PRELIMINARY EVALUATION OF *ANOPHELES* MOSQUITO LARVICIDAL EFFICACY OF MANGROVE ACTINOBACTERIA

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ABSTRACT: Totally 30 actinobacteria isolates were screened for their larvicidal activity against *Anopheles* mosquito larvae. Of them four isolates producing strong larvicidal activity. These isolates were morphologically characterized and identified as the isolate CC17, SM13 as *Streptomyces* sp, isolate SH15 as *Streptosporangium,* isolate S22 as *Micropolyspora.* The present investigation clearly reveals the larvaicidal potentials of selected actinobacterial isolates in the Muthupet mangrove soil and sediments. To exploit these findings for humanwelfare, it is necessary to carryout field trial and strategies for optimizing of large scale production of cellbiomass and larvicidal compounds are suggested as future course of action.

Key words: Larvicidal activity, actinobacteria, mangrove, *Anopheles* mosquito

Introduction

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year. Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations. It has also resulted in the development of resistance (Brown, 1986), undesirable effects on non-target organisms and fostered environmental and human health concern (Hayes and Laws, 1991), which initiated a search for alternative control measures. Microorganisms are considered as a rich source of bioactive chemicals3 and they may be an alternative source of mosquito control agents.

Insect 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 the various mosquitos 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. Vijayan and Balaraman (1991) reported the extracellular secondary metabolites from 350 fungi and 94 actinobacteria were screened for larvicidal activity against *Culex quinquefasciatus, Anopheles stephensi* and *Aedes aegypti*. Of them, 133 fungal metabolites and 35 from actinobacteria were active against mosquitos. Two from *Streptomyces* sp. and one from *Paecilomyces* sp. were highly active with LC50 value of 1-3 µl /ml.

The metabolites were more toxic to *C. quinquefasciatus* than to *A. stephensi* or *A. aegypti* larvae. Control of such a diseases is becoming increasingly difficult because of increasing résistance of mosquitoes to pesticides (Balaraman, 1995). An alternative approach for mosquito control is application of natural products of microbial origin.

The need for new stratagies 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, yellow fever.

Actinobacteria from marine samples have already undergone screening for novel metabolites and there is evidence that actinobacteria usually make up only small proportion of the bacterial flora of marine habitats, with absolute numbers of actinobacteria much lower than in terrestrial habitats (Goodfellow, 1983).

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 mosquitos. A perusal of the literature clearly indicates that reports on the antagonistic actinobacteria from the marine environment are very scanty. The marine soil of Tamil Nadu 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 posse's siginificant larvicidal property.

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

Soil and sediments samples were collected from different locations in Muthupet mangrove during the period of June 2005. The soil samples were collected randomly and brought to the laboratory in sterile polythene bags and for further investigation.

Isolation of actinobacteria

Starch casein agar (Kuster and Williams, 1964) medium was prepared and sterilized at 121°C in 15 lbs pressure for 15 min. Then it was supplemented with Amphotericin B 50 µg/l and Tetracycline 20 µg/l to prevent the bacterial and fungal growth [for the selective isolation of actinobacteria the medium were supplemented with Penicillin and NaCl (30µg/ml)]. The medium was poured into the sterile petri plates. The collected soil samples were diluted upto 10^{-6} and 0.1 ml of the diluted samples was spread over the agar 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 actinobacteria colonies were purified and maintained in starch casein agar medium for further investigation.

Larvicidal activity in actinobacteria

Collection of *Anopheles* larvae

The mosquito larvae were collected from water reservoir in and around the Central Adhiparasakthi Agricultural Farm, G.B.Nagar, Kalavai, Tamil nadu.India

Extraction of extracellular larvicidal compounds from actinobacteria

Totally 30 actinomycete isolates were selected and its larvicidal spectrum was tested 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 cells free culture filtrates were separated bycentrifugation and used for larvicidal activity.

