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Enrichment and Characterisation of Cellulose-Degrading Bacteria

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ABSTRACT

In the current research, a total of 10 bacterial isolates have been collected from municipal waste, vegetable waste, a dumping ground, a rice field, termite waste, cotton dyeing industry waste, forest waste, the soil under a coconut tree, dead decomposed plant waste, and sewage waste.Using soluble carboxy methyl cellulose degradation assays, the 10 bacterial strains have been isolated and examined for cellulolytic activity. In one percentage CMC agar medium, four strains, namely C9D, C9E, C9C, and C9B, efficiently digested cellulose, with a cellulolytic index of 35 *mm*, 25 *mm*, 17 *mm*, and 18 *mm*, respectively. The appearance of a clear zone was utilised to estimate cellulolytic activity. High-cellulolytic-activity isolates were identified up to the genus level.

Keywords: Carboxymethyl cellulose; Cellulolytic activity; Soil sample; 16S rRNA

NOMENCLATURE

CMC	Carboxymethylcellulose
w/v	weight/volume
^{0}C	Degree Celsius
µl	Microlitre
PCR	Polymerase chain reaction
OD	Optical density
DNSA	Dinitro salicylic acid
NCBI	National Centre for Biotechnology Information
BLAST	Basic local alignment search tool
TSI	Triple sugar iron medium
RT	Room temperature

1. INTRODUCTION

Cellulose, a complex polymeric carbohydrate, is most abundant biological component in both terrestrial and aquatic ecosystems, and it also accounts for a large portion of plant biomass.[1,2,3] In nature, cellulose is the major source of plant biomass, hence its degradation is crucial for carbon cycle. All polymers are broken down by various micro-organisms, resulting in synergic activity of enzymes.

From periods it has served as a construction material, involving wood and textile fibres like cotton or flax or paper type. Besides, it is a versatile initial material for chemical alterations, aiming at fabrication of a type of stable cellulose by-products utilised in numerous industries and household life.[4] In addition to plants, cellulose is produced by various bacterial species in nature. Cellulolytic microorganisms play a vital role in biosphere by reprocessing cellulose, as carbohydrate is major form produced by plants. Cellulose is a polymer which are insoluble, crystalline microfibrils extremely resistant towards enzymatic hydrolysis. As it forms an insoluble compound waste management is a major problem. If this type of waste is not degraded; it may get accumulate and cause serious environmental problems. Mostly waste is disposed oby burning which may increase the CO_2 level in the atmosphere and pollute the environment.

Plant biomass is the most common agricultural waste material, manifesting as stalks, stems, and husks. Because of its potential as an energy source, cellulose has sparked researchers' curiosity.[5] It is also one of the significant carbon sources on the earth, and it is generated by both terrestrial and aquatic plants.[6,7] Cellulases are needed for accurate cellulose breakdown.In nature, cellulose degradation and subsequent utilisation are crucial for the global carbon cycle to maintain a balance. Due to the obvious importance of cellulose as a renewable energy source, cellulose hydrolysis is a matter of some debate in academia and industry.[8-10] To date, the preponderance of cellulose degradation research has always been on identifying novel microbes capable of producing cellulase enzymes with high specific activity and efficiency.[5,11,12]

The majority of cellulose breakdown in nature is caused by microorganisms. The primary natural

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agents of cellulose degradation are fungi and bacteria.[13] Aerobic and anoxic haemophilic bacteria, actinomycetes, basidiomycetes, thermophilic bacteria and alkaliphilic bacteria, some protozoa are the principal cellulose-consuming organisms.[14-15] The bacteria responsible for cellulose breakdown enzymatically hydrolyse complex polymers in the first phase. Fungi exhibiting high cellulase activity are perhaps the most common, however bacterial cellulase production is acquiring prominence since bacteria have higher growth rates than fungi, making them ideal for cellulose enzyme synthesis.[19,20]

2. METHODOLOGY

2.1 Collection of Bacterial Samples

Ten soil samples (S1 to S10) had been collected from various sources comprising municipal waste, vegetable waste, a dumping ground, a rice field, termite waste, cotton dyeing industry waste, forest waste, the soil under a coconut tree, dead decomposed plant waste, and sewage waste. Selected soil samples were utilised as an inoculum for the isolation of cellulose-degrading bacteria.[16]

2.2 Media

The soil samples were inoculated in three different carbon-containing media; each media contained 0.5 % of carboxymethylcellulose (CMC) (w/v), 0.5 % of filter paper (w/v), and 0.5 % of absorbent cotton (w/v) as a source of carbon. Medium pH was maintained at 7 (Table 1).

