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Lethal effect of *Streptomyces citreofluorescens* against larvae of malaria, filaria and dengue vectors

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ABSTRACT

Objective: To investigate lethal effect of culture filtrates of *Streptomyces citreofluorescens (S. citreofluorescens)* against *Anopheles stephensi (An. stephensi)*, *Culex quinquefasciatus (Cx. quinquefasciatus)*, and *Aedes aegypti (Ae. aegypti)* larvae vectors for malaria, filarial and dengue. **Methods:** The culture filtrates obtained from *S. citreofluorescens* 2528 was grown in Potato Dextrose Broth (PDB), filtrated and used for the bioassay after a growth of 15 days. **Results:** The results demonstrated that the *An. stephensi* shows mortalities with LC_{50}, LC_{90} values of first instar 46.8 μL/mL, 79.5 μL/mL, second instar 79.0 μL/mL, 95.6 μL/mL, third instar 79.0 μL/mL, 136.9 μL/mL, and fourth instar 122.6 μL/mL, 174.5 μL/mL. Whereas, The *Cx. quinquefasciatus* were found effective on first instar 40.0 μL/mL, 138.03 μL/mL, second instar 80.0 μL/mL, 181.97 μL/mL, third instar 100.0 μL/mL, 309.2 μL/mL, and fourth instar 60.0 μL/mL, 169.82 μL/mL. The *Ae. aegypti* were successfully achieved susceptible with higher concentrations in comparisons of *An. stephensi* and *Cx. quinquefasciatus* larvae. These outcomes of the investigations have compared with the Chitinase of *Streptomyces griseus (S. griseus)* C6137 that shows 90%-95% mortality. **Conclusions:** These new findings significantly permitted that the culture filtrates of *S. citreofluorescens* can be used as bacterial larvicides. This is an environmentally safe approach to control the vectors of malaria, dengue and filariasis of tropical areas.

1. Introduction

Streptomyces are filamentous soil bacteria that produce a wide variety of secondary metabolites[1]. Few species are pathogenic for animals, cause plant diseases and degraders of organic matter in the natural environment, but also as producers of antibiotics and other useful compounds of commercial interest[2-4]. In addition, *Streptomyces* are important for the production of enzymes. Chitinase is originally an enzyme used by insects to degrade the structural polysaccharide "chitin" during the molting process[5]. The largest chitinases activity among bacteria has been observed in species of *Streptomyces, Serratia, Vibrio* and *Bacillus*. Chitinase enzyme is very important in the biological control of insects[6]. Species of *Streptomyces* showed high multiplicity of chitinase genes[7,8] as in the case of *Streptomyces coelicolor* and *Streptomyces griseus (S. griseus)*[9]. There is a lack of published information with regard to the use of *Streptomyces*, as biocontrol agents for mosquito larvae.

Mosquito vectors are solely responsible for transmitting diseases, such as malaria, dengue, Chikungunya, Japanese encephalitis, yellow fever, and lymphatic filariasis[10]. Malaria is an important cause of death and illness in children and adults, especially in tropical countries. Malaria is caused by a parasite that is transmitted from one human to another by the bite of infected *Anopheles stephensi (An. stephensi)*. Half of the world's population is at risk from malaria. Each year almost 250 million cases occur, causing 860 000 deaths[11]. Approximately 3.5 billion people live in dengue endemic countries which are located in the tropical and subtropical regions of the world[12]. Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. The infection occurs when filarial parasites are transmitted to humans through *Culex quinquefasciatus (Cx. quinquefasciatus)*. More than 1.3 billion people in eighty one countries worldwide are threatened by lymphatic

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filariasis[13].

In addition, the mosquito control strategy emphasizes the need for selective and sustainable preventive measures for reducing disease transmission. The options available for present day vector control efforts mainly include chemical, biological, natural plant products, and environmental management. The larval control of mosquitoes is a proven preventive method that has become renewed consideration for mosquito control programs in the next generation tools. Accordingly, in the present study we apply the most promising chitinase producing actinomycetes as bio-control agents *Streptomyces citreofluorescens (S. citreofluorescens)* culture filtrates against larvae of *An. stephensi*, *Cx. quinquefasciatus*, and *Aedes aegypti (Ae. aegypti)* under laboratory conditions.

2. Materials and methods

2.1. Collection and culture of S. citreofluorescens

The strain of *S. citreofluorescens* was obtained from Microbial Type Culture Collection and Gene Bank (MTCC-2528) Institute of Microbial Technology, Chandigarh India. S. citreofluorescens were maintained on autoclaved Potato Dextrose Broth media (infusion of potatoes 200.0g, dextrose 20 g, deionized water 1 000 mL and adjust pH to 7.2 with KOH).

2.2. Mosquitoes rearing

The larvae of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were maintained in the laboratory at a temperature of (25±2) ℃, (70±5)% relative humidity and LD 14:10 h. The different larval instars were maintained in separate enamel containers (25 cm length×15 cm width×5 cm depth), at a density of 200 larvae per container. All larvae were fed 0.4 mL/container of a 5% (w/v) autoclaved suspension of freeze-dried yeast in distilled water on day 1 and day 2. Larvae were reared in double-distilled sterile water at pH 7.0. To counteract evaporation, water was added daily.

