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Screening, Production and Characterization of Potential Lignocellulolytic Actinomycetes from Agricultural Field

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Abstract

Actinomycetes are a suitable microbial group for the synthesis of lignocellulose-degrading enzymes. Enzymes that may degrade organic material, including cellulose, hemicellulose, and lignin, are released by actinomycetes. The aim of this research was to isolate actinomycetes from Rajkot, Gujarat, India's soil and evaluate the activity of their cellulase and Xylanase enzymes. Starch Casein Agar (SCA) was used to identify a total of 30 isolates of actinomycetes. A qualitative plate assay (CMC-Na, Congo red) revealed that the highest zone of catalysis for MMD1 was 36 mm. Five strains were discovered to be effective for quantitative quantification of endoglucanase utilising filter paper and CMC as substrates: MMD1, MMD2, MMD3, MMD4, and MMD8. Following MMD 1 (endoglucanase 5.4 IU; FPase 4.4 IU), MMD 2 (endoglucanase 4.5 IU; FPase 3.4 IU) has demonstrated considerable endoglucanase and FPase activity. Beechwood xylan was used to treat sugarcane bagasse in order to test Xylanase, and 45% of the xylan (hemicellulose) fraction was obtained. MMD1 and MMD2 measured the xylanase enzyme activity (4.8IU and 4.2IU) in quantitative and qualitative assays (34 mm and 22 mm for BWX and 32 mm and 14 mm for agro-waste xylan). The strain MMD1 was identified as *Streptomyces chartreusis* through morphological, biochemical, and finally molecular characterization by 16S rRNA sequencing. It was then submitted to NCBI GenBank with the accession number MT254830.

Keywords: Actinomycetes, Lignocelluloses, Endoglucanase, FPase, Sequencing

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INTRODUCTION

One of the most abundant renewable non-fossil carbon sources on earth is lignocellulose, an essential component of plant cell walls. It is widely distributed in waste from agriculture, fruits and vegetables, forests, and cities. Using less expensive lignocellulosic material as the major carbon source and other energy sources can lead to the economic and environmentally friendly production of bioethanol and other bioproducts. Lignocellulose as one of the most abundant renewable biomasses from green and dry vegetation usually comprised of cellulose, hemicellulose, and lignin.[1] The hydrolysis of cellulose is carried out by endoglucanases (EC 3.2.1.4), β-glucosidase (EC 3.2.1.21) cellobiohydrolase (EC. 3.2.1.91) and exoglucanases (EC 3.2.1.74) respectively.[2] Besides myriad microbial sources as lignocelluloses degraders[3,4] actinomycetes a saprobe[3] and a unique Gram-positive, filamentous prokaryote with high GC content and extracellular enzyme, secondary metabolites secretion properties was also studied as a resource organism for lignocellulose degradation by many researchers[5] and the search still in progress either in culture dependent[6] or independent[7] manner. Efficient hemicellulose degraders generally reduce the need of commercial xylanase other hemicellulose degrading enzymes with specific substrate specificity. The prime aspects which had influenced to carry out the present study was an exploration for an efficient lignocellulose degrading actinobacteria especially *Streptomycetes* capable enough with multi lignocellulose synthesis potential and enhanced degradation ability of the renewable wastes abating pollution and be used as significant strain of choice in biofuel production in a cost-effective manner.

MATERIALS AND METHODS

Sample collection

The two different soil samples were collected from agricultural field longitude 22.2344° N, Latitude 70.8004° E near Rajkot, Gujarat, India. Overnight, soil samples were air-dried and then incubated at 50°C.

Isolation of actinomycetes

Actinomycetes were isolated using serial dilution method on Starch Casein Agar Medium. 10 ml of distilled water taken in test tube, 1 gram of soil was weighed and thoroughly mixed. Then, it was serially diluted up to a 10^{-5} dilution. After that, 0.1 ml taken from the dilution of 10^{-3} and 10^{-5} and spread on the Starch Casein Agar (SCA) plate. The plates were incubated for 7–14 days at 30°C. Actinomycete isolates that have grown on SCA media Containing (g/L) Soluble Starch-10.0, Casein-0.30, $MgSO_4.7H_2O$-0.05, KNO_3 -2.0, K_2HPO_4 -2.0, NaCl-2.0, $FeSO_4.7H_2O$-0.01, $CaCO_3$ -0.02, Agar 18.0 Final pH at 30°C: 7.3±0.2 have been chosen based on their morphology. The chosen isolates were separated by further streaking on additional SCA plates. Then, for further testing, a pure colony of actinomycetes was kept in 10% glycerol at -80°C.[8]

