Full Length Research Article

© 2024, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences
Volume 15; Issue 04 (Jul–Aug 2024); pp 918–921

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Isolation and Screening of Cellulase Producing Bacteria from Sugar Industry Waste

Taniya Sahu[1], Rachana Choudhary*[2] and Pragya Kulkarni[3]

[1] Department of Microbiology, Government V. Y. T. Post Graduate Autonomous College, Durg - 491 001, Chhattisgarh, India
[2-3] Dr. Manrakhan Lal Sahu Government College, Shivpuri Jamul - 490 024, Chhattisgarh, India

Received: 25 Mar 2024; Revised accepted: 22 Jun 2024

Abstract

In the present study, cellulase-producing bacteria were isolated from press mud sample of Sugar Industry (Louh Purus Sardar Vallabhbhai Patel Sahkari Sakker Karkhana Kabirdham, Chhattisgarh). The abundantly available press mud waste obtained during sugar processing was used as a sample for the isolation of cellulase-producing bacteria by using CMC agar. The isolates were further primary screened for cellulase production through the Congo-Red decolorization method. The secondary screening was done by the DNS method for the assessment of enzyme units. Although 24 strains were isolated on CMC agar media. They were further screened for the Congo-Red decolorization test, out of which 07 isolates showed cellulase production activity in terms of Congo-Red decolorization on agar media. Then these isolates were further secondary screened by biochemical activity, and after that, 05 isolates were quantitatively analyzed by their enzyme production by the DNS method. However, all five isolates effectively produced cellulase enzymes and showed good potential, which may be further scaled up by the optimization process.

***Key words*:** Cellulase, CMC, Cellulolytic bacteria, Congo-red decolorization, DNS

Cellulase is an inducible enzyme that is synthesised by a large number of microorganisms, either cell-bound or extracellular, while growing on cellulosic material. Cellulase are a class of hydrolytic enzyme that break the glycosidic bond of macromolecules (Cellulose). Cellulose is a linear polymer of β-D glucosidase units linked by β-1,4 glycosidic bonds. This enzyme is produced and secreted by both bacteria and fungi. The activity of cellulases typically depends on a variety of growth parameters, including pH, temperature, carbon source, and nitrogen source [1-2].

The enzymatic hydrolysis complexity of enzymes requires the synergistic action of three types of enzymes, namely Cellobiohydrolase, Endoglucanase or Carboxymethyl Cellulase (CM Case) and a-glucosidase. An enzyme known as cellulase commonly degrades cellulose. Cellulase refers to a class of enzymes produced by fungi, bacteria and protozoa that catalyse the celluloysis of cellulose. Cellulase have generated significant interest due to the diversity of their numerous applications in the areas of industry and pharmaceuticals [3-4]. The primary applications of cellulase are in the textile industry for "bio-polishing "of fabrics and producing the stone-washed look of denims, in household laundry, detergent for improving fabric softening and brightness [5], in the food, leather, paper, and pulp industries and also in the fermentation of biomass for biofuel production. Cellulase are also used in ruminant nutrients for improving digestibility, in fruit juice processing and another emerging application is the de-inking of paper.

Isolation and characterization of cellulase-producing bacteria remain crucial for biofuel resources, biodegradation and bioremediation. Bacteria have a high growth rate as compared to fungi and good potential for use in cellulase production due to their cellulolytic properties. Cellulolytic properties are shown by some bacterial genera such as *Cellulomonas species*, *Pseudomonas species*, *Bacillus species*, and *Micrococcus species* [6].

MATERIALS AND METHODS

1. *Collection of samples*: Press mud sample was collected from the sugar industry from Louh Purus Sardar Vallabhbhai Patel Sahkari Sakker Karkhana Kabirdham Chhattisgarh and stored in a sterile vessel at room temperature until their processing (Fig 1).

2. *Isolation of bacteria*: The sample was serially diluted and spread on NAM (Nutrient Agar Media) containing 1% CMC and incubated for 24-48 hrs. We found clear bacterial colonies in inoculating plates onto NAM media and analysed for colony characteristics and subculture in minimal medium containing 1% CMC, incubated at 37 °C for 24 hours and then stored at 4 °C [7] (Fig 1).

