International Journal of Botany Studies
ISSN: 2455-541X
www.botanyjournals.com
Volume 1; Issue 2; February 2016; Page No. 47-49

Larvicidal activity of *Streptomyces cacaoi* subsp. *cacaoi*-M20 against *Aedes aegypti*

T. Janaki

Research Scholar, Department of Botany, K. M. Centre for P. G. Studies (Autonomous), Puducherry-8, India.

Abstract
Totally 25 actinomycetes were isolated by dry heat (70 ºC) treatment method on Starch casein agar media, from the soil sample that was collected nearer to the root region of the mangrove *Avicennia marina* from the back water area, Ariyankuppam, Puducherry (UT). Mosquito larvae used in this study was *Aedes aegypti.* Among the 25 isolates, the isolate M20's crude extract was found to better in controlling the larvae of *Aedes aegypti* mosquitoes. The active isolate M20 was identified as *Streptomyces cacaoi sub sp cacaoi* with 98.6% similarity with already reported sp. The isolate M20 was chitinase positive and it inhibits the growth and kills the mosquito larva effectively.

Keywords: Mangroves, Larvicidal activity, *Aedes aegypti, Streptomyces cacaoi* subsp *cacaoi.*M20, chitinase

Introduction

Aedes aegypti is a vector for transmitting several tropical fevers. Its native place is Africa but now it is found in tropical and subtropical regions. Only the female mosquitoes bite for blood, which it needs to lay its eggs (mature). These mosquitoes are very much attracted by chemical compounds secreted by human beings especially right handed (dextrorotatory) octenol molecules. Several research work is going on to find the novel source for controlling the *Aedes aegypti*'s larva. El-Khawagh *et al*., 2011, [4] Sundarapandian *et al*., 2002 [19] did research to control larva of *Culex sp*, Dhanasekaran *et al*., 2010 [2] tried to control the Anopheles mosquitoes through his research work. They used culture filtrates of actinomycetes as the bioinsecticide source. Actinomycetes are potent source of antibiotics, besides vitamins and enzymes, and such antagonistic actinomycetes of marine origin are being regularly reported (Krasilnikov, 1962; Okami *et al*., 1976; Pisano *et al*., 1986: Weyland and Helmke, 1988; Do *et al*., 1991; Farooq Biabani *et al*., 1997: Pusecker *et al*., 1997: Romero *et al*., 1997; Williams *et al*., 1999) [9, 12, 13, 3 22, 5, 14, 17, 23]. Few reports that soil is a major source of actinomycetes (Sivakumar *et al*., 2005; Vijaya kumar *et al*., 2007; Dhanasekaran *et al*., 2008) [1, 18, 20]. Members of actinomycetes which live in marine environment are poorly understood and only few reports are available pertaining to actinomycetes from mangroves (Sivakumar, 2001; Vikineswari *et al*., 1997; Rathna kala & Chandrika, 1993; Lakshmanaperumalsamy, 1978) [2, 15, 11]. Mangrove ecosystem is the most productive ecosystem diversified with variety of microbes (Kathiresan and Bingham, 2001) [8]. Chitinase producing actinomycetes are very potential in killing the insects, based on this, chitinase producing actinomycetes from soil of *Avicennia marina* -mangrove environment of Ariyankuppam, Puducherry was selected. It is an approach to find the bioinsecticide compounds from actinomycetes of mangrove origin.

2. Materials and methods

Collection of soil sample

Soil sample near the root region of the mangrove plant, *Avicennia marina (Forsk). Vierh – (Avicenniaceae)* in Ariyankuppam back water estuary, Puducherry (Lat 11 º46´03'' to 11 º53´40'' North and Longi 79 º49´45" to 79 º48´00" East) was collected, packed in sterile plastic containers and transported immediately to the laboratory. The pH of the fresh soil sample was determined (Reed and cummmings, 1945) [16]. Then the soil sample was air dried for 7-10 days at 40 ºC, Crushed and sieved to remove the shells and debris and stored.

Soil analysis

Physio-chemical nature of soil sample was analysed in soil testing laboratory, Department of Agriculture, Puducherry, India.

Isolation of mangrove actinomycetes

The soil sample was subjected to dryheat (70 ºC for 15 min) (Hayakawa *et al*., 1991) (Janaki *et al*., 2015) pretreatment to enhance the chances of isolating rare and novel actinomycetes. After pretreatment, one gram soil was mixed and serially diluted in sterile water blanks. 0.1 ml of last two dilutions ($10^{-5}$and 10^{-6}) was inoculated by pour plate method (Zheng *et al*., 2000) [24] using Starch casein agar (Kuster and Williams, 1964) [10] supplemented with Fluconazole 80µg/ml and Nalidixic acid 75µg/ml. Plates were incubated at 30 ± ºC for up to 30 days. Plates were periodically examined for actinomycetes colonies. Selected colonies were transferred to Yeast Malt extract agar slants and maintained in the same medium.

