# Isolation, Purification and Characterization of Cellulose-Degrading Bacteria from Soil in Chhattisgarh, India

## ABSTRACT

|  |
| --- |
| Waste mainly contains cellulose, lignin, and hemicellulose and among them cellulose is the most abundant organic polymer on earth, playing a vital role in the structural integrity of plant cells. They are structurally difficult to break which result in their accumulation affecting environment. Biological method involving certain microorganisms, notably cellulose-degrading bacteria is considered as a sustainable method to combat such problems. In this current research work, isolation and characterization of cellulose-degrading bacteria was carried out to evaluate their cellulose-degrading efficiency. Isolation was carried out from dump area and forest soil using CMC agar media plating. Cellulase degrading ability was screened on the basis of halo zone formation by isolates which was performed using Congo red and NaCl solution as staining agent in CMC plate . Out of all, 4 potential isolates were screened and their hydrolytic capacity ranged from 1.2-2.0. Bacterial characterization was done by following Bergey’s manual of determination. All the isolates were found negative for citrate test whereas they had mixed result for other biochemical characters. Isolate GNR-1 and S-17 showed gram positive reaction whereas isolate S-15 and SG-1 showed negative gram reaction. Isolate; GNR-1 & S-17 were found positive reaction for catalase assay. Their morphological study was also carried out that shows variations among all the isolates. The applications of these bacteria in waste management are quite useful, highlighting their potential for improving sustainability. |

***Keywords:*** *Cellulose degrading bacteria; CMC agar plate; colony character; morphology.*

## 1. INTRODUCTION

Each year, approximately 998 million tons of lignocellulosic waste are generated by agricultural activities (Periyasamy *et al.,*2022). Waste mainly contains cellulose, lignin, and hemicellulose and among them cellulose is the most abundant organic polymer on earth, playing a vital role in the structural integrity of plant cells (Bahatkar *et al.*,2023). They are structurally difficult to break which result in their accumulation affecting environment.In certain parts of country, it is seen that this waste is usually burned in fields leading to environmental problem which is an alarming situation. One of the safest way is possible via cellulase enzyme production. Cellulases degrading enzymes can hydrolyze the β (1-4) glycosidic linkages to smaller oligosaccharides and eventually glucose and hence their applications are in high demand (Rawway *et al*.,2018). Cellulose degrading bacteria such as *Bacillus* sp*.,* (Balla *et al.,*2022) *Pseudomonas* spp., *Streptococcus* sp*., Acinetobacter, Cellulomonas,* and *Clostridium* are mostly studied. *Bacillus subtilis* continues to be a dominant workhorse due to its capacity to secrete large quantities of extracellular cellulolytic enzymes (Rawway *et al.,*2018). Some of rumen bacteria such as *Fibrobacter succinogenes, Ruminococcus albus, Pseudomonas, Proteus* and *Staphylococcus* are also involved in degradation (Li *et al*., 2020). Thermophilic bacteria like *Anoxybacillus* sp, *Geobacillus* sp, and *Bacteroides* also exhibit cellulase activity (Dees *et al*.,1995; Rastogi *et al*.,2009; MokaleKognou *et al*.,2022; Li H *et al*.,2023). This study involves the isolation of cellulase degrading microbes, their biochemical characterizationand cellulase degrading ability were screened by CMC with Congo red plate assay.

## 2. METHODOLOGY

**Sample collection:** Mainly the source of isolation of such microorganism is from soil particularly waste dumping area soil, forest area soil, residue incorporated soil etc. Soil samples were collected in clean polyether Ziplock bag followed by keeping them in refrigerator at 40C until further use.

