ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.1.L 51-59

International Journal of Lifescience and Pharma Research

Research Article

Microbiology for health care

Isolation, Screening and Identification of Cellulolytic *Streptomyces corchorusii* (Mn244066) From Soil Sample of Visakhapatnam

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Abstract: Fifty one isolates of cellulolytic bacteria were isolated from cow dung and two soil samples were obtained from Kambalakonda Wildlife Sanctuary and RCD Biodiversity Park in Visakhapatnam, Andhra Pradesh by Enrichment method in basal salt medium with cellulose as substrate for degradation. The cellulolytic activity of the isolated bacteria was determined by the diameter of the zone of hydrolysis by Gram's iodine dye staining method. After primary screening, a total of fifty one isolates showed cellulolytic activity. Out of fifty one strains of cellulolytic bacteria, twenty three isolates from Kambalakonda Wildlife Sanctuary, seventeen isolates from RCD Biodiversity Park and Eleven isolates from cow dung sample obtained from cattle ranch, Visakhapatnam showed cellulase activity. Seven strains showed maximum hydrolytic value greater than 4.0 cm, nineteen strains showed average hydrolytic value between 3.0 and 3.9 cm and twenty one strains showed minimum hydrolytic value between 1.5 and2.9 cm. The potential isolates were obtained from Kambalakonda Wildlife Sanctuary and RCD Biodiversity Park than cow dung sample. The 13 C strain exhibited maximum hydrolytic value of 5.6 cm which was designated as KKV1. The strain KKVI was identified as *Streptomyces corchorusii* (MN244066) by morphological, cultural, biochemical and 16S rRNA sequence. The CMCase and FPase activity of the crude sample were examined by DNS method and found to be 0.21 U/ml and 0.041 U/ml respectively and the specific activity was 4.38 U/mg proteins and 0.86 U/mg proteins respectively. The present study emphasizes that the *Streptomyces corchorusii* have a higher cellulase activity and the soils of bio reserves have a lot of scope for isolating high cellulolytic bacteria which can be exploited for different industrial purposes.

Keywords: Bio reserves, Cellulolytic activity, Hydrolytic value, Cow dung, Soil, *Streptomyces corchorusii.*

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Recieved On 15 November 2019
Revised On 10 December 2019
Accepted On 21 December 2019
Published On 03 January 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation L.Sujatha, K.P.J. Hemalatha* , Isolation, Screening And Identification Of Cellulolytic Streptomyces corchorusii (Mn244066) From Soil Sample of Visakhapatnam.(2020).Int. J. Life Sci. Pharma Res.10(1), L 51-59 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.1.L 51-59

