ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2015; 1(11): 902-905
www.allresearchjournal.com
Received: 14-08-2015
Accepted: 20-09-2015

Shilpa Lokhande
Shri Shivaji College of Arts, Commerce and Science, Akola

M Musaddiq
Shri Shivaji College of Arts, Commerce and Science, Akola

Correspondence
Shilpa Lokhande
Shri Shivaji College of Arts, Commerce and Science, Akola.

Isolation of cellulolytic bacterial strains for bioconversion of municipal solid waste

Shilpa Lokhande, M Musaddiq

Abstract
Cellulose, a major polysaccharide found in agricultural residues and industrial and municipal wastes. In the present study cellulolytic microorganisms were isolated. Eight isolates of cellulose degrading bacteria were isolated from soil, municipal solid waste, plant residues in 10 different villages of saline belt of Akola District, Maharashtra by enriching the basal culture medium with 1% cellulose as substrate for cellulose degradation .To indicate the cellulose activity of the organisms, diameter of clear zone around the colony were measured. Enzyme activity was determined by DNS method. The most potent cellulolytic isolates were identified as *Bacillus subtilis.*

Keywords: Cellulose degrading bacteria, Municipal Solid waste, Saline belt

1. Introduction
Cellulose is biologically renewable resource abundantly found in agriculture waste. The cellulosic waste material can be hydrolysed to glucose and other soluble sugars by using cellulase enzymes of bacteria and fungi. The reducing sugars obtained can be further used for the production of ethanol as biofuel (Acharya *et al*., 2012) [1]. The potential cellulose producing bacteria are *Cellulomonas, Pseudomonas, Thermoactinomycetes* and *Bacillus spp*. (Godana, 2007) [11]. Sustainable resources, which are in need of human being, are derived from plant biomass. Cellulose is the major component of plant biomass (Camassola and Dillon 2007) [5]. Plants produce 4×109 tons of cellulose annually (Coughlan 1990) [6]. Cellulolysis is basically the biological process controlled and processed by the enzymes of cellulase system. Cellulase enzyme system comprises three classes of soluble extracellular enzymes: 1, 4-β-endoglucanase, 1, 4-β-exoglucanase, and β-glucosidase (β-D-glucoside glucohydrolase or cellobiase). Endoglucanase is responsible for random cleavage of β-1, 4-glycosidic bonds along a cellulose chain. Exoglucanase is necessary for cleavage of the non reducing end of a cellulose chain and splitting of the elementary fibrils from the crystalline cellulose, and β-1, 4-glucosidase hydrolyses cellobiose and water-soluble cellodextrin to glucose(Shewale 1982)[21],Woodward and Wiseman 1983) [26]. Only the synergy of the above three enzymes makes the complete cellulose hydrolysis to glucose (Ryu and Mandels, 1982, Wood 1989) [20, 25] or a thorough mineralization to H2O and CO2 possible.
Its crystalline structure and insoluble nature represents a big challenge for enzymatic hydrolysis. Microorganisms are important in conversion of lignocellulose wastes into valuable products like biofuels produced by fermentation (Lynd *et al*., 2002) [15]. Successful bioconversion of cellulosic materials mainly depends on the nature of cellulose, sources of cellulolytic enzyme and optimal conditions for catalytic activity and production of enzymes (Alam *et al.,* 2004) [2]. Cellulose quality, temperature, aeration, carbon sources, incubation period, medium additives, pH of the medium and presence of inducers are important parameters for the optimized production of cellulase enzymes (Immanuel *et al.,* 2006) [13]. Present study is aimed to isolate and screen potential cellulolytic bacteria from saline belt of Akola District, Maharashtra.

2. Materials and Methods
Isolation of cellulolytic bacteria cellulolytic bacterial strains were isolated from soil by using serial dilutions and pour plate technique. The medium used for isolation of cellulolytic

bacteriacontains 1.0% peptone, 1.0% carboxymethylcellulose (CMC), 0.2% K2HPO4, 1% agar, 0.03% MgSO4.7H2O, 0.25% (NH4)2SO4 and 0.2% gelatin at pH 7 for 48 hours of incubation at 30 ˚C (Yin *et al.,* 2010) [27]. Bacterial colonies were purified by repeated streaking. The purified colonies were preserved at 4 ºC for further identification and screening for cellulase production.