3. *Primary screening of cellulase producing bacteria*: The individual microorganisms were grown on NAM media supplemented with 1% CMC medium. The pure cultures were inoculated and incubated at 37 °C until substantial growth was recorded. The Petri plates were incubated at 40 °C for 30 minutes using a shaker incubator. Plates were flooded with

***Correspondence to:** Rachana Choudhary, E-mail: rachanadin@gmail.com; Tel: +91 9770577935

Citation: Sahu T, Choudhary R, Kulkarni P. 2024. Isolation and screening of cellulase producing bacteria from sugar industry waste. *Res. Jr. Agril. Sci.* 15(4): 918-921.

Congo red and allowed to stand for 5–10 minutes. The clear zone was formed around the colony due to the hydrolysis of cellulose by cellulase, which is produced by bacteria [8] (Fig 2).

Fig 1 Collection of press mud samples, isolation and pure culture of bacteria

4. *Secondary screening of cellulase producing bacteria*: On the basis of primary screening, the potential isolates were evaluated for their enzyme activity used for biochemical characterization. The identified isolates were identified by performing several biochemical tests, such as Catalase test, Indole-test, Urease-test, Citrate utilization test, Amylase-test, Cellulase production test.

5. *Cellulase enzyme activity*

5a. *Preparation of crude enzyme*: For enzyme production, bacterial isolates were incubated with minimal agar media containing 1% Carboxymethyl Cellulase (CMC) overnight at 37 °C. After incubation, cultures were centrifuged at 8000 rpm for 15 minutes and the clear supernatant served as a crude enzyme solution. This solution was stored at 4°C and used for enzyme activity measurement using the DNS method. It was also stored for subsequent enzyme purification and activity measurement [9].

5b. *DNS method*: The activity of cellulase was determined by the DNS method, which measured the amount of reducing sugar released from CMC. The CMC substrate was prepared by dissolving in citrate buffer (0.5%), pH 5.0, to stop the reaction, and adding DNS reagent. The samples were boiled for 10 minutes and cooled in water to stabilise the colour. The optical density (OD) was determined at 540 nm, and cellulose activity was measured as a calibration curve based on glucose standards [10] (Fig 2).

RESULTS AND DISCUSSION

Initially, 24 bacterial samples were isolated from press mud sample from the sugar industry (Louh Purus Sardar Vallabhbhai Patel Sahkari Sakker Karkhana Kabirdham) The isolates were further screened for primary screening for cellulase production through Congo-red decolourization in this, only 7 isolates were giving a positive response (PS1 to PS7) (Table 1). The isolates showing a positive response to primary screening were further screened for secondary screening. The secondary screening was done by the DNS method for the assessment of enzymes. Five bacterial isolates (PS1, PS2, PS3, PS5, PS6) were given a positive response on the basis of the concentration of enzyme activity value for further analysis, as shown in (Table 2).

Table 1 Biochemical test of potent cellulases producing bacteria

Bacterial isolates	Catalase test	Amylase test	Citrate utilization test	Indole test	Urease test	Cellulase test
PS1	+	+	+	+	+	+
PS2	+	+	+	+	+	+
PS3	+	+	-	+	+	+
PS4	-	+	+	+	-	-
PS5	+	-	+	-	+	+
PS6	-	+	-	-	+	+
PS7	-	-	-	-	-	-

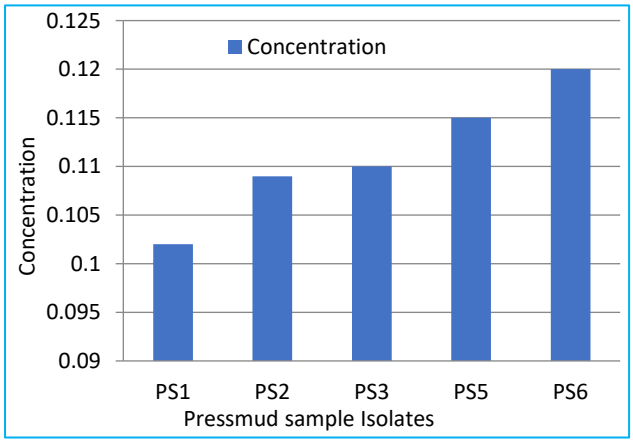

Fig 1 Cellulases activity of different isolates

Table 2 Concentration and enzyme activity of bacteria

Bacterial isolates	Concentration (IU/mg)	Enzyme units (IU/mg)
PS1	0.102	3.0
PS2	0.109	3.2
PS3	0.110	3.8
PS5	0.115	4.0
PS6	0.120	4.24

