Marine red alga *Porphyridium* sp. as a source of sulfated polysaccharides (SPs) for combating against COVID-19

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Abstract

COVID 19, a pandemic spread without any solid anti-viral antidote. Researchers worldwide are currently giving their best to make antidote for this virus with all available literatures and knowledge. Research carried out indicates sulfated polysaccharides (carrageenan) are selective inhibitors of several enveloped and non-enveloped viruses and act predominantly by inhibiting the binding or internalization of virus into the host cells. Polysaccharides produced from *Porphyridium* sp. are sulfated polysaccharides which are promising antiviral agents against respiratory viruses from the family of coronavirus. These biocompatible compounds can be used as a coating material on the sanitary items for COVID-19 prevention.

Key words: Anti-viral, Carrageenan, Coronavirus, COVID-19, *Porphyridium*, sulfated polysaccharides (SPs)

Introduction:

During the last decade, more than 30 drugs of antivirals medicines are approved for clinical use (De Clercq 2004). The first report of the antiviral activity of high-molecular-weight polysaccharides appeared almost 60 years ago (Ginsberg *et al.*,1947). After seventeen years later, it was demonstrated that heparin can act as inhibitors of herpes simplex virus (HSV) (Nahmias and Kibrick 1964). However, as these drugs are not always active and drug-resistant virus strains are rapidly developing, there is still a great demand for further drug development including novel modes of action. A very promising approach is the antiviral screening of products derived from natural sources, such as flora and fauna, bacteria, fungi, and higher plants. Polysaccharides are such natural compounds having tremendous antiviral potential.

Polysaccharides originate from plant (e.g. plant cell walls, tree exudates, seeds, tuber/roots) or animal sources (hyaluronan, chitin, chondroitin sulfate). Additionally, certain types of bacteria and fungi can also produce hydrocolloids (xanthan, gellan, wellan). Algae also contain large amounts of polysaccharides as structural, mucopolysaccharide and storage polysaccharide. Seaweeds (macroalgae) are main sources of these polysaccharides. Increasing market demand of natural polysaccharides for the food, cosmetics, and pharmaceutical industries cannot be met by currently available conventional sources like red and brown macroalgae. Moreover, traditionally these polysaccharides are harvested from natural resources and are dependent on environmental mercy. An attractive alternative can be red microalgae (*Porphyridium*), which can be cultivated round the year in open and close cultivation systems, offering a vast range of potential products. Gaikwad *et al.*, 2009 has reported for first time this alga from India which can be used for bio-refinery approach.

Porphyridium is unicellular microalgae with its cell encapsulated in a sulfated polysaccharide. The external part of this capsule dissolves in the growth medium forming exopolysaccharide. These polysaccharides are complex sugar molecules. They are mainly composed of xylose (38%), glucose (24%), galactose (22%), and glucuronic acid (10%); however, arabinose, rhamnose, and mannose can also be found in minor concentrations (Geresh *et al.,*2002). The molecular weight of the exopolysaccharide was estimated as 5-7 x 10^6 Da (Simon *et al.,* 1992). These are compatible to human body and are easily degraded in environment, they play important roles in medicine, food, and cosmetics. Study carried out by Pujol *et al.*, 2007 also suggested that number of sulfated carbohydrate compounds from marine algae, cyanobacteria, and animal sources have potent inhibitory effects against several human and animal viruses. The aim of this article is to understand the anti-viral potential of sulfated polysaccharides from marine algae in context of currently available data. Our understanding on COVID-19 pandemic is limited and evolving, information contain in the subsequent text is based existing test carried out on various viruses.

Coronavirus

Coronavirus is a family of viruses which are responsible for respiratory illness such as common cold, MERS-CoV and SARS-CoV. Recently identified novel coronavirus (SARS-CoV-2) which is causing COVID-19, is a new strain that has not been identified earlier in humans. Coronaviruses are enveloped virus, large in size and having plus stranded RNA as the genetic material which is the largest among all RNA viruses. This virus has large spike proteins on the envelope which are protruding from the surface of the virus giving it a crown like appearance. These spike proteins are responsible for binding with the host cell receptor and entering inside the cells (FIP Health

Advisory; Weiss, S. R. and Martin, S. N., 2005). These viruses are capable of adapting to the new environment by mutation of its own genetic material. (FIP Health Advisory).

