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Isolation and Identification of Cellulolytic Bacteria from Soil Sample and Their Antibiogram

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Abstract This investigation was focused to isolate and identify the effective cellulolytic soil inhabiting bacteria from the soil of waste disposal site of Noakhali Science and Technology University (NSTU) campus and Maijdee, Noakhali with evaluating their cellulase production ability. Eight cellulolytic bacteria were isolated and identified as potentially effective strain from thirty isolates of twenty samples and their antibiogram was also performed. In this investigation, the maximum carboxymethylcellulose hydrolysis capacities (HC value), for all the isolates, ranged from 1.40 to 2.18 mm whereas maximum clear zone size around the colony ranged from 4.0 mm to 10.0 mm. It was the indication of the highest cellulase production ability of these eight species by degrading cellulose where two isolates sample 2 (10^{-3}) and sample 15 (10^{-3}) displayed the maximum zone of clearance around the colony. The results also revealed that soil of the investigated area can be used, in near future, to produce cellulase enzyme which will be useful for industrial purposes, plant growth promotion and research. Antibiotic sensitivity test was used in the work to determine the sensitivity and resistance pattern of the isolates. The result reported several isolates resistance to commercially used antibiotics. The main reason of this bacterial resistance is the indiscriminate use of the antibiotics. From the microscopic examination, morphological characteristics and various biochemical tests, the isolates were identified as *Bacillus spp, Bacillus cereus, Bacillus megaterium, Clostridium spp, Staphylococcus aureus, Actinomycetes spp, Pseudomonas aeruginosa, Acinetobacter spp*.

Keywords: *cellulolytic bacteria, carboxymethyl cellulose, cellulase, antibiogram, soil, resistance*

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1. Introduction

One of the dominant waste material from agricultural industry is cellulose that remains in the form of stalks, stems and husk. Utilization of cellulose by various microbes helps to obtain the largest material flow in the biosphere by producing cellulase enzyme [1]. The cellulose is made up of D-glucose units linked together to form linear chain via ß-1, 4-glycosidic linkages. Wastes of cellulose may be agricultural, urban or industrial in origin. On the other hand, sewage sludge might also be thought as a source of cellulose. Cellulosic wastes are used as an inexpensive energy resource and feed [2].

In the anaerobic sludge digestion, methane is produced by providing carbon by its cellulosic content [3]**.** Cellulolytic bacteria influences the productivity of the marine environment through mineralization of organic matter. Fungi, bacteria, actinomycetes and protozoa efficiently degrade cellulosic wastes through the biological process, called Cellulolysis and produces cellulase enzyme [4].

1, 4-β-endoglucanase, 1, 4-β-exoglucanase and β-glucosida-se (β-D-glucoside glucohydrolase or cellobiase) are three classes of soluble extracellular enzymes of cellulase enzyme system [5]. Because of potential applicability in various industrial processes such as production of bioethanol, triphasic biomethanation, agricultural and plant waste management, chiral separation and ligand binding studies, cellulase is extensively used [6]. A number of microorganisms synthesizes a large amount of cellulase enzyme. The principal natural agents of cellulose degradation are fungi and bacteria [2].

On account of their applications in industries, starch processing, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, pulp and paper industry and textile industry, researchers have strong interests in cellulases [7]. Aerobic and anaerobic mesophilic bacteria, hemophilic bacteria, filamentous fungi, thermophilic and alkaliphilic bacteria, basidiomycetes, actinomycetes and certain protozoa are the cellulose utilizing population [4]. From the anaerobic, thermophilic spore-forming *Clostridium thermocellum*, the cellulase was first discovered in 1983 [8]. Cellulase production usually depends on variety of growth parameters which

includes inoculums size, pH value, temperature and presence of inducers, medium additives, aeration, growth and time [9]. The activity of cellulase depends on the presence of various metals that acts as activators and inhibitors [2].

Several microorganisms such as *Actinomycetes, Bacteroides succinogenes, Butyrivibrio fibrisolvens, Bacillus cereus, Bacillus megaterium, Staphylococcus aureus, Xanthomonas, Pseudomonas aeruginosa, Acinetobacter species, Clostridium species, Ruminococcus albus, Aspergillus species, Chaetomium species, Fusarium species, Methanobrevibacter ruminantium, Myrothecium species, Penicillium species, Trichoderma* species etc. are reported as potential of cellulase production by degrading cellulose [6]. Particular cellulase producing bacteria are responsible for decomposition of organic matter and composting, also inhabit the other factors. The thoroughly use of cellulases that are produced by different strains of bacteria with multiple exploitable characteristics helps to reduce the time and cost of current bio-conversion processes [2].

