DATE: Day____Month____Year 2018

SUMMARY of

2017 RESEARCH RESULTS REPORT

For International Collaborative Research with IPR, Osaka University

Research Title		**Structure and Functional Analysis of Cellulose Binding Module (CBM) in β-Xylosidase GbtXyl43B of *G.thermoleovorans* IT-08 local strain**
Applicant	**Name**	**Proffesor Ni Nyoman Tri Puspaningsih**
	Affiliation	**Universitas Airlangga**
	Present Title	**-**
Research Collaborator (Host PI)		**Institute of Protein Research (IPR)**

Summary

Hemicellulose, cellulose and lignin are the three major components that build plant cell walls. Mostly hemicelluloses are found in the form of xylan, with variation in side chain. Xylan is a complex polysaccharide consist of a **β**-D-(1→4)-linked xylopyranoside backbone and various functional groups such as acetyl groups, 4-O-methyl glucuronyl groups, or arabinose, therefore complete hydrolysis of xylan requires synergistic action of complex xylanases enzymes including xylosidases (Biely, P. 2003; Saha, B. C, 2003). Cluster of xylanolytic enzymes have been succesfully isolated from Gunung Pancar Hot Spring, Bogor, West Java, Indonesia from *G. thermoleovorans* IT-08. It was then cloned to p-Bluescript plasmid and expressed in E.Coli DH5a, the recombinant plasmid (pTP510) contains five genes : transposase, ABC permease, GbtXyl43A(b-D-Xylopiranoside, xyl43A Genbank no DQ345777), GbtXyl43B (B-D-Xylosidase) and arabinofuranosidase (Abfa, genbank no DQ 387046) (Puspaningsih, 2004). Preliminary investigation of bioinformatics analyses showed that both GbtXyl43A and GbtXyl43B have activity of xylosidase enzymes and composed of Carbohydrate Binding Module (CBM) and Catalytic Module (CM)(Rohman, 2007 and Ratnadewi, 2013). CBM mediates binding of insoluble substrate with the enzyme by bringing the catalytic module into prolonged and intimate contact with substrates and, as a result, increases the catalytic efficiency of the enzyme(Boraston et al, 2004). In the present study, we describe the purification and preliminary crystallization of the protein. Three step purification using Afinity (Ni-NTA), Anion Exchange (His-trap) and Size Exclusion Chromatography produced high level of purification product. The protein of GbtXyl43B was subsequently analyzed for Dinamic Light Scattering (DLS) to check the stability of protein and agregrate formation by edman degradation. No appearance of crystal protein was observed on crystalization procedure both manually and robotic. The data showed that there was a fragmentation of protein which is assumed to inhibit the formation of crystal. Further optimization including reconstruct primer with consideration on his tag position, choice of plasmid will be done on the next study.

***Deadline: May 18, 2018**

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

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