International Journal of Microbiological Research 1 (3): 179-183, 2010
ISSN 2079-2093

Larvicidal Potentiality of Marine Actinomycetes Isolated from Muthupet Mangrove, Tamilnadu, India

[1]R. Vijayakumar, [2]S. Murugesan, [3]A. Cholarajan and [1]V. Sakthi

[1]Department of Microbiology, Bharathidasan University College for Women,
Orathanadu-614 625, Thanjavur District, Tamil Nadu, India
[2]P.G. and Research Department of Botany and Microbiology,
A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613 503, Tamilnadu, India
[3]Department of Microbiology, Meenakshi Chandrasekaran College of Arts and Science,
Karambayam-614 626, Thanjavur District, Tamilnadu, India

Abstract: The marine soil and sediment samples were collected from different locations of Muthupet mangrove, Tamilnadu. A total of thirty different marine actinomycete isolates were isolated by serial dilution plate technique on starch casein agar medium. The isolated actinomycetes were investigated for their larvicidal activity against *Anaphelos* mosquitoes. Of 30 isolates, 23 isolates showed larvicidal activity in preliminary screening, among 23 isolates, 2 isolates exhibited notable larvicidal activity. The potent larvicidal actinomycetes were characterized based on their morphological, biochemical and cultural characteristics the isolate CC11 and SH23 was identified as *Streptomyces* sp. and *Streptosporangium* sp. respectively. This investigation explores the larvicidal potential of actinomycetes for the discovery of novel insecticidal metabolites.

Key words: Marine actinomycetes · Larvicidal activity · Screening · *Anaphelos* mosquitoes

INTRODUCTION

Insect's transmitted disease remains a major source of illness and death worldwide. Mosquitos are one of the major vectors responsible for the transmittance of diseases to more than 700 million people annually. Among various mosquitoes mediated diseases malaria alone kills 3 million people every year including children every 30 seconds. Although mosquito borne diseases currently represent a greater health problem in tropical and sub-tropical countries, no part of the world is immune to this risk [1]. Control of such diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to pesticides [2]. An alternative approach for mosquito control is application of natural products of microbial origin. The need for new strategies for the control of mosquito larva has never been greater for a variety of reasons. Mosquito borne infections fall into two major categories, which includes malaria, filaria, dengue and yellow fever.

Actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive substances. These searches have been remarkably successful and approximately two-thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes [3]. Intensive screening program carried out over the past several decades resulted in the production of actinomycetes which are more abundant in terrestrial soils than in marine sediments showing varying degrees of salt tolerance and produce spores that are undoubtedly washed in large numbers from shore into the sea [4]. Inspite of the fact that they remain active in the marine environment, their role in the production of bioactive compounds is yet to be studied. It needs a screening of which varieties of the species of actinomycetes, that could possibly the potential source of bioactive components.

The secondary metabolites of actinomycetes namely tetranectin, avermectins, faerifungin and macrotetrolides and flavonoids produced were found to be toxic to

Corresponding Author: R. Vijayakumar, Department of Microbiology, Bharathidasan University College for Women, Orathanadu-614 625, Thanjavur District, Tamil Nadu, India, E-mail: rvijayakumar1979@gmail.com.

mosquitoes and the species genera involved are *Micromonospora, Actinomadura, Actinoplanes, Micropolyspora, Nocardiopsis, Oerskonia, Thermomonospora, Sreptoverticillium* and *Chainia.* The secondary metabolites of actinomycetes, tetranectin [5], avermectins [6], faerifungin [7], macrotetrolides [8] and flavonoids [9] produced respectively by *Streptomyces aureus, Streptomyces avermitilis*, *Streptosporangium albidum*, *Streptomyces griseus* and *Streptomyces* sp., were reported to be toxic to mosquitoes.

The marine environment is an untapped source for many useful drugs and an assessment of this potential is imperative. It is well known that the actinobacteria are the potential sources of antibiotics, which could profitably developed in the pharmaceutical industries and the best known example is the product of *Streptomyces.* There is an increasing demand for the new type of antibiotics to control mosquitoes. Perusal of the literature survey indicates that, the reports on the antagonistic actinobacteria from the marine environment are very scanty. The marine soil of Tamil Nadu especially mangrove soils of Muthupet has rich sources of potential microbial diversity. Nevertheless, they have not been extensively explored for the registration of novel actinobacteria.

