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Isolation and Characterization of Cellulase-producing Bacteria from Sugar Industry Waste

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Abstract: Cellulases are inducible enzymes that are synthesized by a large number of microorganisms during their growth on cellulosic materials. This study focuses on the isolation and screening of cellulase-producing bacteria from sugar industry waste (molasses) and characterization by morphological and biochemical analysis. Further, purification of cellulase was carried by ammonium sulfate precipitation and followed by column chromatography and molecular weight determined by SDS-PAGE. The isolated bacterial strains were grown on carboxymethyl cellulose (CMC) agar plate at various optimum conditions like pH, temperature, incubation period, carbon and nitrogen sources and substrate concentration. Three isolated strains showed clear hydrolyzing zone on agar plates containing CMC agar after Congo-red staining were identified as cellulase-producing bacteria. Based on cultural, morphological, and biochemical characteristics, the isolated strains were identified as *Paenibacillus sp*., *Aeromonas sp.,* and *Bacillus sp*. Among the isolated strains, *Paenibacillus sp*. showed the capability for highest cellulase production (0.89 µmol ml^{-1} min^{-1}) at optimal pH 7.0 and 40°C temperature on 24 hour of the incubation period at 1% CMC substrate concentration and was selected for further cellulase purification. In the final step of cellulase purification, the specific activity, purification fold and recovery were 1720 U/mg, 9.74 and 35.6%, respectively. The molecular weight of the purified enzyme was determined 66.9 kDa and the enzyme showed a high specificity to CMC substrate. The bacterial strains present in molasses have the potential for cellulase production. Substrate specificity of the purified cellulase indicates it to be an endo-β-1, 4-glucanase. The cellulase produced from the selected strain may benefit for industrial application.

Keywords: Cellulose, Cellulase, Molasses, Bacteria, Isolation, Optimization, Purification and Characterization

1. Introduction

Cellulose, a homopolymer of D-glucose units linked by β-1, 4 bonds, is the most abundant organic polymer on the earth [1-3]. It is an important structural component of the primary cell wall of green plants and potential renewable resources in the nature [4]. Therefore, it has raised an important economic interest to build up systems for effective management and use of cellulose-containing industrial wastes as inexpensive carbon sources [5]. Both bacteria and fungi have been known to produce a number of cellulases for biological degradation of cellulose [6]. Cellulases hydrolyze the cellulose into glucose molecule through the catalytic actions of 3 types of enzymes, namely endo-β -1, 4 glucanase or carboxymethyl cellulase (CMCase), β-D-glucosidase, and cellobiohydrolase [6, 7].

Cellulases have attracted much interest because of the diversity of their applications. Cellulases are used in the various industrial process, including textile and laundry, food, feed, leather, pulp and paper [4, 8-11]. In fiber modification, biomass fermentation and pharmaceutical sectors cellulases are also widely used [5, 12]. Application of cellulases in like these industries queries the highly stable cellulases that can work at increased pH and high temperature [9]. In recent years, researchers have been paying attention to various bacteria that produce cellulases because of their high growth rate and resistance to an extreme environment when compared to fungi and has a good potential in cellulase production. Bacteria belonging to the genera *Clostridium*, *Cellulomonas*, *Bacillus*,

Ruminococcus, *Bacteroides*, *Acetovibrio*, *Streptomyces*, and *Paenibacillus* have been found to produce different types of cellulase when incubated under aerobic or anaerobic conditions [6]. Cellulases from various sources have distinctive features as they exhibit optimum pH and temperature, solubility depending on the amino acid composition [13]. Thermal stability and exact substrate specificity may also vary with their origin. Numerous microorganisms that are able to degrade cellulose have been isolated, screened and identified. However many studies have put more emphasis on cellulose degrading bacteria from agricultural, industrial and municipal wastes because the cellulases that they produced are easy to extract, and some of the bacterial cellulases have been used as commercial cellulase [14].

