

Biochemical and kinetic characterisation of a novel xylooligosaccharide-upregulated GH43 β -D-xylosidase/ α -L-arabinofuranosidase (BXA43) from the probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12 - DTU Orbit (09/11/2017)

Biochemical and kinetic characterisation of a novel xylooligosaccharide-upregulated GH43 β -D-xylosidase/ α -L-arabinofuranosidase (BXA43) from the probiotic *Bifidobacterium animalis* subsp. *lactis* BB-12

The *Bifidobacterium animalis* subsp. *lactis* BB-12 gene BIF_00092, assigned to encode a β -D-xylosidase (BXA43) of glycoside hydrolase family 43 (GH43), was cloned with a C-terminal His-tag and expressed in *Escherichia coli*. BXA43 was purified to homogeneity from the cell lysate and found to be a dual-specificity exo-hydrolase active on *para*-nitrophenyl- β -D-xylopyranoside (pNPX), *para*-nitrophenyl- α -L-arabinofuranoside (pNPA), β -(1 \rightarrow 4)-xylopyranosyl oligomers (XOS) of degree of polymerisation (DP) 2–4, and birchwood xylan. A phylogenetic tree of the 92 characterised GH43 enzymes displayed five distinct groups (I – V) showing specificity differences. BXA43 belonged to group IV and had an activity ratio for pNPA:pNPX of 1:25. BXA43 was stable below 40°C and at pH 4.0–8.0 and showed maximum activity at pH 5.5 and 50°C. K_m and k_{cat} for pNPX were 15.6 ± 4.2 mM and 60.6 ± 10.8 s⁻¹, respectively, and substrate inhibition became apparent above 18 mM pNPX. Similar kinetic parameters and catalytic efficiency values were reported for β -D-xylosidase (XynB3) from *Geobacillus stearothermophilus* T-6 also belonging to group IV. The activity of BXA43 for xylooligosaccharides increased with the size and was 2.3 and 5.6 fold higher, respectively for xylobiose and xylotetraose compared to pNPX. BXA43 showed clearly metal inhibition for Zn²⁺ and Ag⁺, which is different to its close homologues. Multiple sequence alignment and homology modelling indicated that Arg⁵⁰⁵Tyr⁵⁰⁶ present in BXA43 are probably important for binding to xylotetraose at subsite +3 and occur only in GH43 from the *Bifidobacterium* genus.

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