Table 1. Composition of enrichment medium

Sources of carbon at 0.5% (w/v)		
Carboxmethylcellulose	**Filler paper**	**Absorbent cotton**
K_2HPO_4 0.1g	K_2HPO_4 0.1g	K_2HPO_4 0.1g
$MgSO_4$ 0.1g	$MgSO_4$ 0.1g	$MgSO_4$ 0.1g
Na_2SO_4 0.2g	Na_2SO_4 0.2g	Na_2SO_4 0.2g
$(NH_4)_2SO_4$ 0.2g	$(NH_4)_2SO_4$ 0.2g	$(NH_4)_2SO_4$ 0.2g
$CaCO_3$ 0.2g	$CaCO_3$ 0.2g	$CaCO_3$ 0.2g
CMC 0.5g	CMC 0.5g	CMC 0.5g
Distilled water 100 ml	Distilled water 100 ml	Distilled water 100 ml
pH 0.7	pH 0.7	pH 0.7

The sterile media was obtained by autoclaving medium (15 mins) at 15 lbs pressure and 121 °C. Growth medium chemicals used were purchased from HiMedia® Laboratories in India as Analytical Reagent (AR) grade chemicals. All the glassware used in the investigation was acid washed with HNO_3 (10 % v/v) and then cleansed with detergent and tap water.

2.3 Enrichment

In the present work, selective growing medium containing CMC as the only carbon source was utilised for enrichment.[17] Carboxymethylcellulose (CMC), water-soluble cellulose derivative, is a helpful substrate for the detection of cellulase enzyme producers and is degraded rapidly by microorganisms. Soil samples were enriched in three CMC-based media containing 0.5 % of CMC (w/v), 0.5 % of filter paper (w/v), and 0.5 % of cotton (w/v) as sources of carbon. Slight turbidity was observed in the enrichment media after 72 hours of incubation at room temperature ($28\pm2\ ^0C$), indicating the growth of cellulose degraders. The media were incubated for further enrichment. Enrichment was performed for 45 days at room temperature.

Concentration of carbon sources in the media was increased from 0.5 % to 2 % during the 45 days of enrichment. The inoculum used for enrichment at increased concentrations of the carbon sources was the growth obtained from previous enrichment on the same carbon sources. Next, 25 *ml* of the medium broth was inoculated using 0.25 *ml* of the earlier enriched broth. Each enrichment was carried out for 45 days of incubation at room temperature.

2.4 Isolation of Cellulolytic Bacteria

Utilisation of streak plate technique and incubation for 48 hours at room temperature, cellulolytic bacteria were isolated from enriched samples. Streak plating was used to purify the bacterial culture. Gram staining and analysis of colony characteristics were used to characterize the colonies morphologically. Four of the 10 isolated strains that produced the largest zone of clearance were selected and biochemically characterised. These pure cultures were stored at 4 °C for identification and cellulase production.

2.4.1 Media Composition of CMC Agar

Peptone 1.0 %, CMC 2.0 %. K_2HPO_4 0.2 %, Agar 2 %, $MgSO_4$, $7H_2O$ 0.03 %, $(NH_4)_2SO_4$ 0.25 %, Distilled water 100 *ml*, pH 7

2.5 Screening of Cellulolytic Bacteria

Isolated bacterial cultures were transferred to 2 % CMC agar plates and incubated for 48 hours. The presence of clear zones around colonies formed by bacteria indicates hydrolysis of cellulose. Largest clear zones formed by colonies of bacteria was the character chosen for identification and cellulase production.

The cultures that gave the largest zone of clearance were inoculated in 2 % CMC broth and incubated at room temperature overnight. The culture suspension was then inoculated (50 μl) in the 2 % CMC agar medium plate wells, bored by a 10 *mm* borer. Plate incubation was done at $25\pm2\ ^0$C for 48 hours. Saturated one percent Congo red was applied to visualize clearance zones on CMC media as it is a rapid and sensitive cellulolytic screening test (Fig. 2). A 1M NaCl solution was poured on the plates as a de-stain solution to give a clear contrast. Clear zones around bacterial colonies were observed, indicating cellulose hydrolysis.

2.5.1 Morphological Characterisation and Biochemical Characterisation:

Gram staining screening was conducted for all the bacterial isolates by observing them under the oil immersion lens of a compound microscope (Fig. 3). The strain C9D was chosen since it was showing the biggest zone of clearance. It was then identified morphologically and biochemically and was subjected to molecular identification by using 16S rRNA.

Methyl Red-Voges Proskauer test, indole test, catalase test, oxidase test, citrate utilisation test, triple sugar iron test, glucose and lactose fermentation test, xylose, cellulose, mannitol, sucrose fermentation test, urea hydrolysis test, H2S production test, motility test, and spore staining all were performed on the bacterial strains that produced cellulase enzymes.

The results were matched with Bergey's Manual of Systemic Bacteriology, the second edition. Because it is difficult to correctly identify microorganisms only based on its biochemical tests' further confirmation of the isolated strain to the genus level was done with 16S rRNA.