Although, a few number of bacteria can produce considerably large-scale bioactive compounds that are able to hydrolysis of crystalline cellulose *in vitro*. The key step of an industrial fermentation process development is the isolation of strains that are capable of producing the targeted products in commercial yields. This approach includes an extensive screening to test a big number of bacterial strains for identifying the potential cellulase producer with having some novel properties. Among several types of industrial wastes, the sugar industry wastes (molasses) are generally cellulosic in nature and the microorganisms available there have the capability to degrade large cellulose molecule into smaller monosaccharide units for their usual growth and development. Presence of some species of bacteria, yeast, and fungi in sugar industry wastes has been reported in a recent study [15]. This study aimed to isolate and screen the cellulase producing-bacteria from sugar industry wastes (molasses) and purify and characterize the cellulase produced by the isolated bacterial strains.

2. Materials and Methods

2.1. Sample Collection, Isolation, and Screening for Cellulolytic Bacteria

The samples (molasses) used in this study were collected from Rajshahi Sugar Mills located in Harian, Rajshahi, Bangladesh with discussing the authority maintaining the national guidelines. A serial dilution was made by taken 1 gm of each sample. Then the sample (diluted up to 10^{-6}) was taken into *Luria-Bertani medium (LB).* From each dilution 100 µl of the solution was shifted into 1 L of carboxymethyl cellulose (CMC) agar plates containing 0.5 g of KH_2PO_4, 0.25 g $MgSO_4$, 0.25 g cellulose and 2 g gelatin for increased the bacterial activity. Then the agar plates were incubated at 37°C for 24 h and stored at 4°C [16]. After incubation, the plates were submerged with 0.1% (w/v) Congo-red solution for 15 min and discolored with 1 M NaCl solution. The degradation zone around the bacteria indicated that the strains could hydrolyze CMC. Enzyme activity of the isolated strains was confirmed by several types of assay like iodine solution, Congo-red, and filter-paper degradation assay [17-18]. Those bacteria that formed clear zones on CMC plates were subject to filter-paper degrading activity assay.

2.2. Bacterial Identification by Morphological and Biochemical Characterization

The isolated strains were identified based on morphological examination and biochemical characterizations.

2.2.1. Morphological Characterization

The agar plates were tested by microscopic viewing and Gram staining technique for the identification of isolated strains [19]. Gram staining technique was applied to differentiate between gram-positive and gram-negative bacteria. The isolated strains were divided into two groups based on whether they retain or lose crystal violet color after iodine and alcohol treatment and counterstaining with safranin.

2.2.2. Biochemical Characterization

The parameters investigated for biochemical characterizations were Carbohydrate fermentation test, Catalase test, Motility test, Congo-red test, Methyl-red test, H_2S production, Citrate utilization test, and Voges-Proskauer test by standard protocols [20].

2.3. Optimization of Different Culture Parameters for Cellulase Production

Different culture conditions such as pH, temperature, incubation time, substrate concentration, and carbon sources were optimized for the isolated bacterial strains. The selected strains were placed in CMC media and tested at different parameters. The influence of the parameters on cellulase production was estimated by measuring the enzyme activity at several pH levels ranging from 5 to 11 and temperature varying from 20°C to 45°C and incubation period ranging from 24 to 96 h at 37°C. The CMC substrate concentrations from 0.5 to 2% were used for getting the highest enzyme production. Carbon and nitrogen sources were replaced by several substances. Carbon sources included xylose, birchwood xylan, starch, carboxymethyl cellulose, oat spelt xylan, lactose, rich bran xylan, cellobiose, wheat bran, chitin and xylan. Applied nitrogen sources were yeast extract, peptone, tryptone, urea, casein, asparagines, ammonium, phosphate, potassium nitrate and sodium nitrite. Different type of metal salts like NaCl, KCl, $CaCl_2$, $MnCl_2$ and $MgCl_2$ were used to look their effects on bacterial growth and cellulase production. All factors effects on enzyme production were estimated by measuring cellulase activity.

