World Journal of Environmental Biosciences

Available Online at: **www.environmentaljournals.org**

Volume 9, Issue 2: 8-13

ISSN 2277- 8047

Isolation and Identification of Cellulose-Degrading Bacteria from Different Types of Samples

Riad Mahmood, Nazia Afrin*, Shamima Nasrin Jolly and Rasheda Yasmin Shilpi

Department of Botany, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

ABSTRACT

Microbiological utilization of cellulose is the key factor for the utmost material flow in the biosphere. The present study aimed at the isolation and identification of bacteria with cellulase activity from cellulose containing samples. Samples were collected from soil, domestic kitchen waste, and sawdust. A total of 42 bacterial isolates were isolated through serial dilutions and spread plate method in carboxymethyl cellulose (CMC) agar media. The isolates were then screened using congo red staining on CMC agar plates for cellulolytic activity. Among the 42 isolates, only 24 (57%) isolates showed cellulolytic activity. Morphological and biochemical assays suggested that the cellulose-degrading bacterial isolates were members of the genus Bacillus sp., Pseudomonas sp., Staphylococcus aureus, S. epidermidis, and Salmonella sp. The Salmonella sp. (CDB18) (0.5170 IU/ml/min), Bacillus sp. (CDB20) (0.4890 IU/ml/min) and Bacillus sp., (CDB13) (0.4016 IU/ml/min) showed maximum enzymatic activity. Therefore, these bacterial species can be utilized for effective biodegradation of cellulose-containing substrates.

Keywords: *cellulolytic activity, morphological analysis, biochemical analysis, CMC agar, cellulase*

Corresponding author: Nazia Afrin
e-mail ✉ naziaafrin49 @ gmail.com
Received: 12 January 2020
Accepted: 29 May 2020

1. INTRODUCTION

Cellulose is a major component of plant biomass. It is an abundant, cheap biopolymer, and a renewable resource of energy (Khaleel et al., 2018). Approximately 100 billion metric tons cellulose is produced naturally every year, while the entire biomass is around 280 billion metric tons (Cheng et al., 2010). Cellulose is mainly a polysaccharide having a fibrous crystalline appearance and made up of the repeating units of D-glucose, which is linked by β-1, 4- glycosidic linkage (Zaghoud, et al., 2019). It has a high molecular weight and is soluble in water (Malherbe and Cloete, 2003). It can be changed into glucose and other soluble sugars by the process called cellulolysis. For cellulolysis, a set of enzymes named cellulase are required, which includes endoglucanase (endo-1, 4-β-D-glucanase); cellobiohydrolase or exoglucanase (exo-1, 4-β-D-glucanase), and β-glucosidase (1,4-β-D-glucosidase) (Li and Gao, 2008). Different microorganisms produce this inducible bioactive compound during their growth and development on cellulosic matters (Gomashe et al., 2013). Cellulolytic microorganisms mostly fungi and bacteria are involved in cellulosic compound degradation in soils (Ojumu et al., 2003).

Cellulase has massive applicability in different industrial processes involving biofuels like bioethanol (Vaithanomsat et al., 2009), triphasic bio methanation (Chakraborty et al., 2000); plant and agricultural waste management, ligand binding and chiral separation studies (Lu et al., 2004; Nutt et al., 1998). However, there is a deficiency of microorganisms that can produce a significant amount of cellulase enzyme to efficiently convert cellulose into fermentable products (Maki et al., 2009). Bacteria are now being widely explored for cellulase production because of their extremely high natural diversity and the capability to produce stable enzymes that can be applied in industries (Haakana et al., 2004; Ashjaran and Sheybani, 2019). Bacterial cellulases usually act as a highly effective and a potent catalyst (Gautam et al., 2010) and is widely used because of their rapid growth, expression of multi-enzyme complexes, stability at extremes of temperature and pH, lesser feedback inhibition and capacity to colonize a wide variety of environmental niches (Maki et al., 2009).