Considering these huge significance of cellulase and keeping the above in vision, the present work aimed to isolate and characterize bacterial isolates that were collected from soil of different areas of Noakhali Science and Technology University (NSTU) campus and Maijdee of Noakhali district with high cellulase-producing ability determining by the zone size diameter around the colony degrading cellulose. Identification and characterization of these isolates were done by different biochemical tests; besides this, antibiotic sensitivity and resistance pattern was also checked by applying different antibiotic disc.

2. Materials and Method

2.1. Research Sample

In this work, twenty (20) soil samples were collected from different areas of Noakhali Science and Technology University (NSTU) campus and Maijdee of Noakhali district.

2.2. Collection of Samples

The experiment was carried out at the laboratory of Microbiology department of NSTU campus. For the isolation of cellulolytic bacteria, the soil samples were collected at first. Samples were collected aseptically into twenty sterile test tubes using sterile spoon in the morning. The cellulolytic bacteria isolation program from twenty different soil samples under twenty different trees made use of a stratified sampling design (labeling). The test tubes were immediately covered tightly after collection of soil samples and transported to the laboratory for microbiological analysis, different biochemical tests and antibiogram [10].

2.3. Sample Preparation

To prepare the soil sample, 5g of soil was dissolved in 5ml of sterile saline water, which is then homogenized with vortex machine. From those homogenized sample, 1ml mixed with 9ml sterile saline water by following 10 fold serial dilutions was made. Further dilutions were made from it up to 10^{-6} [11].

2.4. Isolation of Bacteria

After the sample preparation, 1ml plated on carboxymethylcellulose (CMC) ($NH_4H_2PO_4$ 1.0g; KCL 0.2g; $MgSO_4.7H_20$ 1.0g; Yeast extract 1.0g; CMC 26g; Agar 3.0g in a liter) agar from each dilutions of 10^{-3}, 10^{-4} and 10^{-5} for 5 sample and 10^{-3} were plated for next 15 samples, total 20 samples were plated following spread plate technique. For 48 hours at 37˚C, the plates were incubated. By repeated streaking bacterial colonies were purified. For further identification and screening for cellulase production, the purified colonies were preserved at 4°C [10].

2.5. Screening of Cellulolytic Bacteria

In CMC agar plates, pure cultures of bacterial isolates were individually transferred. CMC agar plates were flooded with 1% congo red and allowed to stand for 15 min at room temperature after 48 hours incubation. For counterstaining the plates, one molar NaCl was thoroughly applied. Around growing bacterial colonies, clear zones were appeared that was indicating cellulose hydrolysis taken place. For identification and cellulase production, the bacterial colonies were selected that having the largest clear zone [1].

2.6. Cultural Properties of Plates

Colonies were developed on carboxymethylcellulose (CMC) agar, Bacillus cereus agar, MacConkey agar, Cetrimide agar, Starch casein agar plate after incubation and the results were carefully studied and recorded.

2.7. Microscopic Examination of Bacterial Morphology

2.7.1. Gram Staining

Gram positive (+ve) and Gram negative (-ve) bacteria were distinguished through a differential staining method called Gram staining. Gram (+ve) cells appeared purple and Gram (-ve) cells were pink or red when the cells were examined under the light microscope. The examination of Cell morphology was also done and noted [1].

2.8. Biochemical Characterization

The colonies of the isolates were identified by performing various biochemical tests. Citrate utilization test, methyl red test, Voges-Proskauer (VP) test, MIU (motility/indole/ urease) test, Catalase test, Oxidase test, Triple sugar iron agar (TSI) (acid slant/acid butt/gas) test were performed, for Gram (+ve) bacteria and Gram (-ve) bacteria. All the tests were carried out according to the standard protocol as described in Bergey's Manual of systematic Bacteriology [13].