**Isolation procedure:** Isolation was carried out using CMC agar media whose chemical composition is as follow: 0.25g K2HPO4, 0.25 g KH2PO4, 0.5 g (NH4)2SO4, 0.05 g MgSO4.7H2O, 0.05 g CaCl2, 3 g NaCl, 0 0.05 g Yeast Extract, 5 g cellulose, 9 g agar and 500 ml (Cahyani et al.,2021). Then plating along with use of congo red and NaCl solution as staining agent to identify the isolate. The soil samples were collected from various locations near Ambikapur (C.G.) and other forest areas near Raipur for the isolation of cellulose degrading microbes. 1g of soil sample was taken and dissolved in 9ml of distilled water which was then serially diluted upto 10-6 dilutions from which 1ml aliquot from each dilution of 10-4, 10-5 and 10-6, were spread plated on solidified CMC agar plate, then plates were incubated at 32-37oC for 2-5 days.

**Screening of isolates:** To screen out cellulose degrading bacteria we perform subculturing of the colonies on new CMC agar plates. Subculture here is done by streaking prominent bacterial colonies on CMC plates followed by incubating the plates at 32-37oC for 2-5 days. After incubation, we purify the distinct colonies on CMC agar plates followed by incubation. After period of incubation, the plates are stained with 0.1% Congo red solution for 15min and then destained with 1m NaCl solution to finally acquire the pure culture of cellulose degrading bacterial isolate. Further hydrolytic capacity of each selected isolate is determined i.e., the ratio of halo zone diameter(mm) to colony diameter(mm) of isolates (Gupta *et al*.,2011).

**Characterization:** Bacterial characterization was done by following Bergey's manual of determination. Biochemical characterization like catalase test, amylase test, citrate test was carried out (Dash et.al., 2015).Gram stating of selected isolates were also performed. The bacteria that appeared purple were referred to as Gram positive and those which appeared pink were described as Gram- negative (Aneja, 2003).

## 3. RESULTS AND DISCUSSION

The hydrolytic capacity of isolates implies about its degradation capacity, higher ratio signified its capacity to degrade cellulose present in any waste material or forest soil or residue incorporated soil.Similar result was also obtained by Hatami et al., (2008) in which the hydrolytic capacity of isolate ranged from 1.1 to 4.0. In another study conducted by Rawway *et al.,* (2018) hydrolytic capacity value for isolates from agricultural fields and garden soils were also found in range of 1.3-2.8.

**Biochemical characterization**: The cellulose degrading bacteria was characterized based on the Bergey’s manual of determination. The biochemical characterization like urease, catalase, starch hydrolysis, and gram staining were performed on selected isolate.

**Table 1. Degradation capacity of cellulose degrading bacterial isolates**

|  |  |  |
| --- | --- | --- |
| **Serial no.** | **Isolate code** | **Hydrolytic capacity** |
| 1. | GNR-I | 1.5 |
| 2. | S-17 | 2.07 |
| 3. | S-15 | 2.0 |
| 4. | SG-I | 1.2 |

All the isolates were found negative for citrate test whereas they had mixed result for other biochemical characters. Isolate GNR-1 and S-17 showed gram-positive reaction whereas isolate S-15 and SG-1 showed negative gram reaction. Isolate; GNR-1 & S-17 were found positive reaction for catalase assay.

A previous study demonstrated that excessive inoculation leads to excessive bacterial density, resulting in insufficient nutrients and dissolved oxygen, which ultimately limits the growth of bacteria and reduces the capability of producing enzymes (Li et al., 2020).

**Table 2. Biochemical characteristics of cellulose degrading bacterial isolates**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sl. No.** | **Parameters** | **Observation** |  |
| **SG-I** | **S-15** | **S-17** | **GNR-I** |
| **1** | Morphology | yellow | Slimy white | Slimy white | White colony |
| **2** | Gram staining | +ve | -ve | -ve | -ve |
| **3** | Catalase test | -ve | -ve | -ve | -ve |
| **4** | Citrate test | -ve | -ve | +ve | +ve |
| **5** | Starch hydrolysis test | +ve | -ve | -ve | -ve |

**Fig. 1. Cellulose degrading bacterial isolate in CMC with Congo red plate assay**

****

**Fig. 2. biochemical characterization of the isolates**

## 4. CONCLUSION

The hydrolyzing power was observed among the cellulase degrading bacterial isolates and characterization was carried out by Bergey’s manual of determination. The applications of these bacteria in waste management are quite useful, highlighting their potential for improving sustainability.