Bacterial efficient bioconversion of cellulose-containing materials largely depends on the types of cellulose, sources of the cellulolytic enzyme, and favorable conditions for the production of enzymes and its catalytic activity. Moreover, cellulose nature and quality, aeration temperature, incubation period, carbon sources, pH of the medium, medium additives, and presence of different inducers are also crucial parameters for the maximum production of different cellulase enzymes (Angsana et al., 2009).

Experiments on the isolation and characterization of active cellulase producing microorganisms from various sources, like soil, organic matters, decayed plant materials, feces of ruminants, hot springs, and composts have been continued for many years (Doi, 2008). However, most of the studies emphasized on fungi with less emphasis on bacteria as a source for cellulase production. So, the primary objective of this research was the isolation and identification of bacterial species capable of efficiently hydrolyzing the cellulosic

compounds and acting as an enhancer in the biodegradation of these cellulosic compounds.

2. MATERIALS AND METHODS

2.1 Sample collection

The samples were collected from various habitats (sawdust, kitchen-waste, and soil substances) and were stored at 4°C in sterile containers until inoculation.

2.2 Isolation of soil bacteria

One gram of soil sample was added to 9 mL of distilled water in a sterile test tube and was shaken thoroughly. Dilutions were made up to 10^{-6}. About 0.1 ml of inoculum from serially diluted samples were spread on CMC (carboxymethyl cellulose) agar plate thoroughly and incubated at 37°C for 24 hours.

2.3 Determination of cellulase producing activity of the bacterial isolates

The CMC agar plates incubated with bacterial isolates were then flooded with 0.1% Congo red solution for 20 minutes. After that, the plates were rinsed with 1 M NaCl solution and visualized the hydrolysis zone. A clear hydrolysis zone formation mentions cellulose degradation. The diameter of the bacterial colony (d) and clear zone (D) around the colony after Congo red and NaCl treatment was measured. The ratio (R) of D and d was also calculated to identify the highest cellulase activity of bacterial isolates. On the basis of R values, nine isolates with the highest R values were selected for further quantitative assay of cellulase production. The largest ratios were assumed to have the maximum cellulase producing activity.

2.4 Assessment of carboxymethyl cellulase (CMCase) activity of bacterial isolates

Quantitative analysis for the cellulase production by the isolates was performed by the CMCase assay method for Endo 1, 4-glucanase. DNS method was used for reducing sugar estimation for this purpose. The activity was assessed using a 1% carboxymethyl cellulose (CMC) solution in 0.05 M citrate buffer (pH 4.8) used as a substrate. The reaction was run at 50°C for about 30 minutes, and it was terminated by adding 3ml of DNS solution into the reaction mixture. The mixture was then boiled at 100°C for about 5 minutes. Optical Density (OD) was measured with a systronics119 spectrophotometer at 540 nm. The assessment was done depending on the obtained by-product.

2.5 Characterization & identification of bacterial isolates

These isolates were then chosen to study their morphological, physiological, and biochemical characteristics to identify their genus and species names. Different selective and differential media were used to culture the bacterial colonies such as MacConkey, EMB, BGA, King's B, Bouillon Agar, MSA, etc. Different biochemical tests (Catalase, Indole, Fermentation, Starch hydrolysis) were also conducted. Results of different physiological and biochemical tests were examined following Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

3. RESULTS

3.1 Isolation of bacterial isolates from samples

A total of forty-two bacterial isolates were isolated from different samples and screened for the bacteria with cellulase producing capability through congo red assay, carboxymethyl cellulase assay, and biochemical identification.

3.1.1 Congo red assay for cellulose production

Out of 42 isolates, 24 (57%) showed a distinct clear zone around colonies in the CMC agar plate after Congo red staining. Out of these 24 isolates, only nine bacterial isolates showed zone to colony ratio (R) value within the range of 2.33 and above. Among all, only three bacterial species exhibited the highest cellulose-degrading activity within the range of 3.3-4 ratios, 6 showed high activity range of 2.5-2.88, while ten isolates showed moderate cellulolytic activity within the range of 2.0-2.33 ratios as given in Table 1. The rest 4 isolates showed lower cellulolytic activity within the range of 0.2-1.77. Cellulase production of the isolates and R-value are positively correlated to the results of a study conducted by Hong-li et al. (2015). The highest R-value containing isolates (CDB2, CDB4, CDB6, CDB7, CDB13, CDB15, CDB18, CDB20, and CDB21) were considered to be efficient isolates for cellulase production. The diameters of clear zones (D), colony size (d), and R-values of the cellulase producing isolates are shown in Table 1.

