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Screening, Isolation and Characterization of Cellulolytic Bacteria from Agro-industrial Soils of Bangladesh and Their Optimization for Cellulase Enzyme Production

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ABSTRACT

Cellulase enzymes are capable of degrading cellulose containing industrial pollutants. Nowadays, they have a broad range of applications in different industries including textile, detergent, food and fermentation industries. In the present study, soil samples were collected from different agro-industrial regions of Bangladesh. Eighteen cellulolytic bacteria were isolated from the diluted soil samples. These bacterial isolates were preliminarily screened on Bushnell Haas medium (BHM) agar plate with the help of 0.3% congo red for the qualitative determination of cellulase activity where six isolates made the highest clearing zone around the bacterial colony. The highest clear zone forming bacterial isolate represented the highest cellulase activity. Different morphological (gram staining) and biochemical tests (catalase, indole, urease, oxidase, methyl red, Voges-Proskauer, gelatin hydrolysis, hydrogen sulfide, antibiotic sensitivity and carbohydrate including glucose, sucrose, lactose, xylose, sorbitol, mannitol and myo-inositol utilization tests) were performed to identify cellulolytic bacteria. Isolate 4, 6, 13 and 17 were identified as *Streptococcus sp.*, *Xanthomonas sp.*, *Brucella sp.* and *Bacillus sp.*, respectively while isolate 1 and 9 were identified as *Staphylococcus sp*. In the present study, order (the highest to the lowest) of cellulase activity (U/mL) of bacterial isolate 13, 17, 6, 4, 9, 1 was (1.85±0.05) > (1.56±0.07) > (1.26±0.06), (0.84±0.05) > (0.56±0.04) > (0.52±0.03) at their respective optimum temperature and pH. The findings of this study will help to identify other cellulolytic bacteria that can degrade cellulose containing soil pollutants.

Introduction

Cellulose is the major constituent of plant biomass and is considered to be the most copious biological matter found in both aquatic and terrestrial ecosystems (Shankar *et al*., 2011). Plants are the crucial origin of renewable energy and carbon. Cellulose rules over the wastes coming from agroindustry. In the agroindustry, cellulose can be found in different forms like – husk, stems and stalks. The interest in cellulose is rising day by day as feedstuffs and source of energy (Balachandrababu *et al*., 2012). Basically, cellulose is found as a structural constituent in the cell wall of green plants, different algae and oomycetes. On an estimate, plants manufacture about 4×10^7tons of cellulose per year in the world (Bakare *et al*., 2005). Cellulose is also contemplated as a carbon source that is synthesized by terrestrial and aquatic plants at the rate of about 0.85×10^{11} tons/year (Nowak *et al*., 2005).

Degradation as well as the subsequent use of cellulose as an efficient carbon source is very much important worldwide. The importance of cellulose as a renewable energy source has brought the hydrolysis of cellulose into extended interest for both industrial and research fields (Bhat, 2000). Several research works have been conducted to identify new microorganisms synthesizing cellulase (an enzyme that breaks cellulose) with higher efficiency and specificity (Subramaniyan and Prema, 2000; Bhat *et al*., 2000). Cellulase has a great role in biodegradation where lignocellulosic compounds are effectively disintegrated by several cellulolytic protozoa, actinomycetes, bacteria and most importantly fungi. In industrial application, this enzyme has immense importance for the manufacture of ethanol, fermentable sugars, detergents, organic acids and so on. Cellulase has also the potential to obtain magnificent benefits for the successful utilization of biomass (Wen *et al.*, 2005).

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Numerous microorganisms can produce cellulase enzyme. On the other hand, bacteria and fungi are the major degraders of cellulose in nature (Lederberg, 1992). Cellulose-consuming microflora are aerobic and anaerobic bacteria, mesophilic and thermophilic bacteria, alkaliphilic bacteria, numerous fungi, different protozoa and certain actinomycetes (Alexander, 1961). The aerobic bacterial cells secret different enzymes having different binding domains for different conformations of cellulose molecules. But anaerobic bacteria are found to have a distinct multi-enzyme complex, known as cellulase. The demand for cellulase producing bacteria is escalating as bacteria can grow very fast and have the potential to be utilized in cellulase production industries. The explore of noble and efficient strains of bacteria with higher production of cellulase and higher tolerance against adverse environmental conditions (high pH, high temperature and non-sterile conditions) may be regarded as one step forward to a better economy.