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

**REFERENCES**

Aneja, K.R. (2017). Fundamental Agricultural Microbiology.1st edition, New age Int. pvt. Ltd. Publishers, New Delhi.;560-562

Bahatkar, B.P., Gahukar, S.J., Akhare, A.A., Ingle, Y.V., Rathod, D.R., Charpe, A.M. (2023). Decomposition of Agriculture Farm Wastes by Cellulolytic Bacteria. International Journal of Environment and Climate Change, 13(10),411-21.

Balla, A., Silini, A., Cherif-Silini, H.,Bouket, A.C., Boudechicha, A., Luptakova, L., Alenezi, F.N., Belbahri,L.( 2022). Screening of cellulolytic bacteria from various ecosystems and their cellulases production under multi-stress conditions. Catalysts,12(7),769.

Cahyani, V.R., Purwanto, E., Sakya, A.T., Azzahra, N.Y., Lakshitarsari,K.P.(2021). Effect of lignocellulolytic microorganisms isolated from the peel of cassava, rice straw, and sawdust for the composting process of rice straw. InIOP Conference Series: Earth and Environmental Science , 905( 1), 012114.

Dash, D., Gupta, S.B. and Bajpai, R.K. (2015). Screening *in vitro* of stress tolerant *Rhizobium* inoculants for black gram. Annals of Plant and Soil Research. spcl. Issue, 17 (Special Issue),387-390.

Dees, C., Ringelberg, D., Scott, T.C., Phelps, T.J. (1995). Characterization of the cellulose-degrading bacterium NCIMB 10462. Applied Biochemistry and Biotechnology,51,263-74.

Gupta, P., Samant, K., Sahu, A. (2012). Isolation of cellulose‐degrading bacteria and determination of their cellulolytic potential. International journal of microbiology. 12(1),578925.

Hatami, S., Alikhani, H.A., Besharati, H., Salehrastin, N., Afrousheh, M., Yazdani, Z.J., Jahromi,Z.(2008). Investigation on aerobic cellulolytic bacteria in some of north forest and farming soils. American-Eurasian Journal of Agricultural and Environmental Sciences, 3(5),713-716.

Li, F., Xie, Y., Gao, X., Shan, M., Sun, C., Niu, Y.D., Shan, A.(2020). Screening of cellulose degradation bacteria from Min pigs and optimization of its cellulase production. Electron. J. Biotechn. 48, 29–35

Li, H., Zhang, M., Zhang, Y., Xu, X., Zhao, Y., Jiang, X., Zhang, R., Gui, Z. (2023). Characterization of Cellulose -Degrading Bacteria Isolated from Silkworm Excrement and Optimization of Its Cellulase Production. Polymers. ,15(20), 4142.

MokaleKognou, A.L., Chio, C., Khatiwada, J.R., Shrestha, S., Chen, X., Han, S., Li, H., Jiang, Z.H., Xu, C.C., Qin, W.( 2022).Characterization of cellulose-degrading bacteria isolated from soil and the optimization of their culture conditions for cellulase production. Applied Biochemistry and Biotechnology. 194(11),5060-82.

Periyasamy, S., Karthik, V., Senthil, K. P., Isabel, J.B., Temesgen, T., Hunegnaw, B.M., Melese, B.B., Mohamed, B.A., (2022). Chemical, physical and biological methods to convert lignocellulosic waste into value-added products. A review. Environmental Chemistry Letters, (2),1129-1152.

Rastogi, G., Muppidi, G.L., Gurram, R.N., Adhikari, A., Bischoff, K.M., Hughes, S.R., Apel, W.A., Bang, S.S., Dixon, D.J., 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.

Rawway M, Ali SG, Badawy AS. (2018). Isolation and identification of cellulose degrading bacteria from different sources at Assiut Governorate (Upper Egypt). Journal of Ecology of Health & Environment, 6(1):15-24.