AATCC Review

22 November 2022: Received
10 March 2023: Revised
06 May 2023: Accepted
28 May 2023: Available Online

www.aatcc.peerjournals.net

Research Article Open Access

Isolation and Characterization of Streptomyces from Soil against the Tobacco Caterpillar, *Spodoptera litura* (Fab.)

J. Aswathy[1]*, K. Elanchezhyan[1], L. Allwin[2], T. Abdul Razak[1], M. Gomathy[1] and M. Joseph[1]

[1]Agricultural College and Research Institute, Tamil Nadu Agricultural University, Killikulam, Tuticorin, Tamil Nadu, India
[2]Rice Research Station, Tamil Nadu Agricultural University, Ambasamudram, Tirunelveli, Tamil Nadu, India

ABSTRACT

The tobacco caterpillar, Spodoptera litura (Fab.) was considered the major pest of many crops such as tobacco, cotton, tomato, castor, cowpea, sesbania, etc. A total of 15 indigenous Streptomyces spp. strains were isolated from soil samples of the Western Ghats, Tamil Nadu, India. Isolation of Streptomyces strains was carried out by serial dilution method and plating technique. Purification of strain was done by streaking method on International Streptomyces Project 2 (ISP 2), ISP 4, and starch casein nitrate agar (SCNA) medium. The main challenges in the study was the isolation and purification of Streptomyces strains were difficult with the contamination of cultures. They were overcome by the addition of antibiotics such as Cyclohexamide and Streptomycin sulphate into the media. The morphological and biochemical characterization was performed for identifying the efficient strain. Diet impregnation bioassay was also carried out against the 2nd instar larvae of S. litura. Based on the results of morphological, biochemical, and diet impregnation bioassay, Strain 1 (ST1) was considered as the efficient strain. The ST 1 was molecularly identified by 16S rDNA sequencing and compared with Streptomyces species using NCBI BLAST program. Among the 15 isolated Streptomyces strains, ST 1 (Streptomyces katrae) showed a higher percentage of mortality (73.33 %) of S. litura. ST 1 showed the most efficient entomopathogenic activity against S. litura among the 15 isolates of Streptomyces. The metabolites present in the fermentation broth showed strong larvicidal, pupicidal and toxic effects against the notorious pest S. litura. These findings denote that the fermentation broth of S. katrae strain has the ability to control the S. litura pest populations at a considerable level.

Keywords: *Streptomyces, ST 1 (S. katrae), isolation, purification, fermentation broth, characterization, bioassay, entomopathogenic activity, Spodoptera litura*

INTRODUCTION

The tobacco caterpillar, Spodoptera litura (Fab.) is a notorious and polyphagous pest feeding on several hundreds of host plants around the world wide [1]. The major host plants include Cotton, Castor, Chinese Cabbage, Cowpea, Tomato, Tobacco, Sesbania etc. [2]. Initially, larvae feed gregariously on young leaves of the plant and in later stages, defoliation of plants occurs [3]. S. litura might cause an economic loss ranging from 26-100% [4]. Streptomyces species comes under the category of Actinomycetes and were the best example for the production of antibiotics [5]. The actinomycetes were related to both fungi and bacteria [6]. They are gram-positive, filamentous, aerobic, multicellular, and prokaryotic organisms [7]. [8] reported that Streptomyces from unexplored environmental sources such as deep-sea, desert and volcanic environments have proven to be a source of habitat for Streptomyces with a unique production of a new class of biochemical compounds [9].

Newly discovered pesticides have an extra-ordinary mode of action and attack specific pests but they are harmful to the environment [10]. The management of S. litura becomes a difficult task in many countries; actinomycetes are a good alternative for the management of insect pests and diseases [11]. They are the alternative to chemical pesticides and therefore increasing agricultural production and productivity [12]. Members of Actinobacteria exhibit different physiological, metabolic, and morphological characteristics such as coccoid and branched mycelium with hyphae [13]. The actinomycetes isolated from soil emit some earthy odor due to a compound called 'geosmin' [14]. Bioassay of Streptomyces was performed with the second instar larvae of S. litura [15]. The secondary metabolites of Streptomyces spp. in the culture broth were highly toxic to the S. litura larvae at higher concentrations [16].