2.6 Molecular Identification by Using 16S rRNA

Genotypic characterisation of the bacteria through molecular analysis was achieved using the following procedures:

2.6.1 Total DNA Preparation

The genomic DNA from the potential experimental bacterial isolate was obtained using the QIAmp DNA Mini Kit of Qiagen, Germany. Pure culture bacterial isolates from the CMC plate were selected and mixed with 200 microlitre of phosphate buffered saline within a 1.5 millilitre of microcentrifuge tube followed by centrifugation at 13,500 rpm for 2 minutes for cell recovery. The manufacturer's protocol was used to harvest DNA from the bacterial suspension. For the bacterial isolates, DNA was extracted and saved at 2°- 8 °C until the usage.

2.6.2 Polymerase Chain Reaction

Bacterial universal primers (PSL 5′-AGGATTAGATACCCTGGTAGTCCA-3′ and XB4 5′-GTGTGTACAAGGCCCGGGAAC-3′), the 16S rRNA gene were used from extracted DNA for amplification using Polymerase Chain Reaction (PCR). 2X Kappa Taq Ready-mix (Kapa Biosystems, Massachusetts, US) was used to make a reaction mixture that also included KAPA Taq DNA Polymerase with 0.5 Unit per 25 *µl* reaction, KAPA Taq Buffer, dNTPs of 0.2 *mM* of each dNTP at 1X, magnesium chloride of 1.5 *mM* at 1X, and stabilizers. In an 18 µl reaction container, primers were combined to achieve a total concentration of 0.2 picomoles. Following thereafter, 2 microlitre of DNA template were added to achieve a total reaction volume of 20 microlitre. In a VeritiDx thermal cycler, the PCR reactions were carried out (Applied Biosystems, USA). The PCR program was as follows: denaturation of template DNA at 94 °C (2 minutes), continued by 30 cycles of the entire procedure.

Table 2. Condition for PCR reaction

PCR condition	Temp (◦C)	Time	
Initial Denaturation	95	5 mins	
Denaturation	95	30 sec	
Annealing	60	45 sec	30 cycles
Extension	72	30 sec	
Final Extension	72	7 mins	

3. ANALYSIS

3.1 Detection of Amplicon by Electrophoresis Analysis

Amplification was evaluated using 2.0 % agarose gel horizontal electrophoresis with Tris-borate EDTA (0.5 X TBE) buffer after the PCR reaction. To achieve a final concentration of 2.0 % agarose, agarose was solubilised in buffer (0.5X Tris borate EDTA) and heated in a microwave oven for about 2 minutes 30 seconds to melt the agarose, then permitted to cool down to about 50 °C. To facilitate visualisation under UV light, ethidium bromide (EtBr) was added to the liquified agarose. The agarose was poured into the tray that had already been set with the comb and allowed to be set. Following that, 8 *µl* of PCR product was combined with 2 *µl* of 6X gel loading dye (Takara, Japan) and loaded in specific wells of the gel. The amplicon size range was determined using a 100-bp DNA marker (Takara, Japan). The Endurotm Gds Gel Documentation System had been used to visualise the EtBr-stained DNA bands (Labnet International, Inc., Edison, NJ, USA).

3.2 Measurement of DNA Concentration and Purity

Biophotometer Plus was used to determine the DNA concentration (Eppendorf, Hamburg, Germany). To commence, a blank 3 *µl* of elution buffer in which DNA was eluted was used. Another 3 *µl* of the sample was added, and the DNA strength was estimated in *ng/µl* units. The software also displayed the OD260/OD280 ratio, which was obtained as 1.2, which reflected the sample's purity.

3.3 Sequences of Universal Primers

Two universal primers (PSL 5′-AGGATTAGATACCCTGGTAGTCCA-3′ and XB4 ′GTGTGTACAAGGCCCGGGAAC3′) was utilised to amplify 16S rRNA gene.

3.4 Sequence Analysis of 16S rRNA Gene

The PCR products were sequenced with Sanger sequencing method and BigDye™ Terminator v3.1 Cycle Sequencing Kit of Applied Biosystems, Foster City, CA, USA, on a thermal cycler under following conditions mentioned in table 2.0 below. Sequenced product was

precipitated/purified using 3 M Na acetate (pH 4.5) and 100 % alcohol in a post-sequencing purification process. The 3500 Dx Genetic Analyzer was used to run the purified sequenced product (Applied Biosystems, Foster City, CA, USA).

3.5 Sequence Submission and BLAST Search

The sequences were then submitted to BLAST in FASTA format to identify the organism. Once a query is submitted to BLAST, the search results return that several species in the queried sequence are homologous.

3.6 16S rRNA Gene Sequence Analysis

The ABI Genetic analyser had been used to follow the Sanger sequencing method.

C9D isolate 16S rRNA gene DNA sequence (FASTA format).

Thereafter, the query sequence was submitted to NCBI's nucleotide data bank and BLASTn was performed on it. The top homologous sequences from the NCBI database are displayed here, ranked by percentage identity and E value ($< 10^{-5}$). The FASTA format had been used to download all the subject sequences.