The aim of this study was to isolate and screen cellulase-producing bacteria from pressmud waste. Cellulolytic bacteria were isolated from Louh Purus Sardar Vallabhbhai Patel Sahkari Sakker Karkhana Kabirdham (Chhattisgarh). Appropriate dilutions of each sample were inoculated on Carboxymethyl Cellulase (CMC) Agar Media. Maki *et al.* [11] production on Isolated bacterial colonies with higher cellulase

activity were further screened to obtain a pure culture. The pure culture produced a clear zone when it was flooded with Congo-red dye, giving a sharp and distinct clear zone around the cellulase-producing bacteria colonies within 3 to 5 minutes. Clear zone-producing bacterial isolates were further used for primary and secondary screening and therefore obtained the concentration of cellulase activity at 540nm [12-15].

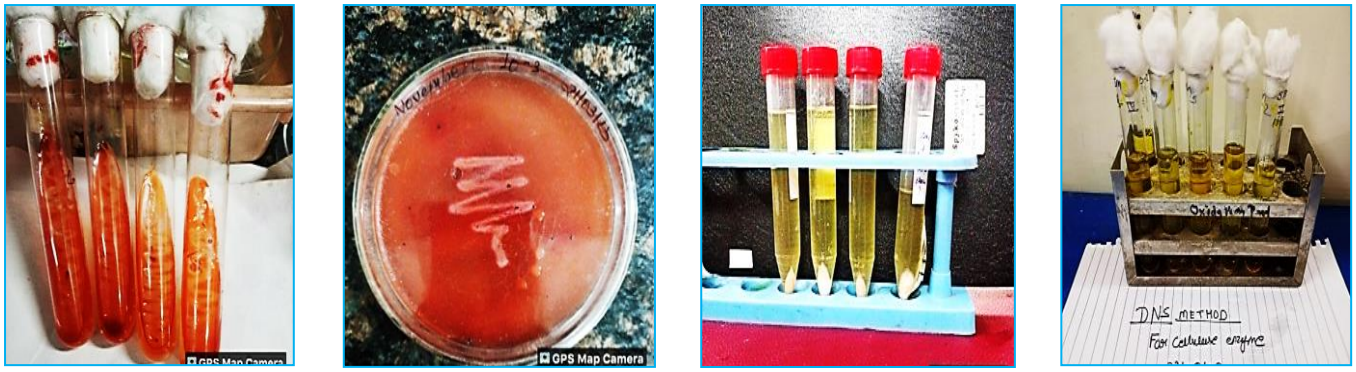

Fig 2 Primary and secondary screening of bacterial isolates

Isolation and screening of the various isolates were performed using serial dilution and the pour plate method. The selection of the isolates was done on the basis of the discrete colonies and was further used for screening. In primary screening, the formation of a clear zone after staining with Congo-red indicates that these colonies are cellulase producers. Isolates producing a transparent zone on a CMC agar plate containing cellulose represent substrate hydrolysis. These isolates were further taken for secondary screening of the cellulase-producing bacteria [16-19].

CONCLUSION

The present study was to screen and isolate the best cellulase-producing bacteria from press mud waste sample. Cellulolytic potential of the isolates was evaluated by quantitative as well as qualitative screening methods. Although 42 isolates were obtained on CMC agar plates, they were further selected for the Congo red decolourization test, out of which 24 isolates were found to have potential activity in cellulase production in terms of Congo red decolourization on agar media. The five best isolates were selected by secondary confirmation by the DNS quantification method PS V (4.24), after 48 hours of incubation, followed by PS IV (4.0). The present work can be extended in the future to enhance cellulase-producing bacteria for optimisation studies and different types of industries. Enzyme-based industries are gaining importance over chemical-based industries due to process safety, low refining costs, high yields, efficient process control and friendly nature. Enzymes, particularly cellulase have potential applications in the paper, pharmaceutical, detergent, and food industries.