The present study was aimed at the further evaluation of isolated Streptomyces strains by various methods of characterization and screening of isolated strains against target organisms such as S. litura.

*Corresponding Author: J. Aswathy
Email Address: aswathyj2016@gmail.com

DOI: https://doi.org/10.58321/AATCCReview.2023.11.03.183

MATERIALS AND METHODS

Isolation, purification and maintenance of the organism

A total of 15 indigenous Streptomyces spp. strains were isolated from soil samples of the Western Ghats, Tamil Nadu, India. Isolation of Streptomyces strains was carried out by serial dilution and plating techniques. For the serial dilution, 1g of soil was added to 9 ml of sterile distilled water. They were mixed vigorously and allowed to stand. Serial dilutions were made up to 10-4 using sterile distilled water with a total volume of 10 ml. An aliquot was plated on a starch casein nitrate agar (SCNA) medium [17]. The plates were allowed for incubation at about 28-30°C for 7 days [18]. After incubation, typically pigmented dry powdery colonies were selected from a mixed culture plate. They were sub-cultured on SCNA, International Streptomyces Project 2 (ISP 2), and ISP 4 medium (Fig.1) Isolated Streptomyces strains were stored at 4°C for further use [19]. A total of 0.5 ml of different isolated strains was taken in sterile labeled Eppendorf® tubes separately. Then 20 per-cent of glycerol was added to each tube and mixed thoroughly by vortexing and stored at -20°C [20].

Characterization of the Streptomyces strains

The following experiments were done to authenticate the isolated Streptomyces strains.

Phenotypic characterization

Gram staining and visual observation of colony morphology such as color, size, shape, texture, elevation, and margin were used to determine the morphological characterization. Some special media such as ISP 2 and ISP 4 were used to determine the growth responses of the isolated strains.

Biochemical characterization

Biochemical tests such as Indole, Methyl Red, Voges, Proskauer, and Citrate (IMViC) test; sodium chloride tolerance test; test for gelatin hydrolysis; test for starch hydrolysis; cellulose degradation test; hydrogen sulfide production test; coagulation of milk test; ability to grow in different pH. The assimilation of different carbon sources, lipolytic activity, protease activity, and chitinolytic activity were also determined.

Molecular characterization

Pure cultures of the isolated Streptomyces strains were grown in ISP 2 and ISP 4 medium. Genomic DNA was isolated as per the protocols of [21]. The amplification of 16S rDNA gene was performed with the primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-GWATTACCGCGGCKGCTG -3'). The 16S rRNA sequence was compared with available sequence data in the GenBank at the National Center of Biotechnology Information (NCBI) using the BLAST program [22].

Screening of Streptomyces against S. litura

The 15 isolated Streptomyces strains were cultured on ISP 2, ISP 4, and starch casein agar plates for seven days at 28°C. After seven days of incubation, dry powdered colonies were developed on the agar plates. Then a loopful of culture was taken and transferred to the ISP 2 broth, allowing them for incubation. All 15 isolates were screened for insecticidal activity against 2nd instar of S. litura larvae using the food utilization assay.

Insect rearing and maintenance

The S. litura larvae were reared by following the standard protocols proposed by [23]. The 2nd instar larvae of S. litura were collected from the castor field at Agricultural College and Research Institute, Killikulam, Tuticorin district, Tamil Nadu, India and they were maintained on the
semi-synthetic diet at laboratory temperature (27-30°C) with relative humidity (60-70%). The artificial diet for S. litura was prepared lablab bean-based diet [24]. The semi-synthetic diet was poured into a sterilized container and kept at room temperature for solidification. The artificial diet for larvae was stored at the refrigerator for further use.

Bioassay

Preliminary bioassays were carried out to screen the efficacy of the virulent strains of isolated Streptomyces against 2nd instar of S. litura

Diet Impregnation Assay

Lablab bean-based semi-synthetic diet was used for carrying out the bioassay by diet impregnation assay. A quantity of 2 ml of artificial diet was taken in micro-centrifuge tubes and pressed inside the vials. The diet was mixed with 0.8 ml of culture broth and dried it inside a laminar air-flow chamber. After drying, individual larvae were released per vial. Larvae were pre-starved for 6 hours. For each treatment, three replications were maintained and for each replication, ten larvae were maintained. The insect mortality was recorded at 12 hours intervals [25].

