PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.3748

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Combating tobacco caterpillar, *Spodoptera litura* (Fab.) through isolated strains of *Streptomyces* from the soils of Kerala and Tamil Nadu

Elanchezhyan K[1*], Aswathy J[1*], Allwin L[1], Rajinimala N[2], Anandhi P[3] & Abdul Razak T[1]

[1]Department of Agricultural Entomology, VOC Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam, Vallanadu 628 252, Tuticorin, Tamil Nadu, India

[2]Department of Plant Pathology, VOC Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam, India

[3]Tamil Nadu Rice Research Institute, Tamil Nadu Agricultural University, Aduthurai, India

*Email: drchezhiyanphd@gmail.com, aswathyj2016@gmail.com

OPEN ACCESS

ARTICLE HISTORY
Received: 09 July 2024
Accepted: 03 November 2024
Available online
Version 1.0 : 21 December 2024

Check for updates

Additional information
Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE
Elanchezhyan K, Aswathy J, Allwin L, Anandhi A, Rajinimala N, Abdul RT. Combating tobacco caterpillar, *Spodoptera litura* (Fab.) through isolated strains of *Streptomyces* from the soils of Kerala and Tamil Nadu. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.3748

Abstract

The tobacco caterpillar, *Spodoptera litura* (Fab.) is a polyphagous pest on agricultural and horticultural crops. Biological control is an effective and sustainable alternative to insecticides for insect pest management. Now-a-days, microbial compounds are used for the management of insect pests and they are the good alternative to inorganic pesticides. The various microbial control agents are obtained from the different microorganisms, actinomycetes specifically the filamentous *Streptomyces* produce metabolites possessing insecticidal activity. The *Streptomyces* is a Gram-positive multicellular bacteria and they possess numerous secondary active metabolites. The soil samples were collected from different locations in Kerala and Tamil Nadu states, India. Isolation of *Streptomyces* was carried out by serial dilution of 10^{-4} and pour plating method. The *Streptomyces* can be cultured in International Streptomyces Project 2 fermentation broth. The *Streptomyces* sp. such as *S. katrae* (ST 1), *S. acidiscabies* (ST 3), *S. andamanensis* (ST 5) and *S. cerasinus* (ST 7) were isolated from the soils of Kerala and Tamil Nadu, India by 16S rDNA sequencing and matched with *Streptomyces* sp. using NCBI BLAST program and were screened against 1st and 2nd instar larvae of *S. litura.* The strain *S. katrae* (ST 1) and a consortium of all the strains (ST 1 + ST 3 + ST 5 + ST 7) exhibited 66 -70% mortality of 1st instar larvae and 70-74% mortality of 2nd instar larvae of *S. litura*. The presence of secondary active metabolites in the ISP 2 fermentation broth exhibited strong larvicidal activities and these findings indicate that the ISP 2 fermentation broth of *S. katrae* and the consortium possess the ability to control the pest populations at the desirable level.

Keywords

consortium; efficacy; *Spodoptera litura*; *Streptomyces strains*

Introduction

The tobacco caterpillar, *Spodoptera litura* (Fab.) was considered the most devouring polyphagous pest of both field and horticultural crops including tobacco, tomato, castor, cotton, okra, chilli, chickpea, cowpea, soybean, etc. (1, 2). The larvae of *S. litura* damage ranges from 80 to 100% (3, 4) and the crop damage varies from 10-25% in the field (5, 6). The small-scale farmers followed the conventional practices such as hand-picking of larvae, indiscriminate use of insecticides, etc. (7).