Isolation of cellulase producing bacteria is already reported in parts of the world. However, in Bangladesh, this type of research works is still in its initial stage. So, isolation and characterization of cellulase producing bacteria will be helpful in different industries of Bangladesh as cellulase has a broad industrial application. The present study was designed to screen and identify cellulose degrading bacteria from different agro-industrial soil samples of Bangladesh and also to measure the cellulase production capability of the bacterial isolates by the submerge fermentation process.

Materials and Methods

Sample collection

The industrial soil samples were collected from different regions of Bangladesh (Osman Agro Industries, Mohadevpur, Naogaon; Ifad Agro-complex Ltd, Bhaluka, Mymensingh; Chittagong Meridian Agro Industries Ltd., Chittagong; Mazim Agro Industries Ltd., Gazipur, Dhaka). Soil samples were collected from 10-15cm depth of soil. Pre-sterilized spatula and plastic bags were used for sample collection and before bacterial isolation the samples were stored at 4°C in the ice box.

Isolation of cellulolytic bacteria

Ten-fold serial dilutions of each soil sample were prepared in sterilized distilled water. One gram of soil sample was suspended in 9 ml of sterile distilled water. After serial dilution (10^{-1} to 10^{-6} times), an aliquot of 200 μL of each dilution was taken and spread plated onto Bushnell Haas medium (BHM) (Bushnell and Haas, 1941). Each agar plate contains the following components (g/L): Carboxymethylcellulose (CMC) (10.0), NH_4NO_3 (1.0), KH_2PO_4 (1.0), K_2HPO_4 (1.0), $CaCl_2$ (0.02), $MgSO_4{\cdot}7H_2O$ (0.2), $FeCl_3{\cdot}6H_2O$ (0.05) and agar (20.0). Then plates were incubated at 37°C for 48hr.

Qualitative screening of cellulolytic bacteria

Morphologically distinct colonies were selected from different dilution plates and streaked on separate BHM agar plate and incubated at 37°C for 72 hr. To perform staining the replica plates were also prepared individually (Ruijssenaars and Hartmans, 2001). The 0.3% congo red was added to the replica plates for 12-15 min. Then congo red solution was poured off and eachagar plate was washed with 1M NaCl (Teather and Wood, 1982). The isolates showing a clear zone around the colonies were selected and taken from the master plate and further used for the enzyme production in a liquid medium. The selected bacterial isolates were cultured on BHM nutrient broth containingthe following components: CMC (10.0 g/L), NH_4NO_3 (1.0 g/L), KH_2PO_4 (1.0 g/L), K_2HPO_4 (1.0 g/L), $MgSO_4{\cdot}7H_2O$ (0.2 g/L), $CaCl_2$ (0.02 g/L) and $FeCl_3{\cdot}6H_2O$ (0.05 g/L). The culture slants were stored at 4°C as inoculum and sub-cultured every 10–15 days.

Characterization of bacteria isolates from soil samples

Morphological characterization

Gram staining is a powerful technique to identify bacteria and it was conducted according to the procedure of Cappuccino and Sherman (2005).

Biochemical characterization

The following biochemical analyses were carried out including catalase (Reiner, 2010), indole (MacWilliams,2009), urease, oxidase (Shields and Cathcart, 2010), gelatin hydrolysis (Cruz and Torres, 2012), H_2S production, antibiotic sensitivity test (Prescott *et al*., 2000),Voges-Proskauer (McDevitt, 2009), methyl red, carbohydrate including glucose, sucrose, lactose, xylose, sorbitol, mannitol and myo-inositol utilization tests (Cappuccino and Sherman, 2005) for proper identification and characterization of the bacterial isolates. Morever, genus of the bacterial isolates was identified using the Bergey's Manual of Determinative Bacteriology (1938).