Table 1: Determination of cellulase enzyme activity of some cellulose-degrading bacterial isolates in CMC agar plates through the halo zone formation

Sl. No	Sample Name	Isolate	Colony size, d (cm)	Zone size, D (cm)	Ratio of zone size and colony size, R= D/d
1	S1C1	CDB1	0.3	0.6	2
2	S1C2	CDB2	1.2	3	2.5
3	S2C2	CDB3	1.8	3.2	1.77
4	S2C5	CDB4	0.9	2.1	2.33
5	S3C2	CDB5	0.2	0.4	2
6	S3C4	CDB6	0.9	2.5	2.77
7	S4C2	CDB7	0.4	1.1	2.75
8	S6C1	CDB8	0.7	1.6	2.28
9	S6C3	CDB9	0.4	0.9	2.25

10	S7C2	CDB10	0.2	0.35	1.75
11	SDC1	CDB11	0.1	0.2	2
12	SDC2	CDB12	0.4	0.8	2
13	SDC3	CDB13	0.2	0.7	3.5
14	SDC4	CDB14	0.2	0.4	2
15	SDC5	CDB15	0.15	0.42	2.8
16	SDC6	CDB16	0.1	0.3	0.2
17	SDC7	CDB17	0.1	0.15	1.5
18	KC4	CDB18	0.4	1.6	4
19	KC5	CDB19	0.4	0.8	2
20	KC6	CDB20	0.6	1.7	2.8
21	KC7	CDB21	0.15	0.6	3.3
22	KC8	CDB22	0.4	0.8	2
23	KC9	CDB23	1	2	2
24	KC10	CDB24	0.25	0.72	2.88

3.1.2 Carboxymethyl cellulase assay of bacterial isolates

The CMCase activity of CDB18 (**0.5170**) was the highest of all the isolates, followed by CDB20 (0.4890) and CDB13 (0.4016) (Table 2).

Table 2: Estimation of enzyme activity (DNS method) value (I.U./ml/min)

Sl. No	Sample Name	Isolate	Absorbance (I.U./ml/min)
1	S2C5	CDB4	0.3120
2	SDC1	CDB6	0.3566
3	SDC2	CDB7	0.3542
4	SDC4	CDB2	0.3170
5	SDC5	CDB15	0.3215
6	KC4	CDB18	0.5170
7	KC5	CDB13	0.4016
8	KC6	CDB20	0.4890
9	KC7	CDB21	0.3523

3.1.3 Biochemical identification of bacterial isolates

In this study, *Staphylococcus aureus, S. epidermidis, Bacillus* sp., *Pseudomonas* sp., and *Salmonella* sp. were presumptively isolated through their typical colony characteristics onto specific culture media. *S. aureus* gave yellow-colored, and *S. epidermidis* gave pink-colored colonies on MSA media. *Bacillus* sp. gave a white-colored colony on Bouillon agar, and *Pseudomonas* sp. showed a greenish-yellow colored colony on King's B media. Its pink-colored colony identified *Salmonella* sp. on EMB agar media. Besides, the results of the performed biochemical tests of different isolates revealed that out of 9 isolates, 6 (67%) isolates were Gram-positive and the rest 3 (33%) were Gram-negative and rod-shaped. Among the Gram-positive isolates, 4 (67%) were cocci and 2 (33%) were rod-shaped. All the isolates were catalase-positive and indole negative. Only 3 (33%) out of 9 isolates showed a positive starch hydrolysis test, and 2 (22%) were positive for endospore staining, indicating the presence of *Bacillus* sp. Among four cocci-shaped bacteria, 2 (50%) were positive for the coagulase test, which confirmed the presence of *S. aureus.* Among nine isolates, 2 (22%) Gram-negative isolates were positive for the lactose fermentation test, which showed a pink-colored colony on EMB media indicating the presence of *Salmonella* sp.