2.9. Antimicrobial Susceptibility Test

By the disk diffusion which is a modification of Kirby-Baur method, bacterial susceptibility to antimicrobial agents was determined in vitro [14]. As per the instructions provided by the manufacturer, the Muller-Hinton Agar (MHA) (Himedia) was prepared. The media was autoclaved at 121°C for 15 minutes and then cooled to 50°C. Approximately 12~15 ml of the solution was poured onto the 15×150 mm Petri dishes. From the CMC agar plate culture, one isolated colony was selected. With a loop, the top of colony was touched and transferred into a vial that containing 5 ml nutrient broth. Incubation of the broth culture was done at 37ºC for 5-6 hrs. By dipping a sterile cotton swab into the cell suspension that was previously stocked in nutrient broth, inoculation was done on the plate of MHA. After that, the turbidity of actively growing broth culture was obtained. The method was carried out with commercially used antimicrobial disks. Antibiotics used in the work includes Erythromycin (E), Azithromycin (AZM), Chloramphenicol (C), Ciprofloxacin (CIP), Gentamycin (CN), Penicillin (P), and Tetracycline (TE). After the disks were applied, the plates were inverted and placed in an incubator set to 37°C within 15 minutes. Each plate was examined after 16-18 hours of incubation. With a confluent lawn of growth, the resulting zone of inhibition was uniformly circular. Diameters of the zones of complete inhibition (judged by the unaided eye) were measured, including the diameter of the disk. Zones were measured to the nearest whole millimeter, using sliding calipers, which were held on the back of the inverted Petri plate. The zone margin was taken as the area showing visible growth that could be detected with the unaided eye. The sizes of zones of inhibition were interpreted by referring to zone diameter interpretive standards from NCCLS 2002 and equivalent minimal inhibitory concentration (MIC) breakpoints are reported as susceptible or resistant to the agents that have been tested and were read as the antimicrobial concentrations at the points where dense colonial growth intersected the discs [15]. The test was performed in quadruplicate for each culture. The antimicrobial activity was checked to determine the treatment of the disease caused by those particular organisms.

2.9.1. Reading Plates and Interpreting Results

Concentration and diffusion zone breakpoints for resistance for antimicrobial agents were tested in this research.

3. Results

This work was aimed to isolate and characterize cellulolytic bacteria from soil samples. Out of 20 samples, 60% samples were collected from Maijdee and 40% from Noakhali Science and Technology University (NSTU) campus of Noakhali district. Dilution, selective plating, biochemical tests had been done for isolation and identification of bacteria with collected samples. Besides, antibiogram of each isolates had been also performed.

3.1. Zone Formation

Cellulolytic bacteria were isolated from those samples which formed zone on CMC media after staining by congo red. Zone formation around the colony denoted the cellulase production ability of the different isolates (cellulolytic bacteria) by degrading cellulose.

3.2. Evaluation of Cellulose Degrading Ability of Isolated Cellulolytic Bacteria

The result showed that maximum clearing zone ranged from 4.0 to 10.0 mm and the maximum HC value, i.e. ratio of zone size to colony diameter ranged from 1.40 to 2.18 mm on CMC assay (Table 1) demonstrating that all the isolates had the ability to degrade the carboxymethyl cellulose and indicating high ability of cellulase production. Distinct cellulase production ability were detected by different size of zone formation produced by different bacterial isolates. As evidenced by sample 2 (10^{-3}) which was isolated from garden soil of girls' Hall (NSTU campus) which maximum clearing zone size was 10.0 mm and HC value 2.18 mm. From the observation, it was clear that the largest zone size of the sample 2 (10^{-3}) was most efficient in producing cellulase enzyme when compared with others, followed by sample 15 (10^{-3}) collected from the soil beneath a Mango tree (Maijdee) produce maximum clearing size of zone 8.95 mm and HC value 1.92 mm, sample 18(10^{-3}) collected from the soil beneath a Coconut tree (campus) produce maximum zone 7.5 and HC value 1.67 mm, sample 5(10^{-5}) collected from the soil of a road side Banyan tree (campus) produce maximum clearing zone 6.4 and HC value 1.64 mm, sample 4(10^{-5}) collected from the soil beneath a Lemon Tree (Maijdee) produce maximum clearing zone size 5.9 and HC value 1.58 mm, sample 8 (10^{-3}) collected the soil beneath a Guava tree (Maijdee) produce maximum clear zone 5.2 and HC value 1.50 mm, sample 3(10^{-3}) collected from the soil of NSTU Park (campus) produce maximum zone 4.9 and HC value 1.45, sample 9(10^{-3}) collected from the Garden soil (Maijdee) produce maximum clearing zone around colony 4.5 and highest HC value 1.41mm, respectively. In Figure 1 and Figure 2, sample 2 (10^{-3}) exhibits the largest clear zone on CMC agar medium by using congo red reagent. The graph in Figure 3 showed the highest cellulase production ability of the isolates extracted from Table 1.