Inoculum preparation and submerged fermentation for the enzyme production

Few bacterial colonies were taken from the pure cultures of the selected bacterial isolates by using a loop. Then colonies were inoculated in inoculum broth containing CMC (10.0 g/L), NH_4NO_3 (1.0 g/L), KH_2PO_4 (1.0 g/L), K_2HPO_4 (1.0 g/L), $CaCl_2$ (0.02 g/L), $MgSO_4{\cdot}7H_2O$ (0.2 g/L), $FeCl_3{\cdot}6H_2O$ (0.05 g/L) and incubated at 37°C for 24hr. After 24 hr of the fermentation period, the bacterial cells were used as inoculum source for the production

medium. Bacterial isolates were selected from plate staining culture and then grown in 50mL enzyme production medium (at pH 7.0) containing CMC (10.0 g/L), yeast extract (5.0 g/L), NH_4NO_3 (1.0 g/L), KH_2PO_4 (1.0 g/L), K_2HPO_4(1.0 g/L), $MgSO_4 \cdot 7H_2O$ (0.2 g/L), $CaCl_2$(0.02 g/L) and $FeCl_3 \cdot 6H_2O$ (0.05 g/L). Then 50mL medium containing 2% inoculum was taken in 250 mL Erlenmeyer flask and incubated at 37°C at 180 rpm for 72hr. After every 24hr, the culture was centrifuged at 14000g for 15 min at 4°C. The cell-free culture broth was considered as the crude enzyme. CMCase activity was estimated from this culture broth. Depending on the higher CMCase activity, an isolate was selected for further characterization and identification. To estimate the insight enzyme production from the bacterial isolate, the absorbance was measured with cell growth at 600 nm using a UV-visible spectrophotometer.

CMCase activity assay

The CMCase activity (U/mL) was estimated by measurement of reducing sugars liberated from CMC. The enzyme assay was performed by incubating the enzyme with CMC at 37°C for 15 min. The reaction mixture (100μL) contained 50μL of enzyme and 1.0% (w/v) final concentration of CMC in 50mM phosphate buffer (pH 7.0). The reducing sugar was measured by the method of Nelson and Somogyi (Nelson, 1944; Somogyi, 1945). The absorbance was measured at 500 nm using a UV-visible spectrophotometer against a blank with d-glucose as standard. One unit (U) of cellulase activity is defined as the amount of enzyme that liberates 1μmol of reducing sugar (glucose) in 1min at 37°C and pH 7.0.

Optimization of cellulase enzyme production

The optimum fermentation condition was determined for cellulase production utilizing the selected cellulolytic bacterial isolates. The cellulase fermentation was carried out at different ranges of parameters including temperature, pH, incubation period and substrate concentration. After fermentation at different parameters the crude enzyme sample was collected from each to check the enzyme activity.

Effect of temperature, pH, incubation period and substrate concentration on cellulase enzyme production

To standardize optimum fermentation condition for each of the bacterial isolates, different temperature (30°C, 35°C, 40°C, 45°C), pH (6.0, 6.5, 7.0, 7.5), incubation period (24, 48, 72, 96 hr) and substrate (CMC) concentration (0.2%, 0.5%, 1.0%,1.2% and 1.5%) were studied with the purpose to obtain maximum cellulase enzyme production.

Statistical analysis

All analyses were performed in three replicates and the experimental data were statistically analyzed using the Statistical Package for Social Sciences (SPSS, 2007) database, version 16.0, submitting the data to a simple one-factor Analysis of Variance (ANOVA). ANOVA was performed and mean separation was done by SPSS database ($p<0.05$).

Results

Primary screening of cellulose degrading bacteria

A total of 18 colonies from the dilution (10^{-1}-10^{-6}) plates were obtained (Figure 1) and their ability to produce cellulase was determined by primary screening on Carboxy Methyl Cellulose (CMC) agar to select the potential isolates showing best zone of hydrolysis. The average of the ratio is given in Table 1. In the present study, six (6) bacterial isolates (isolate number 1, 4, 6, 9, 13 and 17) among 18 isolates were qualitatively selected for further morphological and biochemical characterization.

Characterization of bacteria isolates

Morphological characterization (Gram staining)

Morphological features of the isolates were determined by gram staining test. Isolate 1, 4, 9 and 17 retained purple color of crystal violet stain implying that they were gram-positive bacteria while Isolate 6 and 13 retained the purple color of Safranin stain indicating that they were gram-negative bacteria**.**

Biochemical characterization

After gram staining of cellulolytic bacterial isolates, different biochemical tests were performed and their results were shown in Table 2.

