



# Physiological and biochemical characterization cellulolytic bacteria

S. D. Bharambe<sup>1,\*</sup>, Kunvar Gyanendra Kumar<sup>2</sup>, R. P. Singh<sup>3</sup>, A. S. Pawar<sup>4</sup>

<sup>1</sup>Ph.D. Scholar, Dept. Agriculture Biotechnology, Bhagwant University, Rajasthan, India.
<sup>2</sup>Faculty of Agriculture, Bhagwant University, Rajasthan, India
<sup>3</sup>Dean of Agriculture, Bhagwant University, Rajasthan, India
<sup>4</sup>Research Scholar, Dept. Agriculture Biotechnology, Bhagwant University (Rajasthan), India.
Corresponding author: <u>surekhapatil08@gmail.com</u>

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Abstract— Several efficient cellulase producing microorganisms were isolated. The purpose was to identify and characterize those isolates displaying the greatest cellulase activity for the possible use in the large scale biorefining. Cellulases are inducible enzymes that are synthesized by a large number of microorganisms during their growth on cellulosic materials. Cellulases have attracted much interest because of the diversity of their applications. Cellulases are used in the various industrial process, including textile and laundry, food, feed, leather, pulp and paper. The biochemical characterizations of the isolated Bacterial strains from termite gut. Isolated strains are efficient namely TG I and TG II. Indicate that they may play a role in cellulose digestion in termite gut.



Keywords—Bacteria, Cellulase, Termites, Biochemical study and Enzyme

# I. INTRODUCTION

Bacteria has high growth rate as compared to fungi has good potential to be used in cellulose production. Some bacterial species viz., Cellulomonas species, Pseudomonas species, Bacillus species and Micrococas have cellulolytic property <sup>(15)</sup>. Large number of bacteria are capable of degrading cellulose, but only a few of them produce significant quantities of cell-free bioactive compounds capable of completely hydrolyzing crystalline cellulose invitro (16). Cellulolytic bacteria have been isolated from diverse habitats like soil, compost, and water (8). Common classification separates different pretreatments into physical, chemical, physicochemical, and biological treatments (18). Enzymatic hydrolysis, pre-treatment of cellulosic material is utmost importance to obtain glucose which can be further converted into bioethanol by microbes (2, 11, 17, and 22). Bacteria are now being widely explored for cellulase production because of their extremely high natural diversity and the capability to produce stable enzymes that can be applied in industries <sup>(3,</sup> 9).

# II. MATERIAL AND METHODS

A. **Sample collection:** Termite Sample was collected from sites which includes cellulose feeding organisms, such as termite residing on woody western ghat region Maharashtra state. Sample dissect in 0.9% saline solution under sterile condition. 1.0 gram of each sample is placed in 9 ml of 0.9% saline, mixed it rapidly and Serial dilution techniques was followed and the dilutions selected for further studies.

**B. Isolation cellulolytic Bacteria`:** The mixture was mixed by vortex for 2-3 mins for removal of microorganisms. One ml of this sample was plated by serial dilution (up to 10<sup>4-</sup> (technique amended with CMC agar and incubated at 37°c for 24 -48 hours. Bacterial cultures grown on CMC slants were cultured on basal mineral salt medium (BSM) as shown Fig 01.

## C. Culture and Biochemical Characteristics

# I. Morphology

Sample	TG I	TG II
Test Shape	Cocci	Cocci
Gram strain	Positive	Positive
Motility	Motile	Motile

## **II.** Colony Characteristics

Sam	TG I	TG II
Test		-
Colour	Dirty white	Dirty white
Elevation	Convex	Convex
Density	Opaque	Opaque
Opti. Temp	30 <sup>0</sup> C	30 <sup>0</sup> C
Opti. pH	7	7

## **III. Biochemical Test**

Sample	TGI	TGII
I) Starch hydrolysis	+	
II) Carbohydrate fermentation		
Arabinose	+	
Maltose	+	+
Lactose	+	•
Mannitol		+
Starch	+	
Cellulose	+	+
Glucose		+
III)H2S production	- (Alkaline)	- (acidic)
IV)Simmon citrate	+	+
V)Caseic hydrolysis	+	-
VI) Catalase	+	+

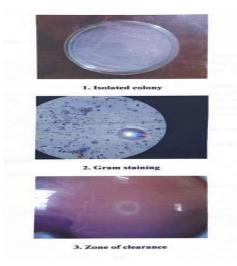


Fig.1 Isolated Bacteria.

