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Study on the oligosaccharides composition of the water-soluble fraction of marine mucilage by electrospray tandem mass spectrometry

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ABSTRACT

The massive accumulation of organic matter, which periodically occurs in the northern Adriatic Sea, and in other locations worldwide, is presently thought to be the results of the aggregation of dissolved organic matter (DOM) into particulate organic matter (POM). This phenomenon is the result of human activities and propitious weather conditions. Although many aspects of the phenomenon are well understood, the trigger mechanisms leading to mucilage formation have not been clarified yet, probably as a consequence of inadequate analytical approaches. In this context, the recent advancements in LC-MS interfacing might contribute in clarifying the mechanism of mucilage formation. In the present paper, hydrophilic interaction liquid chromatography coupled with electrospray tandem mass spectrometry (HILC-ESI-MS/MS) is proposed as an innovative method for the investigation of underivatized oligosaccharides in mucilage samples. Recent findings suggest that the significant presence of these compounds in seawater can play an important role in the initial steps of the agglomeration processes forming gelatinous material. Our results reveal the presence of several maltodextrines in the water-soluble fraction of mucilage macroaggregates, collected in various locations of the northern Adriatic Sea. In our knowledge, the proposed method is the first application of LC-MS in the investigation of marine mucilage.

1. Introduction

The northern Adriatic Sea episodically experiences a massive flocculation of gelatinous material to form aggregates of striking dimensions and abundance (Stachowitsch et al., 1990). Mucilage events have been recorded for over two centuries (Molin et al., 1992), however, during the last two decades, their incidence increased, affecting on at least one occasion (1989) an area of 10,000 km^2 (Marchetti et al., 1992; Stachowitsch et al., 1990; Penna et al, 1993). During the periods of maximum development of the phenomenon the environmental and economical impact on coastal communities has been remarkable: suspended aggregates created serious damages to the fishery industry and gelatinous masses, floating on sea surface, were driven by winds towards the coast, seriously reducing all tourism activities (Penna et al., 2000). Furthermore, the sedimentation of macroaggregates induced the suffocation death of many benthonic organisms (Penna et al., 1993). Small aggregates like marine snow are commonly found in oceanic and marine systems,

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while macroaggregates, morphologically similar to those of the northern Adriatic Sea, has been observed in the Tyrrhenian Sea, in Californian coastal waters and North Sea (Mingazzini and Thake, 1995).

Chemical composition of mucous aggregates, collected at different evolutionary stages, was extensively studied using spectroscopic techniques (Mecozzi et al., 2001; Kovac et al., 2002, 2004). The obtained results revealed that the organic fraction of the northern Adriatic Sea mucilage is mainly composed of carbohydrates and humic acids, although proteins and lipids are also present in small amounts (Mecozzi et al., 2001; Kovac et al., 2004).

Despite mucilage phenomenon in the northern Adriatic Sea is well described, too little is known about the trigger mechanisms leading to the aggregates formation. Mucilaginous masses are presently thought to be the result of the aggregation of dissolved organic matter (DOM) into particulate organic matter (POM) (Kovac et al., 2002). In this framework, dissolved carbohydrates are suspected to play a key role. Recent published data demonstrate that polysaccharides released by plankton can spontaneously assemble to form polymer microgels which can aggregate and anneal to each other, allowing the formation of larger macrogels such as transparent exopolymeric particles (TEP) (Chin et al., 1998; Passow, 2000; Engel et al., 2004). Due to their capability to interact with surface of particles, TEP support coagulation processes allowing the formation of large particle aggregates such as mucilages (Verdugo et al., 2004). The initial release of mucilaginous material seems to be due to an alteration of the nitrates/phosphates ratio, which occurs during periods of high rivers inflow in the northern Adriatic Sea. This imbalance induces a change in the composition and growth rate of diatom algae allowing the release of polysaccharidic extracellular exudates (Myklestad et al., 1995; Mingazzini and Thake, 1995; Penna et al., 1999). A recent hypothesis on mucilage formation suggests that mucilage may be also produced as a result of bacteria–organic matter interactions (Azam et al., 1999). Once high quantities of mucilaginous material have been released in seawater, climatic conditions act to concentrate or disperse the mucilaginous particles in the water mass (Degobbis et al., 1995; Mingazzini and Thake, 1995).

