International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 5, July-August 2021 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Cost-effective Analysis of Different Soil Samples to Check their Potential for Producing Cellulose Degrading Bacteria

Khushboo Pal[1], Anshika Sharma[2], Tanu Sharma[3], Dr. Divya Sharma[*]

[1,2,3]Student, Department of Microbiology,
[*]Assistant Professor, Department of Life Sciences,
[1,2,3,*]Institute of Applied Medicines and Research (IAMR), Ghaziabad, Uttar Pradesh, India

ABSTRACT

The present investigation was undertaken to isolate and screen the cellulase producing bacteria from soil. Cellulose is considered to be the most abundant carbohydrate on earth along with hemicelluloses and lignin, which constitutes the plant cell wall. Cellulolytic enzymes are the backbone of various industries including food, animal feed, brewing, wine, agricultural biomass refining, pulp, paper, textile and ethanol production. In this paper, cellulose degrading bacteria was isolated and screened from different soil samples (from agriculture farm, waste dumping area and termite soil) using Carboxymethyl cellulose (CMC), wheat bran and waste paper plus nutrient agar as a selective medium. After screening, only five isolates (KHU2, KHU8, KHU9, KHU13 and KHU14) were selected due to the maximum diameter of zone of substrate hydrolysis. The diameter of zone of substrate hydrolysis in NAM plates containing wheat bran for the isolates KHU2, KHU8, KHU9, KHU13 and KHU14 was 8±3mm, 21±2mm, 13±1mm, 12±1mm and 10±2mm, respectively, whereas the diameter of zone of substrate hydrolysis in NAM plates containing waste paper for the isolates KHU2, KHU8, KHU9, KHU13 and KHU14 was 13±1mm, 24±1mm, 17±1mm, 14±2mm and 15±1mm, respectively. This was compared with the diameter of zone of substrate hydrolysis in NAM plates containing CMC cellulose for the isolates KHU2, KHU8, KHU9, KHU13 and KHU14 was 16±1mm, 25±1mm, 22±1mm, 19±2mm and 20±2mm, respectively. It was concluded that the diameter of zone of substrate hydrolysis on waste paper (24±1mm) was nearly same as obtained with CMC cellulose (25±1mm). The highest diameter of zone of substrate hydrolysis was seen in isolate KHU8 in NAM plates plus waste paper and CMC cellulose from termite soil sample. So, termite soil has the maximum potential to produce cellulose degrading bacteria.

KEYWORDS: *Cellulase; isolation & screening; wheat bran; waste paper; CMC cellulose; termite soil*

How to cite this paper: Khushboo Pal | Anshika Sharma | Tanu Sharma | Dr. Divya Sharma "Cost-effective Analysis of Different Soil Samples to Check their Potential for Producing Cellulose Degrading Bacteria" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-5, August 2021, pp.799-804, URL: www.ijtsrd.com/papers/ijtsrd43937.pdf

IJTSRD43937

Corresponding Author:
Dr. Divya Sharma
Assistant Professor, Department of Life Sciences, Institute of Applied Medicines and Research (IAMR), Ghaziabad, Uttar Pradesh, India

1. INTRODUCTION

Cellulose is the most abundant biomass on Earth [1]. It is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bioresource produced in the biosphere [2, 3]. Cellulose is commonly degraded by an enzyme called cellulase. This enzyme is produced by several microorganisms, commonly by bacteria and fungi [4–7]. Cellulose is the principal constituent of the cell wall of most terrestrial plants.

The source of cellulose is in plants and it is found as microfibrils ("2–20 nm" in diameter and "100–40,000 nm" long). These form the structurally strong framework in the cell walls.

Despite a worldwide and enormous utilization of natural cellulosic sources, there are still abundant quantities of cellulosic sources containing raw materials and waste products that are not exploited or which could be used more efficiently. The problem in this respect is, however, to develop processes that are economically profitable. Complete hydrolysis of the enzyme requires synergistic action of 3 types of enzymes, namely, cellobiohydrolase, endoglucanase or Carboxymethyl cellulase (CMCase), and beta-glucosidase [8]. Bacteria with high growth rate as compared to fungi have good potential to be used in cellulase production.