Transmission of the COVID-19 is like flu can be spread from infected person to healthy person, particularly by physical contacts, handshake, while coughing, sneezing, and talking the infected person spread droplets thus transmitting the disease. Researchers are carrying out studies to fight against the virus and cure the disease.

Sulfated polysaccharides (SPs)

Polysaccharides from algae are the natural source, cheaply available, non-toxic in nature, safe and biodegradable and in many instances biocompatible. These polysaccharides are the material that have attracted biomedical and pharmaceutical industries to use them in their appliances and medicines. Sulfated polysaccharides are complex group of macromolecules having variety of potential clinical applications (Seedevi, P. *et al.*, 2013). Sulfated polysaccharides are commonly found in marine environment. These are present in marine algae, animals and plants grown in saline soil. According to Aquino *et al.* 2011 the presence of sulfated polysaccharides is in response to the salt adaptation process which might have developed during evolution. When they grew *Ruppia maritima* Loise in its natural saline environment it showed the presence of sulfated polysaccharides whereas when cultivated in absence of salt, SPs were disappeared. Also, they grew *Oryza sativa* in salt stress condition, but they found carboxylated polysaccharides instead of sulfated polysaccharides. This suggests that the negatively charged cell wall of plants help them cope up with the salt stress.

Marine animals including vertebrate and invertebrate animals are rich source of heparin like sulfated polysaccharides (Nader H. B., 2004). Pomin (2010) described that marine invertebrate species can produce sulfated polysaccharides like sulfated fucans and galactans. Silva J. M. C. *et al.* 2012 in their study stated that at their best knowledge there is no report about presence of SPs in land plants, animals, fungi and other organisms. Sulfated polysaccharides are characteristics of only marine organisms and hence are focused more for production of such compounds. There are two major sources of antivirally active sulfated polysaccharides from marine animals one is chondroitin sulfate and another heparin (Ghosh T. *et al.*, 2009).

SPs are widely distributed in marine algae such as Chlorophyta, Rhodophyta and Phaeophyta. These are preferred over animal sulfated polysaccharides due to its vegan source. These are usually found secreted along with the exopolysaccharides (EPS) from algae which are mucilaginous in nature. *Porphyridium* is a marine red algal unicellular genus cultivated commercially worldwide (Fig.1).

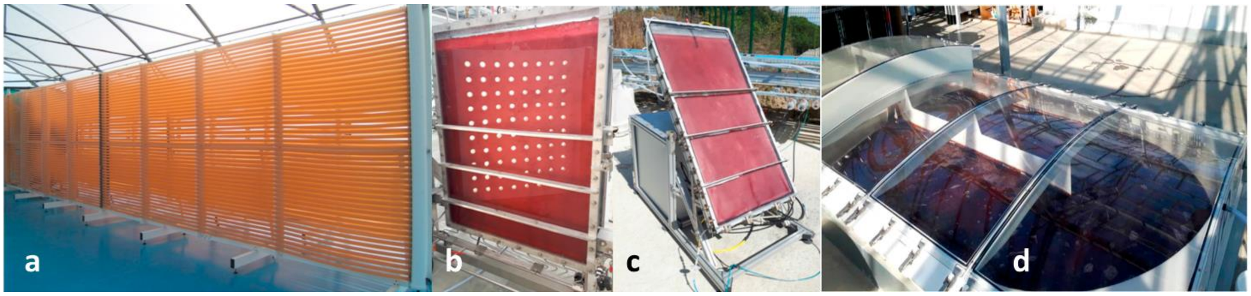

Fig. 1. Commercial cultivation from different companies: a. *Porphyridium cruentum*, vertical tubular photobioreactor (Greensea, Mèze,France); (b) *Porphyridium cruentum*, flat plate

photobioreactor with artificial light; (c) *Porphyridium cruentum*, inclined flat plate photobioreactor with natural light (AlgoSolis, GEPEA, Université de Nantes, CNRS, France) and (d) *Porphyridium cruentum*, closed raceway ponds (AlgoSolis, GEPEA, Université de Nantes, CNRS, France).