Microorganisms are abundant in the ecosystem and present in diverse forms than the organisms belonging to the higher order such as plants, animals, etc. They possess a variety of metabolite compounds and are useful in medicine, industries, agriculture, etc. *Streptomyces* is a gram-positive, filamentous, multicellular bacteria which comes under the category of genus Actinomycetes. They possess both fungal

and bacterial characters (8, 9). The *Streptomyces* produce antibiotics of about 60% for agricultural use. The *Streptomyces* can be cultured in fermentation broth; the purification and formulation of insecticidal secondary metabolites were effectively used against many insect pests. The active metabolites of *Streptomyces* play a vital role in the management of *S. litura* (10) and are eco-friendly in nature (11). The present study was aimed to evaluate the efficacy of *Streptomyces* strains against *S. litura*.

Materials and Methods

Mass culturing of Spodoptera litura (Fabricius)

The 2nd instar larvae of *S. litura* were collected from a castor field at Agricultural College and Research Institute, Killikulam and were maintained on the semi-synthetic diet developed by Tamil Nadu Agricultural University, Coimbatore. The 1st and 2nd instar larvae were used for testing the efficacy of *Streptomyces* strains (12).

Diet impregnation assay

Lablab-based artificial diet was utilized for carrying out the bioassay against the 1st and 2nd instar of *S. litura* by diet impregnation assay. A quantity of 2 mL of artificial diet was taken into insect-rearing vials and the diet was mixed with 800 µL of culture broth and dried inside a laminar airflow chamber. After drying, pre-starved individual larvae were released in each vial with an artificial diet. The bioassay was conducted for individual effective strains as well as for the consortium of effective strains. For each treatment, 3 replications with 10 larvae were maintained. The insect mortality was recorded at 12 h and 48 h intervals (13).

Leaf detached assay

The detached leaf bioassay was carried out with the help of freshly collected castor leaves. Leaves were washed thoroughly in normal water, followed by distilled water to avoid the interference of the exudates released by the plant. These leaves were dipped in 5 mL of the culture broth for 5 min and allowed to dry. After drying, the leaves were placed in the Petri plates and the 1st and 2nd instar larvae were released into the plates. Pre-starved larvae were used for the bioassay. The bioassay was conducted for individual effective strains as well as for the consortium of effective strains. The experiment was carried out with 3 replications and 10 larvae were used for each replication. Observations were recorded periodically at 12 h intervals (13).

% mortality was corrected by standard formula (14):

$$\% \text{ mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

The data on mortality by different isolates were subjected to ANOVA analysis. The % mortality was corrected by standard formula. The mean values of different treatments were separated by the least significant difference (LSD) between them (15).

Results and Discussion

The *Streptomyces katrae* (ST 1) showed a higher % of mortality (70.00%) followed by *S. acidiscabies* (ST 3) (58.66%), *S. andamanensis* (ST 5) (53.00%) and *S. cerasinus* (ST 7) (41.66%) against 1st instar larvae of *S. litura*. The standard culture showed 55% mortality against 1st instar larvae whereas, in the 2nd instar *S. katrae* (ST 1) showed 74 per cent mortality followed by *S. acidiscabies* (ST 3) (60.00%), *S. andamanensis* (ST 5) (52.33%) and *S. cerasinus* (ST 7) (30.00 %) (Table 1). The standard culture showed 51 % mortality against 2nd instar larvae of *S. litura*. The consortium of *Streptomyces* sp. *i.e. S. katrae* (ST 1) + *S. acidisacbies* (ST 3) + *S. andamanensis* (ST 5) and *S. cerasinus* (ST 7) showed 71.66% mortality of 1st instar larvae of *S. litura* (Table 2) whereas, in the 2nd instar larvae of *S. litura,* the consortium of *Streptomyces* sp. (ST + ST 3 + ST 5 + ST 7) exhibited 72% of mortality by diet impregnation method (Table 2). The

Table 1. Effect of *Streptomyces* against *S. litura* by diet impregnation assay.