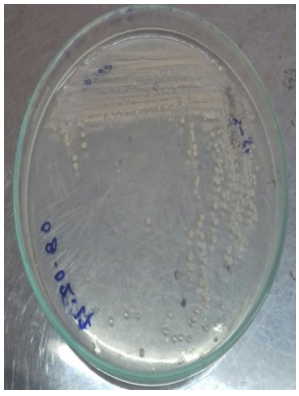

Figure 1. Growth on CMC agar plate (streaking) before staining with congo red, sample 2 (10^{-3})

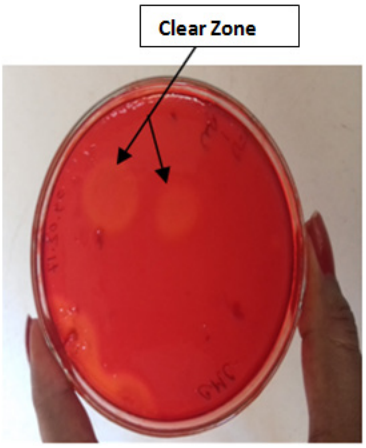

Figure 2. Formation of clear zone around colonies after staining with congo red and Nacl, sample 2 (10^{-3})

3.3. Isolation of Bacteria from Sample

A total number of 30 isolates were selected from 20 samples enrolled in the research and then inoculated on four different media namely Bacillus Cereus agar, Mac Conkey agar, Cetrimide agar, Starch Casein agar plates. Selective colonies gave positive result on selective media. The samples contained many different isolates that were confirmed by different biochemical test.

3.4. Identification of the Isolates

3.4.1. Cultural Characteristics of the Isolated Colonies and Biochemical Identification

In order to identify the isolates, the cultural characteristics on solid media, microscopic examination followed by various biochemical tests were undertaken.

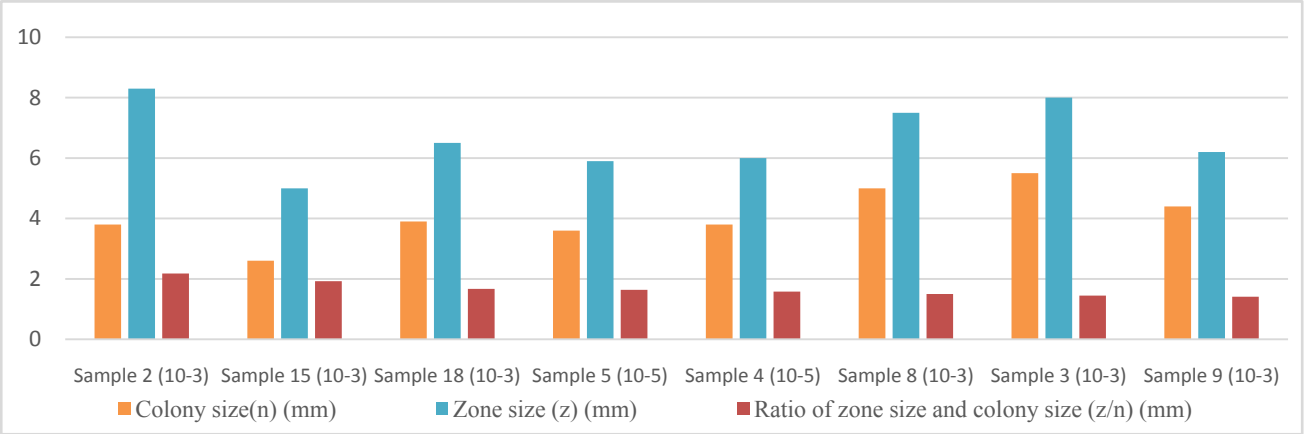

Figure 3. Highest cellulase production ability of different isolates (sample 2, 3, 8, 9, 15, 18 (10^{-3}), 4, 5(10^{-5}))

Table 1. Zone of hydrolysis of different isolates

Sample N0.	Dilution	CMC	Colony size (n) (mm)	Zone size (z)(mm)	Ratio of zone size and colony size (z/n) (mm)
1	10^{-3}	+	4.01	6.5	1.62
	10^{-4}	+	4.13	9.0	2.18
	10^{-5}	+	4.11	6.7	1.63
2	10^{-3}	+	4.59	10.0	2.18
	10^{-4}	+	4.56	8.7	1.91
	10^{-5}	+	4.70	10.0	2.13
3	10^{-3}	+	3.38	4.9	1.45
	10^{-4}	+	4.75	9.5	2.00
	10^{-5}	+	4.50	8.2	1.82
4	10^{-3}	+	3.55	5.5	1.55
	10^{-4}	+	3.17	4.6	1.45
	10^{-5}	+	3.73	5.9	1.58
5	10^{-3}	+	3.68	6.0	1.63
	10^{-4}	+	4.04	6.5	1.61
	10^{-5}	+	3.90	6.4	1.64
6	10^{-3}	+	4.20	7.0	1.67
7	10^{-3}	+	4.23	8.0	1.89
8	10^{-3}	+	3.47	5.2	1.50
9	10^{-3}	+	3.19	4.5	1.41
10	10^{-3}	+	4.13	6.6	1.60
11	10^{-3}	+	3.59	5.6	1.56
12	10^{-3}	+	4.94	8.8	1.78
13	10^{-3}	+	4.23	6.9	1.63
14	10^{-3}	+	2.86	4.0	1.40
15	10^{-3}	+	4.66	8.95	1.92
16	10^{-3}	+	3.33	5.0	1.50
17	10^{-3}	+	4.66	8.9	1.91
18	10^{-3}	+	4.49	7.5	1.67
19	10^{-3}	+	4.52	8.5	1.88
20	10^{-3}	+	4.25	7.0	1.65

3.4.2. Microscopic Observation of the Isolates

All the isolates were examined microscopically after Gram staining to determine whether the isolates were Gram (+ve) or Gram (-ve) and to observe their arrangements. The results showed that samples(1,4,5 (10^{-3}, 10^{-4}, 10^{-5}); 2 (10^{-3}, 10^{-5}); 3 (10^{-3}, 10^{-4}); 6, 9, 10, 11, 13 14, 18, 20 (10^{-3})) were Gram (+ve) and samples (2 (10^{-4}), 3 (10^{-5}), 7, 8, 12, 15, 16, 17, 19 (10^{-3})) were Gram (-ve) bacteria (Table 2). The percentage of Gram (+ve) and Gram (-ve) bacteria in the total cellulolytic bacteria showed in Figure 4. The colony characteristics of the isolates were found variable. The size of the colonies of the isolates ranged from 2.86 mm to 4.94 mm. Microscopic observation of the isolates revealed that most of them are rod shaped and motile. By Gram staining, morphological characteristics of different types of colonies of each samples were recorded. Isolates were identified by their microscopic examination and biochemical reaction.