This alga has many medicinally important components in its exopolysaccharides. The alga when reaches stationary phase secrete EPS with sulfated polysaccharides. Research studies have been carried out to show the potential of this alga in antibacterial, antiviral and anti-inflammatory action. The alga has high growth rate of 0.48 h^{-1} (Csogör Z. *et al.*, 2001) and high production of exopolysaccharides up to 2.5 g/L (Soanen N. *et al.*, 2016). (Fig 2.)

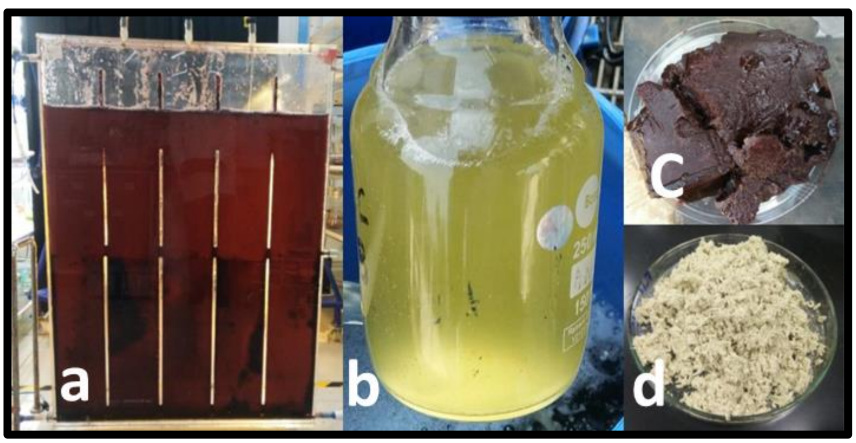

Fig. 2. Cultivation of *Porphyridium* and EPS in-house facility a. Growth in photobioreactor, b. supernatant after centrifugation, c. biomass slurry after centrifugation, d. dried EPS powder

The SPs prevent the virus from attaching to the target molecules on the cell surface. The SPs possess a binding site on the CD4 that is relatively like the HIV–gp120 binding region. Hence, by binding to this lymphocyte, the SPs inhibit the binding of the monoclonal antibodies to the initial two domains of the CD4, thereby disrupting the CD4–gp120 interaction. The anti-HIV activity of the SPs is by shielding off the positively charged sites on the V3 loop of the gp120 protein, thereby preventing the virus attachment to the cell surface (Parish *et al.*, 1990).

Sulfated polysaccharides from *Porphyridium*

Number of studies have been carried out from the algae derived polysaccharides such as agar, alginates, fucoidan, carrageenan, rhamnan sulfate and many more (Almeida 2011). Glycan are the abundant form of carbohydrates in the nature which are also termed as polysaccharides. Arad, S. and colleagues in 1985 carried out cultivation of *Porphyridium aeruginum* because it is unicellular and have faster growth rate than other red macroalgae. The phycocolloids, also known as EPS, are formed outside the cells and get dissolved in water making it highly viscous. In the log phase of the culture the rate of formation of phycocolloids is lesser than at the stationary phase. He also stated that the solubility of these exopolysaccharides is lesser than its rate of production because of which the liquid appears viscous. These phycocolloids are mixture of many sulfated sugars and hence also termed as sulfated polysaccharides. The polysaccharide of *Porphyridium* sp. is composed of xylose, glucose, galactose, and sulfate esters, with several minor additional constituents, including hexuronic acids (Ramus J. 1976; Arad, S. 1985; Raposo M. F., 2013) and fractions of glucuronic acid and galacturonic acid (Gaignard C., 2019). The polysaccharide of *Porphyridium* is a heteropolymer like the Carrageenan as sulfated galactans of other red seaweeds. The specific viscosity of *Porphyridium* polysaccharides is much more than lambda and kappa carrageenans and hence is preferred over others (Fig 3.) (Arad, S., 1985).