1. INTRODUCTION

Cellulose is a homopolymer containing glucose residues bonded with β-1, 4 linkagesand is the main structural component of the cell wall of plants and the most profusely obtained carbohydrate in nature[1]. Enormous amounts of cellulosic waste obtained from agriculture, industry and municipality are accumulated or inefficiently used because of high cost during the utilization processes[2]. Successful utilization of abundant cellulosic materials as renewable carbon sources requires the development of technologies for economic process. These technologies help in the production of cellulase that produce low molecular weight components like hexoses and pentoses by enzymatic hydrolysis of cellulosic materials[3].Cellulase production was the most expensive step during ethanol production from cellulosic biomass and it accounted for approximately 40% of the total cost[4]. Most of the cellulose degradation occurring in nature is done by microorganisms with the help of a multi-enzyme complex. Cellulase is an enzyme that hydrolyzes the β-1, 4-glycosidic bonds in the polymer to release glucose units[5]. These enzymes are produced by different groups of microorganisms like fungi, bacteria, protozoa, some termitesand the microbial intestinal symbionts of other termites. Cellulase enzyme system comprises three classes of soluble extracellular enzymes: 1, 4-*β*-endoglucanase, 1, 4-*β*-exoglucanase, and *β*-glucosidase (*β*-D-glucoside glucohydrolase or cellobiase). Endoglucanase randomly cleave *β*-1, 4-glycosidic bonds along with the homopolymer chain of cellulose. Exoglucanase is necessary for cleavage of the non reducing end of a cellulose chain and splitting of the elementary fibrils from the crystalline cellulose, and *β*-1, 4-glucosidase hydrolyses cellobiose and water-soluble cellodextrin to glucose[6]. The activity of the complete cellulase complex (cellobiohydrolase, endo-l,4-β-glucanase, and β-glucosidase or cellobiase) can be measured using crystalline celluloses such as cotton fiber, filter paper, or Avicel[7]. Cotton fiber or filter papers prepared from cotton fiber are considered to be the best substrates by many but some workers favour Avicel. The cellulose degrading enzymes can be efficiently used in the formulation of washing powders for extraction of juices from fruits and vegetables and for processing of starch[8], commercial food processing in coffee, in the textile industry, laundry detergents, in the processing of pulp and paper for various purposes, and they are also used for pharmaceutical applications. Cellulases are also used in the fermentation of biomass into biofuels. Although most of the organisms can degrade cellulose but only a few of the microorganisms can produce free enzyme in large quantities which can degrade crystalline cellulose completely[9].The actinomycetes were recorded for expansive secondary metabolite production, but very less research have been carried on hydrolytic enzyme production. Hence this study was undertaken to isolate, identify and characterize the cellulase producing *Streptomyces corchorusii* so that it can be efficiently used in the bioconversion of cellulosic waste and for industrial exploitation.

2. MATERIALS AND METHODS

2.1 Collection of soil and cow dung samples

Three soil samples from different areas of each site had been collected and mixed to analyse the overall microflora. Three cow dung samples from different cattle ranch was collected and mixed. Top soil samples (0 to 10 cm depth) were collected in sterile polythene bags from Kambalakonda Wildlife Sanctuary and RCD Biodiversity Park in Visakhapatnam, Andhra Pradesh. The soil samples were sieved through a 2.0 mm width sterile mesh to remove stones and plant debris. Fresh cow dung sample was collected in a sterile polythene bag and brought to the lab for testing.

2.2 Isolation of cellulose degrading bacteria

One gram of collected soil samples and cow dung were suspended in labelled 9ml of sterile saline solution and uniformly mixed in a vortex. One millilitre from each sample was serially diluted upto 10^{-6}. About 0.5 ml of solution from each serial dilution was taken and spread on sterile basal salt media plates ($NaNO_3$ 2.5 gm; KH_2PO_4 2 gm; $MgSO_4$ 0.2 gm; NaCl 0.2 gm; $CaCl_2 \cdot 6H_2O$ 0.1 gm, agar- 15-20 gm in a litre) containing 0.2 % cellulose for the isolation of cellulolytic bacteria. This step was repeated for other two serially diluted samples. These cultures were incubated for 3-5 days at 37°C. Different colonies obtained for each sample were streaked on sterile cellulose agar media plates(KH_2PO_4 0.5 gm, $MgSO_4$ 0.25 gm, cellulose 2.0 gm, agar 15 gm, gelatin 2 gm in 1 L, pH 6.8–7.2) to get pure cultures of Cellulose Degrading Bacteria(CDB) and analyzed for cellulose degradation.

2.3 Screening of Cellulose degrading bacteria

The pure cultures obtained native isolated colonies were streaked in a circular manner as a spot on cellulose agar medium plates. The plates were incubated at 37°C for 48 h and observed for growth. Following the incubation, the plates were flooded with Gram's iodine solution (0.133 g Potassium iodide and 0.067 g Iodine were dissolved in 20 ml distilled water) to check the cellulolytic activity and the zone of hydrolysis was observed around the line of growth and the zone of diameter was determined. The cellulose degrading bacteria can also be identified by staining the cellulose agar plate with 1% Congo red for 15- 20 min. The Congo red stain was removed and the plate was counter stained with 1M NaCl. The cellulose hydrolysis is the ratio of overall clear zone diameter to colony diameter and it is measured in order to select the highest cellulase producer[10]and highest the cellulase enzyme production, greater the zone of hydrolysis for an isolate. Consequently, they were graded based on hydrolysis and the results were tabulated. Bacterial colonies were repeatedly streaked to get a purified culture. The purified cultures were preserved at 4°C for further analysis.

