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Grant Title: Identification of Glycosyltransferases Involved in Pectin Biosynthesis
Abstract

Pectin, a component of plant cell walls, is composed of three regions: homogalacturonan, rhamnogalacturonan I (RG-I), and RG-II. Approximately 30 glycosyltransferases are thought to be involved in their biosynthesis, but few have been identified. Therefore, the role and function of pectin remain largely unexplored. Our group has previously identified genes for rhamnosyltransferase involved in RG-I biosynthesis (Takenaka et al., 2018). Based on this experience, in order to elucidate the function of pectin, we started the study to identify the genes of two enzymes, RG-II apiosyltransferase and RG-I galactosyltransferase. Because the genetic approach for identification of genes encoding pectin-biosynthetic glycosyltransferases has not been successful, we attempted to identify these genes by biochemical approaches.
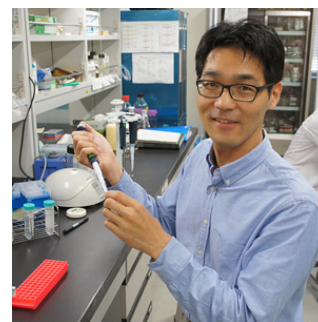
RG-II apiosyltransferase is a critical enzyme that synthesizes RG-II side chain. The apiose residue of RG-II forms a boron diester bond and is involved in the dimerization of RG-II. This apiosyltransferase activity has never been detected so far, because no preparation method for its donor substrate, UDP-apiose, has been established. UDP-apiose is unstable because the hydroxyl group at the second position of the sugar of UDP-apiose is located close to the adjacent phosphate ester and can easily be nucleophilic attacked to form cyclic phosphate. In this study, the stabilization of UDP-apiose was achieved by orienting triethylamine as a counter ion. We prepared UDP-apiose with sufficient amount for using RG-II apiosyltransferase assay. The results were published in the journal *Carbohydrate Research* (Fujimori et al., 2019). Currently, the gene encoding this enzyme is searched from Arabidopsis genome using this biochemical method.

RG-I galactosyltransferase is a key enzyme in the formation of RG-I side chain galactans, which interact with cellulose in the cell wall and are thought to play a role in the firmness and softness of plants. In addition, RG-I with galactan has been shown to be an effective medicinal component with immune-enhancing activity in Chinese herbal medicine. In this study, an acceptor substrate for this enzyme was prepared and an assay method for this enzyme was constructed using the substrate. The RG-I main chain of oligosaccharides of a certain length (>10 sugars) was found to be the best substrate for this enzyme. We also found that this enzyme was superactivated in the presence of certain cationic surfactants and polyelectrolytes. This would suggest that this enzyme has a hydrophobic region and interacts with other proteins. The results were published in the journal *Plant Physiology and Biochemistry* (Matsumoto et al., 2019). As this is the first time that this enzyme activity has been detected, we have applied to Enzyme Nomenclature to issue an enzyme number (EC number). Using this established method for measuring the activity of this enzyme, we are searching for the gene encoding this enzyme. The identification of these genes will lead to elucidation of the role and function of pectin.

References

1. Takenaka et al. (2018) *Nature Plants* **4**, 669-676.

2. Fujimori et al. (2019) *Carbohydr. Res.* **477**, 20-25.

3. Matsumoto et al. (2019) *Plant Physiol. Biochem.* **142**, 173-178.