The isolate with the highest cellulolytic activity was subjected to molecular characterisation. QIAmp DNA Mini Kit has been used to extract DNA from isolated C9D. (Qiagen, Germany). The BigDye™ Terminator v3.1 Cycle Sequencing Kit was used to purify the amplified 16S rRNA PCR product of Applied Biosystems, Foster City, CA, USA. Purified sequenced product were later Sanger sequenced on an Applied Biosystems 3500 Dx Genetic Analyser (Foster City, CA, USA). Using the internet search tool BLAST, a continuous stretch of nucleotide sequence from the 16S rRNA gene was assessed by looking for similar sequences. The isolate C9D had the closest sequence identity of 99.0 % with Acinetobacter given by the NCBI database.

3.7 Cellulase Enzyme Production and Estimation of Cellulase Activity

The selected isolate C9D was grown in a medium containing 1 % CMC, a carbon source, to induce the production of cellulase, which are inducible enzymes synthesised by bacteria during their development on cellulosic materials. The fermentation medium was prepared, and the clear supernatant was acquired after centrifugation was utilised like a crude enzyme source.[8-9] Cellulase activities were primarily assessed using a reducing sugar assay i.e., the DNSA method.

4. RESULTS

4.1 Isolation of Cellulolytic Bacteria

Four of the 10 strains were reported to have higher cellulolytic activity and were allocated the identities as C9D, C9E, C9C, and C9B. Clear zone appeared surrounding bacterial colonies, indicating that cellulose was being hydrolysed (Fig. 1). In a submerged system, bacterial colonies with major clear zone were preferred for identification and cellulase production. The zone of clearance around the cellulose-digesting colonies indicates cellulolytic organisms.

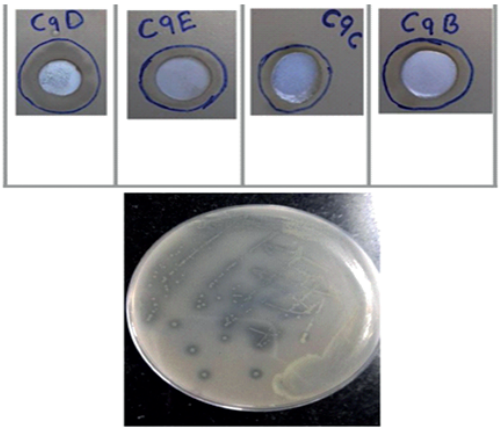


Figure 1. Zone of clearance around the cellulose digesting colonies.

4.2 Screening of the Cellulolytic Bacteria

Pure cultures have been transferred to the CMC agar plates for 48 hours for incubation. Around expanding bacterial colonies, a zone of clearing emerged, indicating cellulose hydrolysis.

4.3 Zone of Clearance on CMC Agar Media After Congo Red Test

Agar plates pre-inoculated with cultures in cups bored with cork borer dimension 10 *mm* were saturated with 1 % Congo red and allowed to remain at RT for 15 min. Plates were thoroughly de-stained with 1M NaCl to give a contrast. The zone of clearance surrounding the colonies indicated cellulose degradation. The use of Congo-Red as a cellulose degradation indicator provides the foundation for a rapid and sensitive cellulolytic bacteria screening test (Fig. 2). The following formula was used to determine the cellulolytic index (CI): CI = (well diameter – clear zone diameter)/well diameter. The CI of C9D, C9E, C9C, and C9B, respectively, was 35 *mm*, 25 *mm*, 17 *mm*, and 18 *mm*.

4.4 Morphological Characterisation and Biochemical Characterisation

Gram staining was used to determine the morphological characteristics of the four isolates and samples C9C, C9D, C9E, and C9B, revealed Gram positive bacilli, Gram negative coccobacilli, Gramp ositive bacilli, and Gram negative coccobacilli, respectively (Fig. 3). Compared to the other strains recovered, isolate C9D was determined to have the best cellulolytic activity.

As a result, the strain C9D was morphologically and biochemically recognised, as well as molecularly characterised using 16S rRNA. The following Fig. 4 and Table 3 illustrate the biochemical characterisation results for C9D.

4.5 Estimation of DNA Band Size

PCR product size was determined by agarose gel electrophoresis to be approximately 762 bp (Fig. 5).

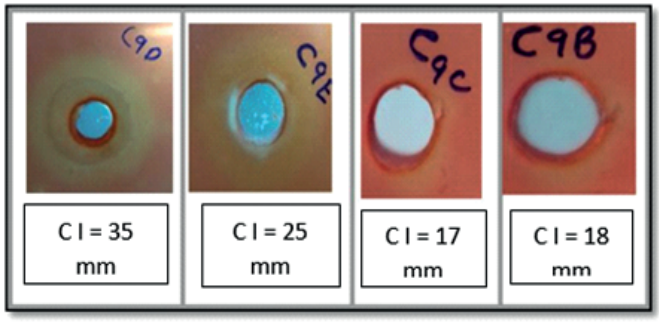


Figure 2. Clearance zone using Congo red.