Percent mortality in the above treatments was corrected by Abbott's (1925) formula [26]

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

Statistical analysis

To analyze the toxicity of different isolated Streptomyces strains against S.litura, Completely Randomized Block Design (CRBD) was followed and the mortality data were analyzed using the analysis of variance (ANOVA) software provided by Wasp 2.0. Per-cent, mortality in the treatments was corrected by using Abbott's (1925) formula. The mean values of different treatments were separated by the least significant difference (LSD) between them [27].

Results and Discussion

A total of 30 soil samples were collected from different locations in Western Ghats, Tamil Nadu, India. Fifteen strains were isolated by following the serial dilution and plating technique. The isolated strains were as named Strain 1 (ST 1), ST 2, ST 3, ST 4, ST 5, ST 6, ST 7, ST 8, ST 9, ST 10, ST 11, ST 12, ST 13, ST 14 and ST 15.

Fig.1. Isolated strains of Streptomyces species

The phenotypic characterization of strains was studied by the Gram staining method. All isolated strains were Gram-positive (Fig.2) and they possess filamentous, branched mycelia. [28] observed that fourteen isolates were found positive after Gram staining in their experiment. All the isolates colonies showed colony color as white, pink, brownish white, or greyish pink; colony size as large, medium, or small; colony shape as irregular; colony margin as entire, erose, undulate, round, or lobate; colony elevation as flat, raised, crateriform or pulvinate; colony texture as rugose or smooth (Table.1)

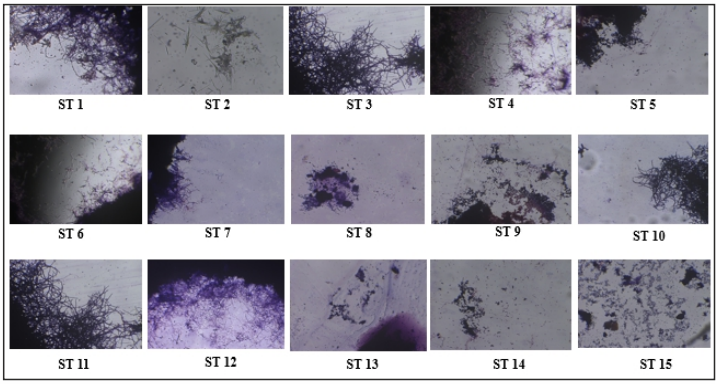

Fig.2. Gram Staining of isolated strains (Gram positive)

Table 1. Morphological characterization of fifteen isolated strains

S.No	Isolates	Colony Colour	Colony Size	Colony Shape	Colony margin	Colony Elevation	Colony Texture
1.	ST 1	White	Moderate	Irregular	Entire	Flat	Smooth
2.	ST 2	White	Large	Irregular	Undulate	Raised	Smooth
3.	ST 3	White	Moderate	Irregular	Erose	Crateriform	Rugose
4.	ST 4	White	Small	Irregular	Undulate	Raised	Smooth
5.	ST 5	Brownish white	Moderate	Irregular	Erose	Flat	Sooth
6.	ST 6	Pink	Moderate	Irregular	Round	Flat	Smooth
7.	ST 7	Brownish white	Small	Irregular	Lobate	Pulvinate	Smooth
8.	ST 8	White	Moderate	Irregular	Round	Flat	Smooth
9.	ST 9	Pink	Moderate	Irregular	Round	Raised	Smooth
10.	ST 10	White	Moderate	Irregular	Lobate	Raised	Smooth
11.	ST 11	Greyish pink	Small	Irregular	Round	Flat	Smooth
12.	ST 12	White	Moderate	Irregular	Erose	Raised	Smooth
13.	ST 13	White	Moderate	Irregular	Round	Flat	Smooth
14.	ST 14	White	Moderate	Irregular	Erose	Flat	Smooth
15	ST 15	White	Moderate	Irregular	Entire	Flat	Smooth

