Study of the mode of action of a polygalacturonase from the phytopathogen Burkholderia cepacia - DTU Orbit (01/01/2019)

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We have recently isolated and heterologously expressed BcPeh28A, an endopolygalacturonase from the phytopathogenic Gram-negative bacterium Burkholderia cepacia. Endopolygalacturonases belong to glycoside hydrolase family 28 and are responsible for the hydrolysis of the non-esterified regions of pectins. The mode of action of BcPeh28A on different substrates has been investigated and its enzymatic mechanism elucidated. The hydrolysis of polygalacturonate indicates that BcPeh28A is a non-processive enzyme that releases oligomers with chain lengths ranging from two to eight. By inspection of product progression curves, a kinetic model has been generated and extensively tested. It has been used to derive the kinetic parameters that describe the time course of the formation of six predominant products. Moreover, an investigation of the enzymatic activity on shorter substrates that differ in their overall length and methylation patterns sheds light on the architecture of the BcPeh28A active site. Specifically the tolerance of individual sites towards methylated saccharide units was rationalized on the basis of the hydrolysis of hexagalacturonides with different methylation patterns.
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