

Novel Sulfated Trisaccharides Produced from Fucoidan by a Novel Bacterial Endo-fucoidan-lyase Degradation and Elucidation of Long Side Chain of Fucoidan

An electrophoretically homogeneous fucoidan was purified from a brown seaweed, *Kjellmaniella crassifolia* Miyabe. We found that a kind of *Flavobacterium* secreted a novel fucoidan degrading enzyme that digested about 20% of the fucoidan and produced novel sulfated trisaccharides. Each of the oligosaccharides was isolated respectively by anion exchange chromatography, and characterized by component sugar analysis, mass spectrometry, and 500MHz 1D and 2D ¹HNMR spectrometry. The structures of the products were L-Fuc(3-*O*-sulfate) α 1-3(Δ _{4,5}GlcA β 1-2)D-Man and L-Fuc(2,4-*O*-disulfate) α 1-3(Δ _{4,5}GlcA β 1-2)D-Man(6-*O*-sulfate) and so on. The enzyme also produced hexa- or longer saccharides, such as L-Fuc(3-*O*-sulfate) α 1-3(Δ _{4,5}GlcA β 1-2)D-Man α 1-4GlcA β 1-2(L-Fuc(3-*O*-sulfate) α 1-3)D-Man early in the reaction. From the results, the existence of long side chains in the fucoidan was considered because all products contained unsaturated glucuronic acid, that is a trace of enzymatic digestion. To elucidate the existence, the fucoidan was hydrolysed in 0.5M oxalic acid at 100°C for 3h before and after the enzymatic degradation. A polymer of (-4-D-GlcA β 1-2-D-Man α 1-)_{about 40} remained after hydrolysis of fucoidan before enzymatic degradation only. From these results, at least a chain of (-4-D-GlcA β 1-2-D-Man α 1-)_{about 40} that C-3 mannosyl residue substituted by 3-*O*-sulfated L-Fucose or 2,4-*O*-disulfated L-fucose, should exist in one molecule of the fucoidan. The novel endo-fucoidan-lyase cleaved D-Man α 1-4 GlcA in the fucoidan eliminatively and produced the novel sulfated trisaccharides.