2.4 Identification of cellulose degrading bacteria

The potential strain was identified by morphological, cultural and biochemical characterization as per the Bergey's Manual of Systematic Bacteriology[11].

2.5 Morphological Characterization

The bacteria was stained by Gram staining technique and observed microscopically. Arrangement, shape and motility of bacteria were observed by performing SEM (Scanning electron microscope) analysis.

2.5 Cultural Characteristics

The bacterial isolate was cultured on selective and enrichment media like MacConkey agar medium and cellulose

agar medium respectively.The culture was examined for configuration, colour, margin, elevation, surface, texture, pigmentation and density.

2.7 Biochemical Characterization

The bacterial strain was analysed on the basis of different biochemical tests which include Indole test, Methyl Red test, Voges-Proskauer test, Citrate utilisation test, Catalase test, Oxidase test, Sugar fermentation test, Urease test, Nitrate test, Gelatin hydrolysis, Starch hydrolysis and Casein hydrolysis. The results were compared with Bergey's Manual of Determinative Bacteria[12].

2.8 Molecular characterization

The molecular analysis of the strain KKV1 was authenticated by theInstitute of Microbial Technology (IMTECH), Chandigarh, India. Culture was sent to IMTECH for 16s rRNA gene sequencing. Genomic DNA was isolated from the pure culture using ZR Bacterial DNA Miniprep Kit (Make Zymo Research). 16s rRNA gene was PCR amplified using universal 27 F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGTTACCTTGTTACGACTT). PCR product was visualized on 1% Agarose gel. PCR amplicon was gel eluted and purified using QiAquick Gel extraction Kit (MakeQiagen). Purified PCR product was sequenced using Sanger DNA sequencing method. The sequences obtained were visualized and analysed using Finch TV software ver 1.4.Assembled nucleotide sequences of 16S rRNA gene were subjected to similarity using BLAST tool in EzBiocloud portal (http://WWW.ezbiocloud.net/) or (https://blast.ncbi.nlm.nih.gov/). The sequence was analysed for homology and submitted to NCBI GenBank to get accession number. The Phylogenetic analysis was performed to check the evolutionary relationships with the program MEGA 7[13].

2.9 Production of cellulase enzyme

The isolate which showed maximum zone of hydrolysis was cultured for production of cellulase by submerged fermentation in Basal salt medium ($NaNO_3$ 2.5 gm; KH_2PO_4 2 gm; $MgSO_4$ 0.2 gm; NaCl 0.2 gm; $CaCl_2 \cdot 6H_2O$ 0.1 gm in a litre) containing 0.2% cellulose (pH 7.2) and incubated at 37°C at 150 rpm in a shaker incubator for 48 hrs. Broth culture after two days of incubation period was filtered through whatman filter paper and the filtrate was subjected to centrifugation at 10000 rpm for 10 min at 4°C. The Supernatant was collected and stored as crude enzyme preparation at 4°C for further enzyme assays.

2.10 Enzyme activity assay

Cellulase activity was measured by a DNS methodby determiningreducing sugars liberated from CMC and Filter paper[14]. The supernatant was then used as crude enzymesample to assay the activity of carboxymethylcellulose (CMCase) and total cellulose activity (FPase). CMCase (Endoglucanase) activity was assayed by measuring the amount of reducing sugar from CMC. This activity was determined by incubating 0.5 m lof supernatant with 0.5 ml of 1% CMC in 0.05 M phosphate buffer (pH 7) at 50°C for 30 min. FPase (Exoglucanase, Total cellulase activity)was assayed by measuring the amount of reducing sugar from filter paper. The activity was determined by incubating 0.5 ml of supernatant with 1.0 ml of 0.05 M phosphate buffer (pH 7) containing Whatman no.1 filter paper strip—1.0 × 6.0 cm (50 mg) at 50°C for 1 hour.Similarly, the glucose standards(0.2-2 mg of glucose per ml) and controls were prepared. The reaction was terminated after incubation by adding 3 mL of 3, 5-dinitrosalicylic acid (DNS) reagent to 1 ml of reaction mixture. All the tubes were boiled in water bath for 10 min. After cooling, the reducing sugar present in the samples were estimated spectrophotometrically at 540 nm (Shimadzu, UV-1800). Cellulase production was estimated by using glucose calibration curve. The enzymatic activity of total FPCase and CMCase (Endoglucanase) were defined in international units. One unit of enzymatic activity is defined as the amount of enzyme that releases 1 µmol reducing sugar (glucose) per ml per minute.