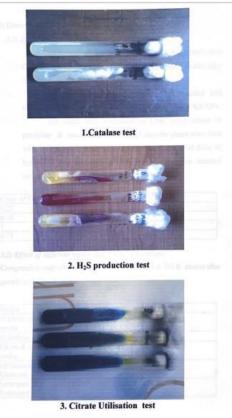


Fig.2 - Biochemical Test

#### III. RESULTS AND DISCUSSION

Gram stain was an empirical method of distinguishing bacterial species into two large groups (Gram-positive and Gram-negative) based on the presence of chemicals, primarily the presence of high levels of peptidoglycan and physical properties of their cell walls. A number of cellulolytic bacterial colonies were isolated from Termite gut. The colony morphology was studied in detail and the results are presented in Tabulation with heading also followed by biochemical tests performed for the isolated microorganisms.as shown fig 02. Clear zone producing bacterial isolates were then subjected to various biochemical test.

#### IV. CONCLUSIONS

With the help of biochemical tests) we could conclude that bacterial isolate belongs to genus belongs to genus Bacillus. A potential cellulose degrading enzyme from B. subtilis was characterized and studied for its possible hydrolyzing capability for disintegrating the cellulosic biomass residues.

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#### REFERENCES

- Acharya, A. Joshi, D. Shrestha, K. Bhatta, D. (2012). Isolation and screening of thermophilic cellulolytic bacteria from compost piles. Scientific world.10: 43-6.
- [2] Arora, S. Khajuria, R and Kaur, L. (2015). Non Alcoholic, Naturally-Carbonated Beverage from Daucus carota Using Saccharomyces cerevisae Isolate. Carpathian Journal of Food Science & Technology, 7(2).
- [3] Ashjaran, A and Sheybani S. (2019).Drug Release of Bacterial Cellulose as Antibacterial Nano Wound Dressing. International Journal of Pharmaceutical Research and Allied Sciences. 8(3):137-143.
- [4] Chilana, H. Arora, S. Khajuria, R and Kaur, L. (2015). Non-Alcoholic, Naturally-Carbonated Beverage from Vitis Vinifera Using Saccharomyces cerevisae Isolated from Cheese Whey. Online Journal of Biological Sciences, 15(3): 184.
- [5] Davinder, A. Kumar, A. Singh, R. Pratap, S and Singh, B. (2017). Impact of zinc and boron on growth, yield and quality of Kinnow (Citrus deliciosa x Citrus nobilis) in sub-tropical conditions of Punjab. Journal of Pure and Applied Microbiology, 11(2): 1135-1139.
- [6] Faridha, B. Meignanalaksmi, S and Pandima, D. (2013).Isolation and Characterization of Cellulase Producing Paracoccus Pantotrophus fmr19 (jx012237) from Goat Rumen Fluid and Its Effects on pH, Temperature and Carbon Sources. International Journal of Advanced Biotechnology and Research. 4: 384-390.
- [7] Ge, X. Chang, C. Zhang, L. Cui, S. Luo, X. Hu, S. Qin, Y and Li, Y. (2018). Conversion of lignocellulosic biomass into platform chemicals for biobased polyurethane application. –In: Li Y & Ge X. (eds.), Advances in Bioenergy, Vol 3, Elsevier, pp.161-213.
- [8] Gupta, P. Samant, K and Sahu, A. (2012): Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential, International journal of microbiology, 2012.
- [9] Haakana, H. Mittinen-Oinonen, A. Joutsjoki, V. Mantyla, A. Souminen, P and Vahmaanpera J.(2004) Cloning of cellulase from Melanocarpus albomyces and their efficient expression in Trichoderma reesei. Enzyme Microbial Technology. 34:159-167.
- [10] Heptinstall, J. Stewart, J. and Seras, M. (1986). Fluorimetric estimation of exocellobiohydrolase and β-dglucosidase activities in cellulase from Aspergillus fumigatus Fresenius. Enzyme Microb. Technol. 8, 70–74. doi: 10.1016/0141-0229(86)90073-6.
- [11] Kaur, J. Kumar, V. Goyal, A.. Tanwar, B. Gat, Y. Prasad, R and Suri, S. (2019). Energy drinks: health effects and consumer safety. Nutrition & Food Science.