The biochemical characterization results showed that strains ST 1, ST 2, ST 5, ST 6, ST 7, ST 8, ST 9, ST 10, ST 11, ST 14 and ST 15 were positive while ST 3, ST 4, ST 12, and ST 13 were negative for Indole test. All the strains showed positive responses for the Methyl Red test except ST 9. For the Citrate Utilization test, positive results were observed for ST 1, ST 2, ST 4, ST 7, ST 8, ST 9 and ST 13, while others were negative. The strains ST 9, ST 11, and ST 15 produced hydrogen sulfide effectively but the others were not produced. [27] [28] examined that the most accurate indicator for H2S-producing streptomycetes was the blackening of lead acetate strip. The strains ST 1, ST 2, ST 7, ST 8, ST 9, ST 10, and ST 14 were tolerant to sodium chloride. Gelatin liquefaction was positive for the ST 1, ST 2, ST 3, ST 4, ST 5, ST 7, ST 11, and ST 14 strains. The strains ST 2, ST 4, ST 5, ST 6, ST 7, ST 9, ST 11, ST 12, ST 13, ST 14, and ST 15 hydrolyzed the starch while the other strains were not. The carbon sources such as dextrose, sucrose, fructose, cellulose, and mannitol were used for the carbon assimilation test and the strains ST 1, ST 2, ST 4, ST 7, ST 8, ST 11, ST 12, ST 13 were utilized the all carbon sources in an effective manner. The tests for carbon utilization tests by the organisms were performed and the carbon sources were utilized well where the glucose was selected as the positive control absence of carbohydrate in basal agar medium were taken as the negative control [28]. The degradation of cellulose were performed by the strains ST 1, ST 2, ST4, ST 5, ST 6, ST 7, ST 8, ST 10, ST 11, ST 12, ST 13, and ST 15. The strains ST 3, ST 9, and ST 14 showed a negative reaction to the lipolytic activity. The protease activity shown by the strains ST 1, ST 2, ST 4, ST 8, ST 10, ST 11, ST 13, and ST 15. In the strains ST 1, ST 4, ST 7, ST 8, and ST 9, chitinolytic activity was observed. The strains ST 2, ST 3, ST 5, ST 10, ST 11, and ST 14 showed a negative response to the coagulation of milk test. The ability to grow in different pH 5, 6, 7, 8, and 9 were also tested in all the strains where ST 1, ST 4, ST 7, ST 9, and ST 19 have the ability to grow in different pH. As comparing all the results of the biochemical test, ST 1 showed the positive result to all the tests except hydrogen sulphide production test. Therefore ST 1 were considered as the best among all the strains and were suggested for further studies. Results for biochemical characterization were represnted in (Fig.3) and (Table.2).

Table.2. Biochemical characterization of strains

S. No	Biochemical test	ST 1	ST 2	ST 3	ST 4	ST 5	ST 6	ST 7	ST 8	ST 9	ST 10	ST 11	ST 12	ST 13	ST 14	ST 15	Control
1.	Indole test	+	+	-	-	+	+	+	+	+	+	+	-	-	+	+	-
2.	Methyl Red test	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
3.	Citrate Utilization test	+	+	-	+	-	-	+	+	+	-	-	-	+	-	-	-
4.	Hydrogen Sulphide production test	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-
5.	NaCl Tolerance test	+	+	-	-	-	-	+	+	+	+	-	-	-	+	-	-
6.	Gelatin Liquefaction test	+	+	+	+	+	-	+	-	-	-	+	-	-	+	-	-
7.	Starch Hydrolysis Test	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	-
	Dextrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
	Cellulose	+	+	-	+	-	+	+	+	-	+	+	+	+	-	-	-
	Sucrose	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-	-
	Fructose	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-
	Mannitol	+	+	-	+	-	+	+	+	-	-	+	+	+	-	-	-
9.	Cellulose degradation	+	+	-	+	+	+	+	+	-	+	+	+	+	-	+	-
10	Lipolytic activity	+	+	-	+	+	+	+	+	-	+	+	+	+	-	+	-
11.	Protease activity	+	+	-	+	-	-	-	+	-	+	+	-	+	-	+	-
12.	Coagulation of milk	+	-	-	+	-	+	+	+	+	-	-	+	+	-	+	-
13.	Chitinolytic activity	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-
14.	Ability to grow in different pH																
	pH 5	+	-	-	+	-	+	+	-	+	-	-	-	-	-	+	-
	pH 6	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
	pH 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
	pH 8	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-
	pH 9	+	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-