Table 2. Biochemical test results of isolates

Sample No.	Dilution	Growth on				No.of Isolates	Gram staining	Oxidase test	Catalase test	Citrate utilization	MIU(motility/indole/urease)	MR	VP	TSI (acid slant / acid butt/gas)	Name of the Organism
		MacConkey agar	Bacillus cereus agar	Cetrimide agar	Starch casein agar										
1	10^{-3}	N	+	N	N	1	+	+	+	+	+/-/-	-	+	-/+/-	*Bacillus cereus*
	10^{-4}	N	N	N		1	+	+	+	+	+/-/-	-	+	+/+/-	*Bacillus spp.*
	10^{-5}	N	+	N		1	+	+	+	+	+/-/-	-	+	-/+/-	*Bacillus cereus*
2	10^{-3}	N	N	N	N	1	+	+	+	+	+/-/-	-	+	+/+/-	*Bacillus spp.*
	10^{-4}	+	N	+		1	-	+	+	+	+/-/-	-	-	-/-/-	*Psedomonas aeruginosa*
	10^{-5}	N	N	N		1	+	+	+	+	+/-/-	-	+	+/+/-	*Bacillus spp.*
3	10^{-3}	N	N	N	N	1	+	+	+	-	+/-/-	+	-	+/-/-	*Bacillus megaterium*
	10^{-4}	N	N	N		1	+	+	+	+	+/-/-	-	+	+/+/-	*Bacillus spp.*
	10^{-5}	+	N	+		1	-	+	+	+	+/-/-	-	-	-/-/-	*Psedomonas Aeruginosa*
4	10^{-3}	N	N	N	N	1	+	-	-	-	+/-/-	+	-	-/+/+	*Clostridium spp*
	10^{-4}	N	N	N		1	+	+	+	-	+/-/-	+	-	+/-/-	*Bacillus megaterium*
	10^{-5}	N	N	N		1	+	-	-	-	+/-/-	+	-	-/+/+	*Clostridium spp*
5	10^{-3}	N	+	N	N	1	+	+	+	+	+/-/-	-	+	-/+/-	*Bacillus cereus*
	10^{-4}	N	+	N		1	+	+	+	+	+/-/-	-	+	-/+/-	*Bacillus cereus*
	10^{-5}	N	+	N		1	+	+	+	+	+/-/-	-	+	-/+/-	*Bacillus cereus*
6	10^{-3}	N	N	N	+	1	+	+	+	+	-/-/+	+	+	-/+/-	*Actinomycetes spp.*
7	10^{-3}	+	N	+	N	1	-	+	+	+	+/-/-	-	-	-/-/-	*Psedomonas Aeruginosa*
8	10^{-3}	+	N	N	N	1	-	-	+	+	-/+/+	-	-	-/-/-	*Acinetobacter spp*
9	10^{-3}	N	N	N	N	1	+	-	+	-	-/-/+	+	+	+/+/-	*Staphylococcus Aureus*
10	10^{-3}	N	+	N	N	1	+	+	+	+	+/-/-	-	+	-/+/-	*Bacillus cereus*
11	10^{-3}	N	N	N	N	1	+	-	-	-	+/-/-	+	-	-/+/+	*Clostridium spp*
12	10^{-3}	+	N	+	N	1	-	+	+	+	+/-/-	-	-	-/-/-	*Psedomonas Aeruginosa*
13	10^{-3}	N	+	N	N	1	+	+	+	+	+/-/-	-	+	-/+/-	*Bacillus cereus*
14	10^{-3}	N	N	N	N	1	+	-	+	-	-/-/+	+	+	+/+/-	*Staphylococcus Aureus*
15	10^{-3}	+	N	+	N	1	-	+	+	+	+/-/-	-	-	-/-/-	*Psedomonas Aeruginosa*
16	10^{-3}	+	N	N	N	1	-	-	+	+	-/+/+	-	-	-/-/-	*Acinetobacter spp.*
17	10^{-3}	+	N	+	N	1	-	+	+	+	+/-/-	-	-	-/-/-	*Psedomonas Aeruginosa*
18	10^{-3}	N	N	N	+	1	+	+	+	+	-/-/+	+	+	-/+/-	*Actinomycetes spp.*
19	10^{-3}	+	N	+	N	1	-	+	+	+	+/-/-	-	-	-/-/-	*Psedomonas Aeruginosa*
20	10^{-3}	N	N	N	+	1	+	+	+	+	-/-/+	+	+	-/+/-	*Actinomycetes spp.*

Note: N for not applied.