Figure 1. Different bacterial isolates from diluted soil samples cultured on BHM agar media at 37°C for 48 hr

Table 1. The ratio of the clear zone diameter to the colony diameter of 18 isolates. Ratio indicates the hydrolysis of cellulose

Isolate number	Clear zone diameter (cm)	Colony diameter (cm)	Ratio of Clear zone diameter and Colony diameter
1	2.6	1.5	**1.73**
2	No hydrolysis	No hydrolysis	No hydrolysis
3	2.0	1.3	1.54
4	3.0	1.7	**1.76**
5	2.1	1.3	1.62
6	2.8	1.5	**1.87**
7	2.7	1.6	1.69
8	2.9	1.7	1.71
9	3.3	1.9	**1.74**
10	1.0	0.6	1.67
11	No hydrolysis	No hydrolysis	No hydrolysis
12	1.9	1.3	1.46
13	2.9	1.4	**2.07**
14	No hydrolysis	No hydrolysis	No hydrolysis
15	2.9	1.7	1.71
16	2.4	1.5	1.60
17	1.8	0.9	**2.00**
18	1.8	1.3	1.38

Bold data represent the highest hydrolysis among 18 isolates; The highest ratio indicates the bacterial isolate which has the highest cellulose degrading capacity.

Table 2. Results of different biochemical tests of cellulolytic bacterial isolates

Biochemical tests	Isolate 1	Isolate 4	Isolate 6	Isolate 9	Isolate 13	Isolate 17
Catalase test	+	–	+	+	+	+
Indole test	+	–	–	–	–	–
Gelatin hydrolysis test	+	+	–	+	+	+
H_2S production test	–	–	–	–	–	–
Urease test	+	–	–	+	–	+
Oxidase test	–	–	+	–	+	–
MR test	–	+	+	–	+	–
VP test	+	+	+	–	+	–
Glucose utilization test	+	+	+	+	+	–
Sucrose utilization test	+	+	+	–	+	–
Sorbitol utilization test	+	+	+	–	+	+
Myo-inositol utilization test	+	–	–	–	–	–
Xylose utilization test	+	–	+	+	+	+
Lactose utilization test	–	–	–	–	–	–
Mannitol utilization test	+	–	+	+	+	+
Cellulolytic bacteria	*Staphylococcus sp.*	*Streptococcus sp.*	*Xanthomonas sp.*	*Staphylococcus sp.*	*Brucella sp.*	*Bacillus sp.*

'**+**'indicates a positive result and '**–**'indicates a negative result. Genus of the bacterial isolates were identified using the Bergey's Manual of Determinative Bacteriology (1938).

Antibiotic sensitivity

Antibiotic sensitivity test was performed to select the antibiotic that will be the most effective against specific types of bacteria. Inhibition zone diameter interpretation was shown as a reference in Table 3 and Results of different antibiotic sensitivity tests are shown in Table 4.

Optimization of cellulase enzyme production

Cellulase enzyme activities were determined at different conditions of temperature (30°C, 35°C, 40°C and 45°C), pH (6, 6.5, 7, 7.5 and 8), incubation time (24, 48, 72 and 96 hr) and substrate (CMC) concentrations (0.2%, 0.5%, 1.0%, 1.2% and 1.5%).

Table 5 shows that the optimum temperature for cellulase enzyme production of isolate 1, 4, 6, 9, 13 and 17 was 35°C, 40°C, 30°C, 35°C, 40°C and 45°C, respectively; while the optimum pH for cellulase enzyme production was 6.5, 6.5, 6.5, 6.5, 7 and 7, respectively; and the optimum incubation time of isolate 1, 4, 6 and 9 was 48 hr while optimum incubation time of isolate 13 and 17 was 72 hr. The optimum CMC concentration of isolate 1, 6, 9, 13 and 17 was 1.5% while the optimum CMC concentration of isolate 4 was 1.0% for cellulase enzyme production.