The cellulolytic property of some bacterial genera such as *Cellulomonas*, *Cellvibrio, Pseudomonas* sp [9]. *Bacillus* and *Micrococcus* [7] has been reported. Enzyme production is closely controlled in microorganisms and for improving its productivity these controls can be ameliorated. The production of cellulase depends upon a complex relationship involving various factors like inoculums size, pH value, temperature, presence of inducers, medium additives, aeration and growth time [7]. A large amount of agricultural, industrial, and municipal cellulosic wastes has been accumulating or used in efficiently due to the high cost of their utilization processes [10]. Cellulose, a polymer of glucose residues connected by β-1, 4 linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature [11]. Therefore, it has become considerable economic interest to develop processes for effective treatment and utilization of cellulosic agrowastes as inexpensive carbon sources. Cellulase is the enzyme that hydrolyses the β-1, 4 glycosidic bonds in the polymer to release glucose units [12]. Cellulose containing wastes may be agricultural, urban, or industrial in origin; sewage sludge might also be considered as source of cellulases in its cellulosic content provides the carbon needed for methane production in the anaerobic digestion of sludge. Agricultural wastes include crop residue, animal excreta and crop-processing wastes, slashing generated in logging, saw dust formed in timber production, and wood products in forestry originated activities. The previous negative attitude in which wastes were viewed self consciously as valueless and even offensive and for disposal only has been replaced in large part by a positive view in which wastes are recognized as raw materials of potential value [13]. Cellulase is used in the formation of washing powders, extraction of fruit and vegetable juices, and starch processing [14]. Cellulase is produced by a large number of microorganisms. They are either cell bound or extracellular. Although a large number of microorganisms can degrade cellulose, only a few of them produce significant quantities of free enzymes capable of completely hydrolyzing crystalline cellulose [15]. Cellulase is used in the textile industry for cotton softening and denim finishing; in laundry detergents for colour care, cleaning; in the food industry for mashing; in the pulp and paper industries for drainage improvement and fibre modification, and they are even used for pharmaceutical applications [16]. In nutshell, the cellulase enzyme is commonly used in many industrial applications and the demands for more stable, highly active and specific enzymes are also growing rapidly. So, as enzymes are the future of many industries and need attention to develop methodologies for the isolation, screening and production of cellulase (such as cost, substrate specificity and specific activity). By keeping this in mind the present work was carried out to isolate and screen the cellulose degrading bacteria. A comparative study was done in order to observe the potential of various soil samples using agrowaste (wheat bran), waste paper and commercial Carboxymethyl cellulose (CMC) to check their potential of producing cellulase enzyme.

2. MATERIALS AND METHODS

2.1. Chemical requirements

Carboxymethyl cellulose (CMC), Beef extract, Peptone, NaCl, Agar, Congo red dye and all other chemicals were procured from Himedia. Wheat bran was purchased from local market of Ghaziabad and waste paper was collected from printing shops.

2.2. Collection and cleaning of soil samples

Bacteria were isolated from the soil sample collected from different places of Ghaziabad District, Uttar Pradesh, India. The soil samples were collected from the different areas such as termite soil, agricultural farm and waste dumping area. These samples were collected in sterile container and brought up to the laboratory. In the laboratory, samples were put in separate sterile polybags, after that these soil samples were passed through the sieve in order to remove all the impurities of the soil.

2.3. Preparation of nutrient agar media

Nutrient agar media was used for the isolation of cellulase producing bacterial isolates and the composition of nutrient agar media was agar (15g), beef extract (3g), NaCl (5g), peptone (5g) per 1000 ml of distilled water. The nutrient agar media was prepared and enriched with CMC cellulose (1%),

wheat bran (1%) and waste paper (1%) separately. The pH of the media was adjusted to 7 and after that the media was autoclaved at 121°C for 15-20 minutes. Media was cool down and poured into the sterile petriplates.

