Degradation of plant cell wall by *Bacillus subtilis*: Enzymatic characterization and preliminary X-ray crystal analysis of rhamnogalacturonan lyase YesW

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Bacillus subtilis is a typical member of Gram-positive and saprophytic bacteria. A kind of bacilli is utilized for production of soybean-fermented foods, i.e. natto, in Asia. Studies on the interaction between *bacilli* and plants are thus important for clarification of the bacterial ecosystem in nature and for application in biomass-recycling and food industries.

Bacillus subtilis strain 168 showed a significant growth on rhamnogalacturonan type-I (RG-I), a component of pectin from plant cell wall, as a sole carbon source. RG-I contains alternating L-rhamnose (Rha) and D-galacturonic acid (GalA) as a main chain. The DNA microarray analysis suggested that a gene cluster plays an important role in degrading RG-I. The cluster consists of at least 12 putative ORFs coding ABC transporters (YesO, YesP, and YesQ), RG unsaturated galacturonyl hydrolase (YesR), RG acetylesterases (YesT and YesY), RG lyases (YesW and YesX), beta-galactosidase (YesZ), arabinose operon control protein (YesS), and function-unknown proteins (YesU and YesV). Recently, we have reported that YesR is a novel glycoside hydrolase catalyzing a hydrolytic reaction of unsaturated RG disaccharide (Δ GalA-Rha) produced from RG-I by RG lyases. This presentation deals with the enzymatic characterization and preliminary X-ray crystal analysis of *Bacillus subtilis* strain 168 RG lyases.

YesW and YesX show 68.6 % identity in primary structure each other and belong to polysaccharide lyase family PL-11. The recombinant YesW and YesX expressed in *Escherichia coli* cells were purified and characterized. Both enzymes cleaved the glycosidic bond between Rha and GalA of RG-I main chain through β-elimination reaction. Distinct from RG lyases so far analyzed such as *Aspergillus aculeatus* RhgB, YesW and YesX released ΔGalA-Rha from RG-I as a final product, indicating that they are novel type of RG lyases with a different action mode.

In order to clarify the structure/function relationship, YesW was crystallized at 20℃ by means of the sitting-drop vapor-diffusion method with 2-methyl-2,4-pentanediol (MPD) as a precipitant. Preliminary X-ray analysis revealed that a YesW crystal belongs to a space group $P2_1$ and diffracted to 2.40 Å resolution, with cell unit parameters of $a = 56.7$, $b = 105.6$, $c = 101.4$ Å, $\beta = 94.9°$.