Treatments	Isolates	Mortality of *S. litura* (%)	
		1st instar	2nd instar
T1	*Streptomyces katrae* (ST 1)	70.00 (56.81)^{a}	74.00 (59.39)^{a}
T2	*Streptomyces acidiscabies* (ST 3)	58.66 (50.01)^{b}	60.00 (50.77)^{b}
T3	*Streptomyces andamanensis* (ST 5)	53.00 (46.73)^{b}	52.33 (46.34)^{bc}
T4	*Streptomyces cerasinus* (ST 7)	41.66 (40.11)^{c}	30.00 (39.21)^{d}
T5	Standard culture (*S. griseus*)	55.00 (47.88)^{b}	51.00 (45.57)^{c}
T6	Control	0.00 (0.29^{d}	0.00 (0.29)^{e}
		CD (0.05) = 5.83	CD (0.05) = 4.58
		SEd = 2.67	SEd = 2.10
		CV = 8.13	CV = 6.39

Table 2. Effect of *Streptomyces* consortium against *S. litura* by diet impregnation assay.

Treatments	Isolates	Mortality of *S. litura* (%)	
		1st instar	2nd instar
T1	ST 1 + ST 3	64.33 (53.37)^{ab}	54.56 (47.62)^{bc}
T2	ST 1 + ST 5	55.00 (47.97)^{bcd}	58.11 (49.69)^{b}
T3	ST 1 + ST 7	61.67 (51.84)^{abc}	58.33 (49.83)^{b}
T4	ST 3 + ST 5	53.00 (46.79)^{bcd}	45.67 (42.50)^{c}
T5	ST 3 + ST 7	47.67 (43.66)^{cd}	51.00 (45.58)^{bc}
T6	ST 5 + ST 7	42.00 (40.39)^{d}	51.67 (45.97)^{bc}
T7	ST1 + ST 3 + ST 5 + ST 7	71.66 (54.80)^{a}	72.00 (58.06)^{a}
T8	Control	0.00 (0.286)^{e}	0.00 (0.29)^{d}
		CD (0.05) = 8.44	CD (0.05) = 6.20
		SEd = 3.98	SEd = 2.92
		CV = 11.40	CV = 8.44

polyketide metabolite from isolated *Streptomyces* strains showed antifeedant activities and larvicidal activities against *S. litura* and *Helicoverpa armigera* (10). The concentration of 1600 µg/mL with *S. hydrogenans* DH16 strain showed adverse effects as larval, pre-pupal and pupal mortality (16).

In the leaf detached assay, the isolate *S. katrae* (ST 1) showed a higher % of mortality (66.00%) followed by *S. acidiscabies* (ST 3) (60.33%), *S. cerasinus* (ST 7) (54.66%) and *S. andamanensis* (ST 5) (51.67%). The standard culture exhibited 51.67% mortality of 1st instar larvae whereas, in the 2nd instar, the strain *S. katrae* (ST 1) showed 70.00% mortality. The isolates *S. cerasinus* (ST 7) (57.67%), *S. andamanensis* (55.00%) (ST 5) and *S. acidiscabies* (ST 3) (45.00%) showed mortality (Table 3). There was no mortality observed in the control. The consortium of all the *Streptomyces* sp. (ST + ST 3 + ST5 + ST 7) recorded 71.66% mortality of 1st instar larvae of *S. litura* (Table 4). The consortium of all *Streptomyces* sp. produced 72% mortality of 2nd instar larvae of *S. litura* (Table 4).

The strain *Streptomyces* spp. ERI-04 was identified and the obtained strain exhibited strong antifeedant and antifungal activities against *S. litura* and *H. armigera* (17). Three isolates of *S. bacillaris* (Krasilnikov) (CAI-155), *S. albolongus* (Berdy) (BCA-698) and *S. griseoplanus* (Waksman) (SAI-25) showed the entomopathogenic effects against the early instar larvae of *S. litura*, *H. armigera* and *Chilo partellus* Swinhoe with the mortality of 92 % for Extracellular Metabolites (ECM), 77% for Intracellular metabolites (ICM) and 60% for Whole culture (WC) (13). The strain *S. griseoplanus* (Krainsky) SAI-25 showed activities such as 70% antifeedant, 67% larvicidal and 59 % pupicidal mortality against *H. armigera* (18).

