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Enzymatic Screening, Antibacterial Potential and Molecular Characterization of Streptomycetes Isolated from Wayanad District in Kerala, India

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ABSTRACT

Actinomycetes play an important role in the decomposition of various organic materials and replenish the supply of nutrients in the soil. They also produce many secondary metabolites possessing antimicrobial properties. The present study was conducted to determine the enzyme- producing and antibacterial potential of soil actinomycetes isolated from Kuruva Island and Pookot Lake in Wayanad district of Kerala, India. A total of 27 isolates of streptomycetes were recovered from 6 soil samples collected from the two locations and screened for their ability to produce cellulase, pectinase, xylanase, amylase and protease. Screening for the enzyme production was carried out by observing the clear zones surrounding the colonies on agar plates containing specific substrates. The results revealed that 45%, 41% and 33% of the isolates produced cellulase, pectinase and xylanase, respectively, while 26% of the isolates were found to produce all the three enzymes. Amylase and protease production was demonstrated by all the isolates. Screening for antibacterial activity revealed 30% of the total isolates inhibited some Gram positive and Gram negative bacteria. Streptomycete isolate number 23 inhibited Staphylococcus citreus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa and Serratia marcescens. Morphological and biochemical characterization followed by 16S rDNA sequencing of the isolate 23 revealed it as Streptomyces lomondensis strain CK63. The excellent enzymatic potential and antibacterial properties of Streptomyces lomondensis strain CK63 may find various industrial and pharmaceutical applications, respectively.

KEYWORDS: *Antibacterial activity, characterization, sequencing, streptomycetes.*

INTRODUCTION

Actinomycetes constitute a diverse group of microorganisms that are widely distributed in terrestrial, freshwater and marine environments [1]. They play vital roles in decomposition of organic matter and thereby replenish the supply of nutrients in the soil. Among the various genera of actinomycetes identified so far, Streptomycetes are quite significant as they bring about the decomposition of various plant based polysaccharides by the production of wood hydrolytic enzymes, such as cellulases and hemicellulases (xylanases)[2-6]. Cellulases are the extracellular enzymes that hydrolyze cellulose, the biopolymer abundantly found on the earth surface. Xylanases catalyze the breakdown of hemicellulose (xylan) and pectinases or polygalacturonases bring about the depolymerization of pectin, a polysaccharide that is found in the cell walls of plants. These enzymes have received worldwide attention due to their potential applications, especially in the biodegradation of agronomic wastes, and are being increasingly used in textile, paper-pulp and food industries.

Beside enzymes, Streptomycetes are well known as secondary metabolite producers and hence they are of high pharmacological and commercial interest [7,8]. Around 80% of the total antibiotic products are obtained from *Streptomyces sp.* [9]. Their ability to synthesize several broad-spectrum antibiotics has made them interesting subjects of research. The worldwide emergence of multi-drug resistance among several bacterial and fungal

pathogens have necessitated the importance of finding novel strains of Streptomycetes possessing better antimicrobial potential.

Wayanad district in the north-east of Kerala, India, is located on the southern top of the Deccan plateau with lofty ridges covered by dense forest. It is situated on the Western Ghats with high altitudes ranging from 700 to 2100 m. During summer the temperature reaches to a maximum of 35°C (95°F) and during the cold winter the temperature drops to 7°C (45°F). The average rainfall is 2,500 millimetres (98 in) per year. Kuruva Island (10 km from Mananthavady) is a unique and fragile delta system on the Kabini river, while Pookot Lake is a famous tourist spot located near Lakkidi. The diverse flora of Wayanad is characteristic of the Western Ghats and the plantation crops are grown in the cool climate. The soil and climate here are suitable for horticulture on commercial basis. The fertility of Wayanad soil may be obviously attributed to the presence of rich actinoflora.