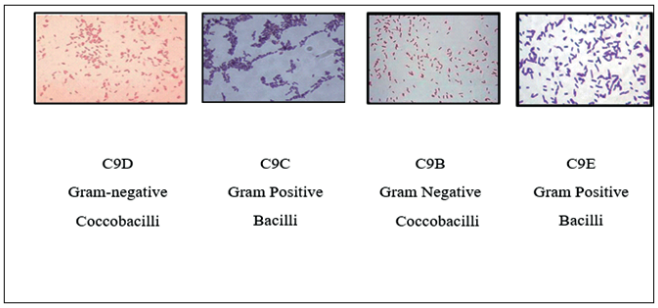


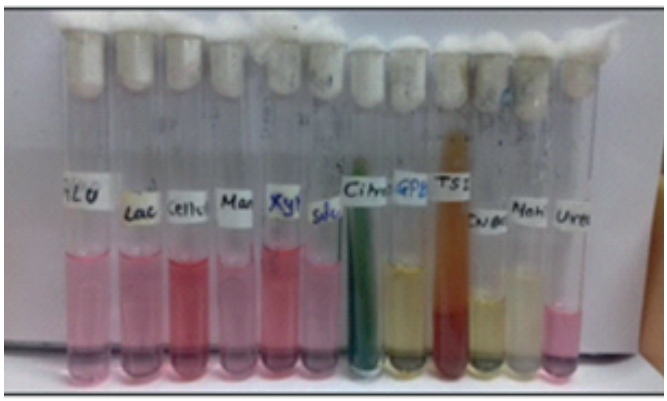
Figure 3. Gram staining of an isolated culture.

Table 3. Biochemical results for C9D

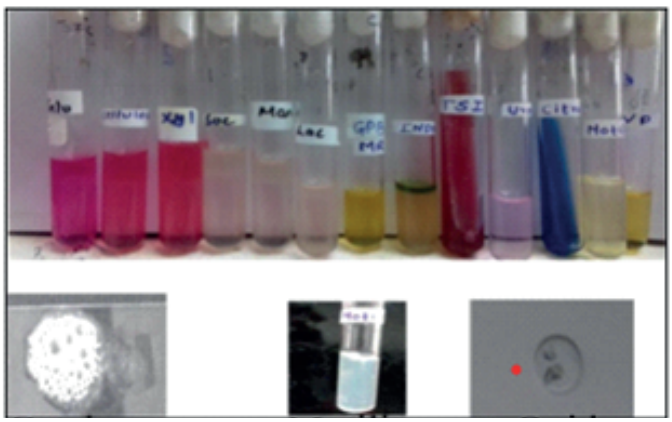
S. No.	Test	Results
1	Glucose	+
2	Xylose	+
3	Sucrose	-
4	Lactose	-
5	Mannitol	-
6	Cellulose	+
7	Methyl red	-
8	Voges Proskauer	-
9	Indole	-
10	TSI	**K/A/-/-**
11	Citrate	+
12	Motility	**Non-Motile**
13	Catalase	+
14	Oxidase	-
15	Urease	-
16	Gram Characteristics	**Gram Negative coccobacilli short rods**

Key: + = Positive; - = Negative; K/A/-/- = Alkaline slant, acidic butt, no gas, no H2S produced

Before Inoculation

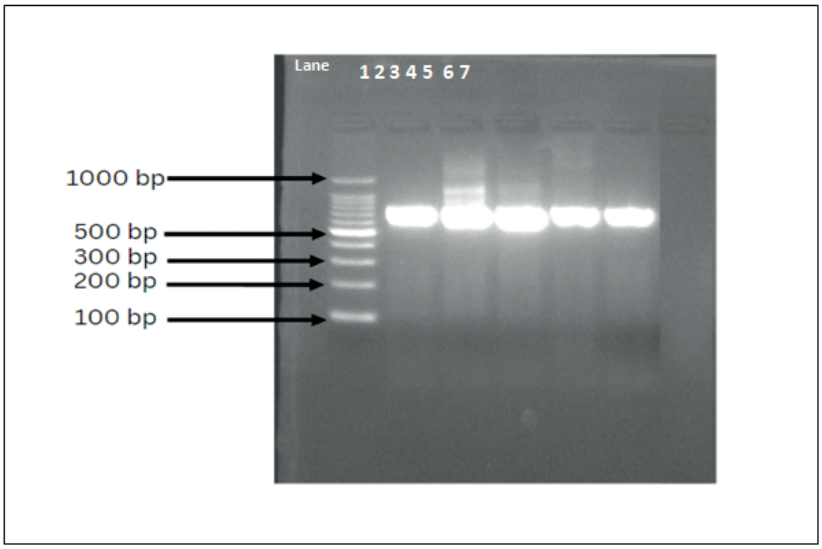


After Inoculation at 30 °C for 24 hrs



Catalase **Motility** **Oxidase**

Figure 4. Biochemical analysis of the selected strain C9D.



Lane 1: DNA Ladder 100bp
Lane 2: Isolate C9D
Lane 3: Isolate C9E
Lane 4: Isolate C9C
Lane 5: Isolate C9B
Lane 6: Positive Control
Lane 7: Reaction Control

Figure 5. PCR product on agarose gel electrophoresis.