Table 3. Inhibition zone diameter interpretation (Tendencia, 2004)

Antibiotics	Disc content	Diameter of inhibition zone (mm)		
		Resistance	Intermediate	Sensitive
Ampicillin (Amp) (For *Enterococcus*)	25 µg	≤15	16-17	≥18
Ampicillin (Amp) (For *Staphylococcus* and *Streptococcus*)	25 µg	≤22	23-25	≥26
Azithromycin (Azm)	30 µg	≤14	15-18	≥19
Kanamycin (K)	30µg	≤13	14-17	≥18
Rifampicin (Rif)	5 µg	≤22	23-25	≥26

Table 4. Antibiotic sensitivity test of six cellulose degrading bacterial isolates. Resistance and Sensitive categories were determined using above reference from Table 3

Antibiotics	Diameter of inhibition zone [Mean±SD, (mm)]					
	Isolate 1	Isolate 4	Isolate 6	Isolate 9	Isolate 13	Isolate 17
Ampicillin (25 µg)	R (0±0)	R (0±0)	R (0±0)	R (0±0)	R (0±0)	R (0±0)
Azithromycin (30 µg)	R (0±0)	R (0±0)	R (0±0)	S (19±0.3)	R (0±0)	R (0±0)
Kanamycin (30 µg)	S (18±0.3)	S (20±0.6)	S (18±0.4)	S (18±0.3)	S (19±0.8)	S (20±0.5)
Rifampicin (5 µg)	R (0±0)	R (0±0)	R (0±0)	R (22±0.8)	R (13±0.4)	R (0±0)

R= Resistance, S= Sensitive, mm = millimeter, Diameter of inhibition zone is expressed as (mean ± SD)

Table 5. Optimization of cellulase enzyme production

Parameters	Values	Cellulase activity of isolates [Mean±SD(U/mL)]					
		Isolate1	Isolate 4	Isolate6	Isolate 9	Isolate13	Isolate17
Temperature (°C)	30	0.46±0.05	0.57±0.03	**1.23±0.03**	0.45±0.04	1.42±0.08	1.19±0.06
	35	**0.51±0.02**	0.65±0.05	1.18±0.04	**0.53±0.01**	1.56±0.05	1.26±0.04
	40	0.40±0.03	**0.77±0.04**	1.09±0.01	0.42±0.02	**1.75±0.09**	1.31±0.02
	45	0.32±0.07	0.59±0.03	0.96±0.04	0.32±0.02	1.59±0.02	**1.39±0.03**
pH	6	0.56±0.09	0.80±0.05	1.20±0.06	0.56±0.04	1.67±0.04	1.36±0.03
	6.5	**0.63±0.02**	**0.95±0.06**	**1.31±0.08**	**0.61±0.06**	1.73±0.04	1.43±0.05
	7	0.57±0.06	0.84±0.01	1.25±0.01	0.55±0.08	**1.82±0.05**	**1.56±0.06**
	7.5	0.49±0.01	0.76±0.02	1.17±0.08	0.46±0.09	1.74±0.09	1.51±0.01
	8	0.43±0.05	0.68±0.06	1.08±0.05	0.41±0.09	1.65±0.01	1.44±0.08
Incubation time (Hour)	24	0.55±0.01	0.79±0.04	1.05±0.02	0.53±0.05	1.63±0.02	1.47±0.03
	48	**0.68±0.03**	**0.91±0.01**	**1.29±0.03**	**0.69±0.04**	1.77±0.04	1.52±0.09
	72	0.60±0.02	0.83±0.03	1.25±0.04	0.57±0.04	**1.88±0.07**	**1.60±0.05**
	96	0.49±0.06	0.77±0.01	1.19±0.04	0.49±0.08	1.73±0.03	1.56±0.07
Concentration of CMC (%)	0.2	0.37±0.05	0.68±0.06	0.97±0.03	0.35±0.03	1.61±0.02	1.37±0.03
	0.5	0.41±0.03	0.77±0.08	1.11±0.05	0.40±0.01	1.66±0.08	1.43±0.01
	1.0	0.54±0.02	0.85±0.03	1.18±0.01	0.52±0.01	1.75±0.03	1.49±0.09
	1.2	0.61±0.02	**0.97±0.02**	1.29±0.01	0.65±0.07	1.82±0.07	1.55±0.05
	1.5	**0.66±0.04**	0.93±0.05	**1.35±0.03**	**0.65±0.06**	**1.89±0.02**	**1.58±0.01**

Bold data indicate the highest/optimum values that determine their respective optimum parameter

Comparison of cellulase enzyme activity among six isolates at their respective optimum conditions

Cellulase enzyme activity among six isolates is shown in Figure 2. In the present study, order (the highest to the lowest) of cellulase activity (U/mL) of 13, 17, 6, 4, 9, 1 was (1.85±0.05) > (1.56±0.07)> (1.26±0.06) > (0.84±0.05) > (0.56±0.04) > (0.52±0.03) at their respective optimum conditions.