Bioassay of culture filtrates of isolate M20 against the larvae of Aedes aegypti

Initially the mosquitocidal activity was tested with larvae of *Aedes* mosquito in the laboratory. Larvae of *Aedes* were collected from K.M.C.P.G.S, Lawspet, and Puducherry-8 and kept under controlled conditions at temperature of 27 ± 2°C. The larvae of aedes were collected in a plastic bucket that had fresh water without chlorine, kept outside the window of laboratory for one week without disturbing it. Larvae of *Aedes* were collected examined under the microscope for its morphological features and they were concluded to belong to

larvae of *Aedes*. Seven test tubes each with 10 ml of sterilized tap water without any chlorine were taken and different concentrations of culture filtrate of isolate M20- 10µl, 25µl, 75µl, 125µl, 250µl and 500µl was added and 6 larvae of *Aedes* were transferred to each tube. Tubes were kept under controlled conditions for 24 hours. The number live larvae were counted and % mortality was calculated after 24–96 hours (Sundarapandian *et al*., 2002, El-Khawagh, *et al*., 2011) [4, 19]. The larvae were observed daily until pupation and adult emergence. The following formula were used

Larval mortality % = Y / X × 100
where X = number of tested larvae and Y = number of dead larvae
Pupation % = X / Y × 100,
where Y = number of pupae and X = number of tested larvae.
Pupal mortality % = X – Y / X × 100
where X = number of produced pupae and Y = number of observed adults.
Adult emergence % = X / Y × 100
where X = number of emerged adults and Y = number of tested pupae.
Pupal malformation % = Z / X × 100
where Z = number of malformed pupae and X = number of tested pupae.

3. Results and Discussion

Bioassay of culture filtrate of isolate M20 against larvae of Aedes aegypti mosquito

The isolate M20 was actively involving in degrading the chitin in chitinase activity tested for checking its efficiency in excellular enzyme production. The mosquitoes have the chitinous covering in their body as the protective layer, also they need chitin for completing its metamorphosis, without chitin the larvae cannot form pupa. Since, the isolate degraded the chitin; the culture filtrate of isolate M20 was bioassay against the larvae of *Aedes* mosquitoes for bio insecticide activity. The collected *Aedes* mosquitoes and larvae were observed under microscope to identify the species. They were identified as *Aedes aegypti* by their morphological differences with other *Aedes sp.* The lethal mortality and pupa abnormality was not observed in control. There was 100% pupa formation and 100% emergence of adults from the control was noted. At the concentration of 10µl culture filtrate 16.7% of lethal mortality, 50% pupa formation, 33.3% adult emergence was noted. In 25µl concentration, 16.7% of lethal mortality, 16.7% pupa formation, 50% pupa abnormality and 16.7% adult emergence were noted. No pupa formation and adult emergence was noted at the concentrations of culture filtrate in 12.5ml of tap water were 75µl, 125 µl, 250 µl and 500 µl. 100% larval mortality was noted at the concentration 500 µl (Table 1).

Table 1: Bioassay of culture filtrate of M20 against larvae of Aedes aegypti

Parameter's tested	Control (Water)	culture filtrate of M20 at different concentration					
		10µl	25µl	75µl	125µl	250µl	500µl
Larval mortality	-	16.7%	16.7%	50%	66.7%	83.3%	100%
Pupa formation	100%	50%	16.7%	-	-	-	-
Pupa abnormality	-	-	50%	50%	33.3%	16.7%	-
Adult emergence	100%	33.3%	16.7%	-	-	-	-

Synthesis of chitin is very essential for insects to get full development and cuticle formation for their protection. Some of the insects act as vectors for spreading harmful diseases on environment where they are surviving, such type of insects can be controlled with the help of chitin lysing enzyme secreted by other organisms that are not harmful. The isolate M20-*Streptomyces cacaoi* subsp. *cacaoi* synthesis such enzyme extracellularly, the compound from the isolate M20 was subjected for mosquito larvicidal activity. Culture filtrate was tested against larvae of *Aedes aegypti.* When the concentration of the culture filtrate was increased, the mortality of larvae of *Aedes aegypti* also increased respectively. Mortality, pupa malformation and abnormality were observed for larvae of *Aedes aegypti*. Since the isolate M20 actively involved in chitin inhibiting activity, this may be a reason for control of larvae of mosquito. Sundarapandian *et al*., (2002) [19] had proved *Culex quinquefasciatus* larvicidal activity of 3 actinomycetes out of 44 screened for the activity, *Streptomyces* -98-7 showed good larvicidal activity; El-Khawgh *et al*. (2011) had proved the larvicidal activity of *Culex pipiens* using a *Streptomyces sp.* It is essential to carry out field trial of larvicidal compounds from *Streptomyces cacaoi* subsp. *cacaoi* for human welfare.

4. Conclusion

Everyday mangroves face lot of fluctuations in their life style. They survive in the high stressed salty, heavy metal stressed environment. Recycling of nutrients take place fastly in these areas. These are the some of the reasons for isolating the novel actinomycetes from the mangrove area to control the vector borne diseases with the help of their bioinsecticide properties.

