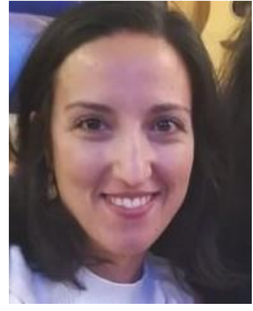

High-throughput substrate specificity analysis of metagenomic-derived arabinoxylan-active enzymes

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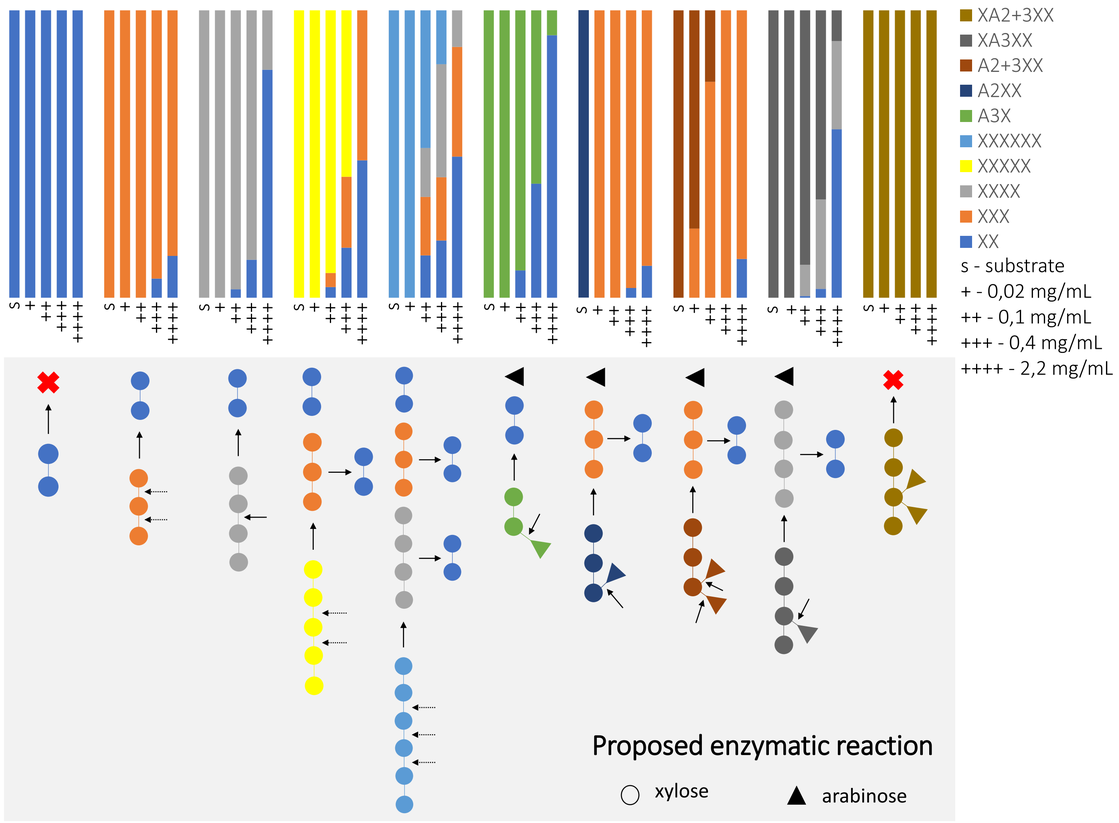
A INTRODUCTION

Functional enzyme screening of the increasing amount of metagenomic data is often cumbersome, especially for the **discovery of enzymes with complex substrate specificities such as arabinoxylan-active enzymes**. We present here our **high-throughput** approach based on DNA sequencer-aided fluorophore-assisted carbohydrate electrophoresis (**DSA-FACE**) for the parallel analysis of **carbohydrate specificities of putative arabinoxylan-active enzymes** present in metagenomics data (Fig. 1).

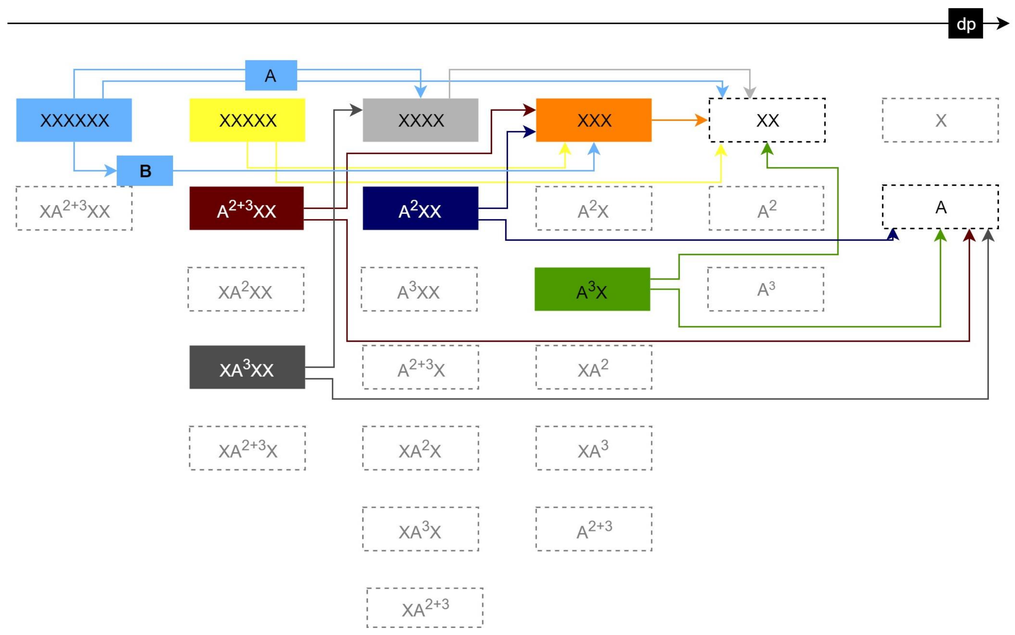
B EXPERIMENTAL DATA

A metagenomic study on the North American beaver (*Castor canadensis*) feces revealed the presence of a putative arabinoxylan-active enzyme from uncharacterized subfamily 28 of the GH43 CAZy family. Specificity scans were done for 4 enzyme concentrations and for 5 XOS and 5 AXOS.

SPECIFICITY SCANS FOR GH43_28 FAMILY MEMBER:



DEGRADATION MAPS FOR GH43_28 FAMILY MEMBER:



C CONCLUSIONS

- By **DSA-FACE** we could **rapidly** perform **specificity scans** for GH43_28 and representative (A)XOS at different enzyme concentrations.
- Based on specificity scans, it seems the preferred substrate for GH43_28 is A^2XX. At low concentration, GH43_28 behaves as a α-L-arabinofuranosidase being able to remove *O*-2 arabinofuranosyl substitutions from mono-substituted A^2XX and *O*-2 and *O*-3 arabinofuranosyl substitutions from $A^{2+3}XX$ when there is no xylose at the non-reducing end.
- At higher concentrations GH43_28 shows endo-xylanase activity.
- GH43_28 seems to be inhibited by non-reducing xylose monomers.

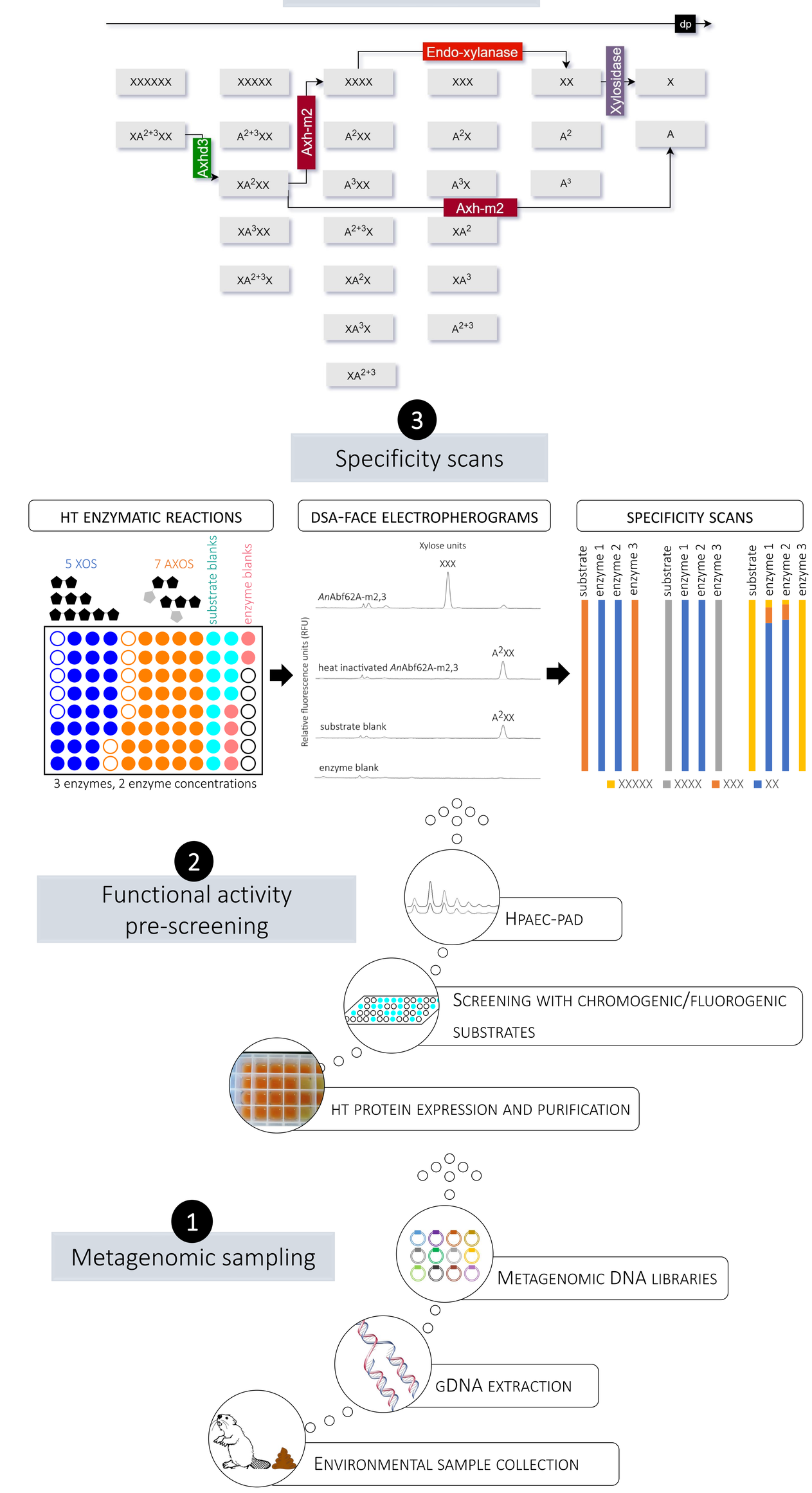


Fig 1. *Blueprint* to discover substrate specificities of new metagenomic putative arabinoxylan-active enzymes with DSA-FACE. ❶ Environmental DNA is extracted from natural sources and used to construct high molecular weight DNA libraries (fosmids) or small insert DNA libraries. ❷ After DNA libraries screening and sequencing, interesting protein coding sequences are cloned and expressed in parallel. Enzymatic reactions with representative arabino(xylan)-oligosaccharides ((A)XOS) are done and analysed by HPAEC-PAD upon prediction of enzymatic activity with chromogenic/fluorogenic substrates. ❸ To be able to reveal complete specificity profiles, parallel enzymatic reactions are done with (A)XOS with different substitution profiles. For example, in a 96-well plate 5 XOS and 7 AXOS are tested with 3 different enzymes at 2 different enzyme concentrations. Substrate and enzyme blanks must be added for posterior interpretation of DSA-FACE electropherograms. Based on electrophoretic mobilities and peak areas, DSA-FACE electropherograms are converted into specificity scans showing reaction products formed and allowing comparison of activities of different enzymes or enzyme concentrations on a substrate. ❹ From specificity scans, degradation maps per enzyme are inferred allowing to better understand substrate specificity pathways.