Novel Enzyme Actions for Sulphated Galactofucan Depolymerisation and a New Engineering Strategy for Molecular Stabilisation of Fucoidan Degrading Enzymes

Fucoidans from brown macroalgae have beneficial biomedical properties but their use as pharma products requires homogenous oligomeric products. In this study, the action of five recombinant microbial fucoidan degrading enzymes were evaluated on fucoidans from brown macroalgae: Sargassum mcclurei, Fucus evanescens, Fucus vesiculosus, Turbinaria ornata, Saccharina cichorioides, and Undaria pinnatifida. The enzymes included three endo-fucoidanases (EC 3.2.1.-GH 107), FcnA2, Fda1, and Fda2, and two unclassified endo-fucoglucurononmannan lyases, FdlA and FdlB. The oligosaccharide product profiles were assessed by carbohydrate-polyacrylamide gel electrophoresis and size exclusion chromatography. The recombinant enzymes FcnA2, Fda1, and Fda2 were unstable but were stabilised by truncation of the C-terminal end (removing up to 40% of the enzyme sequence). All five enzymes catalysed degradation of fucoidans containing α(1→4)-linked l-fucosyls. Fda2 also degraded S. cichorioides and U. pinnatifida fucoidans that have α(1→3)-linked l-fucosyls in their backbone. In the stabilised form, Fda1 also cleaved α(1→3) bonds. For the first time, we also show that several enzymes catalyse degradation of S. mcclurei galactofucan-fucoidan, known to contain α(1→4) and α(1→3) linked l-fucosyls and galactosyl-β(1→3) bonds in the backbone. These data enhance our understanding of fucoidan degrading enzymes and their substrate preferences and may assist development of enzyme-assisted production of defined fuco-oligosaccharides from fucoidan substrates.

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