Screening of larvicidal activity in actinobacteria

To the 9ml of tap water in a test tube, 1ml of actinobacteria culture filtrate was mixed in a test tube. The control tubes were maintained as tap water free from actinobacteria culture filterate. Five *Anopheles* larvae were inoculated in both test and control tubes and inoculated at 28± 2°C for 48 hours. The larvicidal activity was observed for over 30 minutes and death of the larvae conformied the larvicidal activity.

Larvicidal effect of actinobacteria on *Anopheles* mosquito

The potent larvicidal actinobacteria were selected for further study. The different concentration of actinobacteria culture filterate (2%) was taken 500ml beaker with 100ml of tap water. Mosquito (25 numbers) larvae were inoculated and incubated for 48 hours. The control was maintained free from actinobacteria culture filterate. Every 30 minutes larvicidal activity was observed and its percentage of larvicidal activity calculated and recorded.

Characterization and identification of actinobacteria

The selected actinobacteria showing larvicidal activity were further identified by the characterististics of colony morphology (Ghanem *et al*., 2000), and microscopic observations (Coverslip culture technique, Pridham *et al.*, 1958).

Results and Discussion

The antibiotic potentialities of the actinobacteria, especially *Streptomyces* are well estabilished. However, there are both quantitative and qualitative variations in the antibiotics produced by different genera and species. Substrate and micro habitats greatly influence the qualitative and quantitative differences in the production of antibiotics by different isolates of *Streptomyces*. It has been reported that among 47 strains of actinobacteria, only 19 showed antagonistic activity (Moncheva *et al.,* 2002). Thus, there are variations in the quality and quantity of the antibiotics produced even by the different isolates of actinobacteria and fungi.

Totally 30 actinomycete isolates including white, gray, brown, pink and grayish violet coloured colonies with different morphological types were isolated from soil samples four different sites of Muthupet mangrove. Among them 10 and 4 isolates from Chief's corner and Sethukuda soils, 11 and 5 isolates from Sharadi and Xeviermunai soils respectively .The isolates were purified for further studies (Table 1).

Table 1.Cultural characteristics of actinobacteria in Muthupet mangroove environment

S.NO	Station	Culture code	Aerial mycelium	Substrate mycellium	Size (mm)	Pigment production
1	Chief corner soil	CC11	Gray	Grey with black grey	3	-
2	Chief corner soil	CC12	Grey	Grey	5	-
3	Chief corner soil	CC13	Pink around white	Pink	3	+
4	Chief corner soil	CC14	Pink	Pink	6	-
5	Chief corner soil	CC15	Grey with pink	Pink	3	-
6	Chief corner soil	CC16	Grey	White	6	-
7	Chief corner soil	CC17	Pink	Pink	5	+
8	Chief corner soil	CC18	Grey violet	Black	3	-
9	Chief corner soil	CC19	Pink	Pink	2	+
10	Chief 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	2	-
15	Sharadi soil	SH12	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	3	+
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	Seviyar munnai soil	SM11	Dark pink	Pink	5	+
27	Seviyar munnai soil	SM12	Pink	Pink	3	-
28	Seviyar munnai soil	SM13	Creamy pinkish	Pink	7	-
29	Seviyar munnai sedment	SM21	Light pink	Brown	3	-
30	Seviyar munnai sedment	SM22	Pink with white	Pink	5	+

Of the 30 isolates of actinobacteria were screened, only 23 isolates showed the antilarval activity against *Anopheles* mosquitos (Table 2). Among the 23 isolates, only 4 isolates had the potentiality inhibits (100%) the growth of anopheles mosquito larvae. The larvicidal effect of actinobacteria extracts tested in different concentration level such as 2% with 15 larvae. In the low concentration (2%) the CC11 was highly inhibited the larvae (13.3 %) followed by CC17 (6.6%), CC19 and SM13 (6.6 %) and others are not inhibits the larvae after 3 hours of inoculation. Among the actinomycete isolates, three isolates prominently inhibit the larvae and the isolate SM13 showed (Table-3) the weaker inhibitory activity. Similar type of work has been reported by many workers using *Bacillus thuriengiensis* (Balaraman *et al* ., 1979;1983a; 1983b; Manonmani and Balaraman, 2001) , *Bacillus sphaericus* (Rajendiran *et al* ., 1991: Balaraman 1995), *Pseudomonas fluorescence* (Prabhakaran *et al* ., 2005), fungus *Trichoderma viridae* (Geetha and Balaraman, 2001) and actinobacteria(Vijayan and Balaraman,1991)