$$\text{Enzyme activity (U/ml)} = \frac{\text{Amount of glucose produced (mg)}}{\text{0.18 X volume of enzyme X incubation time for reaction}}$$

2.11 *Protein determination*

Protein concentration of crude enzyme was determined using Lowry method with bovine serum albumin (BSA) as a standard[15].

3. STATISTICAL ANALYSIS

All the values presented in the study are the mean ± SEM of three replicates for each test. The statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) software and the significant difference was found to be $P<0.01$.

4. RESULTS AND DISCUSSION

Cellulose is the major fraction of organic carbon present in soil which is mostly obtained from plants. Microorganisms, which live in soil, are accountable for the recycling of soil organic carbon to theenvironment. Degradation of cellulosic materials is a complex process and requires participation of microbial cellulolytic enzymes. Habitats in which these substrates are present are the best sources for isolation of cellulolytic microorganisms[16]. As the bioreserves are the source of efficient cellulolytic bacteria which can produce significant quantities of free enzyme capable of completely hydrolyzing crystalline cellulose, the present study was undertaken to isolate, identify and characterize the cellulase producing bacteria.

4.1 Isolation of cellulose degrading bacteria

In the present study, sixty three bacterial isolates were isolated from two soil samples and cow dung by serial dilution plating method. Depending on the morphological characteristics, the mixed isolates were identified and streaked on sterile cellulose Agar medium to get pure cultures. From these, twelve isolates were not considered due to colonial and morphological similarities. Fifty one strains were used for further analysis. Out of fifty one strains,

twenty three isolates were from Kambalakonda Wildlife Sanctuary, Seventeen isolates from RCD Biodiversity Park and eleven isolates from cow dung was isolated and the results were tabulated in Table 1.

Table 1. Details of the bacterial strains isolated from different samples

S.No	Source of sample	Bacterial strains
1.	Soil sample from Kambalakonda Wildlife Sanctuary	1a, 1c, 2a, 2b,3b, 3c, 3d, 4b, 5a, 5b, 5d,6a, 7a, 8b, 8c,9b, 9c,10a, 11a, 11b, 12a, 13a, 13c
2.	Soil sample from RCD Biodiversity Park	14a, 14b, 15a, 15b, 15c, 15d, 16a, 16b, 16c, 17a, 17b, 18b, 18a, 19a, 19b, 20 a, 20b
3.	Cow dung sample	3,4,5,8,10,11,12,13,14,20,23

4.4 Screening of cellulose degrading bacteria

The 51 bacterial isolates were primarily screened for cellulase production on cellulose agar media. After incubation, the plates were stained with Gram's iodine solution. All the fifty one strains showed cellulose hydrolysis but they varied in their activity. The twenty plates showing Grams iodine staining with zone of clearance are shown in Figure 1. The cellulase producing bacterial strains was identified by the zone of clearance or hydrolysis. All the fifty one strains were graded based on their hydrolysis and the results were tabulated in Table 2. Four strains showed very negligible activity and seven strains showed maximum clear zone around the colony with diameter greater than 4.0 cm. nineteen strains showed average clear zone around the colony with diameter between 3.0 and 3.9 cm and twenty one strains showed minimum clear zone around the colony with diameter between 1.5 and 2.9 cm. The isolate which showed maximum zone of hydrolysis was13 C and it was designated as KKV1 and it was further analysed for identification. The zone of hydrolysis values obtained were similar to the range reported by Pratima et al. and Hatami *et al.*[16,17]who found the hydrolytic value between 28 to 50mm and 4.3 to 9.0 cm for the isolates obtained from macerated gut of termites, book worm, snail, and Caterpillar and lesser hydrolytic value between 1.38 to 2.33 and 0.15 to 13.7 cm of cellulolytic aerobic bacterial isolates from farming and forest soil respectively.

