TITLE: STUDY OF THE ACTIVATION OF A RECOMBINANT FERULOYL ESTERASE FROM *Clostridium thermocellum* BY DEPHOSPHORYLATION.

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ABSTRACT:

Feruloyl Esterases are enzymes that contribute to the degradation of arabinoxylans by hydrolysing the ester bond between phenolic acids and the arabinose linked to the xylose backbone. A recombinant Feruloyl esterase (FAE) from *Clostridium thermocellum* that has previously been described (Blum, et. al, 2000) was subsequently shown to be inactive, and in this ongoing work we validate the hypothesis that a phosphate or sulphate group modifies a catalytic serine in the active site, causing the lack of activity (PRATES, et. al, 2001). The recombinant enzyme was expressed in *Escherichia coli* (STAR) and purified using affinity chromatography. The purified enzyme was treated with a commercial Lambda Phosphatase Protein following the manufacturers´ instructions. Arabinoxylan extracted from sugarcane was incubated with phosphatase treated (FAELPP) and non-treated (FAE) enzymes followed by hydrolysis by a glycosyl hydrolase GH10 for 4h after the first reaction. The total reducing sugar content was measured with the Somogyi-Nelson method and reaction products were analysed with HILIC liquid chromatography - mass spectrometry. Total reducing sugar content did not differ between the samples, as expected for the products released by an esterase, but the chromatograms and mass-spectra showed important differences between the FAELPP and FAE samples at retention time of approximately 12 minutes. The mass-charge peaks of both samples were analysed and compared using an online tool for metabolomics (XCMS online), from which cloudplot maps, PCA, Heatmaps and Venn Diagrams were used to identify the differences in products released under the various experimental conditions. Most of the peaks were identified as oligosaccharides modified with acetate and/or methyl-glucuronic acid or as non-saccharides, of unidentified structure. Our results confirm that the samples with FAELPP release different compounds compared with the FAE samples, leading to the conclusion that after the treatment with a Lambda Phosphatase Protein, FAE had at least part of its activity restored and that the lack of activity in the recombinant protein is the result of phosphorylation.

Keywords: Feruloyl esterase; arabinoxylan; phosphorylation; mass spectroscopy

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