The results of diet impregnation assay and detached leaf bioassay showed morphological abnormalities such as blackened dead larvae, malformed pre-pupae and malformed pupae at the concentration of 800 µL/mL due in the presence of active metabolites in the *Streptomyces* fermentation broth. The different concentrations of *S. hydrogenans* DH 16 exhibited abnormalities such as dead larvae, malformed pre-pupae and pupae in different stages of *S. litura* (16). The *Streptomyces*-treated larvae exhibited deformities in the head, body, pupal case and wings and were also smaller in size (19). At the concentration of 50 ppm, the *S. avermitilis* extract showed morphological changes in treated larvae of *S. litura* (20).

Conclusion

In the present study, *S. katrae* (ST 1) ISP 2 broth showed adverse effects on the life stages of *S. litura* larvae at the concentration of 800 µL/mL where the larval mortality was about 70-74%. The isolates *S. andananensis* (ST3), *S. acidiscabies* (ST 5) and *S. cerasinus* (ST 7) exhibited 30-60% mortality in both diet impregnation and leaf detached assay. There was no larval mortality noticed in the control. The maximum mortality % was observed on the 3rd day of bioassay. As a result of diet impregnation and detached leaf assay, morphological abnormalities such as blackened dead larvae (Fig. 1), malformed pre pupae and malformed pupae were observed in the treated larvae at the concentration of 800 µL/mL. The antifeedant activities of larvae were also observed in the leaves treated with *Streptomyces* sp. The entomopathogenic activity showing *Streptomyces* sp. was confirmed by streaking the haemolymph of the dead cadaver of infected *S. litura* larvae on the ISP 2 agar plate. The results showed the presence of *S. katrae* (ST 1) by small dotted colonies in the agar plates (Fig. 2). The active metabolites in the ISP 2 broth from *S. katrae, S. acidiscabies, S. andamanensis* and *S. cerasinus* were toxic to the larvae, pupae and pre-pupae at the concentration of 800 µL. Therefore, the metabolites showed that they have the potential in pest management to develop a new formulation.

Table 3. Effect of *Streptomyces* against *S. litura* by leaf detached assay.

Treatments	Isolates	Mortality of *S. litura* (%)	
		1st instar	2nd instar
T1	*Streptomyces katrae* (ST 1)	66.00 (54.39)^{a}	70.00 (56.79)^{a}
T2	*Streptomyces acidiscabies* (ST 3)	60.33 (50.97)^{ab}	45.00 (42.04)^{b}
T3	*Streptomyces andamanensis* (ST 5)	51.67 (45.96)^{c}	55.00 (47.91)^{b}
T4	*Streptomyces cerasinus* (ST 7)	54.66 (47.69)^{bc}	57.67 (49.47)^{ab}
T5	Standard culture (*S. griseus*)	51.67 (45.96)^{c}	45.67 (42.50)^{b}
T6	Control	0.00 (0.29)^{d}	0.00 (0.29)^{c}
		CD (0.05) = 4.38	CD (0.05) = 8.77
		SEd = 2.01	SEd = 4.02
		CV = 6.03	CV = 12.38

Table 4. Effect of *Streptomyces* consortium against *S. litura* by diet impregnation assay.