5. References

1. Dhanasekaran D, Panneerselvam A, Thajuddin N. An antifungal compound: 4' phenyl-1-napthyl–phenyl acetamide from Streptomyces spp.DPTB16. Facta Universitatis Series: Medicine and Biology 2008; 15:7-12**.**
2. Dhanasekaran D, Sakthi V, Thajuddin N, Panneerselvam A. Preliminary evaluation of anopheles mosquito larvicidal efficacy of mangrove actinobacteria**,** International Journal of Applied Biology and Pharmaceutical Technology. 2010; 1(2):374-381.
3. DoHK, Kogure K, Imada C, Noguchi T, Ohwada K, Simidu U. Tetrodotoxin production of actinomycetes isolated from marine sediments. J Appl. Bacteril. 1991; 70:464-468.
4. El-Khawagh MA, Hamadah KS, El-Sheikh TM. The insecticidal activity of actinomycete metabolites, against the mosquitoe Culex pipiens. Egypt. Acad. J biolog. Sci**.** 2011; 4(1):103-113.
5. Farooq Biabani MA, Laatsch D, HelmkeE, Weyland H. Δ-Indomycinone: a new memberof pluramycin class of

antibiotics isolated from marine streptomyces sp. J Antibiot. 1997; 50:874-877.
6. Hayakawa M, Nonomura H. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes, J Ferment. Technol. 1987; 66:501-509.
7. Janaki T, Nayak BK, Ganesan T. Different Pre-treatment methods in Selective Isolation of Actinomycetes from Mangrove sediments of Ariyankuppam, Back water Estuary, Puducherry. Int. J Adv. Res. Biol. Sci. 2014; 1(6):154-163.
8. Kathiresan NK, Bingham BL. Biology of Mangroves and Mangrove Ecosystems. Advances in Marine Biology 2001; 40:81-251.
9. Krasil'nikov NA. Antibiotic properties of microorganisms isolated from various depths of world's oceans. Microbiology 1962; 30:545-550.
10. Kuster E, Williams ST. Selection of media for isolation of streptomycetes, Nature 1964; 202:928-929.
11. Lakshmanaperumalsamy P. Studies on actinomycetes with special reference to antagonistic streptomycetes from sediments of Porto Novo coastal zone. Ph.D. thesis, Annamalai University, India, 1978, 192,
12. Okami Y, Okazaki T, Kitahera T, Umezawa H. A new antibiotic aplasmomycin produced by a streptomycete isolated from shallow sea mud. J Antibiot. 1976; 29:1019-1025.
13. Pisano MA, Sommer MJ, Lopez MM. Applications of pretreatments for the isolation of bioactive actinomycetes from marine sediments. Appl. Microbiol. Biotechnol 1986; 25:285-288.
14. Pusecker K, Laatsch H, Helmke E, Weyland H. Dihydropencomycin methyl ester, a new phenazine derivative from a marine streptomycete. J Antibiot. 1997; 50:47-483.
15. Rathna Kala R, Chandrika V. Effect of different media for isolation, growth and maintenance of actinomycetes from mangrove sediments. Indian J mar. sci. 1993; 22:297-299.
16. Reed J, Cummings FS. Soil reaction-glass electrode and colorimetric methods for determining PH values of soil, *Soil Sci* 1945; 59:97-104.
17. Romero F, Espliego F, Baz JP, De Quesada TG, Gravalos D, De La Calle F *et al*. Thiocoraline, a new depsipeptide with anti-tumour activity produced by a marine micromonospora. J Antibiot. 1997; 50:734-737.
18. Sivakumar K, Sahu M, Kathiresan K. Isolation and characterization of streptomycetes producing antibiotic from mangrove environment. Asian Journal of Microbial Biotechnology and Environmental Science. 2005; 7:457-764.
19. Sundarapandian S, Sundaram MO, Tholappian P, Balasubramanian V. Mosquitocidal properties of indigenous fungi and actinomycetes against Culex quinquefasciatus say. J Biol. cont. 2002; 16:89-91.
20. Vijayakumar R, Muthukumar C, Thajuddin N, Pannerselvam A, Saravanamuthu R. Studies on the diversity of Actinomycetes in the Palk Strait region of Bay of Bengal, India. Actinomycetologica 2007; 21:59-65.
21. Vikineswary S, Nadaraj P, Wong WH, Balabaskaran S. Actinomycetes from a tropical mangrove ecosystem - Antifungal activity of selected strains. Asia Pacific J Mol. Biol. Biotec. 1997; 5(2):81-86.
22. Weyland H, Helmke E. Actinomycetes in the marine environment. In: The Biology of Actinomycetes. Okami, Y., Beppu, T. and Nagamura H. (Eds.). Japan Scientific Society Press, Tokyo, 1988, 294.
23. Williams DE, Bernan VS, Ritacco FV, Maiese WM, Holyrines A, possible B. intermediates in staurosporine biosynthesis produced in culture by a marinr actinomycete obtained from the North Atlantic ocean. Tetrahedron Lett 1999; 40:7171-7174.
24. Zheng Z, Zeng W, Huang Y, Yang Z, Li J, Cai H *et al*. Detection of antitumor and antimicrobial activities in marine organism associated actinomycetes isolated from the Taiwan Strait, China. FEMS Microbiol. Lett. 2000; 188:87-91.