Table 3: Results of some major biochemical tests of isolated cellulose-degrading bacterial colonies from different samples

Isolate No.	Gram Reaction	Catalase test	Fermentation test	Starch hydrolysis	Indole test	Endospore staining
CDB2	+	+	_	+	_	_
CDB4	+	+	_	_	_	+
CDB6	+	+	_	_	_	_
CDB7	_	+	+	_	_	_

CDB13	+	+	_	+	_	+
CDB15	+	+	_	+	_	_
CDB18	_	+	+	_	_	_
CDB20	+	+	_	_	_	+
CDB21	_	+	_	_	_	_

4. DISCUSSION

As cellulase is a widely-used enzyme in various industries, the present study deals with screening, isolation, and characterization of cellulose-degrading bacteria and the estimation of the cellulolytic potential of these isolates. Among different cellulose-containing samples used for this study, the study identified nine bacterial isolates having higher enzyme activities and considered as better cellulose degraders and can be used to degrade cellulose from decayed plants more effectively.

Agar media containing cellulose or CMC for the identification of cellulolytic bacteria through the formation of a clear hydrolytic zone have been reported by many researchers (Baharuddin et al., 2010; Gomashe et al., 2013; Das et al., 2014). In the current study, out of 42 bacterial isolates, 24 (57%) isolates showed a hydrolytic zone between 0.3-3.2 cm and colony size of 0.1-1.8 cm. Among the efficient isolates, the maximum cellulolytic zone to colony diameter ratio measured was 4 (CDB18), while the minimum ratio was 2.33 (CDB4). These results indicated that the isolated bacterial species have lower to significant ability to produce cellulase. These results have similarities to the findings by Hatami et al. who also reported the hydrolysis zone value in the range of 1.38 to 2.33 and 0.15 to 1.37 cm while studying with cellulose-degrading aerobic bacterial isolates from farming and forest soil, respectively. *Staphylococcus aureus, S. epidermidis, Salmonella sp., Bacillus sp.*, and *Pseudomonas* sp. were isolated as cellulolytic bacteria from different samples in this study. These results are in agreement with those reported in previous studies where the cellulose-degrading microorganisms have been identified and isolated from different environments like soil (Soares et al., 2012; Sethi et al., 2013), organic waste (Ghio et al., 2012), gut (Dantur et al., 2015), animal waste (Singh et al., 2013), marine sediments and seaweeds (Santhi et al., 2014). Lu et al. observed that bacteria belonging to the genera *Cellulomonas, Clostridium, Thermomonospora, Cellulosimicrobium, Erwinia, Bacillus, Ruminococcus, Streptomyces, Bacteriodes, Acetovibrio, Fibrobacter, Microbispora,* and *Paenibacillus* had been reported to produce different types of cellulase in anaerobic or aerobic conditions.

Gupta et al. reported several cellulolytic bacteria showing CMCase activities within the range of 0.162–0.400 U/mL. This study was in agreement with our current study, where we identified 24 bacterial isolates as capable of yielding cellulase enzymes out of 42 isolates from different cellulose-containing samples. The CMCase activities were within the range of 0.3170 IU/mL to 0.5170 IU/mL, which were higher than the previous study.

Vimal, 2016 identified *Bacillus cereus* and *Bacillus subtilis* by its morphological, cultural, and biochemical characteristics as cellulose-producing bacteria. We also identified *Bacillus* sp. from soil samples and showed relatively good performance in the production of cellulase enzyme. Out of 9 most cellulase producing isolates, 3 (33%) isolates were identified as *Bacillus* sp. and exhibited CMCase activity of 0.4890 IU/ml, 0.4016 IU/ml and 0.3566 IU/ml, respectively. These results coincide with those reported in previous studies, in which many *Bacillus* sp. including *Brevibacillus brevis*, *Bacillus pumilus*, *Brevibacillus* sp. and *Bacillus subtilis* have been used for cellulase production (Singh and Kumar, 1998; Kotchoni et al., 2006; Rastogi et al., 2009; Yin et al., 2010).

