularia oryzae). *Frontiers in Microbiology*, *8*(3), 1–10. https://doi.org/10.3389/fmicb.2017.00003

Poorna, C. A., & Pradeep, N. S. (2016). Identification of novel chitinolytic Streptomyces. spp from a sacred grove and it's in vitro antagonistic activity analysis. *International Journal of Current Microbiology and Applied Sciences*, *5*(8), 916–928. https://doi.org/10.20546/ijcmas.2016.508.103

Salas-Ovilla, R., Gálvez-López, D., Vázquez-Ovando, A., Salvador-Figueroa, M., & Rosas-Quijano, R. (2019). Isolation and identification of marine strains of *Stenotrophomona maltophilia* with high chitinolytic activity. *PeerJ*, *2019*(1), 1–12. https://doi.org/10.7717/peerj.6102

Santhi, R. (2016). Isolation of chitinase producing *Streptomyces albus* FS12, production and optimization of extracellular chitinase. *Int. J. Adv. Res. Biol. Sci*, *3*(4), 229–237. http://s-o-i.org/1.15/ijarbs-2016-3-4-31

Shrivastava, P., Kumar, R., & Yandigeri, M. S. (2017). In vitro biocontrol activity of halotolerant *Streptomyces aureofaciens* K20: A potent antagonist against *Macrophomina phaseolina* (Tassi) Goid. *Saudi Journal of Biological Sciences*, *24*(1), 192–199. https://doi.org/10.1016/j.sjbs.2015.12.004

Soeka, Y. S., & Triana, E. (2016). Pemanfaatan Limbah Kulit Udang untuk Menghasilkan Enzim Kitinase dari *Streptomyces macrosporeus* InaCC A454 U. *J.Kim.Terap.Indones*, *18*(1), 91–101. http://kimia.lipi.go.id/inajac/index.php

Sukalkar, S. R., Kadam, T. A., & Bhosale, H. J. (2018). Optimation of chitinase production from *Streptomyces macrosporeus* M1. *Life Science Informatics Publications*, *4*(1), 106–114. https://doi.org/10.26479/2018.0401.09

Veliz, E. A., Martínez-Hidalgo, P., & Hirsch, A. M. (2017). Chitinase-producing bacteria and their role in biocontrol. In *AIMS Microbiology* (Vol. 3, Issue 3, pp. 689–705). AIMS Press. https://doi.org/10.3934/microbiol.2017.3.689

Vinodhkumar, T., Subhapriya, M., Gamanathan, G., Suresh, J.I., Kalpana. (2015). Screening of bioactive potential for larvicidal activity of marine actinomycetes. *Int.J.Curr.Microbiol.App.Sci,* *4*(2): 972-980. https://www.ijcmas.com/vol-4-2/T.Vinodhkumar,%20et%20al.pdf.

Widiastuti, D., & Marbawati, D. (2016). Efek larvasida bakteri kitinolitik dari limbah kulit udang terhadap larva *Aedes aegypti*. *Aspirator*, *8*(1), 47–54.

https://ejournal2.litbang.kemkes.go.id/index.php/aspirator/article/view/1221

Yotopranoto, S., Kurnijasanti, R., Rohmah, E.A. (2017). Isolation of *Streptomyces* sp. from lapindo mud soil, Sidoarjo, East Java Province, Indonesia as a larvicide candidate against *Aedes aegypti*. *Folia Medica Indonesiana, 53*(2), 118-123. https://doi.org/10.20473/fmi.v53i2.6355.

Zhou, G., Lo, E., Githeko, A. K., Afrane, Y. A., & Yan, G. (2020). Long-lasting microbial larvicides for controlling insecticide-resistant and outdoor transmitting vectors: a cost-effective supplement for malaria interventions. *Infectious Diseases of Poverty*, *9*(1), 1–8. https://doi.org/10.1186/s40249-020-00767-3