



# SEAMARK DELIVERABLE 6.5: STRUCTURAL ANALYSIS OF POLYSACCHARIDE SUBSTRATES AND OLIGOSACCHARIDE PRODUCTS

Public summary of  
confidential report

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6 / T6.5 Structural analysis of polysaccharide substrates and oligosaccharide products

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**Summary:**

This deliverable, D6.5, reports the structural complexity of laminarin and alginate and its derivatives. It is a part of structure-function study where specific immunomodulatory activity is related to specific structural features of the complex saccharides. Laminarin, a storage polysaccharide predominantly found in brown seaweed, is a  $\beta$ -1,3-glucan with  $\beta$ -1,6-branches. In SeaMark, laminarins from three species of brown seaweed were extracted, purified, structurally analysed, and enzymatically modified using the laminaripentaose-producing endo-1,3- $\beta$ -glucanase, LPHase. The aim was to unravel structure-function relationships with immunomodulating activity using a human monocyte-derived dendritic cell (DC) model as described in deliverable report D6.4. The laminarins from the three types of kelp differed with respect to structural complexity, with the laminarin from *Laminaria hyperborea* displaying the simplest structure, with few and short branches. The backbones of the laminarins were confirmed to consist of  $\beta$ -1,3-linkages, with varying percentages of  $\beta$ -1,6-linkages (branches), *L. hyperborea* with ~4%, whereas *Laminaria digitata* and *Saccharina latissima* contained ~10%, and ~21%,  $\beta$ -1,6-linkages, respectively. The molecular sizes were between DP24-25 for the majority of the extracted laminarin. Laminari-oligosaccharide fractions, produced using the laminaripentaose-producing enzyme LPHase, displayed different DP-ranges, degrees of  $\beta$ -1,6-branching and intrachain linkages. The poly- and oligosaccharides were subjected to immunomodulating study described in D6.4. The structure of fucoidan and derived medium molecular weight and low molecular weight fucoidan saccharides will be described in D6.6.

Alginate, a structural polysaccharide found in the cell walls of brown seaweed, is composed of linear chains of  $\alpha$ -L-guluronic acid (GulA) and  $\beta$ -D-mannuronic acid (ManA) residues. This polysaccharide plays a key role in providing strength and flexibility to the seaweed's cell walls. In SeaMark, several alginate lyases with different affinities and specific activities were used to make alginate oligosaccharides of different size and composition. Knowledge from previous studies of enzyme properties and results of simple TLC analyses were used to postulate the structure of the compositions of the enzymatically derived alginate oligosaccharide. The oligosaccharides were submitted to immunomodulating study described in D6.4 were one enzyme-derived product, (from AlyRm3 fully digested), consisting mainly of di-, tri-, and tetrasaccharides, respectively, each with a 4,5-unsaturated non-reducing-end uronic acid showed significant immunomodulation potency.



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