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Fungal Exo-acting Enzymes with Novel Catalytic Properties

Tatsuji Sakamoto[1)]

1) Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Osaka Prefecture University

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Abstract: This article deals with characterizations of two *Aspergillus niger* exo-polygalacturonases (exo-PGs; EC 3.2.1.67) and a *Penicillium chrysogenum* exo-arabinanase (no EC number). Two exo-PGs, termed exo-PG1 and -PG2, purified from a commercial *A. niger* enzyme preparation (Pectinex AR) had molar masses of 82 and 56 kDa, respectively. Exo-PG1 was stable over wider pH and temperature ranges than exo-PG2. Exo-PG1 had a broad specificity towards oligogalacturonates with different DPs, while digalacturonate was the most favorable substrate for exo-PG2. Both enzymes degraded xylogalacturonan from pea hull in an exo manner to produce galacturonic acid (GalA) and Xyl-GalA disaccharide, as identified by electrospray ion trap mass spectrometry (ESI-ITMS). Moreover, exo-PGs split acetylated homogalacturonan in an exo manner, producing GalA and acetylated GalA, as shown by ESI-ITMS. An exo-arabinanase, termed Abnx, was purified from a culture filtrate of *P. chrysogenum* 31B. The enzyme released only arabinobiose from the non-reducing terminus of α-1,5-L-arabinan and showed no activity towards *p*-nitrophenyl α-L-arabinofuranoside or α-1,5-L-arabinofuranobiose. The nucleotide sequence of the *abnx* cDNA gene, which encodes Abnx, was determined. Abnx was found to be structurally distinct from known arabinan-degrading enzymes based on its amino acid sequence and a hydrophobic cluster analysis (HCA). The *abnx* cDNA gene product expressed in *Escherichia coli* catalyzed the release of arabinobiose from α-1,5-L-arabinan. The activity of the recombinant Abnx towards a series of arabino-oligosaccharides, as expressed by k_{cat}/K_m value, was greatest with arabinohexaose. The recombinant enzyme was found to possess trans-arabinobiosylation activity on various acceptors, such as aliphatic alcohols, sugars and sugar alcohols. The

transfer product of glycerol was identified as *O*-α-L-arabinosyl-(1→5)-*O*-α-L-arabinosyl-(1→1)-glycerol on the basis of the spectral data, fast atom bombardment-mass (FAB-MS) and ^{1}H- and ^{13}C-NMR. Unlike endo-arabinanases, Abnx catalyzed the hydrolysis of linear arabinan without inverting the anomeric configuration.

Key words: exo-polygalacturonase, xylogalacturonan, acetylated homogalacturonan, exo-arabinanase, arabinobiose