Fig.3. Biochemical tests of isolated Streptomyces strains

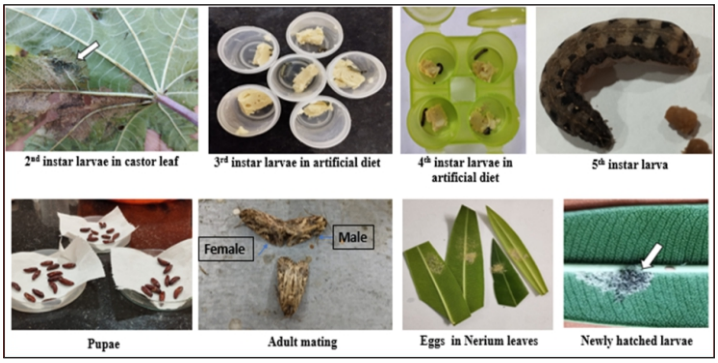

Fig.4. Mass culturing of S. litura under laboratory conditions

The results for molecular characterization were obtained by amplification of 16S rDNA gene with the primers. ST 1 strain was selected for molecular characterization because that strain was considered as best in biochemical characterization as well as the results of bioassay performed against 2nd instar larvae of S. litura showed a higher mortality percentage for that strain. The strain ST 1 was identified as Streptomyces katrae (GenBank Accession No: OR214958). [27] [28] stated that the isolated actinomycetes were molecularly identified by 16S rDNA sequencing and compared with Streptomyces species using NCBI BLAST program.

The field-collected 2nd instar larvae of S. litura were mass cultured in the laboratory conditions (Fig.4) and were used for performing bioassay using S. katrae [35]. [29] found that SAI-25 (S. griseoplanus), BCA-698 (S. albolongus), and CAI-155 (S. bacillaris) showed entomopathogenic activity against Spodoptera litura, Helicoverpa armigera, and Chilo partellus as biocontrol agents.

Table.3. Per cent mortality of Streptomyces strains against 2nd larvae of S. litura

S.No	Strains	Mortality (%)
1.	ST 1	73.33 (55.48)^{a}
2.	ST 2	55.00 (45.95)^{cde}
3.	ST3	46.67 (42.45)^{ef}
4.	ST 4	55.00 (45.95)^{cde}
5.	ST 5	60.00 (46.92)^{bcde}
6.	ST 6	60.66 (50.89)^{abc}

7.	ST 7	40.00 (39.23)^{fg}
8.	ST 8	60.00 (45.00)^{de}
9.	ST 9	66.66 (52.09)^{ab}
10.	ST 10	53.33 (44.28)^{def}
11.	ST 11	50.55 (45.10)^{de}
12.	ST 12	66.66 (48.24)^{bcd}
13.	ST 13	33.33 (35.12)^{gh}
14.	ST 14	25.00 (30.00)^{h}
15.	ST 15	55.00 (45.95)^{cde}
16.	Control	0.00 (0.286)^{i}
CV = 7.52	SEd = 2.58	CD(0.05) = 5.26

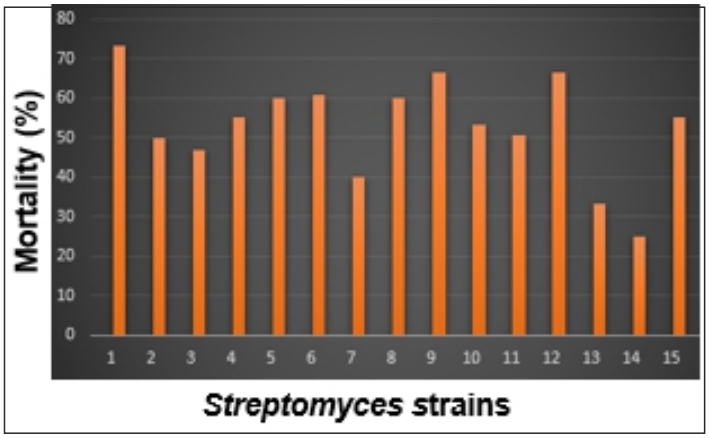

Fig.5. Mortality (%) caused by Streptomyces stains against 2nd instar larvae of S. litura

