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Investigation of the factors influencing the molecular weight of porphyran and its associated antifungal activity

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ABSTRACT

In the present study various extraction methodologies have been adopted to derive sulfated polysaccharides (SPs) called as porphyran (POR) from low tide (LT) and high tide (HT) samples of *Porphyra vietnamensis*. Initially various taxonomical differences among LT and HT samples of *P. vietnamensis* were evaluated. Later on various physico-chemical and antifungal properties were determined. Our results suggested that *P. vietnamensis* collected during LT yields high percentage of SPs with relatively low molecular weight (MW) and high sulfate content than the HT sample. Among various extraction methodologies alkali modified POR yields low MW polysaccharide but surprisingly with high sulfate content which have shown improved physico-chemical and antifungal properties than chitosan (CS) without any toxicological affects. However, extensive research is further required to explore the molecular pathways inhibited or stimulated in this process.

1. Introduction

Sulfated polysaccharides of marine macro algae have been shown to possess a variety of biological activities. Their occurrence among diverse kingdoms such as algal, plant, animal, fungal and in some microbial classes is widely reported. Seaweeds such as ulvans (green algae), fucans (brown algae) and red algae derived galactans such as carrageenans, agarans and porphyrans act as a major source of SPs. Among all sources marine red algae is still recognized as the crucial source of SPs (Costa et al., 2010; Jiao, Yu, Zhang, & Ewart, 2011; Wijesekara, Pangestuti, & Kim, 2011). Red galactans have a linear backbone which is built of 3-linked β-D-galactopyaranose and 4-linked α-galactopyaranose residues. Due to their gelling and several biological properties red galactans are gaining strong attention towards food and pharmaceutical sectors (Costa et al., 2010). Among all red galactans, POR, an emerging SPs, has recently shown a wide array of biological and pharmaceutical properties. These properties are highly dependent on its MW, sulfate content and 3,6 anhydro galactose (Costa et al., 2010). Various factors such as collection place, time, drying of algae and procedures adopted for the extraction (including various hydrolysis methods) of SPs can greatly influence the yield, composition and MW of POR, which further affect the physical and biological properties of the whole fraction. Moreover, certain biological stresses present in the environment (Fig. 1) can also lead to the degradation of these polysaccharides, which can further alter the physical as well as biological properties of

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isolated fraction (Contreras-Porcia, Thomas, Flores, & Correa, 2011). These red galactans posses antimicrobial action, though the intensity of antimicrobial action varies from species to species (Salvador, Garreta, Lavelli, & Ribera, 2007). There is growing demand of new and potent antifungal agent from the different natural sources. Discovering any biopolymer as antifungal agent is an excellent strategy of reducing the ill effects (pathogen-induced resistance and several serious toxicities) associated with the current antifungal agents. CS has recently been explored as a new biopolymeric antifungal agent (Park et al., 2008). This research raises a new scope for those biopolymers that exhibit unexplored antifungal activity. Various reports were available on the strong antifungal properties exhibited by sulfur and its derivatives (Kim, Kubec, & Musah, 2006). Our past experience on seaweed and the presence of high sulfate content and relatively low MW in the LT collected sample of *Porphyra vietnamensis* has encouraged us to work on the antifungal aspect of SPs derived from *P. vietnamensis*. Previous findings supported the direct and indirect antifungal role of degraded SPs (Contreras-Porcia et al., 2011; Vera, Castro, Gonzalez, & Moenne, 2011). In plants as well as in animals these SPs are reported to stimulate several molecular pathways and followed the same mechanism as followed by some well-established anti-fungal agents. Thus primarily it is important to study the factors involved in the degradation of SPs and elevation of sulfate content in *P. vietnamensis* during LT.

