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Review article

Polysaccharides from cyanobacteria: response to biotic and abiotic stress and their antiviral activity

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Present review puts prompt information on polysaccharides secretion from cyanobacteria under biotic and abiotic stress, their applications when released into the surrounding medium (exo- or extracellular polysaccharides, EPS) and different biological activities. In addition, potentials of polysaccharides from marine microalgae to be used as antiviral agents and their mode of action against different viral species (e.g: HIV, HSV, HAV—etc) have also been presented on the current review.

[**Key words:** Blue-green algae, Polysaccharides, Stress factors, Antiviral activity, mode of action].

Introduction

Cyanobacteria are photoautotrophic prokaryotes, include a large number of species, and are among the most successful and oldest life forms present on earth [1-2]. They represent an exceptionally diverse but highly specialized group of microorganisms adapted to various ecological habitats. They can be found in terrestrial, glacial, aerial, marine, brackish and fresh water environments. Cyanobacteria are often the main component of phytoplanktonin many freshwater and marine ecosystems. This widespread distribution reflects a large variety of species, covering a broad spectrum of physiological properties and tolerance to environmental stress. Cyanobacteria produce a range of bioactive compounds (alkaloids, terpenoids, phenolic compounds—etc) [3-4], which are potentially used as feed, food, nutritional, cosmetic, pharmaceutical and neutraceuticals. Different secondary metabolites produced by them are potent toxins, causing health problems for animals and humans when the producer organisms occur in masses (blooms) in water bodies. Cyanobacterial lipopeptides including different compounds have shown potential as like (cytotoxic (30%), antitumor (15%), antiviral (3%), antibiotics (15%) and also as antimalarial, antimycotics, multi-drug resistance reversers, antifeedant, herbicides and immunosuppressive agents [5] (Fig -1).

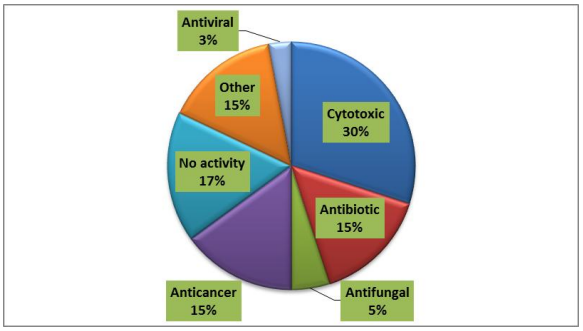

Figure 1: Biological activities of marine cyanobacterial compounds (5)

Polysaccharides are renewable resources representing an important class of polymeric materials of biotechnological interest, offering a wide variety of potentially useful products to mankind. Exopolysaccharides (EPSs) of microbial origin with a novel functionality, reproducible physicochemical properties, stable cost and supply are recognized as better alternative to polysaccharides of algal origin[6]. Cyanobacteria are better suited than macroalgae or higher plants since they exhibit high growth rate and are more amenable to manipulate the conditions for enhancing growth and/or EPS production

Polysaccharides are characterized by an extreme structural diversity; as a result, they play significant diverse roles in nature. Numerous polysaccharides derived from microorganisms are potentially available, known to be involved in pathogenesis, symbiosis, biofilm formation, protection from phagocytic predation and stress resistance; but relatively few have been commercially established [7-8]. Among these microorganisms, cyanobacteria have been known as a potential EPS producer and polysaccharides of cyanobacteria are currently and widely commercially used [9].

In recent years, increasing attention has been paid on exopolysaccharides produced by cyanobacteria (Table 1) [10]. It is interesting that most cyanobacterial polysaccharides are characterized by the presence of uronic acids, pentoses (Table. 2), a polypeptide moiety or other nonosaccharide components, such as organic (e.g., acetyl, pyruvyl, succinyl group) or inorganic (e.g., sulphate or phosphate group) substituents [11-12]. Therefore, cyanobacteria may be regarded as an abundant source of structurally diverse polysaccharides, some of which may possess unique properties for special applications (e.g: increased seed germination and metabolic activity of the seedling of different plants, antiviral, antimicrobial, antioxidant and anticoagulant), not fulfilled by the polymer currently available.