A preliminary bioassay was conducted to screen the entomopathogenic potential of isolated Streptomyces strains against 2nd instar larvae of S.litura. Among the 15 isolated Streptomyces strains, S. katrae (ST 1) showed a higher percentage of mortality (73.33 %). The strains ST 9 (66.66 %), ST 12 (66.66 %), ST 6 (60.66 %), ST 5 (60.00 %), ST 8 (60.00 %) were on par with each other. ST 14 (25.00 %) showed the lowest mortality (%) (Table 3 and Fig. 5). In the present study, S. katrae fermentation broth showed adverse effects on the life stages of S. litura larvae at the concentration of 800 µg/ml where the larval mortality was about 60-70%. There was no larval mortality noticed in the control. [29] also reported that the highest concentration of Streptomyces sp. AP-123 polyketide metabolite, larvae of H. armigera and S. litura. showed larvicidal and growth inhibitory activities in that larval mortality was about 68.41% and 60.02% at 1000 ppm. The Streptomyces treated larvae turned into pupae but the pupal duration was longer till the end of the experiment. Similarly, Adult emergence was delayed and lower whereas the fecundity of emerged adults was also lower. Therefore the S. katrae showed inhibitory effects towards the most notorious pest S. litura. [30] reported the insecticidal and growth inhibitory potential of Streptomyces hydrogenans DH16 on the major pest of India, S. litura. As a result, morphological abnormalities such as blackened dead larvae, malformed pre pupae, and malformed pupae were observed in the treated larvae at the concentration of 800 µg/ml (Fig.6).

Fig.6. Morphological abnormalities in different stages of S. litura fed on diet supplemented with fermentation broth (800 µg/ml) of S. katre

Conclusion

In the present study, the entomopathogenic effects of S. katrae strain on S. litura showed that the metabolites present in the fermentation broth exhibited strong larvicidal, pupicidal and toxic effects against the notorious pest, S. litura by diet impregnation assay. The fermentation broth of S. katrae strain has the ability to control the S. litura pest populations at a considerable level (73.33%).

Future scope of the study:

The results from the study will help to develop a new entomopathogenic formulation using Streptomyces katrae strain against S. litura.

Conflict of interest:

The authors declare that they have no conflict of interest in the publication.

Acknowledgement:

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.

Reference

1. Ahmad M Ghaffar A Rafiq M (2013). Host plants of leaf worm, Spodoptera litura (Fabricius) (Lepidoptera: Noctuidae) in Pakistan. Asian Journal of Agriculture and Biology 1 (1):23-28.

2. Williams S Goodfellow M Alderson G Wellington E Sneath P Sackin M (1983). Numerical classification of Streptomyces and related genera. Microbiology 129 (6):1743-1813.

3. Ramaiah M Maheswari TU (2018). Biology studies of tobacco caterpillar, Spodoptera litura Fabricius. Journal of Entomology and Zoology Studies 6 (5):2284-2289.

4. Ahmad M Arif MI Ahmad M (2007). Occurrence of insecticide resistance in field populations of Spodoptera litura (Lepidoptera: Noctuidae) in Pakistan. Crop Protection 26 (6):809-817.

5. Jain PK Jain P (2007). Isolation, characterization and antifungal activity of Streptomyces sampsonii GS 1322.

6. Waksman S Henrici A (1948). Family III. Streptomycetaceae Waksman and Henrici. Bergey's Manual of Determinative Bacteriology (Edn 6):929-977.

7. Donald L Pipite A Subramani R Owen J Keyzers RA Taufa T (2022). Streptomyces: Still the biggest producer of new natural secondary metabolites, a current perspective. Microbiology Research 13 (3):418-465.

8. Xue M Pang HT Wang HT Li Liu TX (2010). Effects of four host plants on biology and food utilization of the cutworm, Spodoptera litura. Journal of Insect Science 10 (1):22.

9. Cadirci BH Yasa I Kocyigit A (2016). Streptomyces sp. TEM 33 possesses high lipolytic activity in solid-state fermentation in comparison with submerged fermentation. Preparative Biochemistry and Biotechnology 46 (1):23-29.

10. Bano ND Iqbal A Al Othaim Kamal M Albadrani HM Algehainy NA Alyenbaawi H Alghofaili F Amir M Roohi (2023). Antibacterial efficacy of synthesized silver nanoparticles of Microbacterium proteolyticum LA2 (R) and Streptomyces rochei LA2 (O) against biofilm forming meningitis causing microbes. Scientific Reports 13 (1):4150.