Keeping these points in view, the present study has been undertaken to isolate and screen the larvicidal compounds producing actinobacteria from Muthupet mangrove environment of Thiruvarur District, Tamil Nadu, India and also an attempt has been made to characterize the different isolates by analyzing biochemical and larvicidal spectrum of actinobacteria. In order to achieve this goal the present investigation has been planned to find out the potentiality of the production of larvicidal compounds by actinobacteria and characterization of the actinobacteria isolates possess significant larvicidal property against *Anaphelos* mosquitoes.

MATERIALS AND METHODS

Description of Sampling Sites: Muthupet mangrove environment (Lat.10' 20'N and 79' 35'E) is known to be very rich microbial diversity due to high amount of dissolved and particulate organic matter and therefore, different types of microorganisms are found in this type of environment. Microorganisms from these areas play an important role in biodegradation of dead plant material. This area is rich in *Avicennia officinalis, Rhizophora mucaronata. Acanthus illicifolius* and *Excoecaria agallocha* plants.

Soil Sample Collection: The soil and sediments samples were collected from different locations of Muthupet mangrove, Thiruvarur district, Tamil Nadu, India. The soil samples were collected at random brought to the laboratory in sterile polythene bags and stored at 4°C for further study.

Isolation of Actinomycetes: Starch casein agar (SCA) [10] medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 min. Then it was supplemented with amphotericin B 50 µg/ml, penicillin and NaCl 30 µg/ml and tetracycline 20 µg/ml to prevent the growth of bacterial and fungal contaminants. The soil samples were diluted upto 10^{-6} and 0.1 ml of the aliquots was spread over the SCA plates. The inoculated plates were incubated at 28±2°C for seven to ten days. Three replicates were maintained for each dilution. After incubation, the actinomycetes colonies were purified and maintained in starch casein agar medium for further investigation.

Collection of Anopheles Larvae: The *Anopheles* mosquito larva was collected from water reservoir in and around the Central Adhiparasakathi Agricultural Farm, G.B. Nagar, Kalavai, Tamilnadu.

Extraction of Extracellular Larvicidal Compounds of Actinomycetes: Totally 30 different actinomycete isolates were selected and determined for their larvicidal spectrum against the *Anopheles* mosquitos. The selected isolates were inoculated into a 500 ml conical flask containing 200 ml of starch casein liquid medium and shaken at 28±2°C and 200 rpm for seven days. The cell free culture filtrates were separated by centrifugation and used for larvicidal activity.

Screening of Larvicidal Activity in Actinomycetes: Nine ml of sterile tap water was taken in test tubes were mixed with one ml of actinomycetes culture filtrate. The control tubes were maintained as tap water alone. Five *Anopheleses* mosquito larvae were inoculated into the above tubes and incubated at 2°C for 48 h. The larvicidal activity was observed for over 30 min. and death of the larvae confirmed the larvicidal activity.

Larvicidal Effect of Actinomycetes on *Anopheles* Mosquito: The potent larvicidal actinomycetes were selected for further study. The different concentration of actinomycetes culture filtrate (2%) was taken into 500 ml beaker with 100 ml of tap water. Twenty five mosquito larvae were inoculated and incubated for 48 h. The control

was maintained free from actinomycetes culture filtrate. Every 30 min larvicidal activity was observed and its percentage of larvicidal activity calculated and recorded.

Characterization and Identification of Actinomycetes: The potential larvicidal actinomycetes were characterized on the basis of morphological (colony morphology, spore morphology and pigmentation) [11], biochemical properties and identified up to generic level by the standard methods of International *Streptomyces* Project (ISP) [12].

RESULTS AND DISCUSSION

Actinomycetes have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. The activities of bioactive compounds from *Streptomyces* are categorized as pharmacologically, agrobiologically active agents and autoregulators. Thus, it is obvious that the activity profile of *Streptomyces* products is very broad. Hence, intensive research in this area was carried out with special references to morphological, biochemical characterization and screening of antilarval compounds from actinomycetes.