2.4. Preparation of serial dilution

All the soil samples were used for preparing various serial dilutions. For making dilutions, firstly, 1 gm of soil sample was dissolved in 9 ml of distilled water, which denotes the serial dilution as 10^{-1}. After that 1ml of 10^{-1} dilution was transferred to the other tube containing 9 ml of distilled water, which denotes the serial dilution as 10^{-2}. This process was repeated upto the dilutions 10^{-9} i.e. 10^{-1} -10^{-9} from each of the soil sample.

2.5. Inoculation of nutrient agar plates with different dilutions of soil samples

For primary screening, each dilution of various soil samples was transferred to the nutrient agar plates containing wheat bran, waste paper and CMC cellulose, separately. After that, plates were incubated in incubator at 37°C for 24-48 h. Experiments was performed in triplicates to avoid the chances of error.

2.6. Isolation and screening of cellulase producing isolates

After primary screening, plates were observed for the visible growth of bacteria (which was in the form of colonies). For secondary screening, clear colonies were picked and transferred to freshly prepared NAM medium enriched with CMC cellulose (1%), wheat bran (1%) and waste paper (1%) for the selective growth of the colonies. These plates were further incubated at 37°C for another 24-48 h in a bacteriological incubator. All the plates, which have clear zones was stained with Congo red dye for the evaluation of zone diameter.

RESULTS AND DISCUSSION

The complex lignocelluloses present in wheat bran and waste paper were hydrolysed into their monomeric units by the bacteria grows in the NAM plates using these substrates. Moreover, the potential of various soil samples was evaluated for their ability to produce cellulase enzyme. Keeping this in mind, this research was conducted to isolate and screen the cellulolytic bacteria. Out of all the three soil samples (A, B and C), total 15 isolates (**Table 1, 2 &3**) were selected due to the presence of maximum bacterial growth. The selected isolates were further screened using Congo red dye. After staining with Congo red dye only the isolates, which have clear zone of substrate hydrolysis was picked and the remaining isolates with no bacterial growth was discarded.

Table 1. Various isolates from soil sample A

Sr. No.	Soil Sample A (Agriculture farm)	
	Isolates	Serial Dilutions
1	KHU 1	10^{-3}
2	KHU 2	10^{-4}
3	KHU 3	10^{-5}
4	KHU 4	10^{-7}
5	KHU 5	10^{-8}

Table 2. Various isolates from soil sample B

Sr. No.	Soil Sample B (Termite Soil)	
	Isolates	Dilutions
1	KHU 6	10^{-3}
2	KHU 7	10^{-5}
3	KHU 8	10^{-6}
4	KHU 9	10^{-8}
5	KHU 10	10^{-9}

Table 3. Various isolates from soil sample C

Sr. No.	Soil Sample C (Waste dumping area)	
	Isolates	Dilutions
1	KHU 11	10^{-4}
2	KHU 12	10^{-5}
3	KHU 13	10^{-6}
4	KHU 14	10^{-8}
5	KHU 15	10^{-9}

After screening, only 5 isolates (KHU2, KHU8, KHU9, KHU13 and KHU14) were selected due to the maximum diameter of zone of substrate hydrolysis. It was observed that the diameter of zone of substrate hydrolysis in NAM plates containing wheat bran for the isolates KHU2, KHU8, KHU9, KHU13 and KHU14 was 8±3mm, 21±1mm, 13±1 mm, 12±1mm and 10±2mm, respectively (**Table 4**). Whereas the diameter of zone of substrate hydrolysis in NAM plates containing waste paper for the isolates KHU2, KHU8, KHU9, KHU13 and KHU14 was 13±1, 24±1mm, 17±1mm, 14±2mmand 15±1mm respectively. Similarly, the diameter of zone of substrate hydrolysis in NAM plates containing CMC cellulose was also measured for the isolates KHU2, KHU8, KHU9, KHU13 and KHU14 was 16±1mm, 25±1mm, 22±1mm, 19±2mm and 20±2mm. The best cellulolytic bacterial isolate was KHU 8 with highest diameter of zone of substrate

hydrolysis 24±1 mm in NAM plates containing waste paper (**Fig1**), which was compared with NAM plates containing CMC cellulose with diameter of zone of substrate hydrolysis 25±1 mm (**Fig 2**). It was concluded that the diameter of zone of substrate hydrolysis on waste paper was nearly same as obtained with CMC cellulose.

