（学位第３号様式）

学 位 論 文 要 旨

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題　目	Studies on the glycosidases and the assimilative mechanisms of arabinogalactan-protein on bifidobacteria (ビフィズス菌が有するアラビノガラクタン-プロテイン分解酵素の機能解析および代謝機構に関する研究)

Bifidobacteria are the natural inhabitants of the human intestine, and harbor various glycosidases to metabolize several dietary fibers ingested by the host. Although it has been reported that the constituent bifidobacterial species change with weaning, the relationship between adult-type bifidobacteria and dietary fiber remains unclear due to the complexity of microbiota composition and glycan structure. In this study, we focused on arabinogalactan-proteins (AGPs), complex proteoglycans that are widely distributed in plant species, to elucidate the related glycosidases and the assimilative mechanism on bifidobacteria.

Gum arabic AGP has complicated glycan moiety in comparison to other AGPs. Although gum arabic AGP has been reported to increase the concentration of *Bifidobacterium longum* subsp. *longum* (*B. longum*) in the human intestine, the assimilative mechanism has not been clarified. In this study, we first conducted *in vitro* assimilation test and comparative genomic analysis of 12 strains to identify the candidate genes related to assimilation. As a result, we found a gene cluster that was conserved only in the assimilative strain including *B. longum* JCM7052. Cloning and functional characterization of the glycosidases in this gene cluster revealed that 3-*O*-α-D-galactosyl-α-L-arabinofuranosidase (GAfase) released the disaccharides α-D-Gal*p*-(1→3)-L-Ara (GA) and β-L-Ara*p*-(1→3)-L-Ara (AA) from the side chain of gum arabic AGP, which was a novel functional enzyme. Furthermore, intracellular α-D-galactosidase showed high substrate specificity for GA, and the assimilative mechanism of gum arabic AGP in *B. longum* was successfully elucidated.

Subsequently, the author found that α-L-arabinofuranosidase (BlArafE) further degraded GA/AA-free gum arabic AGP which was prepared by GAfase treatment. BlArafE is a multidomain enzyme that possesses two catalytic domains, GH43 subfamily 22 (GH43_22) and GH43_34. Functional analysis of each domain revealed GH43_22 and GH43_34 acted on α1,3-Ara*f* and α1,4-Ara*f*, respectively, indicating that the single enzyme performed the sequential α1,3/1,4-Ara*f* de-arabinosylation from the side chains of gum arabic AGP. Furthermore, after cultivation of *B. longum* JCM7052, AA and α-L-Rha*p*-(1→4)-β-D-Glc*p*A-(1→6)-β-D-Gal*p*-(1→6)-D-Gal (S4) were accumulated in the medium as final products. The cloning and functional characterization of β-L-arabinopyranosidase from *B. adolescentis*, which inhabits adult intestine as *B. longum*, revealed that it showed high substrate specificity for AA, and S4 was found to be metabolized by some *Bacteroides* species.

In this study, the author revealed the assimilating mechanisms of gum arabic AGP on bifidobacteria by the functional analysis of the glycosidases, which suggested the symbiosis between *B. longum* and other commensal bacteria by utilization of the residual oligosaccharides.