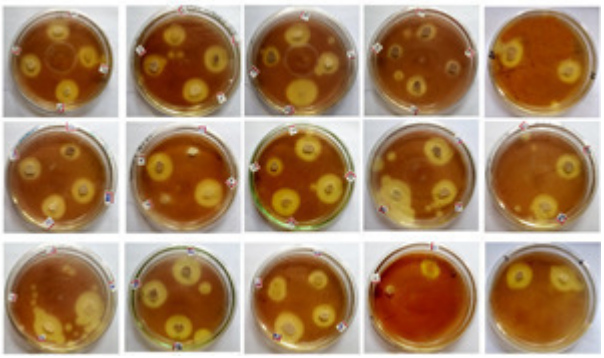

Fig 1.Zone of cellulose utilization indicated by the hydrolysis of ellulose in cellulose agar medium after staining with Gram's iodine.

Table 2. Comparison of zone of hydrolysis of different bacterial isolates on cellulose agar medium after staining with Gram's iodine.

S.No	Bacterial strain	Clear zone diameter (cm)	colony diameter (cm)	Hydrolytic value (cm)
1	1a	2.3	0.9	2.6
2	1c	0.1	0.2	0.5
3	2a	2.4	0.9	2.7
4	2b	2.5	0.6	4.2
5	3b	2.6	0.8	3.3
6	3c	1.9	0.6	3.2
7	3d	2.2	0.8	2.8
8	4b	2.6	0.7	3.7
9	5a	2.1	0.7	3.0
10	5b	1.7	0.6	2.8
11	5d	2.8	0.6	4.7
12	6a	3.1	0.8	3.9
13	7a	1	0.6	1.7
14	8b	2.8	0.7	4.0
15	8c	1.2	0.7	1.7
16	9b	1.8	0.6	3.0
17	9c	1.2	0.6	2.0
18	10a	2.4	0.9	2.7

19	11a	2.3	0.9	2.6
20	11b	2.1	1	2.1
21	12a	2.6	0.8	3.3
22	13a	2	0.8	2.5
23	13c(KKV1)	2.8	0.5	5.6
24	14a	2.4	0.5	4.8
25	14b	2.2	0.5	4.4
26	15a	2.1	0.7	3.0
27	15b	1.8	0.7	2.6
28	15c	2.6	0.9	2.9
29	15d	1.8	0.7	2.6
30	16a	2.9	0.6	4.8
31	16b	1.8	0.7	2.6
32	16c	2.3	0.7	3.3
33	17a	2.1	0.7	3.0
34	17b	2.4	1	2.4
35	18a	2.3	0.8	2.9
36	18b	2.5	0.9	2.8
37	19a	2.1	1.1	1.9
38	19b	2.1	0.6	3.5
39	20a	2.8	0.8	3.5
40	20b	3	1	3.0
41	3	2.7	0.7	3.9
42	4	2.6	0.5	5.2
43	5	-	-	No Zone
44	8	2.4	0.6	4.0
45	10	2.4	0.7	3.4
46	11	-	-	No Zone
47	12	0.2	0.3	0.7
48	13	1.7	0.7	2.4
49	14	2.4	0.7	3.4
50	20	2.6	0.8	3.3
51	23	1.7	0.8	2.1

Hydrolytic Value= Clear zone diameter/Colony diameter

4.3 *Identification of cellulose degrading bacteria*

4.3.1 *Morphological, Cultural and Biochemical Identification*

The morphological and cultural characteristics were studied by growing the potential bacterial strain (KKV1) on cellulose agar medium. The colony morphology of the 13 C strain wasshown in Figure 2 and SEM analysis was given in Figure 3.