2.3. Bioassays

The larvae were collected from a mosquito colony maintained at insectaria at Department of Zoology, Dayalbagh Educational Institute. The bioassays were performed as per the standard procedures recommended by World Health Organization with some modifications[14]. The first, second, third and fourth instars larvae of *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti* have been tested. These experiments were carried out in triplicate using 20 larvae for each replicate assay. The replicates were run simultaneously yielding a total of 60 larvae for each dosage. The larvae were placed in 50 mL disposable plastic cups containing 15 mL of test solution and fed on larval food during all testing. Mortality and survival was established after 24 h of exposure. The number of the dead larvae in the three replicates was expressed as the percentage mortality for each concentration. The negative control was 1% MERCK deionized water while the positive control was the Chitinase of *S. griseus* C6137 purchased form Sigma-Aldrich.

After determining the mortality of larvae in this wide range of concentrations, a narrower range (of 4-5 concentrations, yielding between 10% and 90% mortality in 24h) were used to determine LC_{50} and LC_{90} values. Batches of 20 first, second, third and fourth instar larvae were transferred by means of strainers, droppers to Schott Duran beakers, each containing 200 mL of triple deionized water. Unhealthy or damaged larvae or pupae were removed and replaced. The depth of the water in the cups or vessels should remain between 5 cm and 10 cm, deeper levels may cause undue mortality.

2.4. Statistical analysis

Data from all replicates have been pooled for probit analysis[15]. The LC_{50} and LC_{90} values were calculated from a log dosage probit mortality regression line using computer software programs IBM SPSS 19.0. The bioassays have been repeated at least three times, using new solutions and different batches of larvae each time. Standard deviation or confidence intervals of the means of LC_{50} values were calculated and recorded.

3. Results

The first instar of *An. stephensi* LC_{50} and LC_{90} values of 46.80 μL/mL and 79.50 μL/mL were recorded respectively. The LC_{50} and LC_{90} values of 79.00 μL/mL and 95.60 μL/mL were recorded against second instar. In case of third instar the LC_{50} and LC_{90} values 79.00 μL/mL and 136.9 0 μL/mL were found. While the fourth instar the LC_{50} and LC_{90} values 122.60 μL/mL and 174.50 μL/mL were fond significantly effective (Figure 1). Similarly, the culture filtrates of *S. citreofluorescens* were also found significantly effective against *Cx. quinquefasciatus*. The LC_{50} and LC_{90} values of 40.00 μL/mL and 138.03 μL/mL against first instar, 80.00 μL/mL and 181.97 μL/mL against second instar, 100.00 and 309.20 μL/mL against third instar and 60.00 and 169.82 μL/mL values were calculated against fourth instars of *Cx. quinquefasciatus* (Figure 2). Moreover, these culture filtrates were also evaluated against larvae of *Ae. aegypti*. The LC_{50} and LC_{90} values of 80.00 μL/mL and 181.97 μL/mL against first instar, 100.00 μL/mL and 213.79 μL/mL against second instar, 120.00 μL/mL and 371.43 μL/mL against third instar, and 122.60 μL/mL and 174.50 μL/mL against fourth instar of *Ae. aegypti* (Figure 3, Table 1).

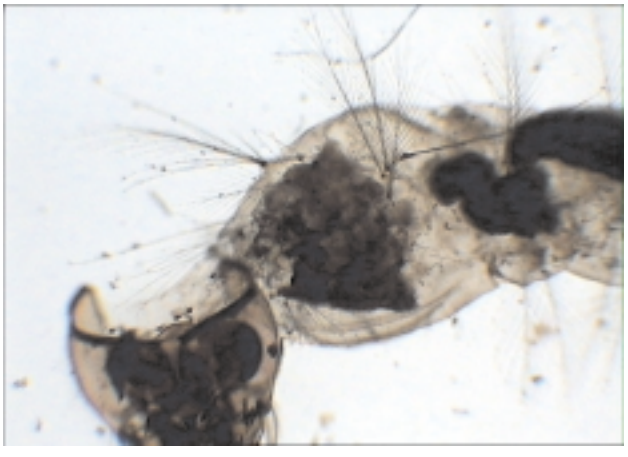

Figure 1. Infected of larvae of *An. stephensi* with the culture filtrates of *S. citreofluorescens* after exposure of 24 hours.

Table 1
Bioassays of *S. citreofluorescens* culture filtrates against *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti* after exposure of 24 hours.