MATERIAL AND METHODS

Collection of soil samples:

Six soil samples were collected from two locations: Kuruva Island (11°49'18"N, 76°5'32"E) and the banks of Pookot Lake (11°32'30"N, 76°1'37"E) of Wayanad district in Kerala, India, during November 2010. The soil samples (rich in lignocellulosic materials) were collected with an auger, (down to 10 cm depth) after removing approximately 5 cm litter of fallen leaves and fruits. Care was taken to see that the points of collection had as widely varying characteristics as possible with regard to the organic matter, moisture content, particle size and colour of soil and to avoid contamination as far as possible. Samples were placed in sterile zip-lock covers and stored at 4°C until processed [10].

Sample processing:

Each soil sample was air dried at room temperature, then mixed thoroughly and sieved through a 2 mm pore size sieve (Retsch, Haan, Germany) to get rid of large debris. The sieved soil was used for the isolation purpose.

Isolation of actinomycetes:

Sieved soil samples of 1 g were suspended in 100 mL sterile distilled water and incubated in an orbital shaker (Orbitek) at 28°C with shaking at 200 rpm for 1 hr [11]. Mixtures were allowed to settle, and then serial dilutions of the spore suspensions were prepared up to 10^{-4}. From each dilution, 0.1 mL was taken and spread evenly over the surface of starch casein nitrate agar (SCNA) plates (supplemented with cycloheximide 50 µg mL^{-1}) (in triplicate) with sterile L-shaped glass rod, then incubated at 28°C for 10 days [12,13]. Dilutions that yielded 30-300 colonies were chosen for further isolation. Actinomycete isolates were purified by streak-plate technique and the pure cultures were maintained on SCNA slants at 4°C for further use.

Screening for cellulase-producing actinomycetes:

Pure isolates of actinomycetes were cultured on cellulose agar containing (g/L): yeast extract, 1; carboxy methyl cellulose (CMC), 10; KH_2PO_4, 4; NaCl, 2; $MgSO_4.7H_2O$, 1; $MnSO_4$, 0.05; $FeSO_4.7H_2O$, 0.05; $CaCl_2.2H_2O$, 2; NH_4Cl, 2 and agar, 20; pH 7.3, and then incubated at 28°C for 5 days. The plates were then flooded with 0.1% (w/v) solution of Congo Red and left for 30 min, washed with 1 mL NaCl (1 M) and left for 15 min. Colonies producing cellulase showed clear zones against red colour of non-hydrolyzed medium. Positive isolates were tested again for confirmation [14].

Screening for pectinase-producing actinomycetes:

Pure isolates of actinomycetes were cultured on pectin agar containing (g/L): yeast extract, 1; pectin, 5; KH_2PO_4, 4; NaCl, 2; $MgSO_4.7H_2O$, 1; $MnSO_4$, 0.05; $FeSO_4.7H_2O$, 0.05; $CaCl_2.2H_2O$, 2; NH_4Cl, 2 and agar, 20; pH 7.3, and then incubated at 28°C for 5 days. Plates were then flooded with 1% (w/v) solution of polysaccharide precipitant CTAB (cetyl trimethyl ammonium bromide), dissolved in 15% alcoholic solution and then used to detect pectinase production. After 1 h of exposure, colonies producing pectinase showed clear zones against an opaque colour of the non-hydrolyzed medium.

Screening for xylanase-producing actinomycetes:

Pure isolates of actinomycetes were cultured on xylan agar containing (g/L): yeast extract, 1; xylan, 10; KH_2PO_4, 4; NaCl, 2; $MgSO_4.7H_2O$, 1; $MnSO_4$, 0.05; $FeSO_4.7H_2O$, 0.05; $CaCl_2.2H_2O$, 2; NH_4Cl, 2 and agar, 20; pH 7.3, and then incubated at 28°C for 5 days [15]. The plates were then flooded with absolute ethanol (99% v/v) and left for 1 h at room

temperature. Colonies producing xylanase enzyme showed clear zones against an opaque colour of non-hydrolyzed medium. Positive isolates were tested again for confirmation.