The highest diameter of zone of substrate hydrolysis was seen in isolate KHU8 (24±1 mm) in NAM plates containing waste paper, which was nearly same as obtained with commercial CMC cellulose from termite soil sample. So, termite soil has the maximum potential to produce cellulose degrading bacteria and waste paper is a good alternative as well as economic source to be used in place of commercial CMC cellulose (expensive).

Table 4. Zone of substrate hydrolysis of selected isolates

Sr. No.	Isolates	Diameter of zone of substrate hydrolysis (mm)		
		Wheat Bran	Waste paper	CMC Cellulose
1	KHU 2	8±3	13±1	16±1
2	KHU 8	21±2	24±1	25±1
3	KHU 9	13±1	17±1	22±1
4	KHU 13	12±1	14±2	19±2
5	KHU 14	10±2	15±1	20±2

Fig.1 The diameter of zone of substrate hydrolysis in NAM plates containing waste paper

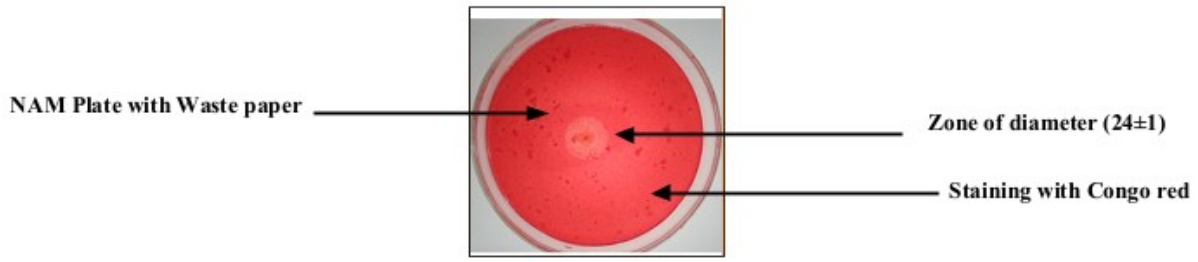
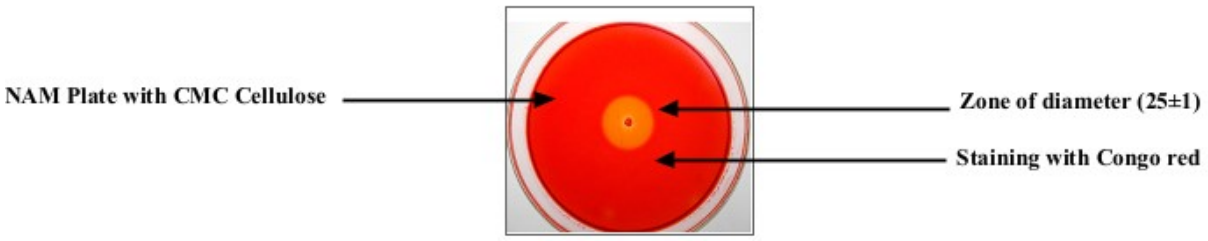


Fig. 2 The diameter of zone of substrate hydrolysis in NAM plates containing CMC cellulose



Similar research using CMC cellulose on bacteria isolated from cow dung was also carried out by Shanmugapriya et al., (2012). Shaikh et al., (2013) has worked on 34 isolates using wood furnishing waste and waste paper, out of which 11 isolates have shown maximum cellulase activity with 14.0mm highest diameter of zone of substrate hydrolysis. Patagundi et al., (2014) has isolated bacteria from the soil sample collected from Botanical Garden, Karnatak University Campus, Karnataka, India using CMC cellulose, in which clear diameter of zones of cellulose hydrolysis were appeared around bacterial colonies. Vipul et al., (2012) have also reported the positive isolates for producing cellulase enzyme from soil sample (agricultural field) on media containing CMC cellulose. The isolation of cellulase producing bacteria from municipal waste using CMC cellulose (out of the 3 isolate) was also observed by Kathiawada et al., (2016). Gopinath et al., (2014) have also worked on cellulase producing bacteria using soil samples collected from paper industry waste, cloth industry waste, kitchen waste and garden, earthworm with CMC cellulose enriched media. Total 32 isolates were obtained after primary screening, out of which 19 isolates have shown the cellulolytic activity. Rasul et al., (2015) have also worked on agrowaste (molasses) for the isolation and screening of cellulase enzyme using CMC cellulose. After screening, total 26 isolates