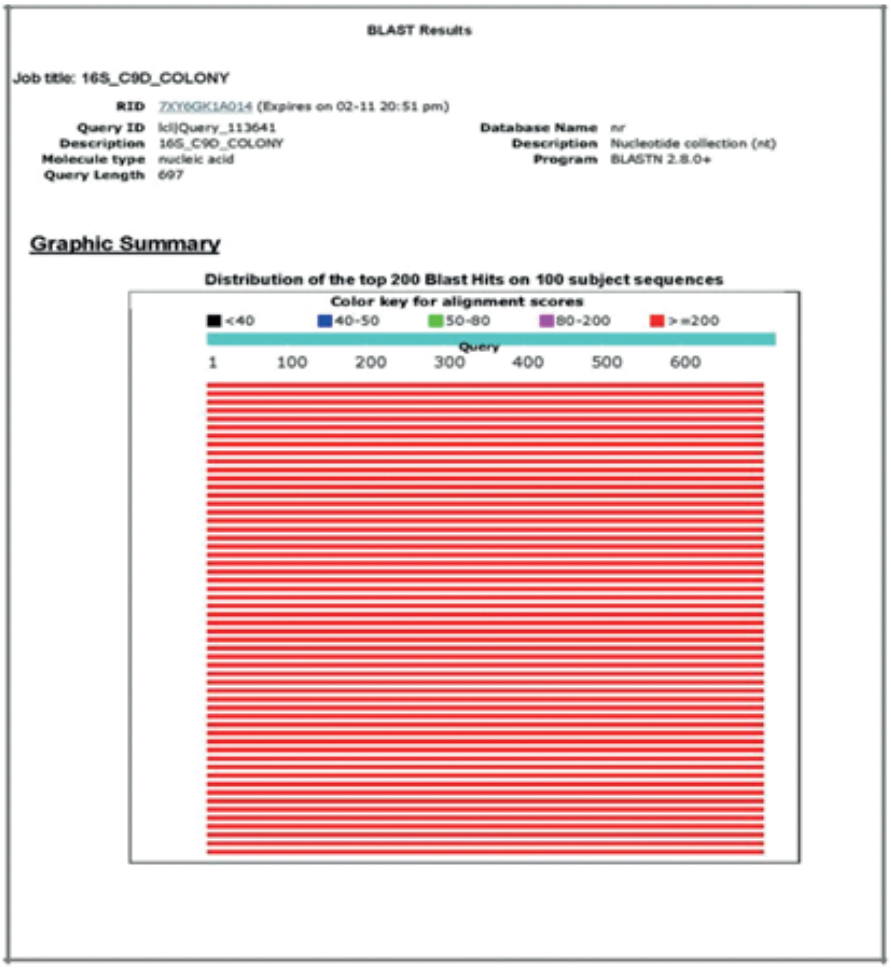


Figure 6. BLAST results.

4.6 Sequence Analysis of 16S rRNA Gene

BLAST Gene sequencing query results (Fig. 6) represent the top sequences in the NCBI list of Isolate C9D with the highest percentage of identity (99 %) and 0.0 E values belonging to the *Acinetobacter* sp. strain.

Thus, by using 16S rRNA, it was concluded that the isolated strain C9D, which showed major cellulolytic activity and clear zone when stained with Congo red and 1M NaCl solution, belonged to the genus *Acinetobacter*. The isolate C9D, which belongs to the genus *Acinetobacter*, was analysed for production of cellulase enzyme and estimation of cellulase activity.

4.7 Cellulase Enzyme Production and Estimation of Cellulase Activity

The isolate C9D had the maximum enzyme activity of 0.288 *U/ml*, which is the quantity of cellulase needed to release reducing sugar (1 *µmole/ml/minute*). The optimum temperature with maximum enzyme activity (0.485 *U/mL*) was determined to be 50 °C. To evaluate the optimum pH, enzyme assays were performed with buffer solution and various carbon sources, including filter paper, cotton, and CMC, at pH values ranging from 4 to 9. Using CMC as a substrate, optimum pH for maximum enzyme activity was determined to be between 6.5 and 7.

5. CONCLUSION

A total of 10 isolates were isolated from various soil samples in this study. Only four of the 10 isolates had cellulolytic activity, and their Gram characteristics were as follows: (C9D) gram-negative coccobacilli (short rods), (C9C) bacilli (rods)- gram positive, (C9B) gram positive bacilli (rods) and (C9E) gram negative coccobacilli (short rods). The cellulolytic activity was visualised using Congo red poured on agar media, and a clearance zone around the wells pre-inoculated with cellulolytic culture suspension indicated positive activity. The cellulolytic activity of the isolates with significant cellulolytic activity was subsequently investigated at the genus level. All four isolates were characterised morphologically and biochemically. The four isolates were identified as C9D-*Acinetobacter sp, C9C-Bacillus megaterium, C9E-Bacillus licheniformis,* and C9B-*Pseudomonas aeruginosa*, respectively. The results were compared to the Bergey's Manual of Systematic Bacteriology-second edition (Vol 2 and 3) to draw a conclusion.