Treatments	Isolates	Mortality of *S. litura* (%)	
		1st instar	2nd instar
T1	ST 1 + ST 3	64.33 (53.37)^{ab}	54.56 (47.62)^{bc}
T2	ST 1 + ST 5	55.00 (47.97)^{bcd}	58.11 (49.69)^{b}
T3	ST 1 + ST 7	61.67 (51.84)^{abc}	58.33 (49.83)^{b}
T4	ST 3 + ST 5	53.00 (46.79)^{bcd}	45.67 (42.50)^{c}
T5	ST 3 + ST 7	47.67 (43.66)^{cd}	51.00 (45.58)^{bc}
T6	ST 5 + ST 7	42.00 (40.39)^{d}	51.67 (45.97)^{bc}
T7	ST1 + ST 3 + ST 5 + ST 7	71.66 (54.80)^{a}	72.00 (58.06)^{a}
T8	Control	0.00 (0.286)^{e}	0.00 (0.29)^{d}
		CD (0.05) = 8.44	CD (0.05) = 6.20
		SEd = 3.98	SEd = 2.92
		CV = 11.40	CV = 8.44

Fig. 1. Blackened dead larvae of *S. litura* caused by *S . katrae.*

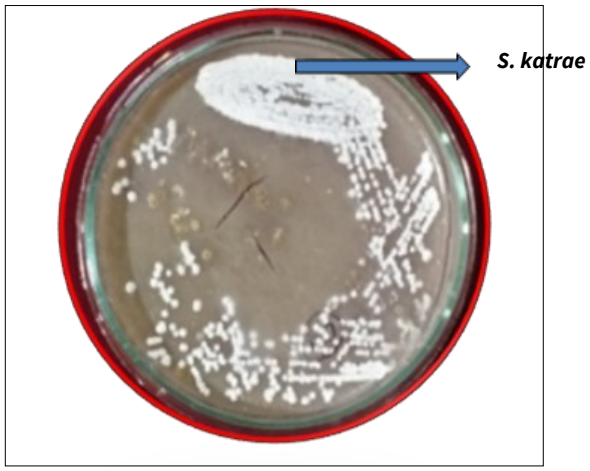

Fig. 2. *S. katrae* confirmation from larval haemolymph by streaking method.

Acknowledgements

We would like to thank Dr. V. S. Saravanan, Assistant Professor (Microbiology), Indira Gandhi College of Arts and Science, Pondicherry for his academic help in gene sequencing and molecular work.

Authors' contributions

KE and JA both contributed equally to this manuscript. TAR gave valuable suggestions and their reviews have helped in improving the contents of this manuscript. The thesis title was rooted in LA and his ideas, comments aided in the completion of this research work. All the authors contributed critically to the draft and gave final approval for publication.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests.