Screening of Cellulolytic Bacteria

Pure cultures of bacterial isolates were individually transferred in CMC agar plates. After incubation for 48 hours, CMC agar plates were flooded with 1% congored and allowed to stand for 15 min at room temperature. One molar NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis (Andro *et al.,* 1984) [3]. The bacterial colonies having the largest clear zone were selected for identification and cellulase production in submerged system.

Identification of Cellulolytic Bacteria

Identification of cellulolytic bacteria was carried out by method as described by Cowen and Steel (Cowan and Steel 1993) [7] and Cullimore (Cullimore2000) [8], which was based on morphological and biochemical tests.

Inoculum development

Pure cultures of selected bacterial isolates were individually maintained on CMC supplemented minimal agar slants at 4 ˚C, until used. Pure cultures of selected bacterial isolates were inoculated in broth medium containing 0.03 % MgSO4, 0.2% K2HPO4, 1% glucose, 0.25% (NH4)2SO4 and 1% peptone at pH 7 for 24h of fermentation period. After 24h of fermentation period these vegetative cells were used as inoculum source.

Estimation of Cellulase activity

Cellulase activity was assayed using dinitrosalicylic acid (DNS) reagent (Miller 1959) [17] by estimation of reducing sugars released from CMC solubilized in 0.05 M phosphate buffer at pH 8 (Bailey *et al.,* 1992) [4]. The culture broth was centrifuged at 14000×g for 10 min at 4 ˚C and the clear supernatant served as crude enzyme source. Crude enzyme was added to 0.5 ml of 1% CMC in 0.05 M phosphate buffer and incubated at 50 ˚C for 30 min. After incubation, reaction was stopped by the addition of 1.5ml of DNS reagent and boiled at 100 ˚C in water bath for 10 min. Sugars liberated were determined by measuring absorbance at 540 nm. Cellulase production was estimated by using glucose calibration curve (Shoham *et al.,* 1999) [22]. One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1µmol of glucose per minute under standard assay conditions.

3. Results and Discussion

The numbers of microbes isolated and details of sample collection from the various locations are shown inTable1 which indicate that the number of microbes were isolated from10 different village of Akola District.

Results presented in Table 2 clearly indicate that screening and cellulolytic microbes were isolated and were measured as zone of clearance in mm. highest cellulolytic activity detected in *Bacillus subtilis* (4.2) followed by *Bacillus spp.* (3.9), *Clostridium thermocellum* (0.5), *Pseudomonas aeruginosa* (0.8), *Staphylococcus aureus* (0.7). The present study indicate that number of microbial forms inhabiting various cellulolytic wastes produce extracellular cellulases. The screened bacterial strains will be used for further studies

Cellulose is the most abundant of all naturally occurring organic compounds, probably comprising at least a third of all the vegetable matter on the earth (Han and Shrinivasan 1968) [12] Cellulose is the main building block of plants and have major fraction of organic carbon in soil. Microorganisms, which live in soil, are accountable for recycling of this organic carbon to the environment (Wang *et al.*, 2008) [24].

Degradation of cellulosic materials is a complex process and requires participation of microbial cellulolytic enzymes. Habitats where these substrates are present are the best sources for isolation of cellulolytic microorganisms (Das *et al.,* 2010) [9]. About one fifth of fresh water and soil samples yield cellulose degrading bacteria after enrichment but some samples did not bear such kind of bacteria (Ivanen *et al*., 2009) [14]. This is due to existence of microenvironments where different growth conditions for cellulose degrading bacteria are present. These bacteria are generally found in well manure soils (Morris *et al*., 2008) [17]. Several microorganisms have been discovered for decades which have capacity to convert cellulose into simple sugars (Perez *et al*., 2002) [19] but the need for newly isolated cellulose degrading microorganism still continues (Niranjane *et al*., 2007) [18].