It has been investigated that high mucilaginous (high moisture content) nature of algae and their dampish environment favored the attack of microorganisms, *e.g.* attack of fungi *Cladosporium sp*. on red alga *Porphyra yezoensis* (Ding, Qin, Li, Chi, & Laatsch, 2008). Previous findings suggested the role of specific fungal species in excreting some carbohydrate-splitting enzymes which can cause the degradation of SPs (Brink & de Vries, 2011; Duan & Kasper, 2011; Johnston, 1966). In Japan seaweeds are deliberately treated with fungi, leading to the breakdown of SPs so that they are more easily digested when eaten. However, it has been found that there are some algal polysaccharides which are likely to withstand the onset of fungal attack (Johnston, 1966). Most of these evidences were only restricted to marine (green and brown algae) and some freshwater algae such as *Nostoc* sp. (Biondi et al., 2004). Only few evidences have been found on the fungal-resistant action of red algae-based galactans though the mechanism/relationship between SPs and involved inhibitory pathways which may lead to this antifungal action is poorly understood yet. More justification can be made on the oxidative burst mechanism induced by the desiccated sample than high tide sample (Contreras-Porcia et al., 2011). Moreover an extensive knowledge regarding physico-chemical properties and their relation with antifungal action could clarify this potential to some extent. Some of our visual observation plays a great role in favoring the antifungal property. During our collection of several algae we observed that few freshwater algae (*Nostoc* sp.) after living in such damp conditions do not have microbial attack. Even this phenomenon persists after packing of moist algae in plastic bags, which clearly demonstrated the strong antifungal action of *Nostoc* sp. (Biondi et al., 2004). While visiting the more damp conditions during LT we have observed that one species of *Porphyra* (*P. vietnamensis*) does not produce foul smell after its packing in plastic bags for four days in comparison with others from different point of collection. As there is a lot of literature available on medicinal, pharmaceutical and nutritional benefits of various *Porphyra* sp., recently we have explored the sun-protective effect, antioxidant and immune-modulatory effects of *P. vietnamensis* (Bhatia et al., 2008, 2010, 2011, 2013; Bhatia, Namdeo, & Nanda, 2010). Therefore it is essential to explore the antifungal potential of edible *P. vietnamensis*. During its association with fungal strains there might be chances of production of low MW antimicrobial compounds by *Porphyra* sp. (Ding et al., 2008). But the cell wall SPs act as the first line of defense against microbial attack; thus perhaps this antifungal activity is attributed to degradation of POR to low MW sulfated oligosaccharides (Vera et al., 2011), under the influence of abiotic stresses during low tide. So there could be differences between the biological activities of HT and LT samples which could be further based on the oxidative burst mechanism (Contreras-Porcia et al., 2011). When *Porphyra* is exposed to long desiccated environment there might be chances of polysaccharide degradation due to excessive radical generation which may lead to depolymerization of SPs, yielding low MW oligosaccharides as depicted hypothetically in Fig. 1. Similarly there are several established methods for the extraction and isolation of POR, which can also lead to depolymerization induced desulfation. The second speculation was based on the existence of high sulfur content, which could be a cause behind the antifungal property as sulfur and its derivatives were reported as potent antifungal agents. Sulfur and its derivatives were proved to exhibit antifungal action. After fungal degradation, leaching of sulfur group from matrix could be a possible mechanism against microbial attack. Various SPs extraction procedures may lead to desulfation of the polysaccharide, and hence loss in antifungal activity. Thus to increase the pharmaceutical value and explore the antifungal potential of this polymer there is a need to determine all physico-chemical properties of POR, which make them much suitable for their utilization in antifungal formulation (synergies of the antifungal action). Hence various extraction procedures have been carried out for the isolation of SPs and a suitable methodology which causes less desulfation and high MW degradation without the transformation of major functional groups was selected (Denis, Jeune, Gaudin, & Fleurence, 2009; Maciel et al., 2008; Navarro, Flores, & Stortz, 2007; Zhang et al., 2013; Zhou, Yu, Zhang, He, & Ma, 2012; Zhao et al., 2006). Our visual observations and previous finding have developed an interest in evaluating the antifungal potential of one of the most commercialized seaweeds of the world, *i.e. Porphyra*. Therefore the current study was designed to evaluate the effect of environment, different time of collection of algae (HT and LT) and different extraction methodologies on the physico-chemical and antifungal properties of different samples of *P. vietnamensis*-derived POR.

2. Material and methods

2.1. Material

P. vietnamensis was collected from the different coastal regions of Maharashtra, India. Collection has been made in various LT and HT seasons in 2012. To prevent the microbial attack the algae were slightly dried under hot air blow (hair dryer machine). Air was blown from a distance of at least