Using response surface methodology, Deka et al. found *Bacillus subtilis* as exhibiting the highest CMCase activity of 0.43 U/mL. Rastogi et al. reported that *Brevibacillus* and *Geobacillus* sp. exhibited the highest CMCase activity of 0.02 and 0.058 U/mL sp., respectively. Das et al. isolated eight bacterial isolates from cow dung samples, and they also identified *Bacillus* sp. as the highest amount of cellulose-producing bacteria. Moreover, *Bacillus* was also recognized as the dominant cellulose-degrading bacteria in different samples collected from organic fertilizers of Red Rock and paper mill sludges of Canada. It was also found in the compost, animal waste slurry as well as soil from Jeju Island (Kim et al., 2012).

Besides, Sheng et al. and Da Vinha et al. reported a maximum CMCase activity of 2.0 and 1.432 U/mL by *Streptomyces viridobrunneus* and *Pseudomonas* sp. respectively using orthogonal experiment design and response surface methodology for medium optimization. *Pseudomonas* sp. was also isolated in our study, and the CMCase activity was 0.3523 IU/mL, which was relatively lower than the previous study. In other studies, Otajevwo and Auyi isolated *Serratia* sp. and *Pseudomonas* sp. from soil samples having a more exceptional capability to produce cellulase enzymes. According to Lynd et al. *Pseudomonas, Aeromonas, Pesturella, Staphylococcus sp., and Bacillus sp.* displayed cellulase-producing activity. Both *Bacillus* and *Pseudomonas sp.* were capable of producing within the range of 0.0038 to 0.2514 IU/ml cellulase in this study. Chen et al. identified *Sphingomonas* sp., *Pseudomonas* sp., *Achromobacter* sp., and *Stenotrophomonas* sp. using a 16S rDNA sequence from cellulose containing samples. Gamache *et al*. also identified *Pseudomonas* spp. and *Thermoactinomycetes* spp. from Nagpur region soil.

Alternatively, some CMCase genes, which were cloned from *Paenibacillus polymyxa*, *Paenibacillus barcinonensis*, *Paenibacillus xylanilyticus*, and *Paenibacillus cookii* have been expressed in *Saccharomyces cerevisiae* and *Escherichia coli* (Shinoda et al., 2012). In our study, no *Paenibacillus* or *E. coli* were identified, but we identified *Salmonella* sp. as maximum cellulose-producing bacteria that was not reported until now in the previous studies.

Staphylococcus aureus and *S. epidermidis* were also identified as cellulose-producing bacteria in this study, and the CMCase activity was 0.0.3170, 0.3120, and 0.3215 IU/ml, respectively. Ahmad et al. also isolated *Staphylococcus* spp. along with *Pseudomonas* spp., *Aeromonas* spp., *Pasteurella* spp. and *Bacillus* from Municipal Waste of Peshawar city and its vicinity.

5. CONCLUSION

Cellulases provide a significant opportunity for attaining tremendous benefits for biomass utilization (Wen et al., 2005). Cellulolytic microorganisms can alter cellulose into different soluble sugars by either enzymatic hydrolysis or acidic reaction. The availability of enough cellulose offers it as an attractive raw material for manufacturing many industrially relevant commodity products. Cellulase has a potentiality to be utilized in biotechnology and industry, viz alcoholic beverage, starch processing, malting, clarify of juice, brewing, pulp bleaching, textile industry, and animal feed (Sreeja et al., 2013; Bakhy et al., 2018). So, in biodegradation, bioremediation and biofuel research, isolation and characterization of cellulolytic bacteria will be continued as an essential aspect. Therefore, the screening of efficient cellulose-degrading organisms, cost-effective operational techniques, and thus useful end-product production from degradation of cellulose would be very beneficial. Using various techniques, further improvement in the case of cellulase performance can be imparted for industrial applications.