Gautam, *et al.,* 2012 [10] isolate total of 250 isolates of these, 165 belonged to 37 fungal species, and 85 to 21 bacterial species. The most frequent fungi were *Aspergillus niger, Curvularia lunata*, *A. nidulans*, *A. fumigatus*, *Penicillium sp., Fusarium roseum*, and *Trichoderma viride,* and bacteria were MRLB #39, MRLB #42, and MRLB #44. Strom, 1985[23] reported that a large majority of the total number of bacterial isolates were members of the genus *Bacillus*.

Table 1: Details of sample collection

Sr. No	Taluka	Name of Village	No. of sample	Types of material and no. of sample			
				soil	Plant residue	compost	Mixed MSW
1	Akola	Rohana	01	01	-	-	-
2	Akola	Ganori	01	-	-	01	-
3	Akola	Dahihanda	01	01	-	-	-
4	Akot	Dhaga	01	-	01	-	-
5	Akot	Karatwadi	01	-	-	01	-
6	Akot	Sawarkhed	01	-	-	-	01
7	Balapur	Manjari	01	01	-	-	-
8	Balapur	Hatrun	01	-	01	-	-
9	Balapur	Singoli	01	-	-	01	-
10	Balapur	Hingana Adsul	01	-	-	-	01

Table 2: Screening and measurement zone of cellulose degradation by different microbes

Sr. No.	Name of Bacteria	Zone of clearance in mm
1	*Bacillus subtilis*	4.2
2	*Bacillus spp.*	3.9
3	*Clostridium thermocellum*	0.5
4	*Pseudomonas aeruginosa*	0.8
5	*Staphylococcus aureus*	0.7

Table 3: Measurement of enzyme activity of bacterial isolates

Sr. No.	Microorganisms	Optical density (540nM)	Enzyme activity IU/ml
1	*Bacillus .subtilis*	0.14	2.2
2	*Bacillus spp.*	0.11	1.9
3	*Clostridium thermocellum*	0.01	0.2
4	*Pseudomonas aeruginosa*	0.9	1.7
5	*Staphylococcus aureus*	0.5	1.5

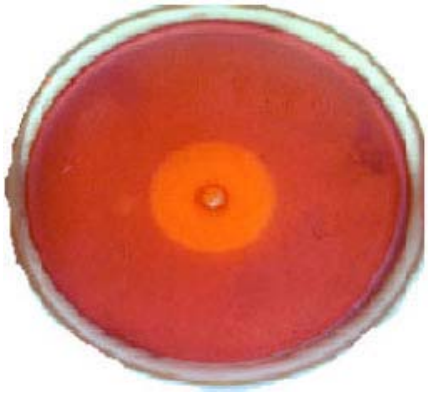

Plate 1: *Bacillus subtilis* showing highest zone of cellulose degradation

From the above findings it is clear that a large no. microorganisms were found in Municipal Solid Waste, compost and soil of saline belt of Akola District. *Bacillus subtilis* had highest cellulose enzyme activity which can be further use for biodegradation of Municipal solid waste.