Despite its importance in the understanding of the processes leading to macroaggregates formation, our present knowledge on the mucilage carbohydrate fraction is incomplete. The major obstacle encountered in the molecular characterization of carbohydrates in macroaggregates is represented by their huge complexity and by their interaction with the other mucilage components. Gas chromatography coupled to mass spectrometry (GC-MS) has been extensively used for the investigation of carbohydrates in mucilages (De Angelis et al., 1993; Mingazzini and Thake, 1995). However, this analytical approach requires both hydrolysis and derivatization of oligo- and polysaccharides, allowing only the determination of their monosaccharides content. In the last few years, the number of publications involving the characterization of oligosaccharides by mass spectrometry has increased, as a consequence on the advances achieved on electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI). Structural information such as sugar composition, sugar sequence, branching and/or type of linkage can be obtained by the study of the fragmentation pathways of the ions formed either by MALDI-MS (Harvey et al., 1999; Garozzo et al., 2000; Spina et al., 2004; Zaia, 2004) and ESI-MS (Reinhold et al., 1995; Zaia, 2004). Based on these findings, liquid chromatography coupled to electrospray tandem mass spectrometry (LC-ESI-MS/MS) is gaining an increasing importance within the growing field of glycobiology as a powerful technique for carbohydrates purification and characterization from biological samples. Several advantages might potentially be found in the application of this analytical approach to the investigation of mucilages carbohydrate content. Unlike GC-MS, the analysis of carbohydrates by LC-ESI-MS/MS does not require hydrolysis and derivatization procedures. In this way, mucilage oligo- and polysaccharides might be analysed in their native form, and the manipulation of the samples might be drastically reduced. Furthermore, the preliminary chromatographic separation allows the purification of carbohydrates from the complex mucilage matrix and an additional parameter such as the retention time, will help in the compound identification step. Finally, the addition of a second stage of mass analysis, in tandem mass spectrometry experiments, can be an essential tool in obtaining structural information regarding the eluted compounds.

Based on these assumptions, the present study has been aimed at applying LC-ESI-MS/MS in the investigation of oligosaccharides belonging to the class of maltodextrines in mucilage samples. The chromatographic separation was achieved using hydrophilic interaction chromatography. Although amino column have been extensively used for derivatized sugar analysis (Neville et al., 2004; Sumiyoshi et al., 2004; Wuhrer et al., 2004a; Maslen et al., 2007) hydrophilic interaction chromatography represents a rare application in LC-ESI-MS/MS for the analysis of derivatized oligosaccharides (Wuhrer et al., 2004b).

2. Experimental

2.1. Samples collection

Five samples of macroaggregates were collected from surface layers using polyethylene bottles at approximately 1 mile off the coast. Samples A and B were collected offshore from the coast of Piran (Slovenia) (45°32′34″N, 13°30′18″E), respectively, at the beginning of summer 2000 and 2004. Samples C and D were picked up in front of the coast of Pesaro (Italy) (43°55′50″N, 12°55′50″E) in the early summer of 2001 and 2003. Sample E was collected offshore from the coast of Marotta (43°45′06″N, 13°11′30″E) at the beginning of summer 2004. In the summer of 2002 and 2005 the phenomenon was not observed. All the samples were immediately frozen at –80 °C until returned to the laboratory.

2.2. Sample preparation

After defrosting, each mucilage sample was isolated from its surrounding seawater by decantation and subsequently centrifuged at 4000 rpm for 20 min at ambient temperature.