were obtained, out of which 6 were selected on the basis of clear zone of substrate hydrolysis with diameter greater than 7.0mm. Lokhande et al., (2017) have also mentioned the isolation of cellulose degrading bacterial from soil sample collected from different villages of saline belt of Akola and Buldhana District, Maharashtra India. Total 146 isolates were observed using CMC cellulose, out of which 37 bacterial isolates were further selected for screening. After screening, only 1 cellulolytic bacterial isolate have shown the highest diameter of zone. Varghese et al., (2017) have also worked in isolating cellulase enzyme from temite gut and termitarium using waste paper and CMC cellulose.

CONCLUSION

Cellulase is one of the most widely used enzymes in industries such as paper, pulp, agriculture, textile, food and animal feed etc. Due to the increased demand in industries, there is a need to produce enzymes with high production titre within short period of time in cost-effective manner. So, in order to fulfill the huge requirement of cellulase enzyme new methodologies has to be developed for isolating novel microbial sources with high cellulose degrading ability. Therefore, the use of wheat bran (agrowaste) and waste paper for isolating cellulase enzyme is one of most promising approach to be used by industries for producing cellulase enzyme.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Institute of Applied Medicines and Research (IAMR), Ghaziabad, Uttar Pradesh for supporting the research.

REFERENCES

[1] P. Tomme, R.A.J. Warren and N.R. Gilkes, “Cellulose hydrolysis by bacteria and fungi,” Advances in Microbial Physiology, vol.37, pp.1–81, 1995.

[2] M. Jarvis, “Cellulose stacks up,” Nature, vol.426, no.6967, pp.611–612, 2003.

[3] Y.H.P. Zhang and L.R. Lynd, “Toward an aggregated understanding of enzymatic hydrolysis of cellulose: Non complexed cellulase systems,” Biotechnology and Bioengineering, vol.88, no.7, pp.797–824, 2004.

[4] A.H. Bahkali, “Influence of various carbohydrates on xylanase production in Verticillium tricorpus,” Bioresource Technology, vol.57, no.3, pp.265–268, 1996.

[5] P. Magnelli and F. Forchiassin, “Regulation of the cellulase complex production by Saccobolus saccoboloides: induction and repression by carbohydrates,” Mycology, vol. 91, no.2, pp. 359–364, 1999.

[6] C.S. Shin, J.P. Lee, J.S. Lee, and S.C. Park, “Enzyme production of *Trichoderma reesei* rut C-30 on various lignocellulosic substrates,” Applied Biochemistry and Biotechnology A, vol.84-86, pp.237–245, 2000.

[7] G. Immanuel, R. Dhanusha, P. Prema, and A. Palavesam, “Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment,” International Journal of Environmental Science and Technology, vol.3, no.1, pp.25–34, 2006.

[8] M.K. Bhat, “Cellulases and related enzymes in biotechnology,” Biotechnology Advances, vol.18, no.5, pp.355–383, 2000.

[9] K. Nakamura and K. Kppamura, “Isolation and identification of crystalline cellulose hydrolyzing bacterium and its enzymatic properties,” Journal of Fermentation Technology, vol. 60, no. 4, pp.343–348, 1982.

[10] Y.J. Lee, B.K. Kim, B.H. Lee et al., “Purification and characterization of cellulase produced by *Bacillus amyoliquefaciens* DL3 utilizing rice hull,” Bioresource Technology, vol.99, no.2, pp.378–386, 2008.