REFERENCES

1. Ahmad, B., Nigar, S., Shah, S. S. A., Bashir, S., Ali, J., Yousaf, S., & Bangash, J. A. (2013). Isolation and Identification of Cellulose Degrading Bacteria from Municipal Waste and Their Screening for Potential Antimicrobial Activity. *World Applied Sciences Journal*, 27(11), 1420-1426.
2. Angsana, R., Warinthorn, S., Annoop, N., & Pawinee, C. (2009). Combination effect of pH and acetate on enzymatic cellulose hydrolysis. *Journal of Environmental Science*, 21(7), 965- 970.
3. Ashjaran, A., & Sheybani, S. (2019). Drug Release of Bacterial Cellulose as Antibacterial Nano Wound Dressing. *International Journal of Pharmaceutical Research & Allied Sciences,* 8(3), 137-143.
4. Baharuddin, A. S., Razak, M. N. A., Hock, L. S., Ahmad, M. N., Abd-Aziz, S., Rahman, N. A. A., Shah, U. K. M., Hassan, M. A., Sakai, K.,&Shirai, Y. (2010). Isolation and characterization of thermophilic cellulase producing bacteria from empty fruit bunches palm oil mill effluent compost. *The American Journal of Applied Sciences*, 7(1), 56-62.
5. Bakhy, E. A., Zidan, N. S., & Aboul-Anean, H. E. D. (2018). The Effect of Nano Materials On Edible Coating and Films' Improvement. *International Journal Of Pharmaceutical Research And Allied Sciences*, 7(3), 20-41.
6. Buchanan, R.E., & Gibbons, N.R. *Bergey's Manual of Determinative Bacteriology*, 8th ed., Williams & Wilkins. Baltimore. 1974.
7. Chakraborty, N., Sarkar, G. M., & Lahiri, S. C. (2000). Cellulose degrading capabilities of cellulolytic bacteria isolated from the intestinal fluids of the silver cricket. *Environmentalist*, 20(1), 9–11.
8. Chen, H. J., Chang, H. J., Fan, C., Chen, W. H., &Lee, M. S. (2011). Screening, isolation, and characterization of cellulose biotransformation bacteria from specific soils. *International Conference on Environment and Industrial Innovation.IPCBEE. IACSIT Press, Singapore*, 12.
9. Cheng, Q., Wang, J., McNeel, J. F., & Jacobson, P. M. (2010). Water Retention Value Measurements of Cellulosic Materials Using a Centrifuge Technique. *BioResources*, 5(3), 1945-1954.
10. Da Vinha, F. N. M., Gravina-Oliveira, P. M., &Franco, M. N. (2011). Cellulase production by *Streptomyces viridobrunneus* SCPE-09 using lignocellulosic biomass as inducer substrate. *Applied Biochemistry and Biotechnology*,164(3), 256–267.
11. Dantur, K. I., Enrique, R., Welin, B., & Castagnaro, A. P. (2015). Isolation of cellulolytic bacteria from the intestine of Diatraeasaccharalis larvae and evaluation of their capacity to degrade sugarcane biomass. *AMB Exp*, 5, 15.
12. Das, A., Bhattacharya, S., & Murali, L. (2010). Production of cellulase from a thermophilic *Bacillus* sp. isolated from cow dung. *American-Eurasian Journal of Agricultural and Environmental Science,*8(6), 685-691.
13. Das, P., Solanki, R., & Khanna, M. (2014). Isolation and screening of cellulolytic actinomycetes from diverse habitats. *International Journal of Advanced Biotechnology and Research*, 5, 438-451.
14. Deka, D., Bhargavi, P., Sharma, A., Goyal, D., Jawed, M., & Goyal, A. (2011). Enhancement of cellulase activity from a new strain of *Bacillus subtilis* by medium optimization and analysis with various cellulosic substrates. *Enzyme* Research, 1-8.
15. Doi, R. H. (2008). Cellulase of mesophilic microbes: cellulosome and non–cellulosome producers. *Ann. N.Y. Acad. Sci,* 1125, 267–279.
16. Gautam, S., Bundela, P. S., Pandaey, A. K., Jamaluddin, Awasthi, M. K., & Sarsaiya, S. (2010). Cellulase Production by *Pseudomonas* spp. Isolated from Municipal waste. *International Journal of Academic Research*, 2(6), 330-333.
17. Ghio, S., Lorenzo, G. S. D., Lia, V., Talia, P., Cataldi, A., & Grasso, D. (2012). Isolation of *Paenibacillus* sp. and *Variovorax* sp. strains from decaying woods and characterization of their potential for cellulose deconstruction. *International Journal of Biochemistry and Molecular Biology,* 3, 352-364.
18. Gomashe, A. V., Gulhane, P. A., Bezalwar, P. M. (2013). Isolation and screening of cellulose degrading microbes from Nagpur region soil. *International Journal of Life Science*, 1, 291-293.
19. Gupta, P., Samant, K., & Sahu, A. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology*, 1-5.
20. Haakana, H., Mittinen-Oinonen, A., Joutsjoki, V., Mantyla, A., Souminen, P., &Vahmaanpera, J. (2004). Cloning of cellulase from *Melanocarpus albomyces* and their efficient expression in Trichoderma reesei. *Enzyme Microbial Technology*, 34, 159-167.
21. Hatami, S., Alikhani, H. A., Besharati, H., Salehrastin, N., Afrousheh, M., & Jahromi, Z. Y. (2008). Investigation on aerobic cellulolytic bacteria in some of north forest and farming soils. *Biotechnology and Bioengineering Symposium.*, 3(5), 193-219.
22. Hong-li, Z., Xiao, Y., Dong-mei, X., Lu, Z., Kai-zhong, T., Xing-yao, X., Yun, L., **&** Xiao-jun, S. (2015). Isolation, Identification and Characterization of Cellulose-Degradation Bacteria from Fresh Cow Dung and