2.4. Enzyme Activity Assay

2.4.1. Preparation of Crude Enzyme

For secondary screening and cellulase production, the bacterial isolates were incubated overnight at 37°C that exhibited a maximum zone of hydrolysis was cultured in *Luria-Bertani medium.* The cultures were then centrifuged for 15 min at 8000 rpm and the clear supernatant was used as a source of crude enzyme solution. The crude enzyme solution (1500 ml) was stored and applied for enzyme purification and measurement of enzyme activity.

2.4.2. DNS Method

Cellulase activity was determined by 3,5-dinitrosalicylic acid (DNS) method through measuring of the amount of reducing sugars released from CMC dissolved in 1N citrate buffer, pH 5.0 [21]. Briefly, 100 µl of crude enzyme and 1 ml of citrate buffer were added into 1 ml of CMC substrate solution. Then the reaction mixture was incubated at 45°C for 30 min. DNS reagent was added to stop the reaction [21]. The treated samples were boiled for 10 min and cooled in water to stabilize the color. The optical density (OD) was determined at 540 nm. Cellulase activity was measured based on glucose standard calibration curve. One unit of enzyme activity was defined as the amount of enzyme that liberated 1µmol of glucose per min [5].

2.4.3. Filter-paper Activity Assay

Those bacterial isolates showing cellulase activity on CMC plates were further screened for quality of cellulase activity by transferring 100 µl of overnight culture to 5 mL of basal salts media ($NaNO_3$ 2.5g; KH_2PO_4 2g; $MgSO_4$ 0.2g; NaCl 0.2g; $CaCl_2 \cdot 6H_2O$ 0.1g in a liter) with a 70 cm wide strip of filter paper (Whatman filter paper no.1) and two drops of 10 nM glucose in glass culture tubes. The cultures were incubated for 7 days and viewed daily for visual evidence of filter paper degradation. Those strains capable of completely degrading the filter paper were selected for further quantitative analysis. This test was done in both aerobic and oxygen-limited conditions by sealing the tubes with parafilm. Filter-paper cellulase (FPase) activity was determined by DNS method. The reaction system was prepared as follows: 500 µL enzymes mixed with 50 mg of filter paper (1.0 x 6.0) in 1 mL buffer for determining the FPase activity. Sodium citrate buffer (100 mM) was used for dissolving or re-suspending the substrate. The mixture was incubated for 1 h. The reaction was then stopped by adding 3 mL of DNS reagent. The mixture was heated in boiling water for 5 min for color development. Then the absorbance was determined at 540 nm.

2.5. Purification of Cellulase

2.5.1. Ammonium Sulfate Precipitation

1500 ml of crude enzyme was lead to 80% saturation with ammonium sulfate. The mixture was kept at 4°C in a magnetic stirrer for overnight. For further purification, the mixture was then centrifuged and the pellet was dissolved in 50 mM of sodium phosphate buffer saline maintaining pH 7.0. Dialysis of partially purified enzyme was done against the phosphate buffer.

2.5.2. DEAE-cellulose Column Chromatography

60 ml of enzyme solution was loaded to the Diethylaminoethyl cellulose (DEAE-cellulose) column which was equilibrated with Tris-HCl buffer (10 mM) at pH 7.0. The unbound fraction was used for determining cellulase activity at 540 nm and measuring protein concentration at 280 nm. The eluted fraction exhibiting maximum activity was pooled and stored for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

2.5.3. CM-cellulose Column Chromatography

The unbound solution (55 ml) from DEAE was loaded to the CM-cellulose column and equilibrated with 10 mM Tris-HCl buffer at pH 7.0. Then proteins were eluted gradually by increasing the NaCl gradient (0.0 to 0.3M) with using same buffer solution. The eluted fractions having cellulase activity (25 ml) were collected and kept for enzyme activity assay and electrophoresis.

2.6. Protein Concentration and Molecular Weight Determination

Protein concentrations in crude solution were measured by Lowry's Method with bovine serum albumin (BSA) as a reference marker [22]. The molecular mass of the purified cellulase was measured using SDS-PAGE [23]. In SDS-PAGE, standard proteins marker was applied next to the purified protein, followed by the dialyzed and crude sample.