Ethical issues: None

References

1. Ashok K, Pavithran S. Biology and morphometrics of *Spodoptera litura* (Fab.) on castor. 2022;456-58. https://doi.org/10.55446/IJE.2021.261
2. Armes NJ, Wightman JA, Jadhav DR, Ranga Rao GV. Status of insecticide resistance in *Spodoptera litura* in Andhra Pradesh, India. Pesticide Science. 1997;50(3):240-48. https://doi.org/10.1002/(SICI)10969063(199707)50:3%3C240::AID
3. Yadav D, Kamte A, Jadhav R. Bio-efficacy of cyantraniliprole, a new molecule against *Scelodonta strigicollis* Motschulsky and *Spodoptera litura* Fabricius in grapes. Pest Management in Horticultural Ecosystems. 2012;18(2):128-34. https://api.semanticscholar.org/CorpusID:76651763
4. Chari M, Bharpoda T, Patel A. Bioefficacy of fluvalinate against *Spodoptera litura* in tobacco nursery. Pestology. 1986;10(1):21-24. https://www.researchgate.net/publication/328419688
5. Patil, Ranjeet A, Deepak M, Mehta, Babu Lal Jat. Studies on life fecundity tables of *Spodoptera litura* Fabricius on tobacco *Nicotiana tabacum* Linnaeus. Entomol Ornithol Herpetol. 2014;3:1000118. https://doi.org/10.4172/2161-0983.1000118
6. Latha M, Shivanna B, Manjunatha M, Kumaraswamy M. Biology of *Spodoptera litura* on chewing tobacco *in vitro*. Journal of Eco-friendly Agriculture. 2014;9(1):43-47. https://doi.org/10.14719/pst.3078
7. Sharma HC. Climate change effects on insects: implications for crop protection and food security. Journal of Crop Improvement. 2014;28 (2):229-59. https://www.researchgate.net/publication/263611092
8. Kutovaya OA, Watson SB. Development and application of a molecular assay to detect and monitor geosmin-producing cyanobacteria and actinomycetes in the Great Lakes. Journal of Great Lakes Research. 2014;40(2):404-14. https://www.researchgate.net/publication/261998971
9. Hwang BK, Ahn SJ, Moon SS. Production, purification and antifungal activity of the antibiotic nucleoside, tubercidin produced by *Streptomyces violaceoniger*. Canadian Journal of Botany. 1994;72 (4):480-85. https://www.researchgate.net/publication/237165270
10. Arasu MV, Al-Dhabi NA, Saritha V, Duraipandiyan V, Muthukumar C, Kim SJ. Antifeedant, larvicidal and growth inhibitory bioactivities of novel polyketide metabolite isolated from *Streptomyces* sp. AP-123 against *Helicoverpa armigera* and *Spodoptera litura*. BMC Microbiology. 2013;131:6. https://www.researchgate.net/publication/236738655
11. Montesinos E. Development, registration and commercialization of microbial pesticides for plant protection. International Microbiology. 2003;6:245-52. https://www.researchgate.net/publication/10582115
12. Saljoqi AUR, Khan J, Ali G. Rearing of *Spodoptera litura* (Fabricius) on different artificial diets and its parasitization with *Trichogramma chilonis* (Ishii). Pakistan Journal of Zoology. 2015;47(1). https://cabidigitallibrary.orgby2409:4073:310:c362:38b9:7118:c993:8ee5
13. Vijayabharathi R, Kumari BR, Sathya A, Srinivas V, Abhishek R, Sharma HC, Gopalakrishnan S. Biological activity of entomopathogenic actinomycetes against lepidopteran insects (Noctuidae: Lepidoptera). Canadian Journal of Plant Science. 2014;94(4):759-69. https://doi.org/10.4141/cjps2013-298
14. Abbott WS. A method of computing the effectiveness of an insecticide. The Journal of Economic Entomology. 1925;18(2):265-67. https://pubmed.ncbi.nlm.nih.gov/3333059/
15. Gomez KA, Gomez AA. Statistical procedures for agricultural research: John Wiley and Sons. 1984.
16. Kaur T, Manhas RK. Antifungal, insecticidal and plant growth promoting potential of *Streptomyces hydrogenans* DH16. Journal of Basic Microbiology. 2014;54(11):1175-85. https://www.researchgate.net/publication/237822910
17. Valanarasu M, Kannan P, Ezhilvendan S, Ganesan G, Ignacimuthu S, Agastian P. Antifungal and antifeedant activities of extracellular product of *Streptomyces* spp. ERI-04 isolated from Western Ghats of

Tamil Nadu. Journal de Mycologie Medicale. 2010;20(4):290-97. https://www.researchgate.net/publication/260291397

18. Sathya A, Vijayabharathi R, Kumari BR, Srinivas V, Sharma HC, Sathyadevi P, Gopalakrishnan S. Assessment of a diketopiperazine, cyclo (Trp-Phe) from *Streptomyces griseoplanus* SAI-25 against cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). Applied Entomology and Zoology. 2016;51:11-20.

19. Dhar A. Plant protection practices by using botanicals for sustainable agriculture. Plant Archives. 2020;20(2):3741-46. https://doi.org/10.4081/ija.2021.1851

20. Prakash VA, Sermalatha G, Selvarathinam T. Extraction of bioactive compounds from *Streptomyces avermitilis* and *Azadirachta indica* and evaluation against *Spodoptera litura*: A green approach. Journal of Entomology and Zoology Studies. 2022;10(1):143-52. https://www.researchgate.net/publication/358118270