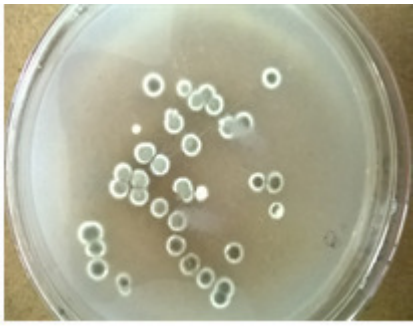

Fig 2. Colony morphology of Pure culture of 13C strain grown on cellulose Agar medium

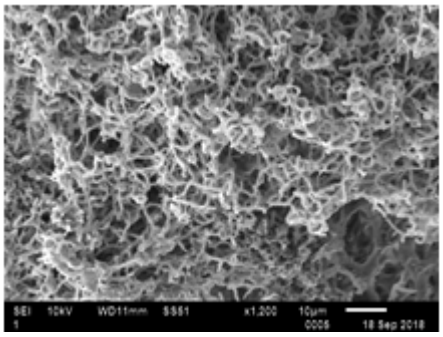

Fig 3.SEM image of 13C strain

The biochemical characterization for KKV1 strain was done and the results were analysed. The colony of KKV1 on cellulose agar medium was observed in green at the centre with white margin in color, surface was powdery, margin was entire, flat and round with opaque density at 37°C and no growth was observed on MacConkey agar medium. Microscopic examination of this isolate revealed that it was gram positive, non motile with highly branched chains of hyphae. It showed negative results for indole production, methyl red test, VogesProskauer test, starch hydrolysis, gelatin hydrolysis, oxidase test, H_2S gas production, urease test and positive for citrate test,catalase, casein test, nitrate reduction and esculine hydrolysis. It could ferment glucose, fructose, salicin, mannitol, raffinose, sucrose, maltose and arabinose.

4.3.2 *Molecular characterization*

Based on the 16s rRNA gene sequencing (Figure-4) and Phylogenetic tree analysis (Figure -5) authenticated by IMTECH, Chandigarh, India, the strain KKV1 showed highest homology (99.85 %) with *Streptomyces corchorusii*. The 16S rRNA sequence was submitted to NCBI Genbank with Accession Number - MN244066. The evolutionary history was inferred using the Neighbor-Joining method[18].The analysis involved 26 nucleotide sequences. The evolutionary distances were computed using the Maximum Composite Likelihood method[19] and are in the units of the number of base substitutions per site. The strain KKV1 was in the same cluster of phylogenetic tree with different strains of *Streptomyces corchorusii.*

ACTTCGGTGGGGATTAGTGGCGAACGGGTGAGTAACACGTGGGCAATCTGCCCTTCACTCTGGG
ACAAGCCCTGGAAACGGGGTCTAATACCGGATACCACTCTCGCAGGCATCTGTGAGGGTTGAAA
GCTCCGGCGGTGAAGGATGAGCCCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAG
GCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCACACTGGGACTGAGACACGGCCCAGA
CTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCC
GCGTGAGGGATGACGGCCTTCGGGTTGTAAACCTCTTTCAGCAGGGAAGAAGCGAAAGTGACG
GTACCTGCAGAAGAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAA
GCGTTGTCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGCTTGTCACGTCGGGTGTGAAAGC
CCGGGGCTTAACCCCGGGTCTGCATTCGATACGGGCTAGCTAGAGTGTGGTAGGGGAGATCGGA
ATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCGGATCT
CTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGT
AGTCCACGCCGTAAACGGTGGGAACTAGGTGTTGGCGACATTCCACGTCGTCGGTGCCGCAGCT
AACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGG
GGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAACGCGAAGAACCTTACCAAGG
CTTGACATACACCGGAAAACCCTGGAGACAGGGTCCCCCTTGTGGTCGGTGTACAGGTGGTGCA
TGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCC
TGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCACAGGAGACCGCCGGGGTCAACTCGGA
GGAAGGTGGGGACGACGTCAAGTCATCATGCCCCTTATGTCTTGGGCTGCACACGTGCTACAAT
GGCCGGTACAAAGAGCTGCGATACCGTGAGGTGGAGCGAATCTCAAAAAGCCGGTCTCAGTTC
GGATTGGGGTCTGCAACTCGACCCCATGAAGTCGGAGTTGCTAGTAATCGCAGATCAGCATTGC
TGCGGTGAATACGTTCCCGGGCCCTTGTACACACCGCCCGTCACGTCACGAAAGTTGGTAACAC
CCGAAGCCGGTGGC