Screening for antibacterial potential:

Primary screening for the antibacterial activity of the pure isolates were determined by perpendicular streak method on nutrient agar (NA) [16]. The bacterial pathogens used were *Bacillus cereus*, *Staphylococcus aureus*, *S. citreus*, *Streptococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Serratia marcescens.* All the test bacterial cultures were obtained from the Department of Microbiology, Genohelix Biolabs, Bangalore. The active isolates of actinomycetes were inoculated in SCN broth and incubated in an orbital shaker for 10 days at 130 rpm. The broth cultures were filtered using Whatman No. 1 filter paper and centrifuged at 5000 rpm for 30 min at 4°C (Hettich Universal 32R, Germany). The supernatants were aseptically transferred to sterile tubes and partially purified by adopting the method specified by Muiru *et al.* [17]. Secondary screening was performed by well diffusion method on Mueller Hinton agar (Himedia) plates swabbed with the test bacteria. Following incubation at 37°C for 24 h, the diameter of the zones of complete inhibition was measured to the nearest whole millimeter.

Morphological characterization of the isolates:

Only actinomycete isolates that gave a positive result for any of the three above enzymes, were characterized morphologically and physiologically following the directions given by the International *Streptomyces* Project (ISP) according to Shirling and Gottlieb [18]. Morphological characters of the isolates were observed by smears from colonies up to 10 days, stained by Gram's method as described by Hucker and Conn [19].The spore chain morphology was determined by direct microscopic examination using the 10 days old cultures under a compound light microscope (Nikon, Japan) using 1000X magnification power. The observed structures were compared with Bergey's Manual of Determinative Bacteriology, ninth edition [20] and the organisms were identified. Color of aerial mycelia was determined from mature, sporulating aerial mycelia of the actinomycetes colonies on SCNA [21]. Color of the substrate mycelia (reverse of the plate) was also observed along with diffusible pigments if any [22].

Biochemical characterization of the isolates:

Various biochemical tests performed for the identification of the potential isolates were indole production, methyl red, Voges Proskauer, citrate utilization, casein hydrolysis, starch hydrolysis, urea hydrolysis, gelatin hydrolysis, H_2S production, acid production from sugar, NaCl resistance and temperature tolerance [23].

Extraction of genomic DNA from actinomycete isolate:

The potent actinomycete isolate was cultured in SCN broth and incubated at 28°C for 7 days in an orbital shaker at 130 rpm. Genomic DNA was extracted using Fungal Genomic DNA Isolation Kit RKT 41/42 (Chromous Biotech Pvt. Ltd., Bangalore, India) according to the manufacturer instructions and visualized using 0.8% (w/v) agarose gel electrophoresis.

PCR amplification:

The PCR amplification reactions were performed in a total volume of 25 µl. Each reaction mixture contained the following solutions: 1.5 µl genomic DNA, 1 µl 10 pmol forward 16S rDNA primer (5'-AGAGTTTGATCCTGGCTCA-3'); 1 µl of 10 pmol reverse 16S rDNA primer (5'-ACGGCTACCTTGTTACGACT-3'); 1 µl of 30 mM deoxyribonucleoside 5'-triphosphate (N= A,T,G,C) (dNTP's); 2.5 µl of 10X PCR buffer and 1 µl Taq polymerase (1 U) (Chromous Biotech Pvt. Ltd., Bangalore, India) and water was added up to 25 µl. The thermal cycler (MJ Research PTC 200, USA) was programmed as follows: 2 min initial denaturation at 94°C, followed by 30 cycles that consisted of denaturation for 1 min at 94°C, annealing for 30 s at 57°C and extension at 74°C for 1 min and a final extension of 5 min at 74°C. The PCR amplified product was analyzed by 1.2% agarose gel electrophoresis using TAE buffer. The resulting DNA patterns were examined with UV light under transilluminator, photographed and analyzed using gel documentation system (Herolabs, Germany).

Partial 16S rDNA sequencing and analysis of sequence data:

The partial 16S rDNA sequencing of the amplified product was performed at Chromous Biotech Pvt.

Ltd., Bangalore, India. The 16S rDNA sequences were aligned manually with the available nucleotide sequences retrieved from the NCBI database by using BLASTN [24].