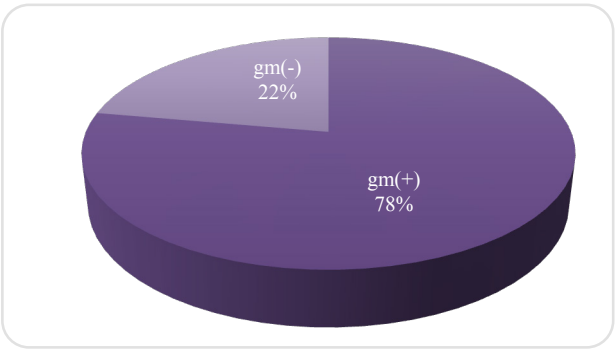

Figure 4. Percentage of Gram (+ve) and Gram (-ve) cellulolytic bacteria

3.4.3. Biochemical Identification

In this work, 30 isolates were found from 20 samples. All the isolates were subjected to different biochemical tests for their identification (Table 2). On the basis of their morphological, biochemical characterization these isolates were confirmed as *Bacillus spp* (Sample 1 (10^{-4}), 2 (10^{-3}, 10^{-5}), 3 (10^{-4}); *Bacillus cereus* (Sample 1 (10^{-3}, 10^{-5}), 5 (10^{-3}, 10^{-4}, 10^{-5}), 10 (10^{-3}), 13 (10^{-3})); *Bacillus megaterium* (Sample 3 (10^{-3}), 4 (10^{-4})); *Actinomycetes spp* (sample 6, 18, 20 (10^{-3})); *Clostridium spp* (Sample 4 (10^{-3}, 10^{-5}), 11 (10^{-3})); *Staphylococcus aureus* (Sample 9, 14 (10^{-3})); *Pseudomonas aeruginosa* (sample 2 (10^{-4}), 3 (10^{-5}), 7, 12, 15, 17, 19 (10^{-3})); *Acinetobacter spp* (Sample 8, 16 (10^{-3})). From the investigation, eight cellulolytic bacteria were isolated and identified from total sample of 30 isolates, where 7 were *Bacillus cereus*, 7 were *Pseudomonas aeruginosa*, 4 were *Bacillus spp*, 3 were *Clostridium spp*, 3 were *Actinomycetes spp*, 2 were *Bacillus megaterium*, 2 were *Staphylococcus aureus*, 2 were *Acinetobacter spp,* respectively (Figure 5).

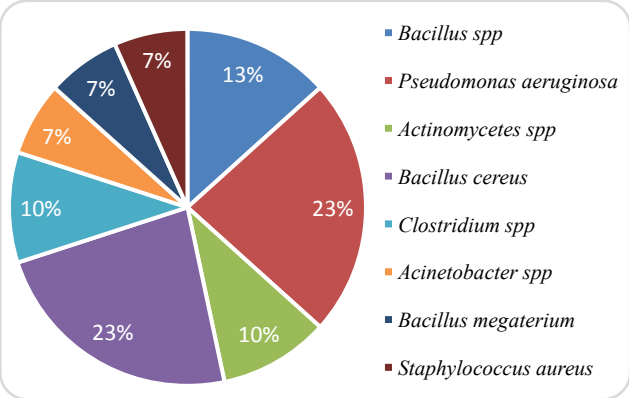

Figure 5. Percentage of different isolates in total isolates

3.5. Antibiogram

Table 3. Antibiotic sensitivity and resistance pattern of detected isolates

Name of the Organism	Zone of inhibition (diameter in mm)						
	E	**AZM**	**C**	**CN**	**CIP**	**P**	**TE**
Bacillus spp	24	21	19	17	22	31	R
Bacillus cereus	27	18	19	19	23	30	R
Bacillus megaterium	28	18	18	15	23	29	R
Acinetobacter spp.	23	19	18	15	22	32	R
Clostridium spp.	25	18	19	16	23	29	21
Actinomycetes	29	22	18	17	29	31	19
Psedomonas aeruginosa	23	20	R	16	27	30	R
Staphylococcus aureus	27	18	18	17	22	29	19

Note: R indicates Resistance.

Eight cellulolytic bacteria that were isolated from soil sample and identified by performing different biochemical test chosen to determine the pattern of antibiotic susceptibility. Seven commonly used clinical purpose antibiotics namely Erythromycin, Azithromycin, Ciprofloxacin Gentamycin, Chloramphenicol, Penicillin, Tetracycline were used for this test. The result of antibiogram indicated that *Clostridium spp, Staphylococcus aureus, Actinomycetes spp* were sensitive to all the used antibiotics. On the other hand, *Bacillus spp, Bacillus cereus, Bacillus megaterium, Acinetobacter spp* showed resistance to Tetracycline whereas sensitive to all other antibiotics. *Pseudomonas aeruginosa* showed resistance to Chloramphenicol and Tetracycline but given sensitivity to all other test antibiotics. Table 3 and Figure 6 showed the results of antibiotic sensitivity and resistance pattern of the inspected bacteria.

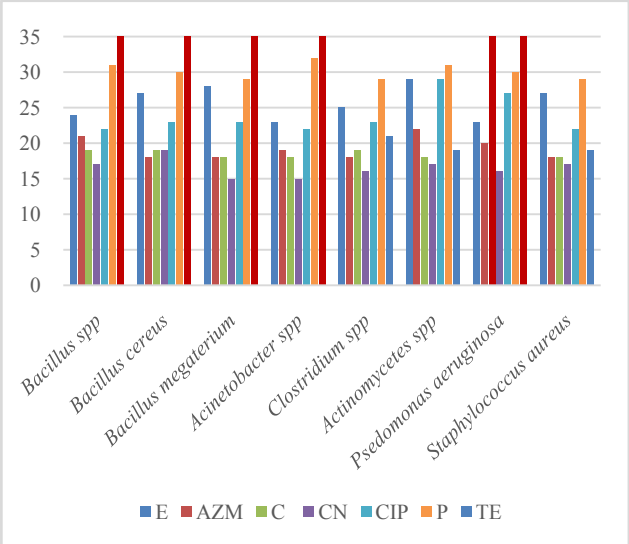

Figure 6. Antibiotic sensitivity and resistance pattern of isolated and identified eight isolates (Red Bars indicates Antibiotic resistance)

