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Study of substrate specificity of two recombinant fucoidanase from marine bacteria *Wenyingzhuangia fucanilytica*

Key words:marine bacteria, Wenyingzhuangia fucanilytica, fucoidan, Fucus evanescens, fucoidanase, substrate specificity

Among polyanionic polysaccharides of brown algae possessing a wide spectrum of biological action (antiviral, antitumor, immunomodulatory, anti-inflammatory, anticoagulant, antiadhesive, anti-angiogenic), fucoidans are of the greatest interest for study [3]. In addition, fucoidans can be used for targeted drug delivery [2]. Fucoidans are sulfated homo- and heteropolysaccharides.

Enzymes that catalyze the cleavage of glycosidic bonds between sulfated fucose residues in fucoidan molecules are called fucoidanases. Interest in the study of fucoidanases, which catalyze certain transformations of fucoidans, is constantly increasing. This is due to the possibility of using them as tools for establishing the detailed structure of fucoidans. One of the most important properties of fucoidanases is specificity. The detailed specificity of the action of fucoidanases has not been sufficiently studied so far, it probably depends not only on the types of O-glycosidic bonds between fucose residues in sulfated polysaccharides, but also on the position of sulfate groups, like the specificity of carrageenases and heparinases [1, 4].

In this work, we studied the specificity of two recombinant fucoidanases from the marine bacterium *Wenyingzhuangia fucanilytica*. Genes of fucoidanases *fwf1* and *fwf2* were cloned in truncated forms without predicted N-terminal signal sequences and C-terminal sorting domain (secretion system). Genetic constructions coding fucoidanases were obtained by restriction free method. The recombinant fucoidanases FWF1 and FWF2 were produced in *Escherichia coli* strain Arctic Express.

To establish the substrate specificity of enzymes, information on the structure of the hydrolysis products of the substate is usually used. We obtained products of enzymatic hydrolysis of fucoidan from *Fucus evanescens* by fucoidanases FWF1 and FWF2. The obtained oligosaccharides were analyzed by NMR spectroscopy using one- and two-dimensional techniques (1H, 13C, COSY, TOCSY, HSQC, HMBC). Based on the analysis of the obtained spectra, was made a conclusion on the structure of the obtained oligosaccharides. The oligosaccharides obtained by the action of FWF1 fucoidanase differed in structure from the products of the action of fucoidanase FWF2, namely the degree of polymerization and the position of sulfate groups with fucose residues. The

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obtained oligosaccharides were used as substrates to study of more detailed specificity of FWF1 and FWF2. The obtained data indicate that these enzymes have different substrate specificity.

Thus, the specificity of fucoidanases is characterized not only by the type of glycosidic bond cleaved by them, but also by the position of sulfate groups.

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