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SUMMARY of FY2025 RESEARCH RESULTS REPORT For International Collaborative Research with IPR, The University of Osaka

Research Title		Crystallization and X-ray analysis of a thermostable glycoside hydrolase family 43 β -xylosidase (GbtXyl43B) from <i>Geobacillus thermoleovorans</i> IT-08
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Research Collaborator (Host PI)		Prof. Genji KURISU
Summary This study was conducted as part of an international collaborative research program between the University-CoE Research Center for Biomolecule Engineering, Universitas Airlangga, Indonesia, and the Protein Crystallography Laboratory, Institute for Protein Research (IPR), The University of Osaka, Japan. The research focuses on the structural characterization of lignocellulose-degrading enzymes, particularly a novel halophilic cellulase, CelGH5, which exhibits thermostability and enhanced catalytic activity under high-salt conditions. To ensure focused progress, this study prioritized the completion of the three-dimensional structure determination of CelGH5, while the crystallization study of GbtXyl43B was temporarily postponed. CelGH5 was identified from oil palm empty fruit bunch (OPEFB) decomposition soil using a metagenomic approach. Biochemical characterization revealed that the enzyme retains approximately 52% residual activity after prolonged incubation at 55°C and shows significantly increased catalytic activity in the presence of NaCl, exceeding 200% activity at salt concentrations of 1–4 M. These properties indicate that CelGH5 is a halophilic and thermostable enzyme with strong potential for industrial applications, particularly in processes involving extreme environmental conditions. The primary objective of this study was to determine and refine the three-dimensional structure of CelGH5 using X-ray crystallography. Diffraction data were collected at SPring-8 with a resolution range of 48.08–2.13 Å. The crystal was assigned to the orthorhombic space group P2₁2₁2, with unit cell parameters a = 166.85 Å, b = 286.88 Å, and c = 50.11 Å. The final refined structure was obtained at a resolution of 2.20 Å. Structural validation using the wwPDB validation server indicates that the model is of good overall quality, with excellent completeness (99.8%), acceptable refinement statistics (R-work 0.236 and R-free 0.273), low clashscore (5), and a high percentage of residues in favored regions of the Ramachandran plot (~96%), along with minimal Ramachandran and sidechain outliers. Despite this, the structure exhibits relatively high B-factor values (~72 Å²) and several regions of weak electron density, suggesting structural flexibility that may be associated with adaptation to high salinity and temperature. Overall, the structure provides important insights into the molecular architecture and functional properties of CelGH5 and serves as a foundation for further. As a major outcome of this research, a draft manuscript entitled “<i>Purification, Crystallization, and Preliminary X-ray Crystallographic Analysis of Halophilic Cellulase from the Metagenomic Approach of Oil Palm Waste Soil</i>” has been prepared and is currently being finalized for submission to a scientific journal.		

***Deadline: May 11, 2026**

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