Isolation and characterization of biopolymer from the ascidian *Styela clava*

U.R. Varela[1], J. Valcarcel[1], P. Ramos[2], J.A. Vázquez[1]

[1]Group of Recycling and Valorisation of Waste Materials (REVAL), Marine Research Institute (IIM-CSIC), Spanish Council for Scientific Research, Spain

[2]Group of Food Biochemistry, Marine Research Institute (IIM-CSIC), Spanish Council for Scientific Research, Spain

Abstract

Ascidians, also known as sea squirts, are marine invertebrate filter feeders characterized by a unique tough outer tunic made of cellulose. The highly crystalline celulosic nanofibrils present in the tunic are endowed with extraordinary mechanical properties, showing potential as nanometer-size fillers into polymers to produce reinforced nanocomposites. Furthermore, sulfated polysaccharides in the body of certain species have shown to attenuate metastasis, thrombosis and inflammation, to promote neurite outgrowth and to act as anticoagulants. Anticoagulant activity has also been reported in sulfated galactans extracted from the tunic. The aim of the present study is to isolate biopolymers from *Styela clava*, an invasive Asian ascidian species widely distributed around the world. Microcellulose has been reported in this species, but the presence of other biopolymers remains unknown.

To this end, the internal organs and tunic of *S. clava* specimens were separated and treated with proteolytic enzymes to release sulfated glycosaminoglycan analogs from the body and sulfated galactans from the tunic. Both polysaccharides were further purified by precipitation and ultrafiltration. The remaining solids from the tunic were subjected to acid and alkaline hydrolysis and bleaching to obtain microcellulose. Molecular weight of polysaccharides was determined by Gel Permeation Chromatography (GPC) and saccharide composition by NMR and strong anion exchange chromatography (SAX-HPLC) after enzymatic digestion. Microcellulose was characterized by X-ray diffraction, Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM).