DATE: Day 18 Month April Year 2024

SUMMARY of

FY2023 RESEARCH RESULTS REPORT

For International Collaborative Research with IPR, Osaka University

Research Title		**Structure determination for beta-glycosidases including accessory domains**
Applicant	**Name**	**James Robert Ketudat Cairns**
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	Present Title	**Professor**
Research Collaborator (Host PI)		**Prof. Genji Kurisu**

Summary

In this project, we investigated the structures of plant and archaeal beta-galactosidases from different glycoside hydrolase families. The rice OsBGal1 structure determination was attempted by crystallization and by single particle cryo-electron microscopy. The protein was produced in *Pichia pastoris* and tested for crystallization after deglycosylation with Endoglycosidase H. However, no protein crystals were observed. The protein was further diluted and spread onto carbon-coated copper EM-disks, blotted and vitrified at low temperature. The initial particle appearance and 2D analysis looked hopeful, but the 3D analysis could not be completed to date. It appears that we will need to further optimize the blotting conditions and particle designations in order to achieve 3D images of the protein.

We further tried to express two archaeal family GH10 enzymes that had beta-galactosidase activity rather than the expected beta-xylanase activity. These proteins were expressed in *Escherichia coli* and purified, then tested for crystallization. However, we have as yet not obtained well diffracting protein crystals, although some conditions appeared to possibly contain microcrystals and will be optimized further in the future.

These projects gave us leads on the future structure determination of the rice and archaeal beta-galactosidases, but it will require further optimization to determine whether the structures can be obtained.

***Deadline: May 10, 2024**
***Please submit it to E-mail: tanpakuken-kyoten@office.osaka-u.ac.jp.**
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***This summary will be published on the web.**