Structure-Functional Analysis of Bacterial Expansins from *Pectobacterium carotovorum* and *Bacillus subtilis*

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Introduction. Expansins are proteins, with activities better characterized in plants where they have roles in cell enlargement and other developmental events requiring cell wall loosening (1). Related sequences are found in phylogenetically diverse bacteria that infect plants. Gene knock-out studies indicate that these "bacterial expansins" promote plant infection or root surface colonization by bacteria, possibly by modifying plant cell walls (2).
Expansins are non-hydrolytic proteins, and it is believed that the disrupting activity of expansins and expansin-like proteins on cell wall components confers a synergistic effect to the enzymatic hydrolysis of cellulose by enabling it to be more accessible to the enzyme (3). Polysaccharides disruptive activity of *B. subtilis* $EXLX1_{Bs}$ has been shown on cellulosic materials. Key conserved residues have been identified, some amino acids are involved in the loosening activity whereas others have a role on substrate binding (4). Through BLAST searches, we have identified a sequence from the plant pathogen *Pectobaterium carotovorum* ($EXLX_{Pc}$) that is highly similar to $EXLX1_{Bs}$. However, we speculate that differences between the two proteins (pI) might account for the specific action on cell walls of different composition. Here, we report the construction and expression of activity- and binding-mutants of $EXLX_{Pc}$ and $EXLX1_{Bs}$ to determine their contribution to the effect on cell wall material of different composition and origin.

Methods. *Cloning and expression:* Wild type genes, *EXLX1Bs* and *EXLXPc,* were cloned in plasmid pET22. Mutant variants were generated by site-directed mutagenesis using the Stratagene QuikChange Lightning Multi Site-Directed Mutagenesis kit. Expression was carried out in *E. coli BL21*. *Circular Dichroism (CD)* spectra were determined and compared to the 3D structure of $EXLX1_{Bs}$ (Protein Data Bank code 3D30 structure). *Binding Assays:* Binding of EXLX1 and mutant variants to cellulose, insoluble arabinoxylans, and cell walls and sequentially extracted cell walls from wheat coleoptiles was determined by incubation and SDS-PAGE (4).

Results. Residues involved in the activity and substrate binding were identified in $EXLX_{Pc}$ by sequence alignment to $EXLX1_{Bs}$ (4). The following mutants were constructed, and their sequence verified:

Species	Mutant	Function
P. carotovorum	D83A	Activity
	Y126A/W127/Y158A	Binding
B. subtilis	D82A	Activity
	W125A/W126A/Y157A	Binding
	R173Q/K180Q/K183	Binging

Wild type and mutants Pc-D83A and Bs-D82A have been expressed. *P. carotovorum* proteins are approximately 22 kDa and B. subtilis proteins are 23 kDa in SDS-PAGE gels. Cultures were grown for 16 h in LB broth with ampicillin, and induced with 1 mM IPTG at 16°C and 37°C, respectively.

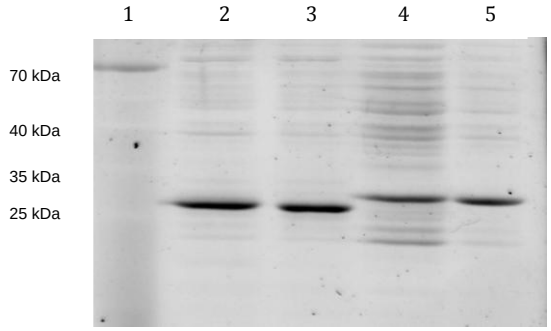

Fig.1 SDS-PAGE gel of expansin variants. 1, PageRuler Prestained Protein Ladder; 2, PcWT; 3, Pc-D83A; 4, BsWT; 5, Bs-D82A.

Conclusions. Key residues have been modified in expansins $EXLX_{Pc}$ and $EXLX1_{Bs}$. Successful expression and purification of the protein variants has been achieved. The contribution of these amino acids to the expansin activity on cell wall polysaccharides will be determined.

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