2.7. Substrate Specificity Determination

The hydrolytic ability of purified cellulase (from *Paenibacillus sp.*) against CMC, cellulose, xylose, lactose, oat spelt xylan, rich bran xylan, and wheat bran xylan was measured for assessing the specificity to substrates [16].

3. Results

3.1. Isolation, Screening and Identification of Cellulase-Producing Bacteria

Three bacterial strains marked as C_1, C_2 and C_3 were isolated from molasses and cultured in CMC agar media. These strains showed hydrolyzing zones on agar plates after Congo-red staining (Figure 1).

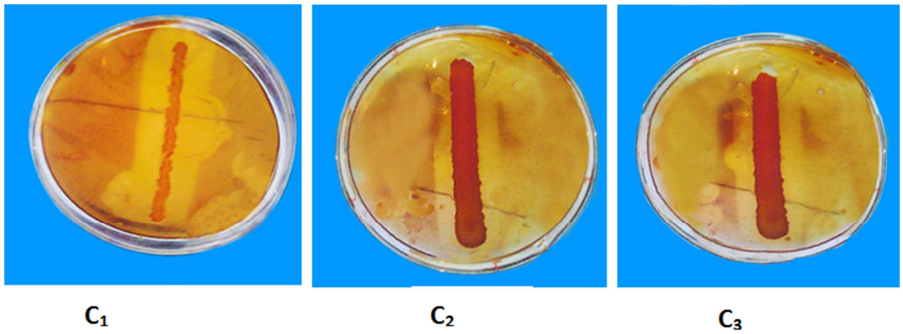

Figure 1. *Congo red test for confirmation of Cellulolytic activity by the isolated bacterial strains: C_1 (Paenibacillus sp.), C_2 (Bacillus sp.) and C_3 (Aeromonas sp.).*

A microscopic observation indicated that the C_1 and C_2 strain were rod-shaped and C_3 strain was short rod-shaped bacteria (Figure 2). In Gram staining technique, the two isolates C_1 and C_2 showed a minimum zone of clearance and were found to be gram-positive and C_3 showed the maximum zone of clearance and was found to be gram-negative bacteria. Physiological and biochemical characteristics for the bacterial isolates are presented in Table 1.

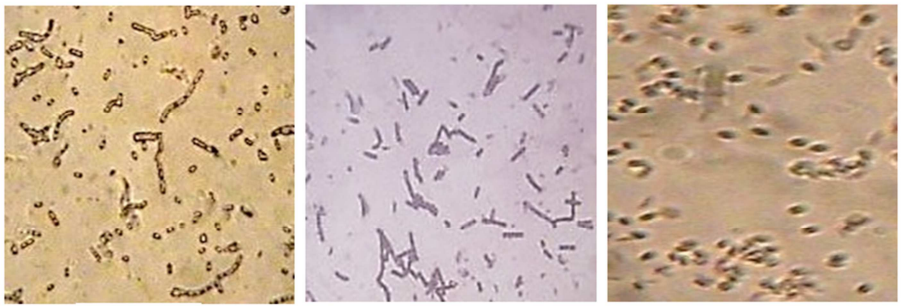

Figure 2. *Microscopic view of gram-positive (C_1, Paenibacillus sp. and C_2, Bacillus sp.) and gram-negative (C_3, Aeromonas sp.) rods.*

Table 1. *Physiological and biochemical characteristics of the bacterial isolates.*

	C_1 strain	C_2 strain	C_3 strain
Morphology	Rod	Rod	Short rod
Gram Staining	+	+	-
Motility test	+	+	+
Glucose Fermentation	+	+	+
Galactose Fermentation	+	+	+
Sucrose Fermentation	+	+	+
Catalase test	+	+	+
Citrate Utilization	-	+	+
Methyl-Red test	+	-	+
Voges-Proskauer test	-	+	-
H_2S Production test	-	-	-

According to morphological and biochemical tests, the selected strains were identified to be *Paenibacillus sp.* (C_1), *Bacillus sp.* (C_2), and *Aeromonas sp.* (C_3), respectively. In filter-paper degradation test, these strains were observed to complete degrade the filter-paper which indicates the cellulolytic properties of the isolated bacterial strains (Figure 3).