RESULTS AND DISCUSSION

Isolation of Actinomycetes:

Wayanad district located in the state of Kerala, India, consists of rugged terrain of the Western Ghats, which is marked by dense forests, deep valleys and rivers. A large area of the district is covered by green forests, which are rich in lignocellulosic materials derived from the thick vegetation of medicinal plants [22]. Naturally, these fertile soils would have rich assemblage of actinomycetes. In this study, a total of 27 isolates of actinomycetes were recovered from 6 soil samples collected from Kuruva Island and the banks of Pookot Lake in Wayanad district of Kerala, India. All of these isolates were selected based on their colony morphology on SCN agar producing dry, compact, chalk-like colonies (**Figure 1** and **Figure 2**). It has been observed that the environment of the soil such as the humidity, pH and diversity of plants species grown on that particular soil influence the growth rate of microorganisms [11, 25]. Majority of the isolates in our study were recovered from the Kuruva island soil. This may be attributed to the presence of thick vegetation on the island and partly saline soil rich in humus content.

Screening of streptomycetes producing hydrolytic enzymes:

Actinomycetes are important not just to the pharmaceutical industries but also the agriculture. Actinomycetes interact in various ways with the higher plants. Plant materials like fallen twigs, barks, leaves and flowers provide nutrients to both microbes and plants through microbial degradation of various polysaccharides. These degradation processes yield simple sugars, fatty acids and amino acids, and eventually lead to mineralization [22]. Cellulose comprises the highest of the carbon sources in plants apart from lignin [26]. Actinomycetes have the potential to utilize different plant polysaccharides such as cellulose, pectin and xylan as carbon sources by being capable of producing the hydrolytic enzymes [27].

In the present study streptomycete isolates were screened for detection of cellulase, pectinase and xylanase hydrolytic enzymes. Among the 27 soil isolates only 17 strains were able to produce the three enzymes (cellulase, pectinase and xylanase) suggesting that almost 63% of the isolated population of streptomycetes possessed the plant-fibre hydrolyzing ability. Amylase and protease production was demonstrated by all the isolates. Results revealed that among the 27 isolates, 33% were able to produce at least one enzyme, 4% produced two different enzymes and 26% were able to produce all the three enzymes. However, 37% of the 27 isolates were not able to produce any of these. The results revealed that 45%, 41% and 33% of the isolates produced cellulase, pectinase and xylanase, respectively. This indicates that actinomycetes possess the potential to secrete broad range of enzymes, which may be the results from the natural selection of the microorganisms in order to survive in a competing environment [26]. The detailed results of the enzymatic potential of the streptomycete isolates have been presented in **Table 1**.

In a previous study, a new bacterium, *Streptomyces* sp. LX, was isolated from soil, which was aerobic Gram-positive and could decompose crystalline cellulose completely [28]. The relative enzymatic potential of the isolates ranged from weak, moderate to strong depending on the diameter of the clear zones of hydrolysis formed surrounding the colonies in the screening process. Among the active isolates producing all the three enzymes, strain no. 23, 25, 26 and 27 demonstrated broad zones of hydrolysis. The streptomycete isolate no. 27 was the highest cellulase producer with a hydrolytic zone of 37 mm on CMC agar medium. Isolate no. 23 was the highest producer of pectinase enzyme with a zone size of 31 mm (**Figure 3**) and isolate no. 26 yielded maximum xylanase production with a zone of 25 mm.

TABLE 1: Screening for enzymatic potential of different Streptomycete isolates.