- [12] Kuhad, R.Gupta, R and Singh, A. (2011) Microbial cellulases and their industrial applications. Enzyme Res.vol.2011, pp.1–10,
- [13] Kumar, A. Joshi, V and Kumar, V. (2020). Systematic investigation on production and quality evaluation of lugdi: a traditional alcoholic beverage of Himachal Pradesh, India. Journal of Microbiology, Biotechnology and Food Sciences, 9(4): 1307-1311.
- [14] Li, X and Gao, P. (2008) Isolation and partial properties of cellulose-decomposing strain of Cytophaga sp. LX-7 from the soil. J. Appl. Microbiol. 82: 73-80.
- [15] Nakamura, K and Kappamura K. (1982). Isolation and identification of crystalline cellulose hydrolyzing bacterium and its enzymatic properties. J Ferment Technol, 60 (4): 343-8.
- [16] Patagundi, B. Shivasharan, C and Kaliwal, B. (2014). Isolation and Characterization of Cellulase producing bacteria from Soil. International Journal of Current Microbiology and Applied Science, 3(5): 59-69.
- [17] Pramanik, T and Maji, P. (2015). Microwave assisted green synthesis of pharmaceutically important dihydropyrimidinones in fruit juice medium. Int J Pharm Pharm Sci, 7, 376-9. Pramanik, T.; & Padan, S. K. (2016a). Microwave irradiated "green biginelli reaction" employing apple, pomegranate and grape juice as eco-friendly reaction medium. Pharmacology, 1, 4.
- [18] Ravindran, R and Jaiswal A. (2016). A Comprehensive Review on Pre-treatment Strategy for Lignocellulosic Food Industry Waste: Challenges and Opportunities. *Bioresour. Techn.* 199, 92–102.
- [19] Sangma, C. Kumar, V. Suri, S. Gat, Y. Kaushal, M and Kumar, A. (2019). Preservation and evaluation of spiced chayote juice using hurdle technology. Brazilian Journal of Food Technology, 22.
- [20] Soares, F. Melo, I. Dias, A and Andreote, F. (2012). Cellulolytic bacteria from soils in harsh environments. World J. Microbiol. Biotechnol. 28, 2195–2203. doi: 10.1007/s11274-012-1025-2.
- [21] Mijena, D., Getiso, A., & Felecho, J. (2024). Assessing Artificial Insemination Service Effectiveness and Evaluation of Semen Quality in West Arsi Zone of Oromia Region, Ethiopia. In International Journal of Forest, Animal And Fisheries Research (Vol. 8, Issue 3, pp. 01– 18). https://doi.org/10.22161/ijfaf.8.2.1
- [22] Teather, R. and Wood, P. (1982). Use of Congo red Polysaccharide Interactions in Enumeration and Characterization of Cellulolytic Bacteria from the Bovine Rumen. Applied and Environmental Microbiology, 43(4):777-780.
- [23] Shyamsunder, B., Menon, Dr. S., Walia, Dr. U. S., Prasanna, T., Raju, G. S. K., & Nawabpet, P. (2024). Effect of intercropping wheat (triticum aestivum.L.) with mustard (brassica juncea) on yield and economics under organic system of cultivation. In International Journal of Environment, Agriculture and Biotechnology (Vol. 9, Issue 2, pp. 218–223). https://doi.org/10.22161/ijeab.92.24
- [24] Nyanyoh, B. B., & Wanie, C. M. (2024). Categorisation of Rural Development Stakeholders in Bui Division, North

West Region of Cameroon. In International Journal of Rural Development, Environment and Health Research (Vol. 8, Issue 2, pp. 44–53). https://doi.org/10.22161/ijreh.8.2.5

- [25] Ranjbaranghaleh, A. (2024). Policy and Regulatory Frameworks Addressing Methane Emissions in Dairy Farming. In International Journal of Horticulture, Agriculture and Food science (Vol. 8, Issue 3, pp. 53–56). https://doi.org/10.22161/ijhaf.8.3.7
- [26] Zhang, H. Huang, S. Wei, W. Zhang, J and Xie, J. (2019): Investigation of alkaline hydrogen peroxide pretreatment and Tween 80 to enhance enzymatic hydrolysis of sugarcane bagasse, Biotechnology for biofuels, 12(1): 107.