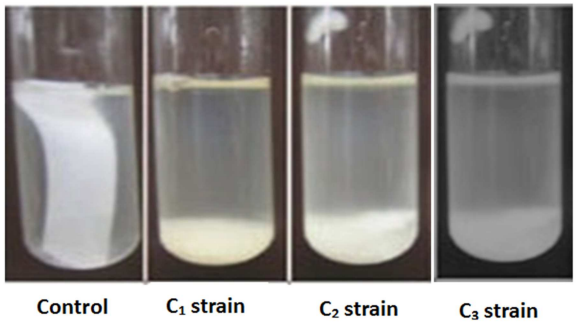

Figure 3. *Qualitative test for filter paper degradation by the isolated bacterial strains: C_1 (Paenibacillus sp.), C_2 (Bacillus sp.) and C_3 (Aeromonas sp.).*

3.2. Optimization of Culture Conditions and Enzyme Production

The optimized culture conditions and cellulase production for the isolated strains are presented in Table 2. The bacterial growth for strain C_1 (*Paenibacillus sp.*) was better in neutral pH 7.0 and exhibited maximum enzyme activity (0.89 µmol ml^{-1} min^{-1}), while C_2 and C_3 strains showed moderate enzyme activity (0.70 and 0.38 µmol ml^{-1} min^{-1}) at pH 6.0 and pH 7.0, respectively. The optimum temperature for the growth of all strains was found to be 40°C. Cellulase production by C_1 strain was gradually increased and showed the maximum enzyme activity (0.98 µmol ml^{-1} min^{-1}) on 24 h of the incubation period and other two strains (C_2 and C_3) showed moderate enzyme activity (0.77 and 0.67µmol ml^{-1} min^{-1}) on 36 h of the incubation time and gradually declined afterward. The C_1 isolate produced the large amount of cellulase and exhibited the highest activity (0.97 µmol ml^{-1} min^{-1}) at 1% of CMC, whereas, C_2 and C_3 isolates showed maximum activity (0.72 µmol ml^{-1} min^{-1} and 0.35 µmol ml^{-1} min^{-1}) at 1.5% and 2% of CMC substrate concentration, respectively. Among various types of carbon sources, CMC was the prominent source for the bacterial growth and enzyme production. As a nitrogen source, peptone was the most suitable one for bacterial growth and better cellulase production and among different types of metal salts, $CaCl_2$ showed the profound effect on cellulase production for the strain C_1 (data not shown). The optimum temperature and pH of the purified cellulase from C_1 strain was same (40°C and pH 7.0) as observed early culture conditions.

Table 2. *Optimaization of culture conditions for the isolated bacterial strains.*

Different parameters	Different values	Enzyme Activity (µmol ml^{-1} min^{-1})		
		C_1	C_2	C_3
pH	5	0.55	0.47	0.20
	6	0.75	0.70	0.25

Different parameters	Different values	Enzyme Activity (µmol ml^{-1} min^{-1})		
		C_1	C_2	C_3
	7	0.89	0.60	0.38
	8	0.70	0.55	0.30
	9	0.55	0.35	0.22
	10	0.21	0.12	0.09
	11	0.11	0.09	0.07
Temperature (ºC)	20	0.25	0.20	0.09
	25	0.45	0.30	0.15
	30	0.70	0.50	0.19
	35	0.80	0.60	0.29
	40	0.93	0.70	0.39
	45	0.40	0.24	0.15
Incubation period (h)	12	0.67	0.57	0.23
	24	0.98	0.60	0.57
	36	0.88	0.77	0.67
	48	0.55	0.33	0.22
	60	0.17	0.09	0.07
	72	0.09	0.05	0.04
	96	0.07	0.03	0.02
Substrate concentration (%)	0.5	0.60	0.45	0.10
	1.0	0.97	0.60	0.19
	1.5	0.50	0.72	0.25
	2.0	0.30	0.20	0.35
Carbon sources	Xylose	0.40	0.40	0.05
	Birch wood xylan	0.42	0.35	0.12
	Oat spelt xylan	0.41	0.44	0.14
	Rice bran xylan	0.42	0.37	0.19
	Starch	0.35	0.32	0.19
	Carboxymethyl cellulose	0.85	0.67	0.29
	Cellobiose	0.80	0.55	0.25
	Lactose	0.12	0.09	0.08
	Wheat bran xylan	0.38	0.21	0.18
	Chitin	0.05	0.04	0.03