Screening of Cellulase producing actinomycetes strain

Qualitative screening was performed with the Carboxy Methyl Cellulose (CMC)(g/L) Containing carboxymethyl cellulose (CMC) 10.0, K_2HPO_4 -2.0, peptone 10.0, $MgSO_4.7H_2O$-0.3, $(NH_4)_2SO_4$-2.5, gelatine 2.0 and agar 18 and pH was adjusted at 6.8-7.2, By the point inoculation 30 Plates were incubated at 30°C for 7 days after catalytic zone was observed and intensified with consecutive flooding by 1% (w/v) Congo-red solution[9] for 15 minutes and destaining with 1 M NaCl for 15 minutes.[10] The area surrounding the selected colonies was measured.[11]

Synthesis of cellulase enzyme

Cellulase Producing Isolates are (MMD1, MMD2, MMD3, MMD4 and MMD8). This production culture assay for checking endo glucanase, FPase activity. These organisms were inoculated liquid culture containing 1% CMC, 0.1% K_2HPO_4, 1% $NaNO_3$, 0.05% $MgSO_4.7H_2O$, 0.1% KCL,0.05% Yeast extract (pH 7). The developing culture was then moved into the production medium of the same composition and incubated for a week at 30°C and 120 rpm in an orbital shaker.[10] The Production media was centrifuge for 10 minutes at 10,000 rpm to get cell free

supernatant. This cell-free supernatant is used for cellulase activity. At every interval of 24 hr crude enzyme extracts of all the selected isolates were undergone assay for CMCase and FPase.[11]

Cellulase assay

The Cellulase activity was carried out using the method described by Maravi and Kumar[12] performed by a reaction mixture was made up of 0.1 ml of crude enzyme extract and 1 ml of 0.5% CMC that had been produced in 50 mM phosphate buffer (pH 7). To create a volume of 2.0 ml The tube was filled with 0.9 ml of 50 mM phosphate buffer pH 7.0. The mixture was then further incubated in a water bath for 30 minutes at 50°C and then add 3.0 ml of dinitrosalicylic acid (DNSA)[13] reagents to stop the reaction. For FPase Whatman filter paper (No.1) strip was used as a substrate the absorbance was recorded at 540 nm. Unit of endoglucanase or Fpase activity defined as amount of enzyme that liberated 1 µmol of glucose per minute. Unit of endoglucanase or Fpase activity defined as amount of enzyme that liberated 1µmol of glucose per minute. The absolute amount of glucose (0.5 mg/ml) plotted against 540 nm was used to create a linear graph of glucose for the enzyme unit calculation. A glucose standard curve was created using a standard formula to measure the concentration of released glucose and the activity of the enzymes.[11]

CMCase Activity= 0.185/(Enzyme concentrtion to release 0.5 mg glucose) × units ml^{-}

Enzyme activity (FPU/ml)= (Sugar Concentration)/(MW of glucoseXT) × H/E

Where MW of Glucose is (180g/mol), incubation time t (minutes), and E is volume of enzyme (ml), H is total volume of enzyme-substrate (ml)

Determination of lignocellulose Composition in Sugarcane bagasse and ground nutshell

Sugar cane bagasse (SCB) and ground nutshell were collected from APMC Yard (Rajkot, Gujarat). Approximately 1 gm (Weight A) of SCB and ground nutshell was dried. The sample was allowed to air dry before being combined with 150 ml of distilled water and benzene alcohol. The combination was then refluxed at 100°C for one hour in a water bath. After filtered out, 300 ml of hot water was applied to rinse the reflux products. After the filtering procedure, Once the residue achieved a constant weight (weight B), it was dried in an oven. After adding 150 ml of 1 N H_2SO_4 to the dry residue, the mixture was refluxed once more for one hour at 100°C in a water bath. After the reflux products were filtered, the residue was cleaned with distilled water until the pH was neutral. After that, the residue was dried until it reached a constant weight (Weight C). After the addition of the dried residue, 100 ml of 72% H_2SO_4 were added, and the combination steeped at room temperature (27°C) for four hours. After then, the residue was filtered again. After adding 150 ml of 1 N H_2SO_4, the filtered residue was refluxed for one hour at 100°C in a water bath. The reflux products were filtered and then washed with distilled water after their pH reached a neutral level. After the residue reached a consistent weight, it was dried in an oven at 105°C (Weight D). The cellulose and hemicellulose content of ground nutshell and SCB can be calculated using the following formulas.