Sanger sequencing methods were utilised to sequence amplified 16S rRNA PCR products, which were then processed on a thermal cycler. A 3500 Dx Genetic Analys er was used to analyse the pure sequencing product. The PCR product had a size of around 762 bp, as determined by agarose gel electrophoresis. The nucleotide data bank was subjected to BLASTn, and the query sequence was submitted to NCBI. For identification, the NCBI database's top homologous sequence with the highest percentage identity and E value ($< 10^{-5}$), as well as the top homologous sequence on the NCBI list, are considered. The strain C9D had the highest sequence identity (99.0 %) with the *Acinetobacter* sp. The isolate C9C had the highest sequence similarity to *Bacillus megaterium* (99.0 %). The isolate C9E had the highest sequence similarity (100 %) to *Bacillus licheniformis*. Finally, isolate C9B exhibited a sequence identity (99.0 %) closest to *Pseudomonas aeruginosa*.

Thus, by analysing 16S rRNA, it was concluded that the four isolated strains, namely, C9D, C9C, C9E and C9B, which showed the highest cellulolytic activity and largest clearance zones when stained with Congo red and 1M NaCl solution, were a species from genus *Acinetobacter*, *Bacillus* and *Pseudomonas*. Isolate C9D, which was a species from genus *Acinetobacter,* showed a larger clear zone than the other three isolates; consequently, was selected C9D for studies on cellulase enzyme production and estimation of cellulase activity.

The *Acinetobacter* isolate was used for cellulase production, and the activity of the crude enzyme produced was assayed. The DNSA method was used to evaluate cellulase activity using a reducing sugar test. The *Acinetobacter* isolate had the greatest enzyme activity of 0.288 *U/ml*, where one unit (U) is specified as the quantity of cellulase required to liberate one micromole of reducing sugar per millilitre per minute. The optimum temperature for the *Acinetobacter* isolate was 50 °C, and maximum enzyme activity was 0.485 *U/ml*. To determine optimum pH, enzyme assays were performed with several buffer solutions and carbon sources, such as filter paper, cotton, and CMC, at pH ranging from 4 to 9. The optimum pH of the *Acinetobacter* isolates with highest enzyme activity was 6.5 to 7 when CMC was the substrate.

The current research is part of a larger study into biogas production. In a small biodigester setup, the isolates from this investigation were evaluated for biogas production, and the generated biogas was quantified after qualitative analysis.[18] Different substrates, namely wood pulp, rice straw, and dead leaves, were used as sources of carbon for biogas production. A culture suspension, called consortium, was prepared using all four isolates together (*Acinetobactersp, Bacillus licheniformis, Bacillus megaterium,* and *Pseudomonas aeruginosa)*.

The biogas obtained was analysed qualitatively for the content of methane and other gases through chromatography. The use of wood pulp as a substrate resulted in the highest methane content. Furthermore, a comparison between the biogas produced by pure culture of the *Acinetobacter* species and that produced by the consortium showed that the *Acinetobacter* species is the largest contributor to the cellulolytic activity of the consortium.

The cellulase-producing bacteria identified in present work were species from genus *Acinetobacter, Bacillus* and *Pseudomonas. Acinetobacter sp* showed the highest cellulolytic activity, and it efficiently degraded cellulosic material in 10–15 days; consequently, it may be used

for waste management and for biogas production. The sludge produced after the emission of biogas can be utilised as manure for plants. Hence, the clearance of waste from the environment, production of biogas, and creation of biofertilisers can all be achieved through the present study.

