Expansins from *Pectobacterium carotovorum* and *Bacillus subtilis* bind differentially to cell wall components

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Introduction

Expansins are small proteins that bind and loosen the plant cell wall polysaccharides. *Bacillus subtilis* expansin, EXLX1, is constituted by 208 amino acids arranged in two domains (D1 and D2), preceded by a signal peptide from about amino acid 15 to 25 (1). $EXLX1_{Bs}$ is responsible for the loosening of the cell walls during root colonization, and similar sequences have been found in organisms that show interaction with plants. Here, we report the characterization of the protein $EXLX_{Pc}$ from *Pectobacterium carotovorum* sp. an economically important plant pathogen. $EXLX_{Pc}$ is highly similar to the $EXLX1_{Bs}$. A striking difference between the two proteins is the abundance of positive residues in $EXLX1_{Bs}$ in comparison to $EXLX_{Pc}$ resulting in different isoelectric points (9.2 *vs.* 4.8, respectively), suggesting differences in the ability to bind to cell wall components between the two proteins.

Methods

pET22 vector was used for heterologous expression of the 6x-His tagged $EXLX_{Pc}$ and $EXLX1_{Bs}$ in *E. coli*. Proteins were purified by IMAC, using a Ni2+ column; imidazole was removed by dialysis. Isoelectric point was determined by 2D gel electrophoresis, thermal stability by calorimetry, and secondary structure content by circular dichroism. Substrate binding was determined using whole cell walls and extracted cell wall-fractions from wheat coleoptiles as described in reference (3).

Results

In accordance with the 3D structure of expansins, circular dichroism of purified $EXLX_{Pc}$ confirmed the presence of high content of β-sheets. Calorimetric analysis showed a Tm of 53.3° C, and that $EXLX_{Pc}$ denaturation is irreversible (Fig 1).

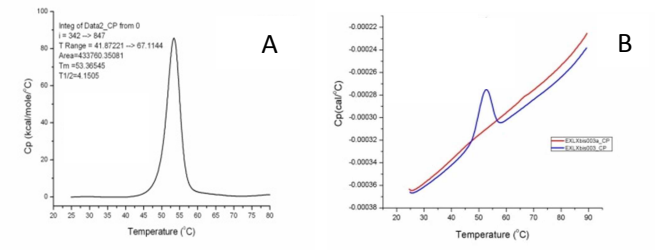

Fig. 1 Calorimetric analyses show the tm of $EXLX_{Pc}$ (A) and its irreversible denaturation (B).

Substrate binding experiments resulted in similar levels of binding of $EXLX_{Pc}$ and $EXLX1_{Bs}$ to whole cell walls (CCW) and cell walls containing cellulose only (CW-P-HC), or Avicel (a commercial crystalline cellulose) (Fig. 2). Strikingly, a clear difference was observed when pectin was initially removed from cell walls (CW-P, Fig. 2), suggesting higher affinity of $EXLX_{Pc}$ to hemicellulose.

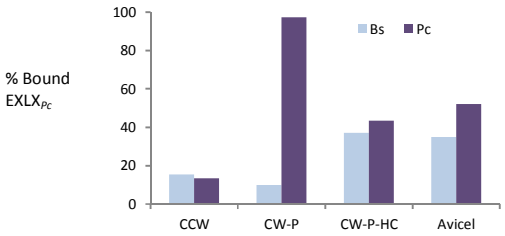

Fig. 2. Binding of $EXLX_{Pc}$ and $EXLX1_{Bs}$ to different substrates. CCW: whole cell walls; CW-P: pectin extracted fraction of cell walls; CW-P-HC: pectin and hemicellulose fraction of cell walls; A: Avicel.

Conclusions.

$EXLX_{Pc}$ expansin was successfully expressed and purified from *E. coli*. Circular dichroism and calorimetry were characteristic of bacterial expansins. Experimental pI determination confirmed that $EXLX_{Pc}$ is an acidic protein (pI=5.9) whereas $EXLX1_{Bs}$ is basic (pI=8.9). Binding to whole cell walls and cellulose is similar for both species, however pectin and hemicellulose seem to be differently recognized by each expansin, probably due to the different mode of interaction of each organism with its host plant.

References.

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