References

1. Acharya A, Joshi DR, Shrestha K, Bhatta DR. Isolation and Screening of Thermophilic Cellulolytic Bacteria from Compost Piles, scientific world, 2012, 10.
2. Alam MZ, Manchur MA, Anwar MN. Isolation Purification, Characterization of cellulolytic Enzymes Produced by the Isolate Streptomyces omiyaensis. Pak J Biol Sci. 2004; 7:1647-1653.
3. Andro T, Chambost JP, Kotoujansky A, Cattano J, Barras F. Mutants of Erwinia chrysanthemi defective in secretion of pectinase and cellulase. J Bacteriol. 1984; 160:1199-1203.
4. Bailey MJ, Biely P, Poutanen K. Inter-laboratory testingof methods for assay of xylanase activity. J Biotechnol 1992; 23:257-270.
5. Camassola M, Dillon AJP. Production of cellulases and hemicellulases by P. echinulatum grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation. J Appl Microbio. 2007; 103:2196-2204.
6. Coughlan M. Cellulose degradation by Fungi. Elsevier Applied Science, London, UK, 1990, 1-36.
7. Cowan ST, Steel KJ. Manual for the identification of medical bacteria 3rd edn; Cambridge University press, USA, 1993, 150-152.
8. Cullimore DR. Practical Atlas for Bacterial Identification. Lewis Publishers, Boca Raton, London, New York, 2000, 209.
9. Das A, Bhattacharya S, Murali L. Production of cellulase from a thermophilic Bacillus sp. isolated from cow dung. American- Eurasian. J Agric Environ Sci. 2010; 8(6):685-691.
10. Gautam SP, Bundela PS, Pandey AK, Jamaluddin Awasthi MK, Sarsaiya S. Diversity of Cellulolytic Microbes and the Biodegradation of Municipal Solid Waste by a Potential Strain International Journal of Microbiology 2012; V:1-12.
11. Godana B, Mitra R, Singh S. Production of Enzymes for Application on Animal Feeds. Submitted in partial fulfillment of the requirements for the degree of Master of Technology (Biotechnology), Department of Biotechnology, Faculty of Science, Engineering and the Built Environment, Durban University of Technology, Durban, South Africa, 2007.
12. Han YW, Shrinivasan VR. Isolation and characterization of a cellulose utilizing bacterium. Applied microbiology, 1968, 1140-1145.
13. Immanuel G, Dhanusha R, Prema P, Palavesam A. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. Int J Environ Sci Tech. 2006; 3:25-34.
14. Ivanen DR, Rongjina NL, Shishlyannikov SM, Isaeva-Ivanova LS, Shabalin KA, kulminskaya AA *et al.* Novel precipitated fluorescent substrates for the screening of celluloly-tic microorganisms. J Microbiol Methods. 2009; 76(3):295-300.
15. Lynd LR, Weimer PJ, Van Zyl WH, Pretorius IS. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Mol Biol Rev 2002; 66(3):506-577.
16. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959; 31(4):426-428.
17. Morris HJ, Almarales A, Carrillo O, Bermudez RC. Utilisation of Chlorella vulgaris cell biomass for the production of enzymatic protein hydrolysates. Bioresour Technol 2008; 99(16):7723-7729.
18. Niranjane AJ, Madhou P, Stevenson TW. The effect of carbohydrate carbon sources on the production of cellulase by Phlebia gigantean. Enzyme Microb Technol 2007; 40:1464-1468.
19. Perez J, Munoz-Dorado J, de la Rubia T, Martinez J. Bio- degradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int Microbiol 2002; 5(2):53-63.
20. Ryu DDY, Mandels M. Cellulases: biosynthesis and applications, Enzyme and Microbial Technology 1980; 2(2)91-102
21. Shewale JG. Glucosidase: its role in cellulase synthesis and hydrolysis of cellulose, International Journal of Biochemistry. 1982; 14(6)435-443.
22. Shoham Y, Lamed R, Bayer EA. The cellulosome concept as an efficient microbial strategy for the degradation of insoluble polysaccharides. Trends Microbiol 1999; 7(7):275-281.

23. Storm PF. Identification of thermophilic bacteria in solid waste composting. Applied Environmental Microbiol 1985; 50(4):906-913.
24. Wang CM, Shyu CL, Ho SP, Chiou SH. Characterization of a novel thermophilic, cellulose-degrading bacterium Paeni bacillus sp. strain B39. Lett Appl Microbiol 2008; 47(1):46-53.
25. Wood TM. Synergism between enzyme components of Penicillium pinophilum cellulase in solubilizing hydrogen ordered cellulose, Journal of Biochemistry. 1989; 260:37-43
26. Woodward J, Wiseman A. Fungal and other β-d-glucosidases: their properties and applications, Enzyme and Microbial Technology 1983; 4(2)73-79.
27. Yin LJ, Huang PS, Lin HH. Isolation of Cellulase Producing Bacteria and Characterization of the Cellulase from the Isolated Bacterium Cellulomonas Sp. YJ5. J Agric Food Chem. 2010; 58:9833-9837.