REFERENCES

1. Demura, T. & Ye, Z.H. Regulation of plant biomass production. C*urrent Opinion Plant Biolo.*, 2010, **13**(3), 298-303. doi: 10.1016/j.pbi.2010.03.002.
2. Shankar, T. & Isaiarasu, L. Cellulase production by Bacillus pumilus EWBCM1 under varying cultural conditions. *Middle-East J. Scientific Res.*, 2011, **8**(1), 40-45.
3. Ahmad, R.A.; Bashir, S. & Tabassum, R. Evaluation of cellulases and xylanases production from Bacillus spp. isolated from buffalo digestive system. *KafkasÜniversitesiVeterinerFakültesi Dergisi*, 2019, **25**(1), 39–46.
4. Klemm, D.; Philpp, B.; Heinze, T.; Heinze, U. & Wagenknecht, W. Comprehensive cellulose chemistry. Volume **1**: *Fundamentals Analytical Methods*. Wiley-VCH Verlag GmbH, 1998.
5. Li, F.; Xie, Y.; Gao, X.; Shan, M.; Sun, C.; Niu, Y. D. & Shan, A. Screening of cellulose degradation bacteria from min pigs and optimisation of its cellulase production. *Electronic J. Biotechnol.*, 2020, **48**, 29-35. doi: 10.1016/j.ejbt.2020.09.001.
6. Nowak, J.; Florek, M.; Kwiatek, W.; Lekki, J.; Chevallier, P.; Zięba, E.; Mestres, N.; Dutkiewicz, E.M. and Kuczumow, A., Composite structure of wood cells in petrified wood. *Mater. Sci. Eng.*: C, 2005, **25**(2), 119-130. doi: 10.1016/j.msec.2005.01.018.
7. Merbach, W.; Mirus, E.; Knof, G.; Remus, R.; Ruppel, S.; Russow, R. & Schulze, J. Release of carbon and nitrogen compounds by plant roots and their possible ecological importance+. *J. Plant Nutrition Soil Sci.*, 1999, **162**(4), 373-383.
8. Bhat, M.K. Cellulases and related enzymes in biotechnology. *Biotechnol. Advances*, 2000, **18**(5), 355-383. doi:10.1016/S0734-9750(00)00041-0 PMid:21531609.
9. Chen, X.; Cheng, W.; Li, S.; Tang, X. & Wei, Z. The "quality" and "quantity" of microbial species drive the degradation of cellulose during composting. *Bioresource Technol.*, 2021, **320**, 124425. doi: 10.1016/j.biortech.2020.124425.
10. Srivastava, N.; Srivastava, M.; Mishra, P.K.; Ramteke, P.W. & Singh, R.L. New and future developments in microbial biotechnology and bioengineering: From cellulose to cellulase: Strategies to improve biofuel production. Elsevier. 2019 eds. eBook ISBN: 9780444642240 Paperback ISBN: 9780444642233.
11. Reczey, K.; Szengyel, Z.S.; Eklund, R. & Zacchi, G. Cellulase production by T. reesei. *Bioresource Technol.*, 1996, **57**(1), 25-30. doi:10.1016/0960-8524(96)00038-7.
12. Heck, J.X.; Hertz, P.F. & Ayub, M.A. Cellulase and xylanase productions by isolated Amazon Bacillus strains using soybean industrial residue based solid-state cultivation. *Brazilian J. Microbiol.*, 2002, **33**, 213-218. doi:10.1590/S1517-83822002000300005.
13. Huang, C.; Feng, Y.; Patel, G.; Xu, X. Q.; Qian, J.; Liu, Q. & Kai, G.Y. Production, immobilisation and characterisation of beta-glucosidase for application in cellulose degradation from a novel Aspergillus versicolor. *Int. J. Biolog. Macromolecules*, 2021, **177**, 437-446. doi: 10.1016/j.ijbiomac.2021.02.154.
14. Imran, M.; Anwar, Z.; Irshad, M.; Asad, M.J. & Ashfaq, H. Cellulase production from species of fungi and bacteria from agricultural wastes and its utilisation in industry: A review. *Advances in Enzyme Res.*, 2016, **4**(2), 44-55. doi: 10.4236/aer.2016.42005.
15. Kaushal, R.; Sharma, N. & Tandon, D. Cellulase and xylanase production by co-culture of Aspergillus niger and Fusarium oxysporum utilizing forest waste. *Turkish J. Biochem./Turk BiyokimyaDergisi*, 2012, **37**(1). doi:10.5505/tjb.2012.43434.
16. Wilson, D.B. Microbial diversity of cellulose hydrolysis. *Current Opinion Microbiol.*, 2011, **14**(3), 259-263. doi: 10.1016/j.mib.2011.04.004 PMid:21531609.
17. Wright, S. The importance of soil microorganisms in aggregate stability. *In* North Central Extension Industry Soil Fertility Conference Proceedings, 2003, November.
18. Gohel, H.R.; Contractor, C.N.; Ghosh, S.K. & Braganza, V.J. A comparative study of various staining techniques for determination of extracellular cellulase activity on Carboxy Methyl Cellulose (CMC) agar plates. *Int. J. Curr. Microbiol. App. Sci.,* 2014, **3**(5), 261-266. ISSN: 2319-7706 http://www.ijcmas.com.
19. Satari, B.; Karimi, K. & Kumar, R. Cellulose solvent-based pretreatment for enhanced second-generation biofuel production: A review. S*ustainable Energy & Fuels*, 2019, **3**(1),11-62. doi: 10.1039/C8SE00287H.
20. Lee, S.M. & Koo, Y.M. Pilot-scale production of cellulase using Trichodermareesei rut C-30 fed-batch mode. *J. Microbiol. Biotechnol.,* 2001, **11**(2), 229-233. 1017-7825(ISSN).
21. Kubicek, C.P.; Messner, R.; Gruber, F.; Mandels, M. & Kubicek-Pranz, E.M. Triggering of cellulase biosynthesis by cellulose in Trichoderma reesei. Involvement of a constitutive, sophorose-inducible, glucose-inhibited beta-diglucosidepermease. *J. Biological Chemistry*, 1993, **268**(26), 19364-19368.

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