Isolation, Production and Characterization of Bacterial Cellulase from *Bacillus sp.*

Shumaila Samad Khan*, Shafaq Aiyaz Hassan
Department of Biotechnology, University of Karachi, Karachi, Pakistan.
*E-mail: shumaila.samadkhan@uok.edu.pk

ABSTRACT

Introduction: Cellulose, the most abundant renewable biopolymer on Earth, is a significant component of plant biomass. Its transformation into value-added products depends on its degradation into fermentable sugars which is essential to sustainably convert lignocellulosic waste into value products. Microbial cellulases are important in this process and have acquired great industrial value across biofuels, textiles, paper, detergents, food as well as pharmaceuticals. However, the low stability of most cellulases under industrial conditions has driven the identification of new and strong sources of enzymes. The current research is concerned with the isolation, production and characterization of cellulase, a local isolate of Bacillus sp.

Objectives: There were two main objectives of this study:

1. To evaluate the potential of the indigenous bacterial isolates for the production of industrially valuable enzymes
2. To explore the cellulosic waste valorization aspect of the cellulase enzyme produced by locally isolated thermostable *Bacillus* sp.

Methodology: The soil sample was obtained from the Department of Biotechnology, University of Karachi and the dilutions of soil sample were plated on carboxymethyl cellulose (CMC) agar plates to select the most active isolate CpC-10 that showed a clear hydrolytic zone. The bacterial isolate was identified through morphological analysis, biochemical tests and 16S rDNA sequencing which confirmed its classification as *Bacillus*. The production of cellulase was done through submerged fermentation using CMC-enriched media while the enzyme activity was measured through DNS assay.

Key Findings: The cellulase produced had maximum activity at 50°C with stability over a wide temperature range indicating moderate thermostability while optimal activity of cellulase was noted at pH 6-7. Substrate hydrolysis experiments revealed that wheat straw and sugarcane bagasse generated more reducing sugars compared to CMC and filter paper, indicating the enzyme's suitability for utilizing the agricultural residues as cost-effective sources of carbon. Metal ion profiling revealed that Ca^{2+} was activating, whereas Hg^{2+} and Cu^{2+} were inhibitory suggesting possible applications in industrial formulation of media.

Conclusion: The study shows that Bacillus sp. is an effective, thermostable, and safe source of cellulase for the environment. The findings highlight the enzyme's great potential for waste valorization and development of sustainable bioprocesses for enzyme-based industries.

Keywords: *Bacillus Species*, Thermostable Cellulase, Bioprocessing, Industrial Enzyme, Thermostability.

REFERENCES

1. Imran M, Anwar Z, Irshad M, Asad MJ, Ashfaq H. Cellulase production from species of fungi and bacteria from agricultural wastes and its utilization in industry: a review. Advances in Enzyme Research. Vol. 4, No. 2, 2016, pp. 44–55.
2. Kuhad RC, Gupta R, Singh A. Microbial cellulases and their industrial applications. Enzyme Research. 2011.
3. Menendez M, Garcia-Fraile P, Rivas R. Biotechnological applications of bacterial cellulases. AIMS Bioengineering. Vol. 2, No. 3, 2015, pp. 163–182.
4. Sanders ER. Aseptic laboratory techniques: plating methods. Journal of Visualized Experiments. No. 63, 2012, e3064.
5. Yadav V, Biswas S, Goyal A. Enzymes of Industrial Significance and Their Applications. Industrial Microbiology and Biotechnology: An Insight into Current Trends. Springer, 2024, pp. 277–307.
6. Truong TQ, Nguyen KD. Isolation, characterization, and identification of cellulolytic bacteria from household compost for cellulase production. Ukrainian Biochemical Journal. Vol. 97, No. 1, Jan–Feb 2025, pp. 90–102.