Table 2. Screening of larvicidal activity of actinobacteria

S.NO	Isolates	Time (Hrs)	Total number of larva	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	2	66.6
14	S11	20	3	2	66.6
15	SH12	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	1	33.3
28	SM13	16	3	3	100
29	SM21	10	3	1	33.3
30	SM22	20	3	2	66.6

Table 3. Larvicidal activity of actinobacteria (Low concentration 2%)

S.No	Isolates	Total number of larva	Time (Hrs)	Number of death	% of death
1	CC17	15	3 6 9 12 15 18	1 3 8 10 12 15	6.6 20 53.3 66.6 80 100
2	S22	15	3 6 9 12 15 18 21 24	- 2 3 5 7 11 13 15	0 13.1 20 33.3 46.6 73.3 86.6 100
3	SH15	15	3 6 9 12 15 18 21 24	- 3 3 5 7 10 12 15	0 20 20 33.3 46.6 60.6 80 100
4	SM13	15	3 6 9 12 15 18	1 3 5 8 12 15	6.6 20 33.3 53.3 80 100

Since only four isolates of actinobacteria *were* found to possess antilarval activities, they were justifiably chosen for the taxonomic characterization. The different parameters namely, morphological, biochemical characteristics were used for the characterization and identification of actinobacterial isolates (Fig.1). Based on the morphological characteristics, the actinobacteria were identified as the isolate CC17, SM13 as *Streptomyces* sp, isolate SH15 as *Streptosporangium,* isolate S22 as *Micropolyspora.* Thus the present study gives an idea about the larvicidal actinobacteria *biodiversity* in different soils of Muthupet mangrove soil.

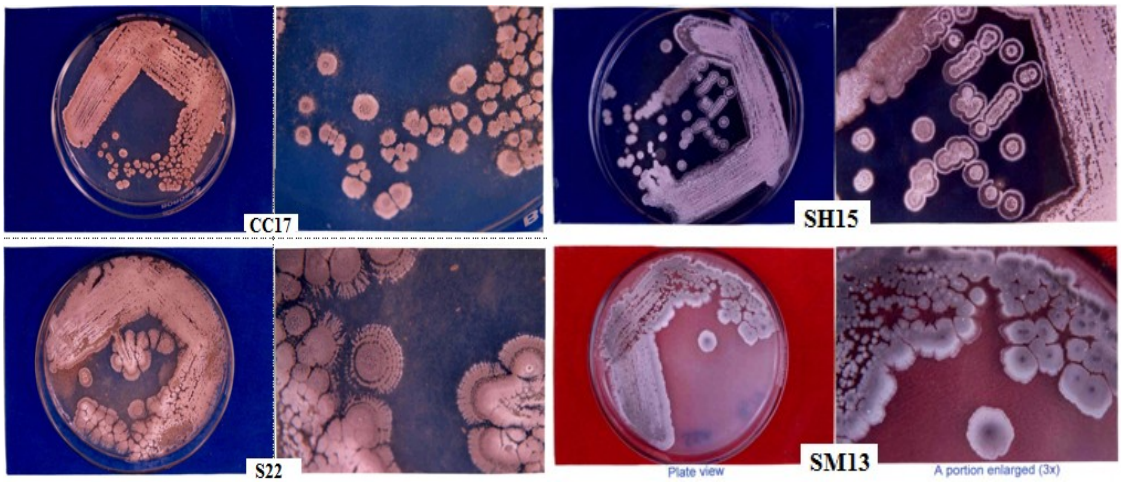

Fig.1. Cultural characteristics of Actinobacterial isolates

Conclusion

Actinobacteria could be an alternative source for mosquitolarvicides because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these microbial derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. Further studies on identification of active compounds, toxicity and field trials are needed to recommend the active fraction of these plant extracts for development of eco-friendly chemicals for control of insect vectors.

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