3.3. Cellulase Purification

Summary for the purification of cellulase is shown in Table 3. The unrefined enzyme extract consists 301 mg of protein exhibited 81790 U in terms of entire activity.

Table 3. *Summary of the purification of cellulase from crude sample of Paenibacillus sp.*

Procedure	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (Fold)	Recovery (%)
Enzyme crude extract	301	81790	272	1.00	99.9
Ammonium sulfate saturation (80%)	160	75125	470	1.72	91.9
DEAE-cellulose column chromatography	40	64530	1613	5.91	78.8
CM-cellulose column chromatography	17	29250	1720	9.74	35.6

Due to the forwarding steps of purification, the amount of total activity and total protein were going to be decreased and the specific activity and purification fold was increased gradually. In the first stage of cellulose purification, the specific activity and purification fold were 272 U/mg and 1.0, respectively. In the final step of purification with CM-cellulose column chromatography, the specific activity, purification fold and recovery were 1720 U/mg, 9.74 and 35.6%, respectively.

3.4. Molecular Weight Determination

According to the SDS-PAGE profile, molecular weight of the purified cellulase was found to be 66.9 kDa comparing with the marker proteins (Figure 4).

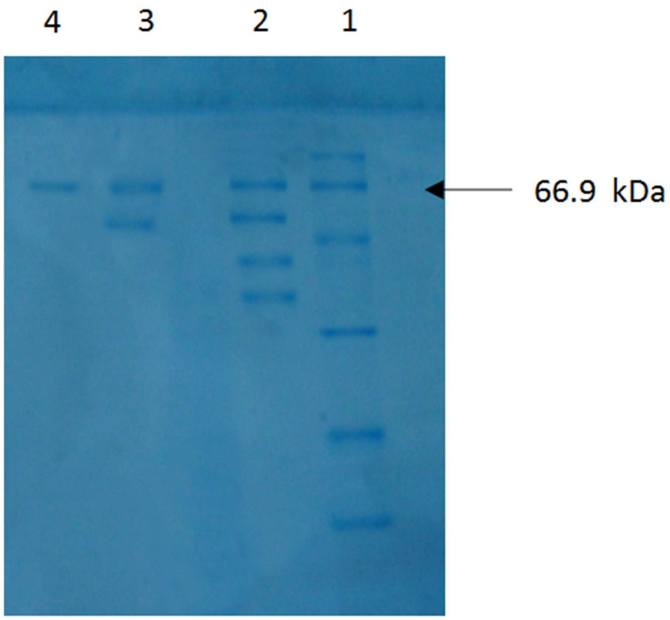

Figure 4. *Determination of molecular weight by SDS-PAGE analysis. Lane 1, marker proteins: [top to bottom: Phosphorylase B (97 kDa), Bovine serum albumin (67 kDa), Ovalbumin (45 kDa), Carbonic anhydrase (29 kDa), Trypsin inhibitor (20 kDa) and Lysozyme (14.6 kDa)]. Lane 2, crude enzyme. Lane 3, DEAE-cellulose unbound fraction and Lane 4, CM-cellulose bound (purified protein). The migration position of cellulase is indicated as 66.9 kDa.*

3.5. Substrate Specificity

The cellulase purified from strain C_1 exhibited the highest activity against CMC substrate. There was moderate hydrolytic activity against, lactose wheat bran xylan, rice xylan, and low activity to xylose and oat spelt xylan (Figure 5). The purified enzyme was found to be stable at pH 7.0 and 40°C temperature against CMC and at this stage, the enzyme didn't lose any activity (Figure 5). Based on substrate specificity, the purified cellulase showed maximum activity on CMC which indicated it to be an endo-β-1, 4-glucanase.

