

MS009.P27

*Structural and functional characterization of ulvan lyase enzymes*

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Ulvan is a complex sulfated polysaccharide biosynthesized by marine green algae and constitutes one of the two major polysaccharides of their cell wall. This water-soluble polysaccharide is composed predominantly of 3-sulfated rhamnose (R3S), glucuronic acid (GluA), iduronic acid (IdoA) and xylose. The physicochemical and biological properties of ulvan make it of interest for a variety of industrial applications.

Bacteria cohabiting with the green algae contain enzymes able to degrade ulvan by a lytic  $\beta$ -elimination mechanism. Genes coding such lyases have been discovered in the genomes of several bacteria. Pseudoalteromonas sp. strain PLSV gene PLSV\_3936 encodes an ulvan lyase that cleaves the glycosidic bond between 3-sulfated rhamnose (R3S) and glucuronic acid (GluA) or iduronic acid (IdoA). Another ulvan lyase, discovered in Alteromonadales and encoded by the gene LOR\_107, degrades ulvan endolytically cleaving the bond between the rhamnose-3-sulfate and glucuronic acid. We have characterized biochemically these two lyases and determined their three-dimensional structures. They represent the first structures of lyases capable of degrading ulvan. In spite of only 17% sequence identity, these two enzymes share the same 7-bladed  $\beta$  propeller fold. The putative active site was identified from structure conservation and confirmed by mutagenesis and structures of these enzymes with bound tetrasaccharide substrates. The catalytic residues are histidine and tyrosine while the substrate acidic group is neutralized by an arginine. Metal ions were detected in both lyases but they play only structural roles and are not involved directly in the catalysis.

Moran Kopel. et al.(2016) The Journal of Biological Chemistry. Vol.291,No.11,5871-5878

**Keywords:** [Ulvan](#), [Polysaccharide lyase- PLSV3936](#), [LOR107](#)