The peculiar features of the polysaccharides released by the cyanobacteria in comparison to those released by other microbial sources

Many cyanobacterial polysaccharides are characterized by an anionic nature, many of them contain two different uronic acids, a feature rarely found in the polymers released by strains belonging to other microbial groups [13]. Cyanobacterial polysaccharide often show the presence of one or two pentose sugar that are usually absent in other polysaccharide of prokaryotic origin [13]. The moiety protects the neighboring glycosidic bonds from the more common glycan hydrolases. Most of the polysaccharides synthesized by cyanobacteria are quite complex, being composed of six or more monosaccharide's. This is the striking difference from the polymers synthesized by other bacteria or by microalgae, in which the number of monomers is usually less than four.

Biological systems must continuously adapt to changing environmental stimuli. A plant cell response to changeable light conditions is particularly important, since this environmental cue induces a different energetic status, which, in turn, leads to the accumulation or degradation of reserve polysaccharides. Starch and glycogen are major storage products of photosynthesis in plants and bacteria, respectively [14-16]. At least 30% of the carbon photo- assimilated by plants is channeled to starch under optimal conditions of light, water, temperature and CO_2 concentration [17].

Table 1: Concentration of extracellular, cell bound and total polysaccharide in different cyanobacterial isolates (10).

Cyanobacteria	Extracellular polysaccharide (mg l^{-1})	Cell bound polysaccharide (mg l^{-1})	Total polysaccharide (mg l^{-1})
Calothrix sp.[b]	$24 \pm 1 \times 10^{-2}$	$117 \pm 4 \times 10^{-2}$	$141 \pm 5 \times 10^{-2}$
Nostoc calcicola RDU-3[b]	$105 \pm 5 \times 10^{-2}$	$256 \pm 2 \times 10^{-2}$	$361 \pm 4 \times 10^{-2}$
Nostoc calcicola RDU-2[b]	$62.50 \pm 5 \times 10^{-2}$	$495 \pm 5 \times 10^{-2}$	$557.5 \pm 4 \times 10^{-2}$
Nostoc muscorum[b]	$26 \pm 3 \times 10^{-2}$	$365 \pm 1 \times 10^{-1}$	$391 \pm 1 \times 10^{-1}$
Nostoc punctiformae[b]	$21 \pm 2 \times 10^{-2}$	$389 \pm 8 \times 10^{-2}$	$410 \pm 5 \times 10^{-2}$
Nostoc carneum RDU-10[b]	$20 \pm 1.4 \times 10^{-2}$	$131 \pm 2.8 \times 10^{-2}$	$217 \pm 2.8 \times 10^{-2}$
Nostoc carneum RDU-11[b]	$31 \pm 3 \times 10^{-2}$	$250 \pm 1 \times 10^{-1}$	$281 \pm 6 \times 10^{-2}$
Anabaena sp. RDU-9[b]	$9.50 \pm 5 \times 10^{-2}$	$135 \pm 5 \times 10^{-2}$	$144.5 \pm 4 \times 10^{-2}$
Anabaena oryzae[b]	$10 \pm 2.8 \times 10^{-2}$	$76 \pm 2.8 \times 10^{-2}$	$86 \pm 4.2 \times 10^{-2}$
Anabaena sp. ACC-3229[a]	$96 \pm 7 \times 10^{-2}$	$118 \pm 7 \times 10^{-2}$	$214 \pm 7 \times 10^{-2}$
Nostoc sp. ACC-3141[a]	$31 \pm 2 \times 10^{-2}$	$72.50 \pm 5 \times 10^{-2}$	$103.5 \pm 8 \times 10^{-2}$
Cylindrospermum sp. RDU-4[b]	$60 \pm 5 \times 10^{-2}$	$163 \pm 4 \times 10^{-2}$	$223 \pm 4 \times 10^{-2}$
Cylindrospermum sp. RDU-5[b]	$50 \pm 5 \times 10^{-2}$	$124 \pm 6 \times 10^{-2}$	$174 \pm 2 \times 10^{-2}$
Nostoc sp. RDU-6[b]	$4.50 \pm 1.4 \times 10^{-2}$	$39 \pm 2.8 \times 10^{-2}$	$43.5 \pm 1.4 \times 10^{-2}$
Anabaena sp. RDU-8[b]	$45.50 \pm 1 \times 10^{-2}$	$79 \pm 8 \times 10^{-2}$	$124.5 \pm 9 \times 10^{-2}$

