**Short Research Article**

**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. This study aims to isolate cellulose degrading bacteria and evaluate its hydrolyzing capacity. This study involves their biochemical characteristics, cellulase degrading ability was screened by CMC with Congo red plate assay. Isolation was carried out from dump area and forest soil using CMC agar media plating along with use of congo red and NaCl solution as staining agent to identify the isolate (halo zone formation). Hydrolytic capacity of selected isolates ranged from 2.0-1.2. 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 , bacteria, CMC agar, colony character, morphology*

**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 [11]. 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. They perform degradation via enzymatic machinery i.e. cellulase enzyme production. Cellulose degrading bacteria such as *Bacillus sp.,* (Balla et al.,2022) *Pseudomonas spp*., *Streptococcus sp., Acinetobacter, Cellulomonas,* and *Clostridium* are mostly studied. Some of rumen bacteria such as *Fibrobacter succinogenes, Ruminococcus albus, Pseudomonas, Proteus,* and *Staphylococcus* are also involved in degradation [6]. Thermophilic bacteria like *Anoxybacillus* sp, *Geobacillus* sp, and *Bacteroides* also exhibit cellulase activity [7-10]. This study involves the isolation of cellulase degrading microbes, their biochemical characteristics, and cellulase degrading ability was screened by CMC with Congo red plate assay.

**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 dilution of 10-4, 10-5 and 10-6 dilutions were taken for isolation purpose. Then 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 each selected isolate’s hydrolytic capacity is determined which is ratio between halozone diameter(mm)and colony diameter(mm) of isolates.

**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).

**RESULT 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.

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.

**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 |

**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.

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 |

**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.

**Figure 1: Cellulose degrading bacterial isolate in CMC with Congo red plate assay**

**Figure 2: biochemical characterization of the isolates**

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**REFERENCES**

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. Li, F.; Xie, Y.; Gao, X.; Shan, M.; Sun, C.; Niu, Y.D.; Shan, A. Screening of cellulose degradation bacteria from Min pigs and optimization of its cellulase production. Electron. J. Biotechn. 2020, 48, 29–35
7. Mokale Kognou AL, Chio C, Khatiwada JR, Shrestha S, Chen X, Han S, Li H, Jiang ZH, Xu CC, Qin W. Characterization of cellulose-degrading bacteria isolated from soil and the optimization of their culture conditions for cellulase production. Applied Biochemistry and Biotechnology. 2022 Nov;194(11):5060-82.
8. Rastogi G, Muppidi GL, Gurram RN, Adhikari A, Bischoff KM, Hughes SR, Apel WA, Bang SS, Dixon DJ, Sani RK. 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. 2009 Apr 1;36(4):585.
9. Li H, Zhang M, Zhang Y, Xu X, Zhao Y, Jiang X, Zhang R, Gui Z. Characterization of Cellulose-Degrading Bacteria Isolated from Silkworm Excrement and Optimization of Its Cellulase Production. Polymers. 2023 Oct 19;15(20):4142.
10. Dees C, Ringelberg D, Scott TC, Phelps TJ. Characterization of the cellulose-degrading bacterium NCIMB 10462. Applied Biochemistry and Biotechnology. 1995 Sep;51:263-74.
11. Bahatkar , B. P., S. J. Gahukar, A. A. Akhare, Y. V. Ingle, D. R. Rathod, and A. M. Charpe. 2023. “Decomposition of Agriculture Farm Wastes by Cellulolytic Bacteria”. International Journal of Environment and Climate Change 13 (10):411-21. <https://doi.org/10.9734/ijecc/2023/v13i102658>.