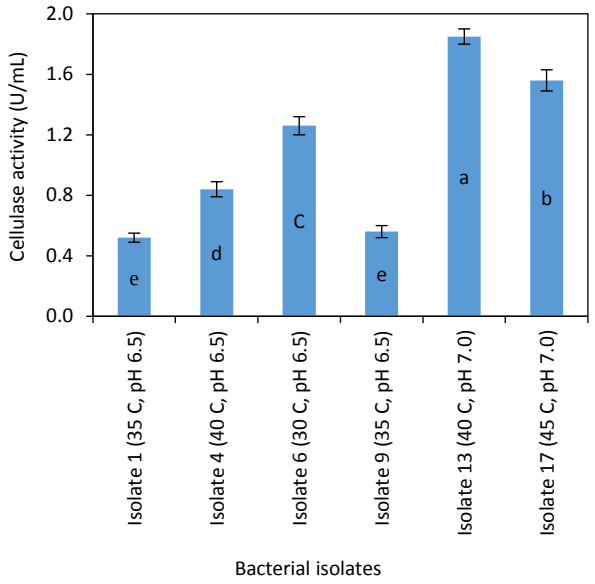

Figure 2. Comparison of cellulase enzyme activity among six isolates at their respective optimum conditions (optimum parameters were also shown in Table 4). Values are presented as mean ± SD (bars), n=3. Different letters indicate the significant difference at $P<0.05$ level while the same letters indicate the non-significant difference at $P<0.05$ level. C designates ^{o}C.

Discussion

Cellulose is the most plentiful renewable plant product in the biosphere and this polymer is converted into glucose units by using several chemical or biochemical processes as it may be utilized for industrial fermentation. There are different methods for cellulose conversions such as enzymatic hydrolysis, acid hydrolysis and pyrolysis where the first one is the most preferred method and environmentally safe and it can be carried out at normal temperature (Bakare *et al*., 2005). Hence, the most studies focus on enzymatic hydrolysis (Lee *et al.,* 2008; Adeleke *et al.,* 2012). In the soil, bacteria and fungi are the crucial cellulose degrading microorganisms. On the other hand, several crayfish, mollusks and insects can synthesize their cellulases which are responsible for cellulose degradation (Watanabe and Tokuda, 2001; Ohkuma, 2003). Six cellulolytic bacterial isolates were collected from different agro-industrial areas of Bangladesh. Efficient cellulase producers like *Humicola*, *Aspergillus*, *Trichoderma*, *Penicillium*, *Acremonium* and *Pellicularia* were isolated from different natural sources and were considered heat sensitive (Fujimoto *et al*., 2011) while the bacterial isolates in our study were found to be mesophilic (maintained at 30°C-45°C). Identification of the six isolates was performed using various morphological and biochemical tests. Four isolates (isolate 1, 4, 9 and 17) were found to be gram-positive while two isolates (isolate 6 and 13) were gram negative. Isolates 4, 6, 13 and 17 were identified as *Streptococcus sp.*, *Xanthomonas sp.*, *Brucella sp.* and *Bacillus sp.*, respectively while isolate 1 and 9 were found to be *Staphylococcus sp.* Our result (Table 2) is almost resembled the result of Bergey's manual of systemic bacteriology (Sneath *et al.*, 1986).