[a] Laboratory strain [b] isolated from saline/alkaline soil

Table 2: Percentage of sulphate, protein, and uronic acids in polysaccharides from different marine microalgae (12).

Microalgae species	Sulphate	Protein	Uronic acids
Spirulina platensis	5-20	6	7
Porphyridium sp	7.6-14.6	1-2	7.8-10
Rhodellasp	8	6	5-7.8
Gyrodinium impudicum	10.3	-	2.9
Navicula salinarum	6.3-11.5	0.5-4.9	7.7-8.0
Cylindrotheseclosterium	0-10.9	7.7-9.2	4.8-21.0

Stress factors on cyanobacteria and biosynthesis of polysaccharides

Polysaccharides production by phytoplankton partly depends on the environment in which they grow [18]. During the last three decades, several important factors that control the production of phytoplankton EPS have been identified. The synthesis of exocellular polysaccharides in microorganisms, including cyanobacteria, plays a major role in protecting cells from stress in extreme habitats and from other harmful conditions. Different studies have focused on the capability of some polysaccharide-producing cyanobacteria to overcome stress caused by desiccation or low water availability in desert or saline environments. For the desiccation-tolerant *Nostoc commune* strain, Hill et al. [19] proposed that the secreted glycan provides a repository for water, thereby acting as a buffer between cells and the atmosphere and representing the key component of the mechanism used by this cyanobacterium to tolerate desiccation.

These findings indicate that a change in EPS production would also contribute to the differences between growing environments in the field and in the laboratory conditions, including different nutrient, light, and temperature conditions (Fig. 2), From these stress factors, we will discuss examples from biotic and abiotic factors and their effects on polysaccharides composition production from cyanobacteria.

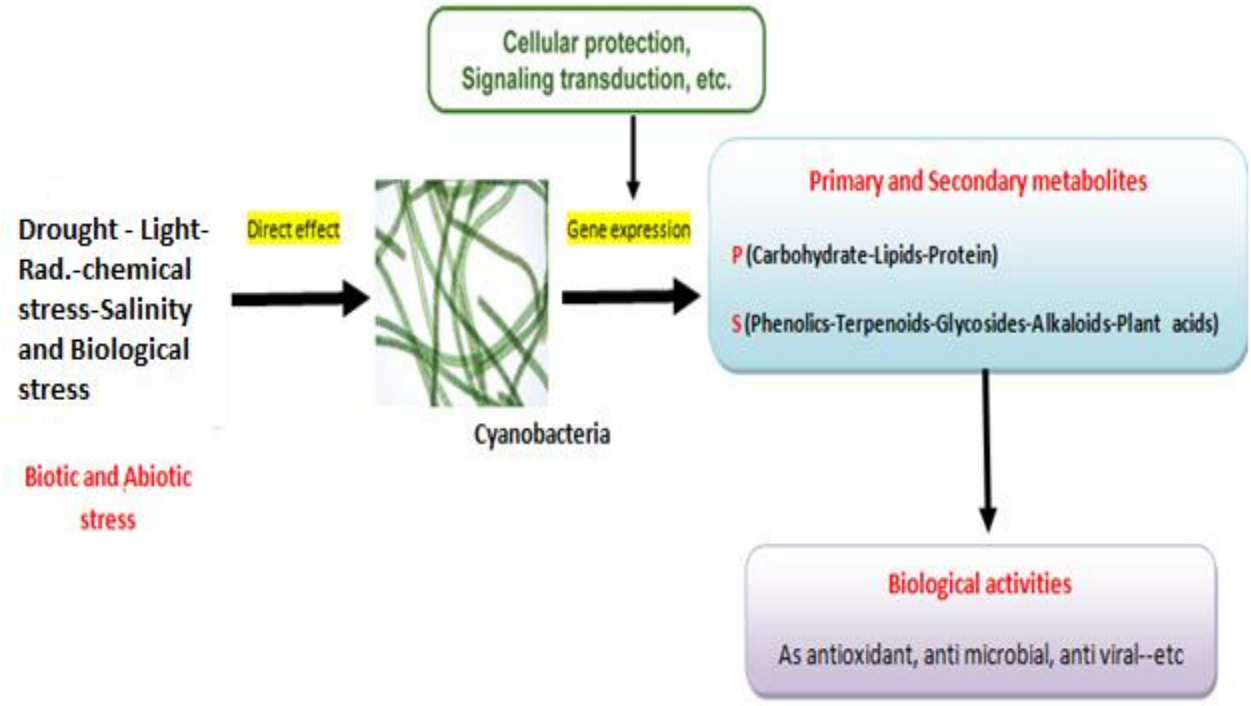

Figure 2: Effect of different biotic and abiotic stress factors on biosynthesis of various metabolites in blue-green algal cells.

Abiotic stress

Light provides the main source of energy for cellular material synthesis in phytoplankton. The net accumulation of EPS in phytoplankton is controlled by the ratio of carbon fixation and utilization [20-21]. The rate of photosynthetic carbon fixation is essentially governed by light intensity and ambient CO_2 density. Protein synthesis predominates in phytoplankton cells under low light intensity because of their low photon saturation [22]. When light intensity exceeds the photon saturation required for protein synthesis, the synthesis of other materials, such as EPS or pigments, will increase as shown in Figure 3 [23].

A study on the effects of light intensity on the components and topographical structures of extracellular polysaccharides (EPS) was carried out on the cyanobacterium Nostoc sp. by Ge et al. [24]. EPS yield was found to increase with light intensity. However, light intensity did not significantly affect the EPS fractions and monosaccharide composition. Higher light intensity generally resulted in higher protein content of EPS in similar fractions. The topographical structure of EPS, investigated by atomic force microscopy, appeared as spherical lumps, chains and networks. The long chains were observed at higher light intensity. Thus, light intensity affected the yield and nature of EPS.

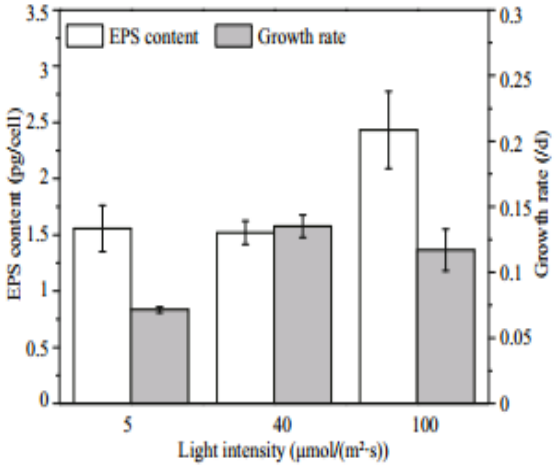

Figure 3: Growth rates and EPS production of M .aeruginosa under different light intensities [23].

The effect of NaCl on EPS production in cyanobacteria is less studied. There are no clear

reports correlating EPS production and NaCl tolerance. The gap in the data about the effect of NaCl on cyanobacterial EPS production was filled by different investigations, and the results of these studies have important implications in both the industrial and environmental arenas. From these studies; Ozturk and Aslim [25] observed a significant correlation between NaCl exposure and EPS production in various species. A similar correlation was reported by Chen et al. [26]. In the desert soil cyanobacterium Microcoleus vaginatus, exposure to NaCl resulted in a nearly 50% increase in extracellular carbohydrate. Also, the enhanced production of EPS under salt stress by some marine algae was determined at some previous studies [27-28]. Sheng et al. [29] reported that the amount of EPS produced by Rhodopseudomonas acidophila increased at high NaCl concentrations. On the other hand, the halotolerant strain *Rhizobium meliloti* EFB1 produced 40% less exopolysaccharides in response to salt [30]. Moreover, under toxic conditions, cyanobacteria generate EPS to act as a diffusion barrier between the cell wall and extreme environments [31]. In the recent study by Shalaby et al. [32], NaCl was revealed as an important stress factor that increases EPS production in cyanobacteria.