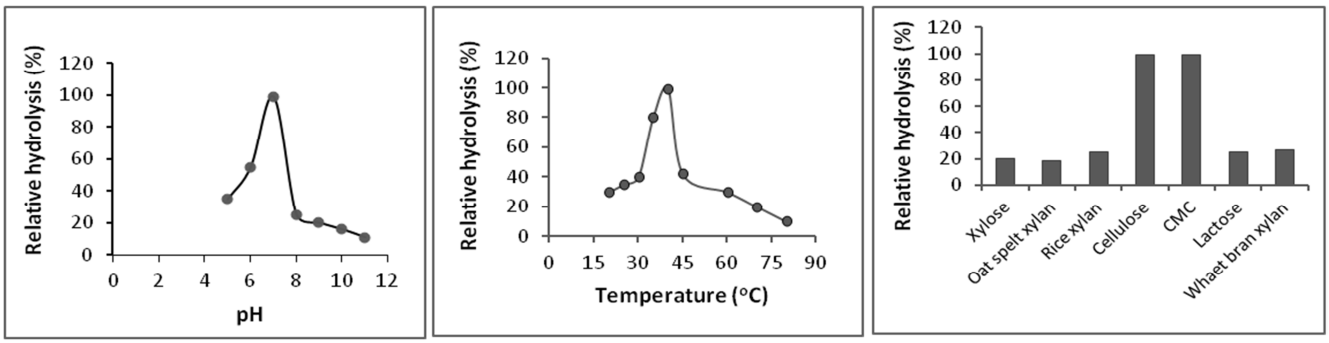

Figure 5. *Characterization of purified cellulase from Paenibacillus sp.: (A) Effect of pH, (B) Effect of temperature and (C) Effect of different substrates on cellulase activity.*

4. Discussion

The biological degradation of cellulose has been investigated for many years. A number of cellulolytic enzymes, especially cellulases from different bacterial strains have been purified and characterized. The cellulolytic activities of the isolated strains depend on the sources and volume of biowaste in nature [24]. In this study, sugar industry waste (molasses) was selected as a source for obtaining desirable cellulase-producing bacteria. The isolated strains (C_1, C_2 and C_3) were cellulolytic bacteria confirmed by filter-paper degradation, Gram's iodine and Congo-red staining assay and cellulase activity of each strain was confirmed and determined by measuring the amount of reducing sugar liberated from cellulose by DNS method [21, 25].

Based on morphological, cultural and biochemical characteristics, the bacterial strains were identified as *Paenibacillus sp*. (C_1), *Bacillus sp*. (C_2) and *Aeromonas sp*. (C_3), where the strains C_1 and C_2 were gram-positive and C_3 was gram-negative. The strains inoculation was performed in the fermentation medium and enzyme production was measured [19, 26]. Cellulase production by the specific strains was tested at various cultural conditions, like pH, temperature, substrate concentration, incubation period, carbon, and nitrogen sources [27-28).

In this study, *Paenibacillus sp*. showed highest cellulase activity at optimal pH 7.0 and temperature at 40°C on 24

hours incubation period at 1% CMC concentration. The other two strains *Bacillus sp*. and *Aeromonas sp*. exhibited comparatively less cellulase activity than *Paenibacillus sp.* at pH 6.0 and 7.0 at 40°C temperature on 36 h incubation period at 1.5% and 2% CMC concentration, respectively. In a previous study, the isolated strain *Bacillus subtilis* from the agricultural fields exhibited highest cellulase activity at culture condition between pH 6.5, and 7.5 at 45°C temperature at 1.5% CMC agar medium [29]. In a recent study, *Bacillus sp.* SM3-M8 was isolated from waste (molasses) and characterized by morphological and biochemical tests and the isolate was found to show highest cellulase activity at pH 7.0, temperature 45°C and CMC concentration at 0.5% after 48 h of incubation period [15]. A similar finding was found in a previous study where the cellulase purified from *Paenibacillus sp.* E2 and E4 in paper mill sludges exhibited maximum enzymatic activity on filter-paper and CMC substrate [30].