In the present study, a total of 30 actinomycete isolates including white, grey, brown, pink and greyish violet coloured colonies with different morphological types were isolated from soil and sediment samples of Muthupet mangrove, Tamilnadu, India. Among them, 10 and 4 isolates from Chief's corner and Sethukuda stations, 11 and 5 isolates from Sharadi and Xeviermunai stations, respectively (Table 1).

Of the 30 screened isolates of actinobacteria, 23 isolates showed the antilarval activity against *Anopheles* mosquitoes (Table 2). Among the 23 isolates, only 9 isolates had the potentiality to inhibit (100%) the growth of *Anopheles* mosquito larvae. The larvicidal effect of actinobacteria extracts tested in different concentration

Table 1: Cultural characteristics of actinomycetes in Muthupet mangrove environment

S. No.	Station	Isolate code	Aerial mycelium	Substrate mycelium	Size (mm)	Pigment Production
1.	Chief's Corner soil	CC11	Grey	Grey with black	3	-
2.	Chief's Corner soil	CC12	Grey	Grey	5	-
3.	Chief's Corner soil	CC13	Pink around white	Pink	3	+
4.	Chief's Corner soil	CC14	Pink	Pink	6	-
5.	Chief's Corner soil	CC15	Grey with pink	Pink	3	-
6.	Chief's Corner soil	CC16	Grey	White	6	-
7.	Chief's Corner soil	CC17	Pink	Pink	5	+
8.	Chief's Corner soil	CC18	Grey violet	Black	3	-
9.	Chief's Corner soil	CC19	Pink	Pink	2	+
10.	Chief's Corner soil	CC110	Pink around grey	Pink	5	-
11.	Sethukuda Sediment	S21	Pink	Pink	4	+
12.	Sethukuda Sediment	S22	Dark Pink	Pink	3	-
13.	Sethukuda Sediment	S23	Light creamy pink	Pink	2	-
14.	Sethukuda Soil	S11	Pure grey	Grey with black	4	-
15.	Sharadi Soil	SH11	Pink	Pink	4	+
16.	Sharadi Soil	SH12	Dark grey	Grey	3	-
17.	Sharadi Soil	SH13	Pink around white	Pink	2	-
18.	Sharadi Soil	SH14	Grey	Black	5	-
19.	Sharadi Soil	SH15	Creamy pinkish	Brown	3	-
20.	Sharadi Sediment	SH21	Pink with grey	Brown	6	-
21.	Sharadi Sediment	SH22	Grey	Black	3	+
22.	Sharadi Sediment	SH23	Pink	Pink	5	+
23.	Sharadi Sediment	SH24	White to grey violet	Brown	2	-
24.	Sharadi Sediment	SH25	Dark grey	Light grey	5	-
25.	Sharadi Sediment	SH26	Grey	Black	3	-
26.	Xeviermunai soil	SM11	Dark pink	Pink	5	+
27.	Xeviermunai soil	SM12	Pink	Pink	3	-
28.	Xeviermunai soil	SM13	Creamy pinkish	Pink	7	-
29.	Xeviermunai sediment	SM21	Pink	Pink	3	-
30.	Xeviermunai sediment	SM22	Pink with white	Pink	5	+