4. Discussion

On earth, cellulose is the most abundant plant biomass and one of the plentiful organic substance. This multiplicity confers to cellulose an immense potential as a renewable source of energy which is degraded to produce cellulase enzyme through enzymatic hydrolysis using cellulolytic bacteria. This research isolated and identified eight cellulolytic bacteria from 30 isolates of 20 samples and all had the cellulose degrading ability by producing cellulase enzyme. This is similar to work of Behera et al. (2014) that worked with 15 isolates of 15 samples where all the isolates had cellulase production ability including *Bacillus spp.* and *pseudomonas aeruginosa*. In a similar work, Tabo and Monsalud (2010), reported the occurrence of *Bacillus cereus, Bacillus licheniformis, Bacillus spp* and *Bacillus pumilus* from philipines mangrove soil. Thatoi et al. (2012), also reported the cellulolytic activity of *Pseudomonas spp, Bacillus spp, Bacillus polymyxa,* and *Bacillus brevis* from mangrove soil of Bhitarakanika, Odisha. Jeffrey (2008) also reported the cellulolytic activity of *Actinomycetes spp* from agriculture soils at Semongok.

Ahmad et al. (2013) conducted a similar research in Municipal Solid Waste, where only 15 isolates were found having cellulase activity out of 108. On the contrary, in our work, all the experimented isolates presented cellulase activity. Rawway et al. (2018), also worked with five different sources and found only 10 isolates out of 120 isolates that showed cellulase activity.

The highest cellulase production ability of eight different cellulolytic bacteria in our work are arranged below: *Bacillus spp >Pseudomonas aeruginosa > Actinomycetes spp >Bacillus cereus > Clostridium spp > Acinetobacter spp>Bacillus megaterium >Staphylococcus aureus.*

In this investigation, eight species exhibited the largest clear zones and the maximum HC value, i.e. ranged from 1.40 to 2.18 mm on CMC assay. In a similar work, Rawway et al. (2018), observed the maximum HC value ranged from 1.33 to 2.87 mm. Hatami et al. (2008), who also observed the ratio of the zone diameter to colony diameter was 0.4 to 2.1. In comparison to the findings, Lu et al. (2005), observed maximum clearing zone ranged from 2.5 to 6.4 cm with maximum HC value of 4.85-13.11 cm. Gupta et al. (2012), exhibited the maximum zone of clearance around the colony with diameter of 28 to 50 mm with the hydrolytic value of 4.3 to 9.8 mm respectively. On the contrary, our observed HC value is lower than the value.

From the antibiogram result, the diameter of inhibition zones showed that all the isolates have sensitivity against bacteria but some species showed resistance to Tetracycline and Chloramphenicol. This is a similar work to Behera et al. (2014), where *Bacillus spp* and *Pseudomonas spp* showed resistance to tetracycline. Patel et al. (2015) also found slightly different result where the cellulolytic bacteria found to be more sensitive to Gentamycin, Streptomycin, Kanamycin, and Tetracycline respectively and resistant to Lincomycin, Chloramphenicol, Rifampcin, and Penicillin G respectively.

5. Conclusion

Treatment of cellulose by cellulase enzyme from different bacterial isolates has attracted the continuing interest of biotechnologists, taxonomists, enzymologists and even some industrialists in their own researches. This research indicates that the garden soil of girls' hall (NSTU campus), followed by soil of Mango tree (Maijdee), Coconut tree (campus), Banyan tree (campus), Lemon Tree (Maijdee), Guava tree (Maijdee), NSTU Park (campus), Garden soil (Maijdee) will be effective in producing cellulase enzyme by different cellulolytic bacteria. The use of these cellulolytic bacteria as bio-inoculants can be incorporated to enhance organic matter decomposition in soil to increase soil fertility and to minimize the fertilizer application in the area of NSTU campus and Maijdee. These bacteria can also be applied to reduce the environmental pollution and promote sustainable agriculture. This antibiogram result can be a useful tool for detecting and monitoring trends in antimicrobial resistance and guide the physicians in selecting the best empiric antimicrobial treatment.

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