Isolate No.	Cellulase	Pectinase	Xylanase
1	+	+	+
2	-	+	-
3	-	+	-
7	+	-	-
8	+	-	-
9	-	-	+
15	+	+	+
16	+	+	-
17	+	+	+
19	-	+	-
20	+	-	-
21	+	-	-
22	-	-	+
23	+	+	+
25	+	+	+
26	+	+	+
27	+	+	+

Screening for antibacterial potential:

Actinomycetes have been well known for the production of various secondary metabolites [26]. Four Gram positive and six Gram negative bacterial pathogens were tested to screen the antibacterial effects of the secondary metabolites produced by the streptomycete isolates. The results of primary screening indicated that 30% of the total isolates demonstrated antibacterial activities. The aqueous extracts of the active isolates were subjected to secondary screening using the same test pathogens. Isolates no. 5, 18, 21, 22 and 23 exhibited higher antibacterial activities against the Gram negative bacteria, whereas isolates no. 21, 22 and 23 possessed antibacterial activities against both Gram negative and Gram positive bacteria. Isolates no. 5, 18, 21, 22 and 23 were quite potent as they inhibited more than one bacterial pathogen. On the other hand, isolate no. 15, 25 and 26 exhibited mild antagonistic effects towards the test bacteria. Interestingly, the cell free culture supernatant of isolate no. 23 revealed a broad spectrum antibacterial activity against both Gram positive and Gram negative bacterial pathogens (**Figure 4**) with the highest activity against *Staphylococcus citreus* (30 ± 0.05 mm). An earlier work reported the isolation of 5 strains of soil actinomycetes showing broad spectrum antimicrobial activity [22]. The significant results of the antibacterial activity of the streptomycete isolates have been listed out in **Table 2**.

Morphological characterization of the isolates:

All the 27 actinomycete isolates obtained in this study demonstrated morphological characteristics similar to that of genus *Streptomyces* as per the description given by Shirling and Gottlieb [18] and Nonomura [29]. Gram's staining revealed the typical filamentous structures of the isolates bearing chains of conidia in verticillate and spiral arrangements. The identification of the streptomycete isolates were further confirmed with reference to the Bergey's Manual of Determinative Bacteriology. After 10 days of incubation, the colonies were observed with white, cream, grey, pale brown, yellow, tan, pink, purple and greenish shades which are the characteristics of actinomycetes. Majority of the isolates were white and grey coloured. The 17 enzyme-producing pigmented *Streptomyces* isolates were categorized into 4 colour series based on the colour of mature sporulated aerial mycelia such as white, grey, yellow and purple colour series (**Figure 5** and **Figure 6**). Besides the aerial mycelia, majority of them produced distinct hues on the reverse of the colonies probably due to the diffusion of pigments and/or secondary metabolites into the media. As reported

previously, 19 isolates of streptomycetes, exhibiting grey, white and brown pigmentation of the aerial mycelia, were obtained from 4 different locations of the Western Ghats region in India[22]. The isolate no. 23, obtained in our study, produced greyish white aerial mycelia on SCN agar (**Figure 7**) and pale yellow pigmentation on the reverse of the colony, suggesting the possible diffusion of some secondary metabolite into the medium. Microscopic observation of the Gram stained smear of isolate no. 23 demonstrated the presence of filamentous structure bearing chains of conidia in open spiral arrangement (**Figure 8**).

Table 2: Antibacterial activity of streptomycete isolates showing diameters of inhibitory zones (in mm).

Bacterial pathogen	I-5	I-15	I-18	I-21	I-22	I-23	I-25	I-26
Staphylococcus aureus	-	-	-	-	-	-	-	-
Staphylococcus citreus	18[a]	-	-	19 ± 0.2	-	30 ± 0.05	15 ± 0.05	13
Streptococcus faecalis	-	-	-	8	11 ± 0.05	9 ± 0.05	-	-
Bacillus cereus	-	-	-	8.5 ± 0.5	12 ± 0.05	-	-	-
Salmonella typhi	-	-	-	-	-	24 ± 0.35	-	-
Proteus mirabilis	22 ± 0.5	12 ± 0.05	21 ± 0.5	23 ± 0.5	21 ± 0.5	24 ± 0.5	-	-
Escherichia coli	13 ± 0.5	-	15 ± 0.2	14 ± 0.1	12 ± 0.05	16 ± 0.05	-	-
Klebsiella pneumoniae	12 ± 0.1	-	13 ± 0.2	13 ± 0.05	15 ± 0.1	17 ± 0.5	-	-
Pseudomonas aeruginosa	15 ± 0.2	-	16 ± 0.5	16 ± 0.5	15 ± 0.2	18 ± 0.5	-	-
Serratia marcescer	14 ± 0.2	-	15 ± 0.2	15 ± 0.5	15 ± 0.2	17 ± 0.5	-	-

Keys: [a], Values are mean of triplicates ± standard deviation.