Acknowledgements

The authors would like to thank the Govt. VYTPG Autonomous College Durg Chhattisgarh and Shri Sankaracharya Mahavidalaya Junwani, Bhilai India for providing the lab facilities.

Conflict of interest

The authors declare that there is no conflict of interest.

Author's contribution

Taniya Sahu carried out the study, collected the data and framed the manuscript. Dr. Rachana Choudhary and Dr. Pragya Kulkarni contributed in scientific planning and review of the manuscript. Taniya Sahu wrote the manuscript. All authors approved and read the final manuscript for publication.

Funding

No funds were availed for this research work.

Data availability

All databases analyzed or generated during this study are included in the manuscript.

Ethics statement

Not applicable.

LITERATURE CITED

1. Bhattacharjee A, Devi S, Pramodini K. 2008. Antibacterial activity and biochemical analysis of *Ipomoea aquatica* forsk. *Int. Jr. Sci. Research* 9(5): 26938-26941.
2. Anagnostakis SL, Hankin L. 2009. Use of selective media to detect enzyme production by microorganism in food products. *Jr. Technology* 38: 570-572.
3. Almeida MN. 2010. Cellulases and Hemicellulase from species of Acremonium Endophytes. M. S. Department of Microbiology and Molecular Biology 2010: 70.
4. Rivera EC, Costa AC, Atala DIP, Maugeri F, Maciel MRW, Filho RM. 2006. Evaluation of optimization techniques for parameter estimation: Application to ethanol fermentation considering the effect of temperature. *Process Biochemistry* 41(7): 1682-1687.
5. Castro AMD, Pereinra NJ. 2011. Production, properties and application of cellulases in the hydrolysis of agro-industrial residues. *Química Nova* 33(1): 181-188.
6. Ceska M. 2012. Enzyme catalysis of solidified media. *Eur. Jr. Biochemistry* 2012: 76-86.
7. Immanuel G, Dhanusha R, Prema P. 2006. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *Int. Jr. Environ. Sci. Technology* 3: 25-34. https://doi.org/10.1007/BF03325904

8. Demissie MS, Legesse NH, Tesema AA. 2024. Isolation and characterization of cellulase producing bacteria from forest, cow dung, Dashen brewery and agro-industrial waste. *PLoS One* 19(4): e0301607.
9. Lynd LR, Weimer PJ, Van Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: Fundamentals and biotechnology. *Microbiology and Molecular Biology Reviews* 66(3): 506-577. doi: 10.1128/MMBR.66.3.506-577.2002
10. Islam F, Roy N. 2018. Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses. *BMC Res Notes* 11(1): 445.
11. Maki A, Carrico AR, Raimi KT. 2019. Meta-analysis of pro-environmental behaviour spillover. *Nat. Sustain* 2: 307-315.
12. Leaw SN Change HC, Sun HF, Barton R, Bouchara JP, Chang TC. 2018. Identification of medicinally important sequence analysis in the large subunit. *Microbial*. 2018: 693-699.
13. Lopes F, Motta F, Andrade CCP, Rodrigues MI, Maugeri-feho F. 2018. Thermo stable xylanases from non-conventional yeast. *Jr. Microbial*. 33: 36-42.
14. Mandels M, Sternberg D. 2019. Recent advance in cellulase technology. *Jr. Ferment. Technology* 54: 267-289.
15. Miller GL. 2019. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31: 426-428.
16. Ogawa K, Toyama H, Toyama N. 2019. Native cellulose hydrolysing cellulase of *Tricoderma reeasei*. *Journal of Ferment. Technology* 60: 349-355.
17. Oliveira NA, Oliveira LA, Andrade JS, Change Junior AF. 2019. Extracellular hydrolytic enzymes in indigenous strains of rhizobia in central Amazones. *Technology* 26: 334-345.
18. Silver MMP, Rocha V, Maeda RN, Pereira NJR. 2021. Evaluation of cellulase production by *Trichderma harzianum* in submerged fermentation. *In Biotech*. 33: 100-104.
19. Chen PJ, Wei TC, Chang YT, Lin LP. 2013. Purification and characteristics of carboxymethyl cellulase from *Sinorhizobium fredii*. *Botanical Bulletin- Academia Sinica Taipei* 45(2): 111-118.