The obtained pellet was then used for Xylanase screening, both qualitatively and quantitatively.[12]

Hemicellulose(%)= b - c/a × 100%

Cellulose(%)= c-d /a × 100%

Screening of xylanase producing actinomycetes strain

Qualitative screening was performed with the Beech wood xylan (SRL) Containing (g/L) KH_2PO_4 3.0; $CoCl_2{\cdot}6H_2O$ 0.02, $MgSO_4{\cdot}7H_2O$ 0.5; $CaCl_2{\cdot}2H_2O$ 0.1; K_2HPO_4 2.0; $FeSO_4{\cdot}7H_2O$ 0.05; $MnSO_4{\cdot}4H_2O$ 0.02; and NH_4Cl 10.0 agar 18 grams supplemented with Beech wood Xylan 1% (w/v) and pH was adjust at 6.8 to 7.0. xylan-degrading Potent colonies were detected by point inoculation and the Plates were incubated at 30°C for 7 days. The zone of hydrolysis was observed by Congo-red solution 0.1% (w/v) for 30 minutes and destaining with 1 M NaCl for 15 minutes. According to the prescribed procedure, the catalytic zone was measured.[12]

Synthesis of xylanase enzyme

Xylanase Producing Isolates (MMD1, MMD2, MMD3, MMD4 and MMD8). This

production culture assay for checking Xylanase activity. These organisms were inoculated liquid culture containing (g/L) KH_2PO_4 3.0; K_2HPO_4 2.0; KH_2PO_4 3.0; $CaCl_2 \cdot 2H_2O$ 0.1; $FeSO_4 \cdot 7H_2O$ 0.05; $MnSO_4 \cdot 4H_2O$ 0.02; $CoCl_2 \cdot 6H_2O$ 0.02; $MgSO_4 \cdot 7H_2O$ 0.5 and NH_4Cl 10.0 and 1% w/v Beech wood xylan as sole carbon source. The developing culture was then moved into the production medium of the same composition and incubated for a week at 30°C and 120 rpm in an orbital shaker. The Production media was centrifuge for 10 minutes at 10,000 rpm to get cell free supernatant. This cell-free supernatant is used as Xylanase activity. At every interval of 24 hr crude enzyme extracts of all the selected isolates were undergone assay for Xylanase.

Xylanase assay

The reaction mixture used in the experiment consisted of 1 ml of 0.5% Beech Wood Xylan and SCB that had been prepared in 50 mM phosphate buffer (pH 7) and 0.1 ml of crude enzyme extract. 0.9 ml of 50 mM phosphate buffer pH 7.0 was added to the tube to make a volume of 2.0 ml. The mixture was then incubated in a water bath for 30 minutes at 50°C. To stop the reaction, 3.0 ml of dinitrosalicylic acid (DNSA)[13] reagent was added to the reaction tube.

Biochemical characterization of MMD1

A few significant biochemical tests, including IMViC (Indole, Methyl red, Voges Proskaur, Citrate utilization), hydrolysis of starch, gelatine liquefication, and nitrate reduction,[14,15] was carried out to identify the genus of MMD1 comparing with criteria stated in Bergey's Manual of Systematic Bacteriology.[16]

Identification and 16S rRNA sequence analysis and phylogenetic tree construction of the Potent Actinomycetes strain MMD1.

The Gram's Staining was used to determine the morphology of the isolated actinomycetes. The Molecular identification of the Cellulase and Xylanase producing actinomycetes isolates was carried out by 16S rRNA Gene sequencing. The 16S rRNA sequence analysis was performed by Gene explore Diagnostics and Research Centre Pvt Ltd. in Ahmadabad. Following established protocols, a number of procedures were carried out, including DNA extraction from MMD1, PCR amplification, sequencing, BLAST analysis against the GenBank database (the first fifteen sequences were chosen and aligned using multiple sequence alignment software based on maximal identity score), and inferring evolutionary history using p-distance and the neighbour joining method. [2,17-19] The phylogenetic tree was generated and drawn to scale using the maximum likelihood approach, which measures branch lengths in the same units as evolutionary distances. Positions with holes and incomplete data were all removed. MEGA7 was used to conduct evolutionary analysis and determine evolutionary divergence between sequences. The proportion of duplicate trees in which the related taxa clustered together is shown next to the branches in the bootstrap test (1000 repetitions).[19] *Streptomyces* sp. MMD1 16S rRNA sequence was uploaded to NCBI GenBank with a modification in coding as F (19)2.

RESULTS

Isolation of actinomycetes

In the current study, from two different agriculture soil sites, a total of 30 Actinomycetes strains were isolated.

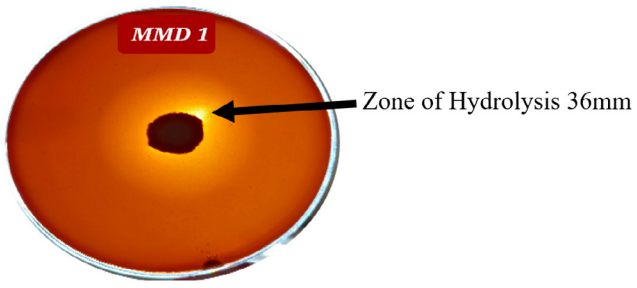

Figure 1. Zone of hydrolysis around colonies of *Actinomycetes* strain MMD1

Screening of Cellulase producing actinomycetes strain by rapid plate assay

The most effective five actinomycetes strains (MMD1, MMD2, MMD3, MMD4, MMD8) developed hydrolytic zones on CMC-Congo red agar plates and were chosen for quantitative analysis out of 30 actinomycetes strains (Table 1). Compared to other strains, the MMD1 strain shown the largest zone of cellulose breakdown (36 mm) (Figure 1). The MMD7 strain shown the lowest zone of cellulose breakdown (0.04 mm)

Cellulase enzyme activity of potent actinomycetes strains

The endo—1,4-glucanase activity of five selected actinomycetes strains was quantified, and MMD1 was found to have the highest activity (5.4 IU), followed by MMD2 (4.5 IU), MMD4 (4.4 IU), MMD3 (4.2 IU), and MMD8 (3.6 IU) during the optimal 72 hours of incubation. The MMD1 enzyme activity profile revealed a rise in activity from 0.3 to 5.4 IU after 24 hours and a subsequent drop (Figure 2). The FPase at an optimal incubation

Table 1. Zone of hydrolysis for cellulase and Xylanase synthesis potent strains of actinomycetes

Actinomycetes Potent Strain	Zone of Hydrolysis of Cellulase (mm)	Zone of Hydrolysis of Xylanase (mm)	Zone of Hydrolysis of Xylanase using SCB (mm)
MMD1	36**	34**	32**
MMD2	18	22	14
MMD3	16	11	14
MMD4	5	8	6
MMD5	6	9	5
MMD6	8	5	6
MMD7	0.4*	0.5*	0.3*
MMD8	31	33	30
MMD9	6	8	7

Footnote- **-highest zone of hydrolysis; * Lowest zone of hydrolysis

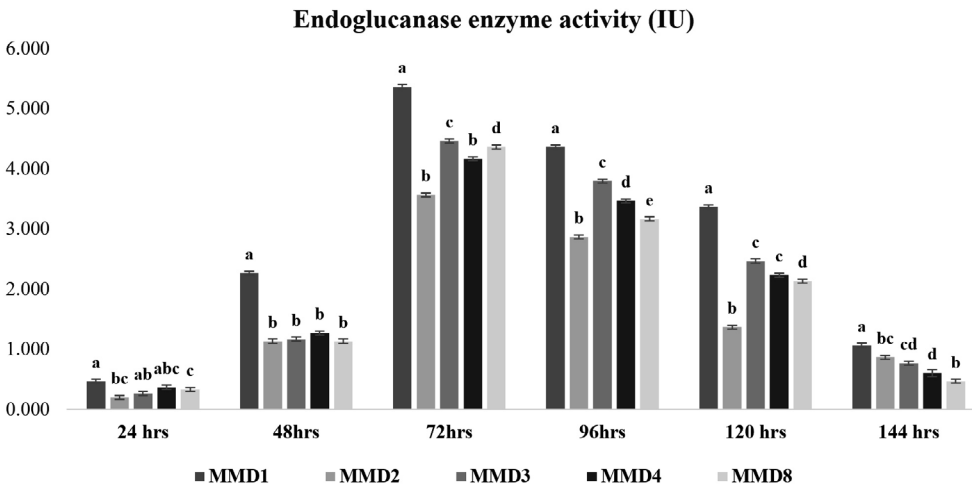

Figure 2. Endoglucanase enzyme activity of isolated potent *Actinomycetes* (MMD1 Streptomyces chartreusis) at 7 days of incubation period. Letters denote significant variations using Tukey's test (P<0.05)

time of 72 hours, the potent actinomycetes strains MMD1, MMD8, MMD2, MMD3, and MMD4 had displayed enzyme activities of 4.4 IU, 3.3 IU, 3.4, 3.9 IU, and 2.9 IU, respectively. While the most effective strain, MMD1, showed enhanced enzyme activity from 24 to 120 hours of incubation (Figure 3).

Screening of xylanase employing actinomycetes strain using beech wood xylan and extraction of treated xylan from SCB

The two potential actinomycetes strains (MMD1 & MMD2) displayed 34 mm and 22 mm, respectively, of xylanase catalysis during 72 hours of fermentation. Similar to this, using hemicelluloses (xylan) residue from treated sugarcane bagasse, MMD1 and MMD2 showed 32 mm and 14 mm of xylanase catalytic zone at 72 hours of fermentation duration, respectively.

Xylanase enzyme activity by selected actinomycete strains from beech wood xylan and extraction of treated Xylan from SCB

After treating sugarcane bagasse, 45% of the hemicellulose was removed (Figure 4). The quantitative experiment using treated SCB xylan fraction showed xylanase activity of 3.7 IU and 3.2 IU by MMD1 and MMD2, respectively, at an optimal 72-hour incubation period (Figure 5). While a quantitative analysis using beech wood

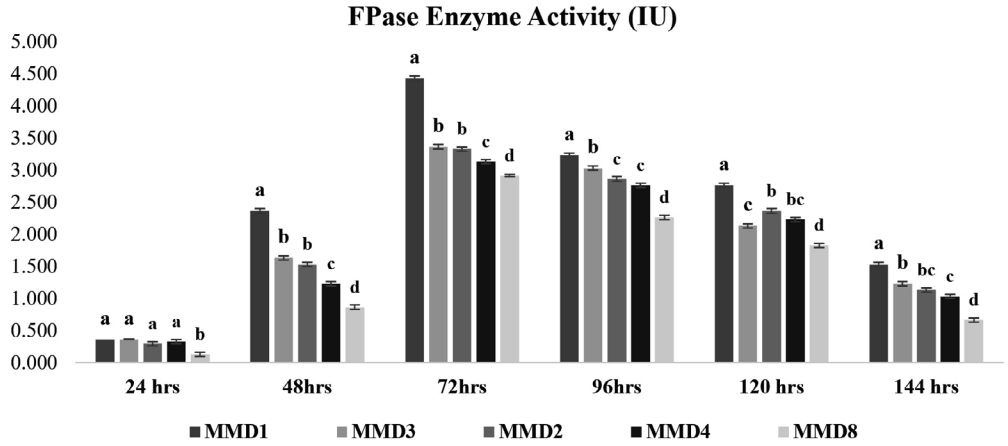

Figure 3. FPase enzyme activity of isolated potent *Actinomycetes* (MMD1 *Streptomyces chartreusis*) at 7 days of incubation period. Letters denote significant variations using Tukey's test (P<0.05)

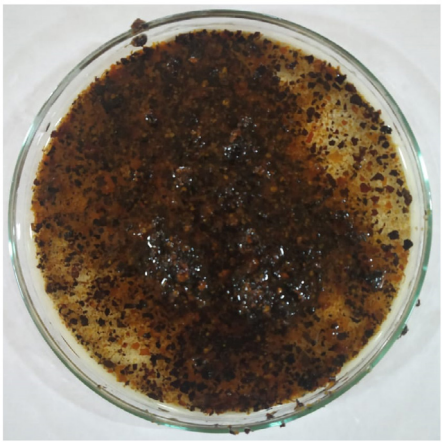

Figure 4. Xylan fraction from treated sugarcane bagasse

Table 2. Morphological Characteristics of *Actinomycetes* strain MMD1

Parameters	Properties
Size	Medium
Shape	Irregular
Margin	Round
Texture	Powdery or Rough
Elevation	Raised
Aerial mycelia colour	Whitish grey
Aerial mycelia morphology	Loose spirals and tight spirals in sporophore morphology
Substrate mycelia	Yellowish white
Spore/conidia	Spore intercalary or terminal
Gram staining	Gram-positive

xylan as a synthetic carbon source with MMD 1 and MMD 2 had found to be recorded with zone of 4.8 IU and 4.2 IU, respectively, at an optimal 72 hours of incubation period (Figure 6).

Morphological and biochemical characteristics of potential actinomycetes MMD1

In the present study, selected potent strains Actinomycetes MMD1 had shown a preliminary characteristic property of the genus *Streptomyces* sp. as being represented in Table 2 and 3 by morphological and biochemical

Table 3. Biochemical Characteristics of Potent Strain MMD1

Biochemical tests	Interpretations
Indole	Negative
Methyl Red (MR)	Negative
Voges Proskauer (VP)	Negative
Citrate Utilization	Negative
Catalase test	Positive
Starch hydrolysis	Positive
Gelatine liquefaction	Positive
Nitrate reduction	Positive
Sugar Hydrolysis	Positive
Nitrogen utilization	Positive

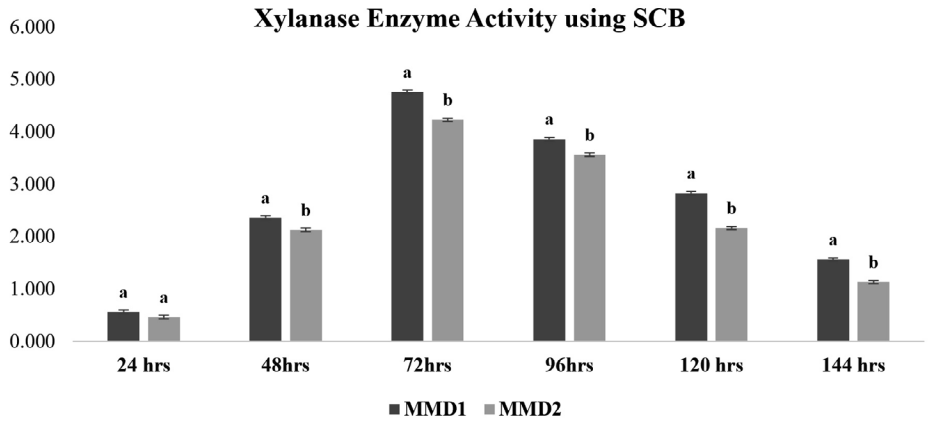

Figure 5. Xylanase enzyme activity using (Treated Sugarcane Bagasse) of isolated potent *Actinomycetes* (MMD1 *Streptomyces chartreusis*) at 7 days of incubation period. Letters denote significant variations using Tukey's test (P<0.05)

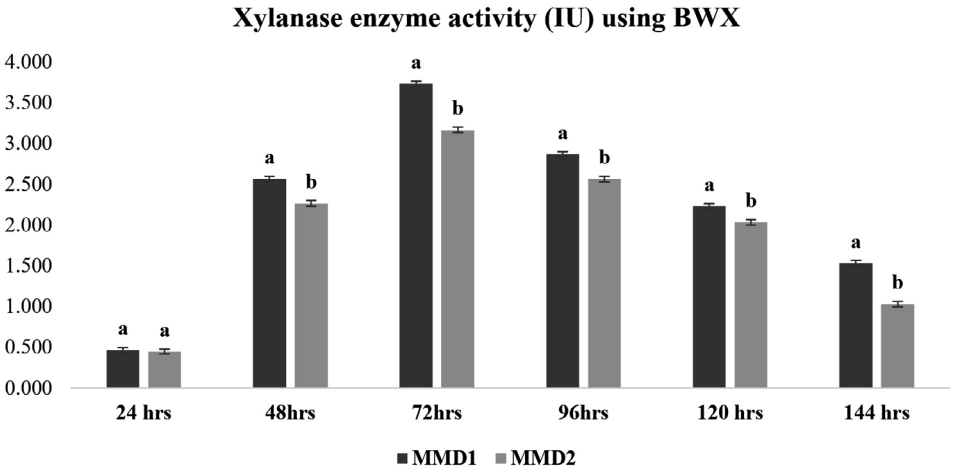

Figure 6. Xylanase enzyme activity using (Beech wood xylan) of isolated potent *Actinomycetes* (MMD1 *Streptomyces chartreusis*) at 7 days of incubation period. Letters denote significant variations using Tukey's test (P<0.05)