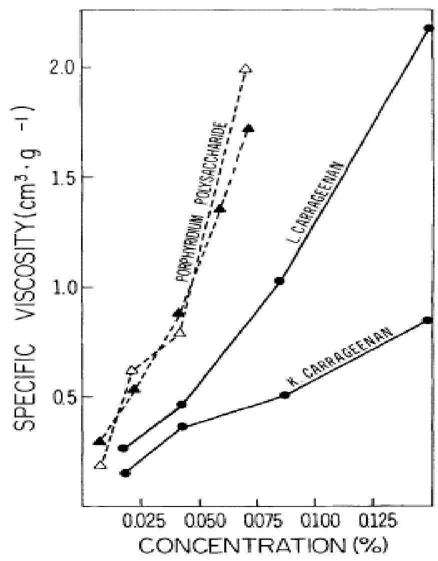

Fig.3: Viscosity of Porphyridium polysaccharide in comparison with that of lambda and kappa carrageenans (Arad S., 1985).

Extraction of Exopolysaccharides

Balti R. *et al.*, 2018 have carried out the extraction of EPS from *Porphyridium* and concentrating using cross flow membrane filtration. Crude EPS can also be extracted using alcohol by concentrating in the liquid. The centrifuged supernatant is mixed with equal volume of 90-100% chilled ethanol. Overnight exposure to 4 °C leads to higher concentration of EPS. The precipitated EPS is collected and dried for further use (Ziadi M. *et al.*, 2018)

Antiviral applications of exopolysaccharides from *Porphyridium*

Recently it was evident that several sulfated polysaccharide materials from marine algae and animals have antiviral activity against many human and animal viruses (Pujol *et al.,* 2007). Raposo M. F. and colleagues in 2013 summarized the antiviral activities of different marine red algae exopolysaccharides. EPS from *Porphyridium* is known to work against herpes simplex virus HSV-1 and HSV-2 (Huheihel M., 2017), varicela zoster virus (VZV), retrovirus (Talyshinsky M. M., 2002) murine sarcoma virus (MuSV-124), MuSV/MuLV (murine leukemia virus), hepatitis B virus (HBV), viral haemorrhagic septicaemia virus (VHSV), African swine fever virus (ASFV), vesicular stomatitis virus (VSV), vaccinia virus VACV and VACV-GFP and ectromelia virus (ECTV). Such a wide range of viruses are being controlled by EPS of *Porphyridium* which is amazing and applicable in medical fields.

Yu J. *et al.,* (2002) demonstrated that EPS from *Porphyridium* significantly improved immunity in mouse. This mechanism of improvement of immunity occurred by improved spleen cell proliferation and increased killer cells. It is reported that the antiviral activity of sulfated polysaccharides depends upon the degree of sulfation. For some semisynthetic polysaccharides is it documented that the higher the sulfation better the antiviral activity of molecules (Ghosh T. *et al.*, 2009).

Lemos D and colleagues presented a work on *Porphyridium purpureum* in IMMR 2018. They extracted EPS from this red alga *Porphyridium* and characterized it to get its composition. They found presence of carrageenan in it along with xanthum gum and agar. Acetylneuraminic acid is a most common sialic acid which act as a surface receptor for most of the influenza viruses. Chemically synthesized pharmaceutical agents from N-Acetylneuraminic acid used to produce nuraminidase inhibitors are being applied against influenza viruses (Chen, J. K., 2010). In addition, N-acetylglucosamine which is found in the mucus membrane of digestive tract are known to bind pathogen with slight modification in the saccharide part. It is also used as a substrate for production of sialic acid (N-Acetylneuraminic acid). All these clinically important sugar molecules are known to be secreted along with exopolysaccharides.

Clinical importance of Carrageenans

There are many reported literatures which show the promising results of bioactive molecules from red algae against many coronaviruses. One such material is carrageenan from the group of sulfated polysaccharides, has antiviral property in human health care. Periera, L. (2018) has stated in his book that carrageenans selectively inhibit binding of many enveloped and non-enveloped viruses.