-Absence of pigment production

Table 2: Screening of larvicidal activity of mangrove actinomycetes

S. No.	Isolate code	Time (h)	Total No. of larvae	Number of death	% of death
1.	CC11	14	3	2	66.6
2.	CC12	48	3	-	0
3.	CC13	10	3	1	33.3
4.	CC14	14	3	1	33.3
5.	CC15	48	3	-	0
6.	CC16	16	3	1	33.3
7.	CC17	16	3	3	100
8.	CC18	48	3	1	33.3
9.	CC19	16	3	2	66.6
10.	CC110	20	3	2	66.6
11.	S21	25	3	1	33.3
12.	S22	20	3	3	100
13.	S23	16	3	3	100
14.	S11	20	3	2	66.6
15.	SH11	16	3	1	33.3
16.	SH12	48	3	-	0
17.	SH13	20	3	2	66.6
18.	SH14	14	3	1	33.3
19.	SH15	16	3	3	100
20.	SH21	16	3	2	66.6
21.	SH22	20	3	1	33.3
22.	SH23	48	3	-	0
23.	SH24	48	3	-	0
24.	SH25	48	3	-	0
25.	SH26	14	3	1	33.3
26.	SM11	48	3	0	0
27.	SM12	20	3	3	100
28.	SM13	16	3	3	100
29.	SM21	10	3	1	33.3
30.	SM22	20	3	2	66.6

level such as 2% with 15 larvae. In the low (2%) concentration of the extracts of the isolates CC11 and SH22 were highly inhibited the larvae (20%) followed by CC110 and SH23 (16%), SH15 (12%), CC19 and S22 (8%) and S21 (4%) 3 h of inoculation. Among the nine larvicidal actinomycetes, the isolate CC11 and the isolate SH23 prominently inhibited the growth of mosquito larvae by 100 and 92% after 15 hours of incubation, respectively (Table 3). The other isolates were showed moderate to minimum (52-76%) larvicidal activity. Similar type of work has been reported by many workers using *Bacillus thuriengiensis* [13-16], *B. sphaericus* [2, 17], *Pseudomonas fluorescence* [18], fungus *Trichoderma viridae* [19] and actinobacteria [1, 20].

Since only two isolates of actinobacteria were found to possess antilarval activities, they were justifiably chosen for the taxonomic characterization. The different parameters namely, morphological (spore morphology, colony morphology and pigmentation) biochemical characteristics were used for the characterization and identification of actinobacterial isolates.

Table 3: Larvicidal activity of mangrove actinomycetes (low concentration 2%)

S. No.	Isolate code	Total number of larvae	Time (h)	Number of death	% of death
1.	CC11	25	3	4	20
			6	9	36
			9	15	60
			12	19	76
			15	25	100
2.	CC17	25	3	2	8
			6	5	20
			9	11	44
			12	14	56
			15	18	72
3.	CC19	25	3	2	8
			6	4	20
			9	7	28
			12	9	60
			15	13	69
4.	CC110	25	3	4	16
			6	7	28
			9	9	36
			12	11	44
			15	13	52
5.	S21	25	3	1	4
			6	4	16
			9	6	24
			12	9	36
			15	13	52
6.	S22	25	3	2	8
			6	5	20
			9	7	28
			12	10	48
			15	17	68
7.	SH15	25	3	3	12
			6	8	32
			9	10	40
			12	16	64
			15	19	76
8.	SH21	25	3	5	20
			6	8	32
			9	10	40
			12	15	60
			15	19	76
9.	SH23	25	3	1	16
			6	3	36
			9	5	60
			12	8	76
			15	12	92

The potent larvicidal actinomycetes were characterized based on their morphological, biochemical and cultural characteristics the isolate CC11 and SH23 was identified as *Streptomyces* sp. and *Streptosporangium* sp., respectively.

In conclusion, the present investigation clearly reveals the biodiversity and distribution of actinomycetes in different soil and their antilarval potentials of selected isolates. To exploit these findings for human welfare, it is necessary to carry out field trials and strategies for optimization of large scale production of both cell biomass and antilarval compounds. In this investigation explores the importance of larvicidal actinomycetes as valuable resource for the discovery of novel insecticidal molecules.

ACKNOWLEDGEMENTS

We thank Dr. P. Sivamani, Director, Microlabs, Arcot, Tamilnadu, India for providing technical assistance and providing the laboratory facilities.