Biochemical analysis revealed that catalase, urease, gelatin hydrolysis, xylose, sorbitol, mannitol and myo-inositol utilization tests were positive for *Bacillus sp.* (Table 2). On the other hand, indole, oxidase, methyl red, Voges-Proskauer, H_2S production, antibiotic sensitivity test and glucose, sucrose, lactose and myo-inositol utilization tests were negative for this species (Table 2) which is supported by the study of Neesa *et al*. (2017). Neesa *et al*. (2017) isolated *B. amyloliquefaciens* and *B. subtilis* from biogas plant effluent and dairy effluent based on their morphological, biochemical and molecular characteristics. Identification results of the present study (Table 2) are similar to the result of Raju *et al.* (2013); Behera *et al.* (2014) and Behera *et al.* (2016). Behera *et al.* (2016) isolated two cellulose-degrading bacteria (*Bacillus licheniformis* and *Brucella sp.*) from mangrove soils. Raju *et al.* (2013) screened and isolated cellulase producing bacteria (*Staphylococcus aureus*, *Bacillus licheniformis* and *Bacillus cereus*) from dump yards of vegetable wastes. In another previous study, Behera *et al.* (2014) identified the cellulose degrading bacteria (*Xanthomonas sp.*, *Bacillus sp.*, *Brucella sp.*, *Micrococcus sp.* and *Pseudomonas sp.*) from mangrove soil.

In our study, the bacterial isolates 1, 4, 6, 9, 13 and 17 were cultured and maintained in 1% CMC containing medium. In previous studies, CMC is considered as a carbon source; capable of being cellulase production ameliorated by cellulose utilization with bacterial growth (Kubicek, 1993; Sang-Mok and Koo, 2001). The supernatant was considered as crude cellulose degrading enzymes and several *Bacillus* genera secreted these enzymes extracellularly (Schallmey*et al*., 2004; Lin *et al.*, 2012). In our study, six isolates showed significantly different levels of cellulase enzyme activity ($P<0.05$). According to Dashtban *et al*. (2010), a reducing sugar assay measured cellulase activities and also determined the end products of the substrate hydrolysis. Therefore, this result is revealed as the hydrolysis capacity of the cellulase. A significant change was found

in enzyme activity with the change in temperature ($P<0.05$). Optimum temperature for cellulase enzyme activity was found at 35°C, 40°C, 30°C, 35°C, 40°C and 45°C for isolate 1, 4, 6, 9, 13 and 17, respectively (Table 5). Microbial cellulases from different sources have been found to have an optimum temperature of approximately 35-50°C (Bakare *et al*., 2005; Aygan *et al*., 2011). A similar temperature profile has been observed in a study using *Bacillus licheniformis* (Bajaj *et al*., 2009).

Enzyme assays to measure the optimum pH were performed in reaction mixtures at different pH (6-8) at the temperature (37°C). In our experiment, for isolates 1, 4, 6, 9, 13 and 17, a significant change was found in enzyme activity with the change in pH ($P<0.05$). The highest cellulase activity was measured at (optimum) pH 7 for isolate 13 and 17 while the highest activity was found at (optimum) pH 6.5 for isolate 1, 4, 6 and 9 (Table 5) implying that the enzyme is an acid cellulase. Each enzyme works its optimum pH and if the pH changes from optimum level, the active site of the enzyme may change decreasing or blocking the production of an enzyme-substrate (ES) complex (Eijsink *et al.,* 2005). Previous studies reported that the Optimum pH range was 4.5-8.0 for cellulase enzyme (Bakare *et al*., 2005; Immanuel *et al*., 2007; Dutta *et al*., 2008). Optimum acidic pH range was found 3.8 to 5.8 for cellulase enzyme (Bajaj *et al.*, 2009; Mojsov, 2012)

Conclusion

Cellulolytic bacteria played an important role for decomposition of cellulose and hemicellulose present in soil. We performed screening and isolation of bacteria showing potential cellulolytic activity from soil samples collected from different agro industrial regions. Different cellulolytic (cellulase producing) bacteria including *Streptococcus sp.*, *Staphylococcus sp.*, *Xanthomonas sp.* and *Brucella sp.* were identified based on their morphological and biochemical characteristics. Production of cellulase enzymes was observed and comparison of enzyme activities of the isolates was also compared. Findings of this study will play an important role for textile, paper, food and agricultural industries as most of these industries import the cellulase from other countries. Cellulase degrading bacteria can also be utilized for degradation of cellulose rich pollutants dumped in soil near agro-industrial regions of Bangladesh.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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