11. Law JWF Tan KX Wong SH Ab Mutalib NS Lee LH (2018). Taxonomic and characterization methods of Streptomyces: a review. Progress In Microbes & Molecular Biology 1 (1).

12. Islam M Aktar M Rahman M Uddin K (2014). Isolation and characterization of Streptomyces spp. collected from Bangladeshi soils on the basis of morphological and biochemical studies. International Journal of Current Microbiology and Applied Sciences 3:734-742.

13. Cavaletti L Monciardini P Bamonte R Schumann P Rohde M Sosio M Donadio S (2006). New lineage of filamentous, spore-forming, gram-positive bacteria from soil. Applied and Environmental Microbiology 72 (6):4360-4369.

14. Kones C Mwajita M Kariuki L Kiirika L Kavoo A (2020). Isolation and characterization of rhizospheric microorganisms from bacterial wilt endemic areas in Kenya. African Journal of Microbiology Research 14 (7):349-360.

15. Reddy KN Paschapur A (2020). Employing environmentally safer and novel synthetic insecticides in organic farming for eco-friendly pest management.

16. Taddei A Rodriguez MJ Márquez-Vilchez E Castelli C (2006). Isolation and identification of Streptomyces spp. from Venezuelan soils: morphological and biochemical studies. I. Microbiological Research 161 (3):222-231.

17. Gomez KA Gomez AA (2010). Statistical procedures for agricultural research

18. Gadelhak,GG El-Tarabily KA Al-Kaabi FK (2005). Insect control using chitinolytic soil actinomycetes as biocontrol agents. International Journal of Agriculture and Biology 7 (4):627-633.

19. Kutovaya OA and Watson SB (2014). 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 40 (2):404-414.

20. Gupta G Rani S Birah A Raghuraman M (2005). Improved artificial diet for mass rearing of the tobacco caterpillar, Spodoptera litura (Lepidoptera: Noctuidae). International Journal of Tropical Insect Science 25 (1):55-58.

21. Arasu MV Esmail GA Al-Dhabi NA Ponmurugan K (2016). Managing pests and diseases of grain legumes with secondary metabolites from actinomycetes. Plant growth promoting actinobacteria: A new avenue for enhancing the productivity and soil fertility of grain legumes:83-98.

22. Altschul SF Gish W Miller W Myers EW Lipman DJ (1990). Basic local alignment search tool. Journal of molecular biology 215 (3):403-410.

23. Chater K (1972). A morphological and genetic mapping study of white colony mutants of Streptomyces coelicolor. Microbiology 72 (1):9-28.

24. Subramanian S Mohanraj P Muthuswamy M (2010). Newparadigm in silkworm disease management using probiotic application of Streptomyces noursei. Karnataka Journal of Agricultural Sciences 22 (3).

25. Ranga Rao G Wightman J Ranga Rao D (1993). World review of the natural enemies and diseases of Spodoptera litura (F.)(Lepidoptera: Noctuidae). International Journal of Tropical Insect Science 14:273-284.

26. Abbot WS (1925). A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18(2): 265-267.

27. Narayanamma VL Sharma H Gowda C Sriramulu M (2007). Mechanisms of resistance to Helicoverpa armigera and introgression of resistance genes into F 1 hybrids in chickpea. Arthropod-Plant Interactions 1:263-270

28. Vijayabharathi R Kumari BR Sathya A Srinivas V Abhishek R Sharma HC Gopalakrishnan S (2014). Biological activity of entomopathogenic actinomycetes against lepidopteran insects (Noctuidae: Lepidoptera). Canadian Journal of Plant Science 94 (4):759-769.

29. Prakash VA Sermalatha G Selvarathinam T (2022). Extraction of bioactive compounds from Streptomyces avermitilis and Azadirachta indica and Evaluation against Spodoptera litura: A green approach. Journal of Entomology and Zoology Studies 10 (1):143-152.

30. Kaur T Vasudev A Sohal SK Manhas RK (2014). Insecticidal and growth inhibitory potential of Streptomyces hydrogenans DH16 on major pest of India, Spodoptera litura (Fab.)(Lepidoptera: Noctuidae). BMC microbiology 14:1-9.