Instars	*An. stephensi*		*Cx. quinquefasciatus*		*Ae. aegypti*	
	LC_{50}	LC_{90}	LC_{50}	LC_{90}	LC_{50}	LC_{90}
First instars	46.80 (38.90–73.10)	79.50 (63.90–116.20)	40.00 (38.86–41.14)	138.03 (136.83–139.23)	80.00 (78.83–81.17)	181.97 (180.72–183.22)
Second instars	79.00 (70.60–89.50)	95.60 (85.40–118.30)	80.00 (78.83–81.17)	181.97 (180.72–183.22)	100.00 (98.80–101.20)	213.79 (212.51–215.05)
Third instars	79.00 (70.60–89.40)	136.90 (105.70–186.30)	100.00 (98.80–101.20)	309.00 (307.64–310.40)	120.00 (118.77–121.23)	371.43 (370.09–372.97)
Fourth instars	122.60 (121.80–124.10)	174.50 (156.20–211.50)	60.00 (58.86–61.14)	169.82 (168.59–171.05)	122.60 (121.80–124.10)	174.50 (156.20–211.50)

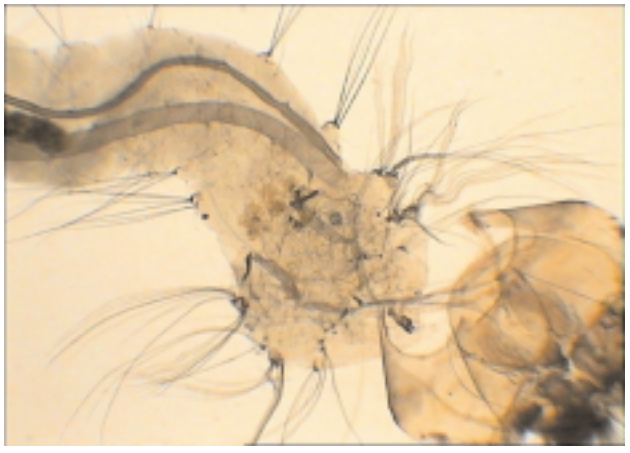

Figure 2. Infected of larvae of *Cx. quinquefasciatus* with the culture filtrates of *S. citreofluorescens* after exposure of 24 hours.

Figure 3. Highly degraded cuticle of larvae of *Ae. aegypti* with the culture filtrates of *S. citreofluorescens* after exposure of 24 hours.

4. Discussion

Several varieties of microorganism like, fungi, bacteria, nematodes and viruses have been reported to biologically control of insect. With this aspect actinomycetes belonging play an important role in the biological control of insects by producing insecticidal active compound[16].

The largest chitinases activity among bacteria has been observed in species of *Streptomyces*, *Serratia*, *Vibrio* and *Bacillus*[6]. Chitinase is originally an enzyme used by insects to degrade the structural polysaccharide "chitin" during the molting process[5]. Chitinase enzyme is very important in the biological control of insects[6]. In the present study the culture filtrates of chitinolytic *S. citreofluorescens* were found significantly effective against mosquito larvae of *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti*. The percent mortalities from selected concentration can be proven that *S. citreofluorescens* secretes chitinase. A macrotetrolide antibiotic recovered from the acetone extract of Staphylococcus aureus exhibited significancant insecticidal activity against *Collosofruchus chinesis*[17]. Bacterial chitinolytic enzymes have been used to enhance the activity of microbial insecticide like *Bacillus thurigiensis*[18]. Similarly the culture filtrates of *S. citreofluorescens* were found effective against *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti* with significant concentrations LC_{50} and LC_{90} against first, second, third, and fourth instar. The process of cuticular chitin deposition is coordinated with the ecdysteroid regulated molting (ecdysis) during insect metamorphosis. Major protein subunits of the chitin-synthase were proven to be integral membrane proteins on the epidermal cell layer underlying the procuticle region of the integument in insects[19]. The cuticle of mosquito larvae consist mainly chitin. It shall be postulate that chitinase present in *S. citreofluorescens* could be involved in larvae control. Therefore, the production of chitinases was used as the criteria for the selection of potential biocontrol agents of *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti* larvae. Microbial chitinolytic enzymes have been considered important in the biological control of many insects because of their ability to interfere with chitin deposition[20-29].

In present investigation the culture filtrates of *S. citreofluorescens* have performed acute potency against selected mosquito larvae after exposure of 24 hours. The effect of *Chrysosporium keratinophilum* and *Fusarium oxysporum* culture filtrates against *An. stephensi* and *Cx. quinquefasciatus* have been significantly increased when applied after chromatographic purification[30,31]. In the present investigation, the culture filtrates of *S. citreofluorescens* found highly pathogenic against *An. stephensi*, *Cx. quinquefasciatus*, and *Ae. aegypti*. The purification of culture filtrates can promote to biotechnological exploitation. Repeated use of insecticides can increase resistance. Whereas, the fungal culture filtrates have significant potential as a new strategy for mosquito control[32-34]. The culture filtrates show significant mortality after exposure for 24 h. The metabolites of *S. citreofluorescens* could be used for larvae control. This new approach can be a significant alternative to control vector borne diseases caused by mosquitoes in many countries especially in tropical and sub-tropical countries.

Conflict of interest statement

We do not have conflict of interest.

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