[11] S. Saha, R.N. Roy, S.K. Sen, and A.K. Ray, “Characterization of cellulase producing bacteria from the digestive tract of tilapia, *Oreochromis mossambica* (Peters) and grass carp, *Cteno pharyngodon idella* (Valenciennes),” Aquaculture Research, vol.37, no.4, pp.380–388, 2006.

[12] Y. Nishida, K. I. Suzuki, Y. Kumagai, H. Tanaka, A. Inoue, and T. Ojima, “Isolation and primary structure of cellulase from the Japanese sea urchin strongylocentrotus nudus, ” Biochimie, vol.89, no.8, pp.1002–1011, 2007.

[13] J. Pranner, “Environmental Microbiology and Waste Utilization,” in G/AMV Proceedings, Emejuaiwe, Ed., pp.67–69, Academic Press, London, UK, 1979.

[14] M. Camassola and A.J.P. Dillon, "Production of cellulases and hemicellulases by *Penicillium echinulatum* grown on pretreated sugarcane bagasse and wheat bran in solid-state fermentation," Journal of Applied Microbiology, vol.103, no.6, pp.2196–2204, 2007.

[15] C. Koomnok, "Selection of cellulase producing thermophilic fungi," in Proceedings of the 31st Congress on Science and Technology of Thailand of Technology, Suranaree University, 2005.

[16] J.R. Cherry and A.L. Fidants, "Direct devolution of industrial enzymes: an update," Current Opinion in Biotechnology, vol.14, no.4, pp.438–443, 2003.

[17] K. Shanmugapriya, P.S. Saravana, Krishnapriya, Malini Manoharan, A. Mythili, SunuJoseph, "Isolation, screening and partial purification of cellulase from cellulase producing bacteria," International Journal of Advanced Biotechnology and Research ,vol.3, no.1, pp. 509-514, 2012.

[18] N.M. Shaikh, A. Patel, S.A. Mehta, N.D. Patel, "Isolation and screening of cellulolytic bacteria inhabiting different environment and optimization of cellulase production," Universal Journal of Environmental Research and Technology, vol.3, no.1, pp.39-49, 2013.

[19] Basavaraj I. Patagundi, C.T. Shivasharan and B.B. Kaliwal, "Isolation and characterization of cellulase producing bacteria from soil," International Journal of Current Microbiology and Applied Sciences, vol.3, no.5, pp. 59-69, 2014.

[20] Vipul Verma, Alpika Verma and Akhilesh Kushwaha, "Isolation & production of cellulase enzyme from bacteria isolated from agricultural fields in district Hardoi, Uttar Pradesh, India," Pelagia Research Library, vol.3, no.1, pp.171-174, 2012.

[21] Prabesh Khatiwada, Jahed Ahmed, Mehadi Hasan Sohag, Kamrul Islam and Abul Kalam Azad, "Isolation, screening and characterization of cellulase producing bacterial isolates from municipal solid wastes and rice straw wastes," Journal of Bioprocessing & Biotechniques, vol.6, no.4, pp.1-5, 2016.

[22] Gopinath S.M, Ismail Shareef, Ashalatha, Shreya Ranjit, "Isolation, screening and purification of cellulase from cellulase producing *Klebsiella variicola* RBEB3 (KF036184.1)," International Journal of Science and Research, vol.3, no.6, pp.1398-1403, 2014.

[23] Faiz Rasul, Amber Afroz, Umer Rashid, Sajid Mehmood, Kalsoom Sughra, Nadia Zeeshan, "Screening and characterization of cellulase producing bacteria from soil and waste (molasses) of sugar industry," International Journal of Biosciences, vol.6, no.3, pp.230-238, 2015.

[24] Shilpa Lokhande and Pethe A.S, "Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production," International Journal of Life Sciences, vol.5, no.2, pp.277-282, 2017.

[25] Libin Mathew Varghese, Sharad Agrawal, Divya Sharma, Rishi Pal Mandhan, Ritu Mahajan, "Cost-effective screening and isolation of xylano-cellulolytic positive microbes from termite gut and termitarium,"3 Biotech ,vol.7, pp.1-7, 2017.