Biochemical characterization of the isolate:

As evidenced by the results of the enzymatic and antibacterial screening, the actinomycete isolate no. 23 was found to produce all the enzymes and demonstrated broad spectrum of antibacterial activity. Therefore, this isolate of streptomycete was subjected to biochemical and molecular characterization. The detailed results of the biochemical tests have been summarized in **Table 3**.

Table 3: Partial biochemical characterization of the potent Streptomycete isolate No. 23.

Biochemical tests	Results
Indole production	_
Methyl Red	_
Vogesproskauer	_
Citrate utilization	+
Starch hydrolysis	+
Casein hydrolysis	+
Gelatin hydrolysis	_
Urea hydrolysis	+
Growth in 0.1% NaCl	+
Growth in 0.5% NaCl	+
Growth in 1% NaCl	+
Growth in 3% NaCl	+
Growth in 5% NaCl	_
Growth in 10% NaCl	_
Growth at 4°C	_

Growth at 25°C	+
Growth at 37°C	+
Growth at 42°C	+
Growth at 45°C	+
Growth at 50°C	_
Sugar fermentation	
Glucose	+/-[a]
Sucrose	+/-
Fructose	+/-
Maltose	-/-
Lactose	-/-
Mannitol	-/-

Keys: [a], acid/gas production.

Figures 1 and 2. Isolates of streptomycetes growing on starch casein nitrate agar plates.
Figure 3. Agar plates showing the hydrolytic activity of cellulase, pectinase and xylanase produced by isolate no. 23.

Fig. 1.

Fig. 2.

Fig. 3.

Figure 4. Plates showing the antibacterial activity of the active isolates of streptomycetes by well diffusion technique.
Figures 5-6. Microscopic observations of the filaments and spore chain morphology of the streptomycete isolates.

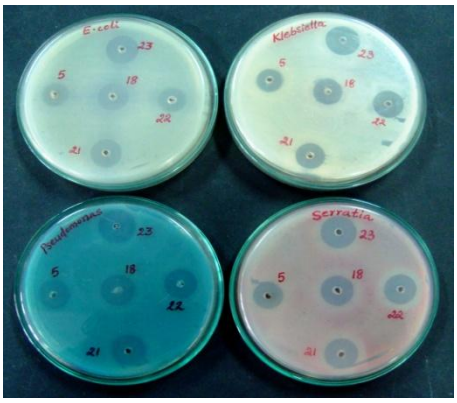

Fig. 4.

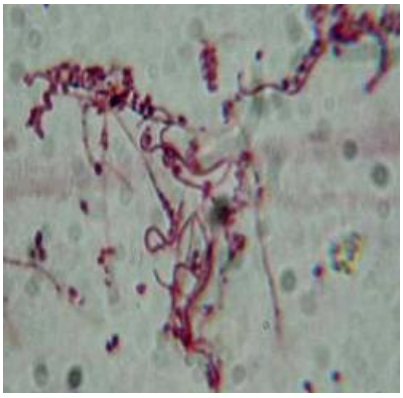

Fig. 5.

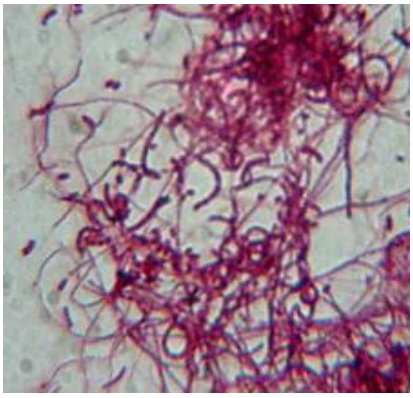

Fig. 6.