REFERENCES

1. Dhanasekaran, D., V. Sakthi, N. Thajuddin and A. Panneerselvam, 2010. Preliminary evaluation of *Anopheles* mosquito larvicidal efficacy of mangrove actinobacteria, Int. J. Appl. Biol. Pharm. Technol., 1: 374-381.
2. Balaraman, K., 1995. Mosquito control potential of *Bacillus thuringiensis subsp. israelensis* and *Bacillus sophaericus*, ICMR Bull., 25: 45.
3. Okami, Y., 1952. Utilization of nitrogen compounds by streptomycetaceae and its applications to classification, Japan J. Med. Sci. Biol., 5: 265-275.
4. Grein, A. and S.P. Meyers, 1958. Growth characteristics and antibiotic production of actinomycetes isolated from littoral sediments and material suspended in Seawater. J. Bacterial., 76: 457-463.
5. Ando, K., 1983. How to Discover New Antibiotics for Insecticidal Use? In: Pesticide Chemistry: Human Welfare and the Environment. Vol. 2. Natural Products (Editors). Takahashi, T., H. Yoshioka, T. Misato and S. Matusunaka), Pergamon Press, New York, pp: 253-259.
6. Pampiglione, S., G. Majori, G. Petrangeli and R. Romi, 1985. Avermectins, MK-933 and MK- 936, for mosquito control. Trans. R. Soc. Trop. Med. Hyg., 79: 797-799.
7. Anonymous, 1990. Biologically active KSB-1939L3 compound and its production- pesticide with insecticide and acaricide activity production by *Streptomyces* sp. culture. Biotechnol Abst., 9: 58. (Japan Patent, No. 273961, 1988).
8. Zizka, Z., J. Weiser, M. Blumauerova and J. Jizba, 1989. Ultra structural effects of macroterrolides of *Sterptomyces griseus* LKS-1 in tissues of *Culex pipiens* larvae- monactin, dinactin, triactin and nonactin preparation; insecticide activity. Cytobios, 58: 85-91.
9. Rao, K.V., S.K. Chattopadhyay and G.C. Reddy, 1990. Flavonoides with mosquito larval toxicity- tangeratin, daidzein and genistein crystal production, isolation and purification; *Streptomyces* spp. culture; insecticide. J. Agric. Food Chem., 38: 1427-1430.
10. Kuster, E. and S.T. Williams, 1964. Production of hydrogen sulphide by *Streptomyces* and methods for its detection. Appl. Microbiol., 12: 46-52.
11. Pridham, T.G., C.W. Hesseltine and R.G. Benedict, 1958. A guide for the classification of streptomycetes according to selected groups. Appl. Microbiol., 6: 52-79.
12. Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol., 16: 312-340.
13. Balaraman, K., U.S.B. Rao and P.K. Rajagopalan, 1979. Isolation of *Metarrhizium anisopliae, Beauveria tenella* and *Fusarium oxysporum (Deuteromycetes)* and their pathogenicity to *Culex fatigans and Anopheles stephensi.* Ind. J. Med. Res., 70: 718.
14. Balaraman, K., M. Balasubramanian and L.M. Manonmani, 1983a. *Bacillus thuringiensis* H-14 (VCRC B-17) formulation as mosquito larvicide. Indian J. Med. Res., 77: 33.
15. Balaraman, K., M. Balasubramanian and P. Jambulingam, 1983b. Field trial of *Bacillus thuringiensis* H-14 (VCRC B-17) against *Culex* and *Anopheles* larvae. Indian J. Med. Res., 77: 38.
16. Manonmani, A.M. and K. Balaraman, 2001. A high mosquitocidal *Bacillus thuringienisis var. thompsoni.* Curr. Sci., 80: 779.
17. Rajendran, G., S. Sabesan, K. Kuppusamy and K. Balaraman, 1991. Evaluation of a *Bacillus sphaericus* formulation, sphaerifix for the control of mansoniodes mosquitoes. Entomon., 16: 213.
18. Prabhakaran, G., V. Padmanaban and K. Balaraman, 2000. Comparative efficacy of UV screens in protecting the activity of a *Bacillus thuringiensis* formulation. J. Biol. Control., 14: 63.
19. Geetha, I. and K. Balaraman, 2001. Biomass and blastospore production in Beauveria bassiana (Bals.) Vuill. as influenced by media components. J. Biol. Control., 15: 93.
20. Vijayan, V.K. and K. Balaraman, 1991. Metabolites of fungi and actinobacteria active against mosquito larvae. Indian J. Med. Res., 93: 115.