

List of Figures

Figure 1-1.	The basic structural components of xylan, and the hemicellulases responsible for its degradation	9
Figure 1-2.	Catalytic mechanism of the Glycohydrolases	10
Figure 1-3.	The three-dimensional structure of bifunctional α -L-arabinofuranosidase and β -D-xylopyranosidase from <i>Aspergillus kawachii</i> IFO4308	11
Scheme 2-1.	The catalytic hydrolysis and transglycosylation of the bifunctional Abf/Xyl	26
Figure 2-1.	The chromatography of the purification of the recombinant of Abf. The absorbance (-○-) was monitored at 280 nm. The fractions with Abf activity (-●-) was eluted within 150-180 mM NaCl (—)	27
Figure 2-2.	SDS-PAGE analysis of Abf obtained from different induction time and the purified protein. Lanes: M, molecular mass markers; 1, recombinant enzyme from day 0 supernatants; 2, recombinant enzyme from day 1 supernatants; 3, recombinant enzyme from day 4 supernatants; 4, pool of active fractions from HiTrap-SP column	28
Figure 2-3.	Thermal stability of the recombinant enzyme assay as Abf (a) and Xyl (b). Enzyme was incubated in various temperature: 25 °C (○), 35 °C (●), 45 °C (□), 55 °C (■), 60 °C (△), and 65 °C (▲)	29
Figure 2-4.	The proposed two-step, double displacement mechanism of Abf/Xyl	30
Figure 3-1.	Proposed reaction mechanism of a retaining α -L-arabinofuranosidase	52
Figure 3-2.	SDS-PAGE (a) and mass spectrometry (b) analysis of the recombinant α -L-arabinofuranosidase. Lanes: M, markers; 1, recombinant wild-type Abf	53
Figure 3-3.	pH activity profiles of wild-type Abf (○) and the D299N mutant enzyme (●). The k_{cat} values of wild-type and D299N mutant were measured at the final pH values: 1.9, 2.0, 2.4, 3.3, 3.9, 4.2, 5.5, 6.5	54
Figure 3-4.	The active site of the GH54-family enzyme from <i>Aspergillus kawachii</i> IFO4308 (1WD4) with arabinofuranose in place. The	55

corresponding amino acids in the *Trichoderma koningii* Abf are labeled in parentheses

- Figure 3-5.** Data from a multialignment exercise, using partial sequences, of family GH54 α -L-arabinofuranosidases. Biology WorkBench 3.2 CLUSTALW (San Diego Supercomputer Center, CA, USA) software was used. All enzyme sequences were derived from published gene sequences. GenBank accession details are: U38661 from *Hypocrea koningii* G-39, AB085904 from *A. kawachii* IFO 4308, Z69252 from *Hypocrea jecorina* RutC-30, AF367026 from *Penicillium purpurogenum*, AB073861 from *Aspergillus oryzae* RIB40, AB073860 from *Aspergillus oryzae* HL15, L23502 from *Aspergillus niger*, U39942 from *Aspergillus niger*, Y13759 from *Emericella nidulans* argB2, AY495375 from *Aureobasidium pullulans*, AJ310126 from *Fusarium oxysporum* f. sp. *Dianthi*, and AF306764 from *Cochliobolus carbonum* 56
- Figure 3-6.** Stereochemical properties and common intermediates of Abf catalysis. (a) Enzymatic reactions, using various substrates, in the presence of methanol. (b) A partial NMR spectrum (chemical shift 3.4–5.4 ppm) of the end-products. Peak assignment is given in the text 57
- Figure 3-7.** Brønsted plots of wild-type Abf (○) and D299G (●) mutant enzyme. (a) Plots of $\log k_{\text{cat}}$ against $\text{p}K_a$ of the leaving phenol. (b) Plots of $\log k_{\text{cat}}/K_m$ against $\text{p}K_a$ of the leaving phenol 58