Fermentation Biogas Slurry. Research & Reviews: Journal of Microbiology and Biotechnology, 4(3), 30-37.
23. Khaleel, H. L., Abd, A. N., & Ali, K. M. (2018). Preparation of Nano-Cellulose from Industrial Waste by Ultrasonic Device. *Journal of Biochemical Technology*, 9(1), 35.
24. Kim, Y. K., Lee, S. C., Cho, Y. Y., Oh, H. J., & Ko, Y. H. (2012). Isolation of cellulolytic *Bacillus subtilis* strains from agricultural environments. *ISRN Microbiology*, 9.
25. Kotchoni, S. O., Gachomo, E. W., Omafuvbe, B. O., &Shonukan, O. O. (2006). Purification and Biochemical Characterization of Carboxymethyl Cellulase (CMCase) from a Catabolite Repression Insensitive Mutant of *Bacillus pumilus*. *Int. J. Agri. Biol.*, 8, 286-292.
26. Li, X., & Gao, P. (2008). Isolation and partial properties of cellulose-decomposing strain of *Cytophaga* sp. LX-7 from the soil. *J. Appl. Microbiol*., 82, 73-80.
27. Lu, W. J., Wang, H. T., & Nie, Y. F. (2004). Effect of inoculating flower stalks and vegetable waste with ligno-cellulolytic microorganisms on the composting process. *Journal of Environmental Science and Health*, 39(5-6), 871–887.
28. Lu, W. J., Wang, H. T., Yang, S. J., Wang, Z. C., & Nie, Y. F. (2006). Isolation and characterization of mesophilic cellulose degrading bacteria from flower stalks-vegetable waste co-composting system. *Journal of General Applied Microbiology*, 51, 353-360.
29. Lynd, L. R., Weimer, P. J., Van,Zyl, W. H., & Pretorius, I. S. (2002). Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiol. Mol. Biol*., 66(3), 506–577.
30. Maki, M., Leung, K. T., & Qin, W. (2009). The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. *Int. J. Biol. Sci*., 5, 500-516.
31. Malherbe, S., & Cloete, T. E. (2003). Lignocellulose biodegradation: fundamentals and applications. *Reviews in Environmental Sciences and Biotechnology*, 1, 105-114.
32. Nutt, A., Sild, V., Prtterson, G., &Johansson, G. (1998). Progress curve: A means for functional classification of cellulases. *Europian Journal of Biochemistry*, 258 (1), 200-206.
33. Ojumu, T., Solomon, V., Bamidele, O., Betiku, E., &Layokun, S. K. (2003). Cellulase Production by *Aspergillus flavus* Linn Isolate NSPR 101 fermented in sawdust, bagasse and corncob**.** *African J. Biotechnol*., 2, 150–152.
34. Otajevwo, F. D., & Aluyi, H. S. A. (2010). Cultural conditions necessary for optimal cellulase yield by cellulolytic bacterial organisms as they relate to residual sugars released in broth medium. *Nigerian J. Microbiol*., 24(1), 2168 – 2182.
35. Rastogi, G., Muppidi, G. L., Gurram, R. N., Adhikari, A., Bischoff, K. M., Hughes, S. R., Sani, R. K. (2009). Isolation and characterization of cellulose-degrading bacteria from the deep subsurface of the Homestake gold mine, Lead, South Dakota, USA. *Journal of Industrial Microbiology and Biotechnology*,36(4), 585-598.