Fig 4 .16S rRNA gene sequence of strain KKV1

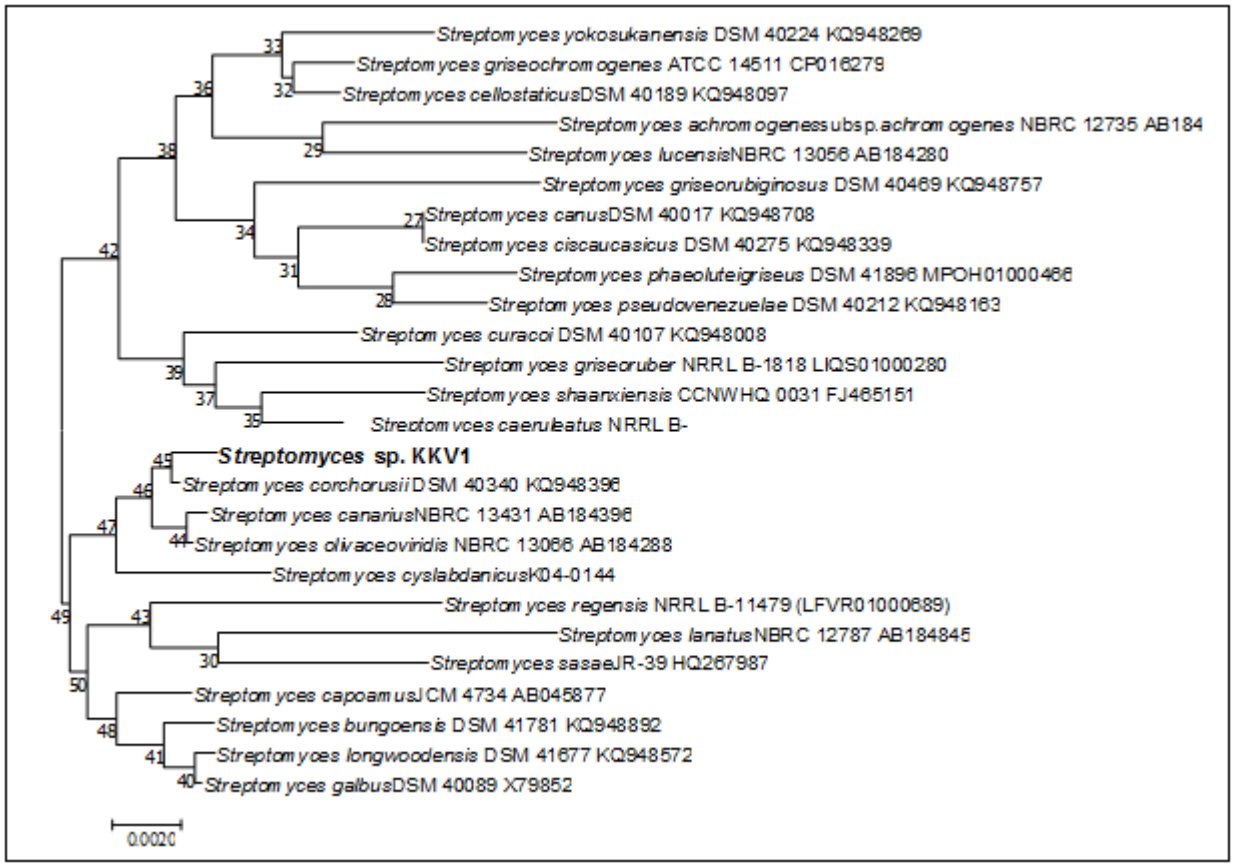

Fig 5. Phylogenetic tree showing evolutionary relationships with nearest *Streptomyces* species.

