DATE: Day 09 Month 05 Year 2025

SUMMARY of
FY2024 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Structural analysis of target proteins involved in the Butyrate cycle from *Faecalibacterium prausnitzii* A2-165
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	Affiliation	**Korea University**
	Present Title	**Professor**
Research Collaborator (Host PI)		**Atsushi Nakagawa**

Summary

1. Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with an imbalance in gut microbiota, particularly a reduction in *Faecalibacterium prausnitzii* strain A2-165. This bacterium is a major producer of butyrate, a short-chain fatty acid with potent anti-inflammatory properties beneficial for gut health and potentially skin conditions like AD. A crucial enzyme in *F. prausnitzii* A2-165's butyrate production pathway is 3-hydroxybutyryl-CoA dehydrogenase (A2HBD). We have successfully determined the three-dimensional structure of A2HBD complexed with its substrate acetoacetyl-CoA and cofactor NAD+ at a high resolution of 2.2 Å and 1.9 Å using BL44-XU Spring-8, Japan. This structural elucidation provides critical insights into A2HBD's functional mechanisms at an atomic level. Understanding how this enzyme works is vital for developing novel therapeutic strategies for AD. These could include next-generation probiotics featuring enhanced *F. prausnitzii* A2-165 activity, prebiotics to boost its growth and butyrate production, or other interventions to modulate the gut microbiome to alleviate AD symptoms (Int. J.Mol.Sci. 25, (19), 2024). Further, we have grown the crystal of BCD, another key enzyme . Butyryl-CoA dehydrogenase (*Fp*BCD) is an enzyme of butyrate cycle and electron-transferring flavoprotein (Etf) complex can convert with Crotonyl-Coenzyme A(CrCoA), results in Butyryl CoA. In order to study structure function relationship of *Fp*BCD, it has been successfully purified and crystallized. The crystal belongs to the tetragonal space group $P4_12_12$, with cell parameters a = b = 100.398, c = 163.832 Å and α = β = γ = 90°. The crystals correspond to 2.46Å^{3}Da^{-1} of Matthews coefficient, 49.9% solvent content and two molecules in an asymmetric unit.

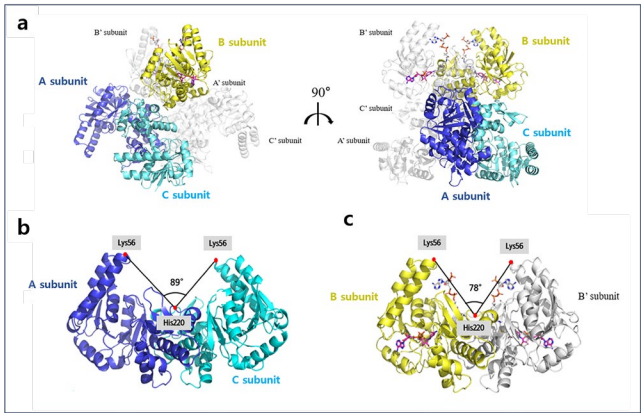

Fig. 1 The overall structure of A2HBD in complex with NAD⁺ and acetoacetyl-CoA. (a) The merged

hexamer of the A2HBD trimer and the symmetric subunit are shown as a cartoon. The trimeric asymmetric unit of the tertiary complex is shown as a colored cartoon. Subunits A and C are labeled as blue and cyan, respectively. (b) Comparison of the cleft angle in subunit dimers. Non-bound subunits A and C dimer are shown as cartoons. Subunits A and C are colored blue and cyan, respectively.

(A) (B) (C)

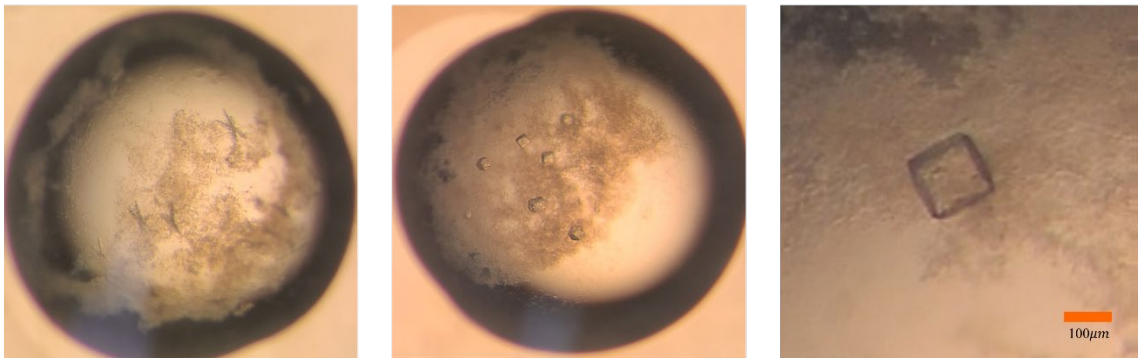

Fig 2. The Photo image of Crystal of FpBCD.

***Deadline: May 9, 2025**
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***Please describe this summary within 1 sheet. Please DON'T add some sheets.**
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