

DATE: Day 1 Month May Year 2024

**SUMMARY of**  
**FY2023 RESEARCH RESULTS REPORT**  
**For International Collaborative Research with IPR, Osaka University**

<b>Research Title</b>		Crystallization and X-ray diffraction analysis of halophilic cellulase (CelGH5) from Indonesia local isolate
<b>Applicant</b>	<b>Name</b>	Prof. Dr. Ni Nyoman Tri Puspaningsih, M.Si.
	<b>Affiliation</b>	(1) Department of Chemistry, Faculty of Science and Technology, Universitas Airlangga, INDONESIA (2) University-CoE-Research Center for Bio-Molecule Engineering, Universitas Airlangga, INDONESIA
	<b>Present Title</b>	Professor
<b>Research Collaborator (Host PI)</b>		Prof. Genji KURISU
<p><b>Summary</b></p> <p>CelGH5 is a lignocellulose-degrading enzyme that has a high novelty in enzyme structure. Understanding the structural interactions between an enzyme and its substrate can be used to engineer the properties of enzymes to improve the ability and capability of enzyme catalytic processes. The three-dimensional structure of the enzyme can elucidate the catalytic mechanism of the enzyme against the substrate, and then how the enzyme interacts with the substrate and the residues involved can be explained. Therefore, this study was conducted to determine the three-dimensional structure of CelGH5 and its interaction with the substrate/ligand using an X-ray crystallographic approach. The specific aims of this research are (1) crystallization of CelGH5 using the optimum condition and (2) exploration of x-ray crystallographic data. The method used in this study consists of enzyme production, enzyme purification, crystallization and X-ray diffraction of enzyme crystals. According to the optimization of crystallization, CelGH5 can be crystallized using 18 mg/mL of protein concentration and crystallization condition of 0.28 M LSM, 23% of PEG 4000, and 0.1 M Tris-HCl pH 8.5 after 7 days of incubation. Some good shapes of single crystals indicated that CelGH5 has the potential to be processed to obtain crystal diffraction data. The crystals were picked and cryoprotected with the solution containing 0.30 M LSM, 25% PEG 4000, 0.1 M Tris-HCl pH 8.5, and 15% glycerol and flash-cooled in a cold nitrogen stream for Spring-8 X-ray diffraction analysis. The resolution of the diffracted CelGH5 crystal was in the range of 48.08–2.13 (2.17–2.13) Å. The molecular replacement analysis demonstrated the space group to be <math>P 2_1 2_1 2</math> and cell constants (a, b, c, <math>\alpha</math>, <math>\beta</math>, <math>\gamma</math>) to be 166.85 Å, 286.88 Å, 50.11 Å, 90°, 90°, 90°. The CelGH5 enzyme derived by the metagenome approach from palm oil waste compost soil has enormous potential to be crystallized and determine its three-dimensional protein structure. Then, the research related to determining the structure of CelGH5 needs to be continued to obtain the final structure of CelGH5 from this research collaboration. This research is still ongoing to obtain the final structure of CelGH5 and can be published scientifically. Thus, the achievement of this research in terms of research papers/articles has not been achieved. The next researcher, who is also a member of our research group, is expected to get the desired results so that the expected joint scientific publications can be achieved.</p>		

\*Deadline: May 10, 2024

\*Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.

\*Please describe this summary within 1 sheet. Please DON'T add some sheets.

\*This summary will be published on the web.