Figure 7. Colonies of *Streptomyces lomondensis* strain CK63 on SCNA plate.
Figure 8. Microscopic observation of spore chain morphology of *Streptomyces lomondensis* strain CK63.

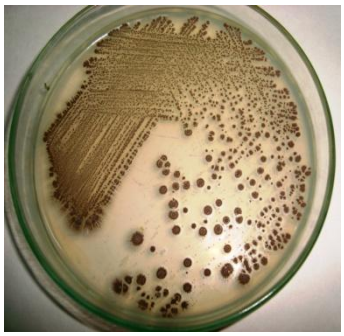

Fig. 7.

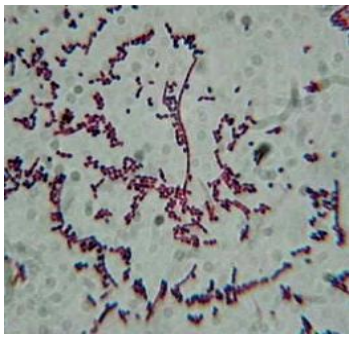

Fig. 8.

Extraction of genomic DNA from *Streptomyces* isolate:

Genomic DNA from the active isolate no. 23 was extracted using commercial genomic DNA isolation kit obtained from Chromous Biotech Pvt. Ltd, Bangalore, India. The extracted DNA was found to be of good quality as visualized by agarose gel electrophoresis. The extracted DNA was intact, clean and free of RNA.

Molecular characterization of the isolate:

With the advancement of technology in molecular study, primers had been developed by researchers to target specifically the 16S rDNA sequence of the actinomycetes [26, 30,31]. Identification of actinomycetes to species level was made possible in a fast and accurate manner.The molecular characterization of isolate no. 23 was carried out after PCR amplification of the 16S rDNA sequence of the genomic DNA using the universal forward and reverse primers. The 1500 bp long PCR-amplified product was then partially sequenced.

Partial 16S rDNA sequencing and analysis of sequence data:

The BLASTN search results using 16S rDNA data as the query sequence revealed that isolate no. 23 belonged to *Streptomyces lomondensis* strain CK63 with GenBank accession no. EU982926.1.The homology value of BLASTN search showed 100% sequence homology with the registered 16S rDNA gene sequence in NCBI data base.

Actinomycetes have always contributed significantly in the areas of soil ecology and industrial exploitation [32]. Therefore, an attempt was made to isolate and identify potential actinomycetes from Wayanad district in search for some bioactive compounds. Our results of antibacterial study and partial sequencing of the active isolate no. 23 are in perfect agreement with the initial findings reported by Johnson and Dietz [33]. *Streptomyces lomondensis*, a soil-dwelling Gram-positive bacterium, had been found to produce a secondary metabolite lomofungin, which possesses a broad spectrum of biological activities including antibacterial and antifungal properties. This natural product was shown to be effective against yeasts, molds, and both Gram-positive and Gram-negative bacteria. The 100% sequence similarity, as evidenced from our Blast result, suggests the possibility for production of similar antibacterial compound by *Streptomyces lomondensis* strain CK63. Recent findings reported that the compound lomofungin is a natural product antagonistic against the botulinum neurotoxin light chain protease[34]. Other works on the lomofungin produced by *Streptomyces lomondensis* suggest that that the compound rapidly inhibits synthesis of ribonucleic acid (RNA) in *Saccharomyces* protoplasts. Further research is required to assess the safety of lomofungin for its therapeutic use.

CONCLUSIONS

Actinomycetes synthesize a broad range of commercially important hydrolytic enzymes and

secondary metabolites. They are ubiquitously found in nature and they play a significant role in the enrichment of soil fertility. The Western Ghats in India is known for its ecological diversity of flora and fauna. Wayanad district situated in the Western Ghats, therefore, offer a suitable habitat for the exploration of many novel actinomycetes. The present findings indicate that the forest and lake soils of Wayanad harbor a diverse population of potent actinomycetes. Our study also reveals the excellent enzymatic potential and antibacterial properties of *Streptomyces lomondensis* strain CK63 which may find various industrial and pharmaceutical applications.

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