Paenibacillus genus is acquainted to contain some better cellulase making strains, E2 and E4 showed high cellulase activities and represent excellent candidates for further cellulase analysis and characterization reported in the study investigated by Maki et al. [30]. To characterize the specificity of the purified enzyme, we initially applied filter paper degradation assay and CMC test. CMC agar helps us to identify the bacterial isolates having cellulase activity on soluble cellulose like CMC thus indicating commonly endoglucanase activities. Then, the isolates showing cellulase activity were screened on CMC substrate for activity on filter paper. In substrate specificity assay, the purified cellulase exhibit maximum activity to CMC and less activity to xylose, wheat bran xylan and rice xylan. This specificity on CMC indicates that the CMCase purified from *Paenibacillus sp*. may be an endoglucanase according to the definition by Coughlan and Mayer [31].

The term CMCase has commonly been regarded as synonymous with endoglucanase which randomly degrades CMC or amorphous cellulose and has no activity or low activity on other substrates [31]. Therefore, in our study, the substrate specificity test indicates that cellulase purified from the selected strain may be an endo-β-1, 4-glucanase. In a related study, researchers have also isolated and characterized a novel endoglucanase (Cel9P) according to CMC substrate specificity from a recently isolated *Paenibacillus* sp. BME-14 [32]. The Endoglucanase Cel9P showed 65% of highest activity at 5°C, which can be useful for industries that have processed at lower temperatures [32]. In another study, a thermophilic, cellulose-degrading bacterium *Paenibacillus sp*. strain B39 was isolated from the poultry manure compost exhibited high activity on CMC was indicated as endoglucanase based on CMC substrate activity [33].

Taking into account the maximum cellulase activity, the strain *Paenibacillus sp*. was chosen for enzyme purification, enzyme characterization and molecular weight determination [34]. In the first step of enzyme purification, the specific activity and purification fold were 272 U/mg and 1.0, respectively and in the last step of purification, the specific activity, purification fold and recovery were 1720 U/mg, 9.74 and 35.6%, respectively. The molecular weight of the purified enzyme was found to be 66.9 KDa and the enzyme was characterized at optimized conditions against several substrates. In various substrates, CMC was found as an important source for highest CMCase (endoglucanase) production and the purified cellulase was stable and retained the activity at optimized conditions. All the cellulase-producing strains isolated and identified in present study have the potential for further use and future works, such as looking at individual enzyme activities to isolate efficient cellulases with unique characteristics.

5. Conclusion

This study identifies some good cellulase producing bacteria from waste (molasses) of sugar industry. Among the isolated strains, *Paenibacillus sp*. exhibited the highest potentiality for maximum cellulase production at optimized conditions and the molecular weight of the purified enzyme was found to be 66.9 kDa. Based on substrate specificity test, the purified cellulase showed high specificity on CMC which indicated it to be an endo-β-1, 4-glucanase. Bacterial isolates with cellulase activity might be an important part of our future research to build up effective cellulase producing system that can be favored for industrial applications.

Limitation of the Study

In present study, we could not perform the molecular characterization of the isolated strains because of our limited laboratory facilities. In our future study, we would like to perform 16s rRNA sequencing for confirming the species identification of individual strain and the structure-function relationship of the purified enzyme by determining N-terminal and C-terminal amino acid sequence.

Abbreviations

CMC: Carboxymethyl cellulose; DNS: 3, 5-dinitrosalicylic acid; LB: *Luria-Bertani;*

Availability of Data and Materials

All data is given in the main body of the manuscript. Data and materials are also available from the corresponding author.

Competing Interests

The authors declare no conflict of interest.

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This research did not receive any external funding.

Authors' Contributions

FI: Conceptualization, data analysis and interpretation, methodology, manuscript writing. NR: conceptualization, supervision, and critical revision of the manuscript. All authors read and approved the final manuscript.

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