Capillary electrophoresis for the functional annotation of carbohydrate-active enzymes: a preliminary case-study

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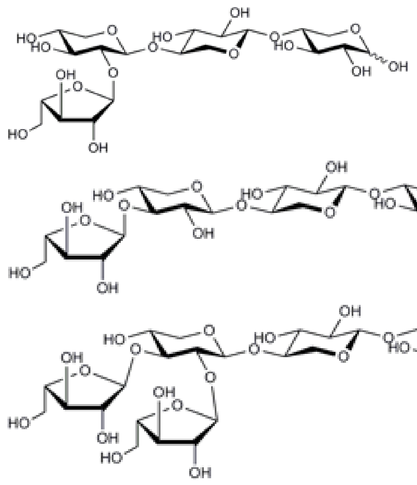
1. Introduction

The **DNA-sequencer aided fluorophore assisted capillary electrophoresis** (DSA-FACE) platform is being exploited for the high-throughput qualitative and quantitative analysis of carbohydrates[1]. Due to its high resolution and sensitivity DSA-FACE enables the resolution of stereoisomers with the same degree of polymerization (DP) and the detection of products in the fmol range. The reliable qualitative analyses are particularly attractive for the analysis of glyco-active enzymes substrate specificity.

Arabinoxylan (AX) consists of a β-1,4-linked xylose backbone which can be heavily substituted, namely by α-1,2 and/or α-1,3 arabinose residues. **Arabinofuranosidases** are exo-acting enzymes that contribute for AX depolymerisation because they are responsible for the hydrolysis of the arabinose monomers. Since arabinose residues can be present at different positions in AX, the knowledge of arabinofuranosidases substrate specificity is of major importance.



2^3-α-L-Arabinofuranosyl-xylotriose (A^2XX)

3^3-α-L-Arabinofuranosyl-xylotriose (A^3XX)

2^3, 3^3-di-α-L-Arabinofuranosyl-xylotriose ($A^{2+3}XX$)

Figure 1. Representation of the possible linkages between arabinose and xylose residues on arabinoxylan oligosaccharides.

2. Assumptions

a) DSA-FACE is able to resolve arabinoxylan oligosaccharides (AXOS) with α-1,2- and/or α-1,3-arabinose substitutions even with the same DP.

b) α-L-arabinofuranosidase (EC 3.2.1.55) from *Apergillus nidulans*[2] belongs to Glycoside Hydrolase family 62 (GH62). As for other members of GH62[3], this enzyme is expected to hydrolyse α-1,2- and α-1,3-arabinose residues exclusively from monosubstituted xylose residues.

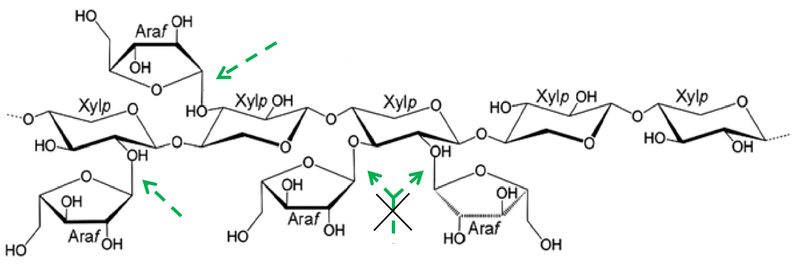
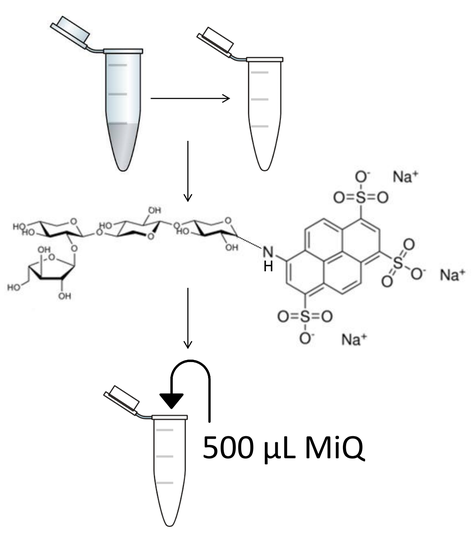


Figure 2. Possible scheme for the hydrolysis of arabinoxylan by GH62 α-L-arabinofuranosidase from *Aspergillus nidulans.*

3. Methods

Step 1

Label AXOS with the fluorophore 8-aminopyrene-1,3,6-trisulfonic acid (APTS, $\lambda_{excitation/emission}$ = 488/512 nm).



Evaporate 1 µM AXOS to dryness (at 60 °C).

Addition of APTS to AXOS by a reductive amination reaction[1]: 10 mM APTS and 500 mM $NaBH_3CN$.
Incubate overnight at 37 °C.

500 µL MiQ

Stop reductive amination reaction.