The genus *Streptomyces* is known for its novel bioactive compounds and has been also previously explored to detect cellulases (endoglucanases, exoglucanases, cellobiases)[20-22], although in the majority of the studies *Streptomyces* have been investigated for its well-known hemicellulase production ability[23-25]. Recently, one gene from *Streptomyces coelicolor* A3(2), recombinantly expressed in *Streptomyces lividans* TK24, was discovered to encode a protein with strong hydrolyzing activity toward Avicel and filter paper, yielding cellobiose as the final product and moderate activity toward CMC (~0.4 U mL^{-1})[26]. Noura El-Ahmady El-Naggar*et al*., (2014)[27] tested the culture supernatant from submerged fermentation of *Streptomyces albogriseolus* subsp. *cellulolyticus* under optimized conditions for the production of endoglucanase that release reducing sugars from agro-industrial residues as substrates.

4.3.3 *Enzyme activity assay*

The cellulase was produced by submerged fermentation in Basal salt medium containing 0.2% cellulose (pH 7.2) at 37°C at 150 rpm in a shaker incubator for 48 hrs. The filtrate was centrifuged at 10000 rpm for 10 min at 4°Cand the supernatant was used as crude enzyme. The crude enzyme was utilised to determine the CMCase and FPase activities by DNS method with glucose as standard. The CMCase and FPase activities of the crude sample were found to be 0.21 U/ml and 0.041 U/ml respectively. The specific activities of CMCase and FPase of the crude enzyme were 4.38 U/mg protein and 0.86 U/mg protein respectively. Previous workers reported that when CMC was used as the substrate for enzyme activity, the activity was highest. It is assumed that this is due to the less complexity when compared to other cellulosic substrates[7].The CMCase activity and FPase activity are in agreement with previous studies, who reported that CMCase activity is greater than FPase activity [6,28,29] and the activity reported in this study is higher than that exhibited by some isolates [6,16,22].

4.3.4 *Protein determination*

Protein concentration of crude enzyme sample was determined by the Lowry method (1951) with bovine serum albumin (BSA) as a standard and it was found to be 0.048 mg/ml.

5. CONCLUSION

In the present study the potential strain was isolated from Kambalakonda Wildlife Sanctuary, Visakhapatnam by enrichment technique in basal medium with cellulose. The strain is characterized by morphological, cultural, biochemical and molecular analysis. The 16s rRNA sequence showed 99.85% similarity with *Streptomyces corchorusii.* The cellulase was produced by submerged fermentation and filtered to get the filtrate which was used as crude sample for assaying enzyme activity. The enzyme activity for CMCase and FPase activities were assayed by DNS method. The CMCase and FPase activities of the crude sample obtained after fermentation were found to be 0.21 U/ml and 0.041 U/ml and the specific activities were 4.38 U/mg protein and 0.86 U/mg protein respectively. The protein concentration of the crude enzyme sample was found to be 0.048 mg/ml. To our knowledge, this is the first report on functional cellulase enzyme obtained from *Streptomyces corchorusii* and also from Visakhapatnam by using crystalline cellulose as substrate for fermentation. Further this strain has to be optimized for the production of cellulase in large scale by fermentation

technology and purification strategies has to be carried out to obtain the enzyme so that it can be efficiently used for research and industrial purposes.

6. ACKNOWLEDGEMENT

The authors are grateful to the Principal, the Director and the Management of Gayatri Vidya Parishad College for Degree and P.G Courses (A), Visakhapatnam for permitting and providing facilities to carry out the research work.

7. AUTHORS CONTRIBUTION STATEMENT

Mrs Sujatha L performed the experiments, conceptualized, gathered and analyzed the data. Prof. K.P.J. Hemalatha has given necessary inputs in designing the manuscript. Both the authors discussed the methodology and results and contributed to the final manuscript.

8. CONFLICT OF INTEREST

Conflicts of interest declared none.

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