Jiao, G. *et al.*, (2011) reviewed sulfated polysaccharides from marine algae and their bioactivity. Agarans and carrageenans are structurally similar except the agarans have galactans with 4-linked α-galactose residues of the L-series and those in carrageenans with D-series. Carrageenans are high molecular weight molecules classified according to their sulfation patterns and presence or absence of AnGal on D-units.

Koenighofer, M. *et al.*, (2014) carried out study on patients suffering from common cold caused by coronaviruses. They used nasal spray with carrageenan in those patients who showed early symptoms of cold (up to 36 hrs in children and 48 hrs in adults). Half of the patients were given iota-carrageenan nasal spray thrice a day for seven days and half were given placebo nasal spray. It was observed that those received carrageenan spray showed reduced duration of the cold than those received placebo. Apart from this a significant difference between the two treatments were observed in terms of relapses of the cold. It was found that there was significantly more relapses in case of placebo. Patients treated with carrageenan showed significantly lower viral titer than placebo treated patients. Morokutti-Kurz, M., (2017) worked on patients with sore throat with Amylmetacresol/2,4-dichlorobenzyl alcohol, hexylresorcinol, or carrageenan lozenges as active components of the treatment. They observed that only carrageenan containing lozenges were highly active against all the virus tested including HRV1a, HRV8, Influenza virus A H1N1n, Coxsackievirus A10, and human coronavirus (hCoV) OC43. On the similar lines Graf, C. *et al.* (2018) also carried out studies on patients with rhinitis and sinusitis caused by human rhinovirus 1a and human coronavirus OC43. This study suggests that the two active components used in the formulation of treatment, xylometazoline HCl and iota-carrageenan, do not influence each other and exhibit their original activity of decongestion and effectiveness against coronavirus respectively.

According to Andreas *et al.*, 2011, at the concentration of 400 µg/ml carrageenan from red algae leads to inhibition of cell death caused by coronavirus infection. At 4 µg/ml concentration of iota carrageenan a significant inhibition was observed but on the other hand lambda and kappa carrageenan do not show any positive result at such a low concentration. They also found that if the cells are pretreated with carrageenan they are well protected against the infection due to coronaviruses. The results suggest that to achieve a significant protection against coronavirus infection the antiviral active agent, carrageenan, must be applied as coating or impregnation on to a solid surface of a hygiene or sanitary item such as sanitary glove, tissue or paper, a nasal tissue or paper, a cotton swab, dust mask or sanitary or medical facial mask (Andreas *et al.*,2011).

Extraction of Carrageenan

Ramalingam and colleagues (2003) carried out commercial extraction of kappa, iota and lambda carrageenan from seaweed. For iota and lambda carrageenan the dried algal material is bleached in sunlight and washed. This is further processed through process like hot washing, filtration, evaporation, alcohol precipitation and centrifugation. The slurry after centrifugation is dried and powdered to get iota or lambda carrageenan.

Carrageenan Safety Aspects

The use of carrageenan for food applications started almost 600 years ago. Due to its long and safe history of use, carrageenan is generally recognized as safe (GRAS) by experts from the US Food and Drug Administration and is approved as a food additive (Carthew 2002).

Conclusion

As evident from the various analysis reports worldwide on antiviral activity of SPs from *Porphyridium* supported with the immunity boosting property, we can say that this microalga can be a versatile player in the treatment of many viral diseases. The role of carrageenan from different biological sources including *Porphyridium* in controlling coronaviral respiratory infection is commendable. The recent pandenic of COVID-19 and its spreading rate are serious problem throughout the world. The treatment of this diseases under rapid research. To support this research, we can also attempt *Porphyridium* EPS along with carrageenan and sulfated polysaccharides in clinical trial studies to reduce its proliferation. Since there are multiple molecules in exopolysaccharides of *Porphyridium*, this organism can make a positive difference in the treatment of COVID-19. Sulfated polysaccharides from these algae can be used as a coating material on the sanitary items and also for the production of antiviral drugs. The present review can benefit the researchers to use the SPs from algae and provide an antiviral pharmaceutical composition suitable for the prevention or treatment of respiratory tract infections caused by coronavirus.

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