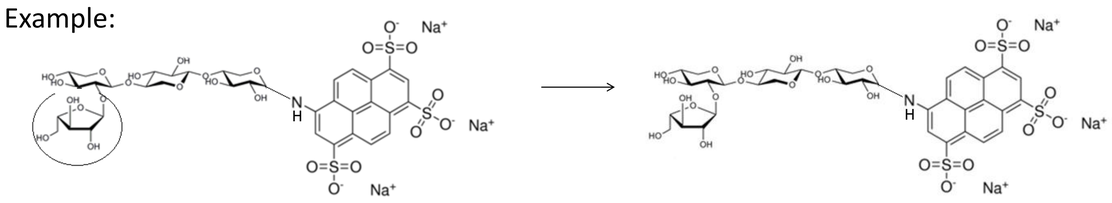
24 h

Step 2

Hydrolysis of A^2XX, A^3XX and $A^{2+3}XX$ by α-L-arabinofuranosidase from *Apergillus nidulans.*

600 pM AXOS + 2 µL supernatant of α-L-arabinofuranosidase *Pichia pastoris* expression strain + 50mM $NaC_2H_3O_2$.
At 50 °C and 600 rpm.

Example:

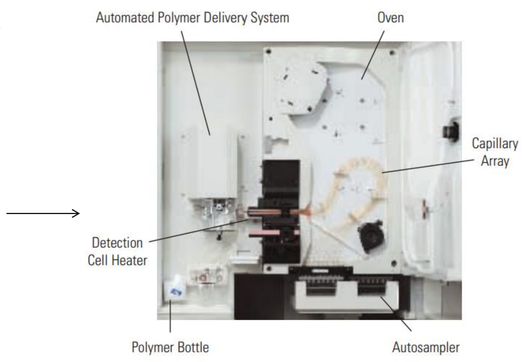


24 h

Step 3

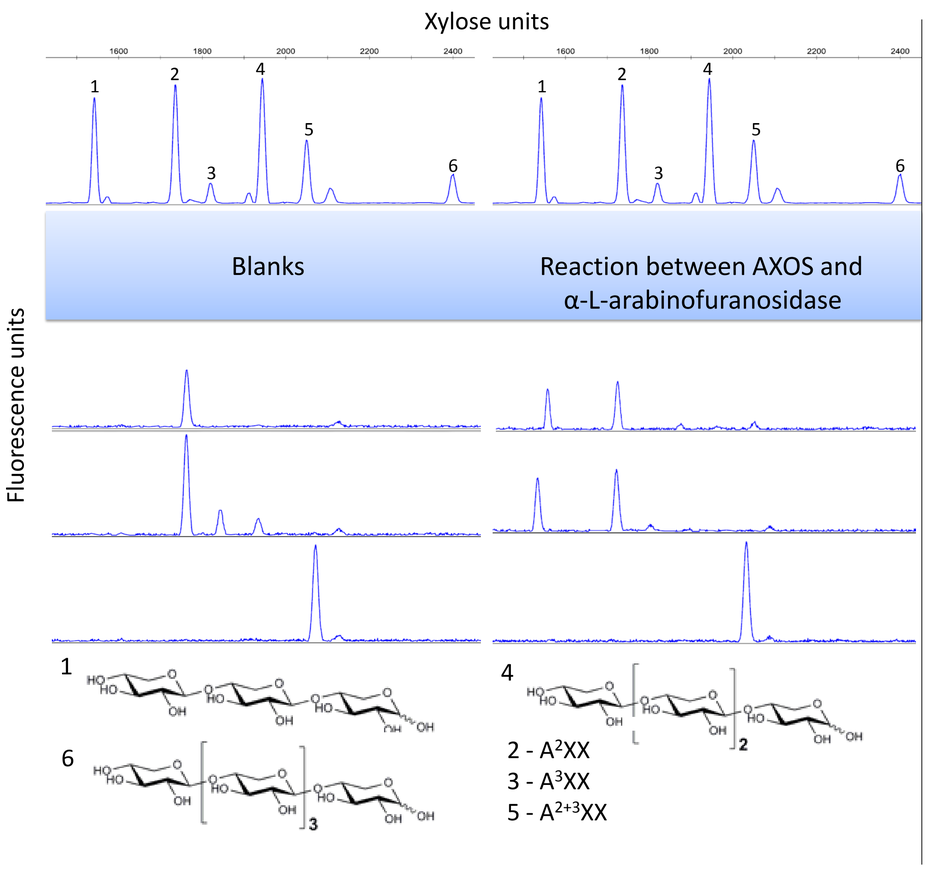
Analysis of AXOS by DSA-FACE.

Concentrate the hydrolysates to a maximum AXOS concentration in the nM range.
Inject 10 µL of each hydrolysate in the Applied Biosystems 3130 Genetic Analyzer.
Analyse the data with GeneMapper® software.



30 min per 4 samples

4. Results and Conclusions



✓ DSA-FACE can separate xylan- and arabinoxylan- oligosaccharides with a good resolution.
✓ It is shown that the α-L-arabinofuranosidase under study is capable of hydrolysing α-1,2 and α-1,3 arabinose residues only from single substituted xylose residues.

Aknowledgments

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References

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