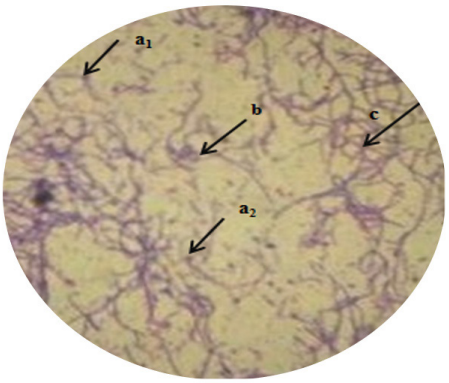

Figure 7. Light microscopic image of Gram staining view of *Actinomycetes* strain MMD1 under 100x (a1, a2- Position of spore; b-Coiling of aerial mycelia; c-Branched aerial mycelia)

features. The colony characteristics were found to be recorded medium size, irregular in shape, round margin, powdery and rough consistency, and texture, raised elevation respectively. The aerial mycelia were whitish grey, and no reverse pigmentation observed. Light microscopic view of Gram staining of MMD1 in Figure 7 has shown the characteristic properties of *Streptomyces* sp. by the presence of branched aerial mycelia, sporophore in varied spirals and terminal of spore (Figure 8). Table 3 has exhibited the positive observation of different biochemical tests, like catalase, starch hydrolysis, gelatine liquification and nitrate reductions, while negative for Indole, MR, VP, and citrate utilization respectively.

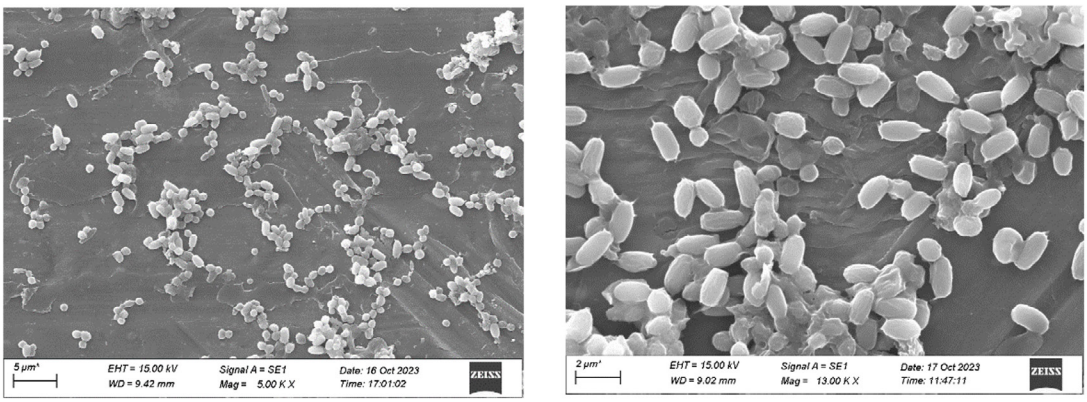

Figure 8. Scanning electron microscopy (SEM) of potent actinomycetes strain MMD1(A Magnification 5000x, Cell width 9.42 mm, B- Magnification 13000x, Cell width 9.02 mm)

Taxonomic characterization of *Streptomyces* sp. MMD1

In the present study of 16S rRNA sequence analysis a total data set of 16 nucleotide sequences with codon positions (1st+2nd+3rd+Noncoding) were recorded along with a total final dataset of 592 positions. The optimal tree with the sum of branch length = 4.91337812 is shown in Figure 9. The phylogenetic tree revealed that, the test isolate *Streptomyces* MMD1 had got 99.50% similarity with the type of strain *Streptomyces chartreusis* strain NBRC 12753 & confirmed the potential strain as *Streptomyces chartreusis*. NCBI GenBank accession number for the nucleotide sequence of the potential isolate is MT254830.

DISCUSSION

Streptomyces along with other actinomycetes, are commonly mentioned as efficient degraders of lignocellulosic biomass. In our study we have used Agro waste, broth culture, and synthetic medium-based rapid plate tests. The Two soil samples was collected from agricultural fields and dumping site for actinomycetes isolation and 30 actinomycetes colonies were isolated. Sukmawaty *et al.*[20] reported the 15 actinomycetes colonies isolated from the soil of Malindo pine forest, South Sulawesi. MMD1 able to produce higher zone of hydrolysis (36 mm) of endoglucanase compare with the Lenni

Figure 9. Phylogenetic tree of the potential isolate of *Streptomyces* sp MMD1 employing neighbour joining method

et al.[21] reported the zone of hydrolysis 2.93 mm of endoglucanase by *streptomyces* spp. The formation of a clear zone surrounding the colony showed actinobacteria's capacity to produce cellulase enzymes. Hydrolysing cellulose into glucose is possible with isolates that generate cellulase. High cellulase production was indicated by the large clear zone that appeared.[22] The process of Quantitative screening of Cellulase from actinomycetes strain MMD1 able to produce much higher levels of endoglucanase (5.4 IU/ml) and FPase (4.4 IU/ml) similar results were also found in the Mohanta *et al.*[23] they have reported 0.734±0.001 IU/mL for FPase and 1.381±0.024 IU/mL for CMCase. Budihal *et al.*[24] have reported under optimized condition sorghum stover was found to be better production of cellulase under submerge 38 IU/ml and solid state 44 IU/ml bioprocess. According to the all-parameter *Streptomyces* spp having good efficiency to degrade cellulose.

The process of quantitative screening of both the artificial beech wood xylan and the agrowaste (treated sugarcane bagasse) xylan fraction had demonstrated quite satisfactory xylanase activity at 72 hours of incubation, which is also thought to be the beginning of the sporulation phase and the production of secondary metabolites (enzymes) in actinomycetes. The same isolate MMD1 has demonstrated positive confirmation for xylanase comparable cellulase and is also suggested by Brito *et al.*[25], owing to the capacity of plant polymer (as a carbon source) degradation. Sporck *et al.*[26] support the increased xylanase activity using xylan residue that has been recovered from heat- or alkali-treated agricultural wastes like rice straw, sugarcane bagasse, etc. Actinomycetes strains MMD1 and MMD2 were found to have 3.7 IU/ml and 3.2 IU/ml of xylanase activity, respectively, when hemicellulose (a source of xylan) was subjected to consecutive acid, alkali, and heat treatment in sequence in the current study. This is in stark contrast to beechwood xylan (a synthetic source), which had 4.8 IU/ml and 4.2 IU/ml. While the Xiuting *et al.*[27] reported under optimized condition xylanase activity of 12 U/ml) with birch wood xylan and *Streptomyces chartreusis* strain L1105, which is quite higher than the present result of the study for Xylanase enzyme production.

Using a 100x magnification, isolates were examined, and Figure 7 shows the microscopic observations. Different forms of aerial mycelium were detected in isolates of Actinomycetes

under a microscope reported Dhanasekaran et al.[28] According to Maheswari *et al.*[29], the aerial mycelium of the genus *Streptomyces* is flexible, spiral, straight, or open loops. Spores of *Streptomyces* are non-motile. Initially smooth, *Streptomyces* colonies can develop into aerial mycelium that can have a variety of appearances, including floccose, granular, powdery, or velvety.[30] Our result shows the similarity with these *Streptomyces chartreusis* mycelium is straight, spores are non-motile, and colonies are powdery.

The isolate of actinomycete MMD1 was tentatively assigned to the genus *Streptomyces* based on morphological and biochemical characteristics for confirmation of genus identification by 16S rRNA Sequencing. current investigation to confirm the *Streptomyces* MMD1 species as *Streptomyces chartreusis* MMD1 matching (99.50) with *Streptomyces chartreusis* BRC12753T. In previous research by Wang *et al.*[31], this strain was also investigated for drought tolerance in sugarcane fields using *Streptomyces chartreusis* WZS021. With this strain, laccase, endo alpha 1,3 glucanase, etc. have all been previously examined.

CONCLUSION

Actinomycetes are recognised as a powerful source of a number of significant commercial enzymes, including cellulase and xylanase. *Streptomyces chartreusis* MMD1 (NCBI GenBank submission number MT254830) has been identified and characterised as an effective cellulose and xylan degrader after a series of experiments exploring significant lignocellulose degraders that started with five of the best actinomycetes strains and culminated in one strain, MMD1. A series of treatments on sugarcane bagasse resulted in 45% hemicellulose yield, and the use of xylanase activity that was good but not as effective as Beechwood xylan (BWX) was discovered. Future applications for the lignocellulolytic degradation property include bioethanol generation, solid waste management, consortia formation for increased degradation, comparative omics level investigations and bioremediation of contaminated sites· application studies in pulp, paper, and petroleum industries and so on.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

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