36. Santhi, V. S., Bhagat, A. K., Saranya, S., Govindarajan, G., Jebakumar, S. R. D. (2014). Seaweed (*Eucheuma cottonii*) associated microorganisms, a versatile enzyme source for the lignocellulosic biomass processing. *Int. Biodeterior. Biodegradation*, 96, 144-151.
37. Sethi, S., Datta, A., Gupta, B. L., & Gupta, S. (2013). Optimization of cellulase production from bacteria isolated from soil. *ISRN Biotechnol*., 985685.
38. Sheng, P., Huang, S., Wang, Q., Wang, A., & Zhang, H. (2012). Isolation, screening, and optimization of the fermentation conditions of highly cellulolytic bacteria from the hindgut of *Holotrichia parallela* larvae (Coleoptera: Scarabaeidae). *Applied Biochemistry and Biotechnology*, 167(2), 270–284.
39. Shinoda, S., Kanamasa, S., & Arai, M. (2012). Cloning of an endoglycanase gene from *Paenibacillus cookii* and characterization of the recombinant enzyme. *Biotechnology Letters*, 34(2), 281–286.
40. Singh, S., Moholkar, V. S., & Goyal, A. (2013). Isolation, identification, and characterization of a cellulolytic *Bacillus amyloliquefaciens* strain SS35 from rhinoceros dung. *ISRN Microbiology*, 1-7.
41. Singh, V. K., & Kumar, A. (1998). Production and purification of an extracellular cellulase from *Bacillus brevis* VS-1. *Biochem. Mol. Biol. Int*., 45, 443-452.
42. Soares, F. L., Melo, I. S., Dias, A. C., & Andreote, F. D. (2012). Cellulolytic bacteria from soils in harsh environments. *World Journal of Microbiology and Biotechnology*, 28(5), 2195-2203.
43. Sreeja, S. J., Malar, P. W. J., Joseph, F. R. S., Tiburcius, S., Immanuel, G., & Palavesam, A. (2013). Optimization of cellulase production by *Bacillus altitudinis* APS MSU and *Bacillus licheniformis* APS2 MSU, gut isolates of fish Etroplussuratensis. *Int. J. Adv. Res. & Tech*., 2(4), 401-406.
44. Vaithanomsat, P., Chuichulcherm, S., & Apiwatanapiwat, W. (2009). Bioethanol production from enzymatically saccharified sunflower stalks using steam explosion as pretreatment—*Proceedings of World Academy of Science, Engineering and Technology*, 37, 140–143.
45. Vimal, J., Venu, A., & Joseph, J. (2016). Isolation and identification of cellulose degrading bacteria and optimization of the cellulase production. *International Journal of Research in Biosciences*. 5(3), 58-67.
46. Wen, Z., Liao, W., & Chen, S. (2005). Production of cellulase by *Trichoderma reesei* from dairy manure. *Bioresource Technology*, 4, 491-499.
47. Yin, L. J., Lin, H. H., & Xiao, Z. R. (2010). Purification and characterization of a cellulase from *Bacillus subtilis* YJ1. *J. Marine Sci. Technol.*, 18, 466-471.
48. Zaghoud, L., Gouamid, M., Benmenine, A., & Khanblouche, A. (2019). Kinetic and Thermodynamic of Gentian Violet Removal by 2, 3-Dialdehyde Nanocellulose. *Journal of Biochemical Technology*, 10(2), 38-42.