Also, Ozturk and Aslim [25] reported that the amount and composition of EPS in three Synechocystis sp. were strongly influenced by the concentration of NaCl in the growth medium. Moreover, differences in the monosaccharide composition and ratios of EPS may promote NaCl tolerance in these microorganisms. The alternative monosaccharide composition in polysaccharides may be important for industrial applications.

During the last three decades, several important factors that control the production of phytoplankton EPS have been identified. Lower nitrogen level result in more EPS production in *Chlamydomonas mexicana* [33], Cyanothece sp. [34], Anabaena sp. [35], *Cylindrotheca closterium* [36], and *Microcoleus vaginatus* [26]. Because excessive carbon is used for polysaccharide synthesis first, the increased concentration of carbon in the culture caused an increase of EPS production in *Cyanospirac apsulata* [37].

Zhen and Fanxiang [23] mentioned that lower nitrogen concentrations exerted a positive influence on EPS production as shown in Figure 4, which might contribute to the C:N ratio increase, thus promoting the incorporation of carbon into polymers [38-39].

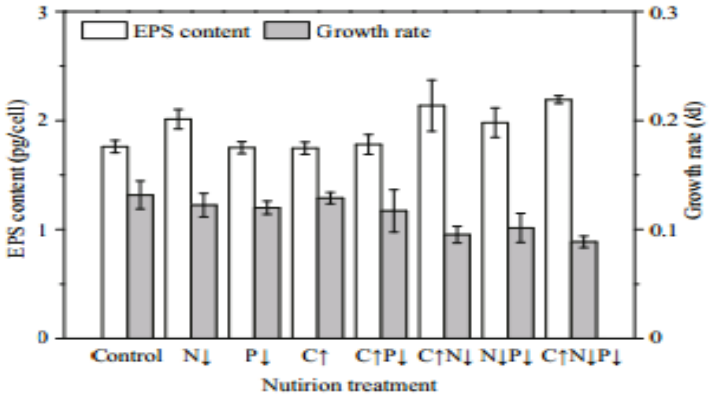

Figure 4: Growth rates and EPS production of *M .aeruginosa* under different nutrition conditions [23].

The possibility of stimulating polysaccharide release by means of an optimization of the culture conditions has been poorly considered. Most of the available studies were mainly devoted to assay the ejects of nitrogen deficiency, which has been shown to stimulate polysaccharide synthesis in some producing microalgae [40-41]. However, the response of cyanobacteria to nitrogen starvation is not univocal. Some strains, like *A. nidulans* [42] and several Cyanothece sp [43], released larger amounts of polysaccharides under conditions of nitrogen limitation and others, like *A. cylindrica* [44] and *A. flos-aquae* [45], the amount of polymer produced were varied depending on the nitrogen source used. On the other hand, in strains like Synechocystis [46], some Cyanothece [47], *C. capsulata* [48] and Phormidium [49], nitrogen starvation did not eject the exocellular production of polysaccharides. In the nitrogen-fixing cyanobacterium C. capsulate, when the metabolic carbon flux was ejected by cultivating the organism under conditions of nitrogen deficiency due to the presence of an argon atmosphere or to the use of inhibitors of nitrogen assimilation (like O-diazoacetyl-L-serine, D,L-7-azatryptophan or methionine-D,L-sulfoximine) [50], an accumulation of intracellular carbon reserves instead of an increase in the production of RPS was observed. In contrast, when the metabolic carbon flux was directly

stimulated by the addition of glyoxylate, a stimulator of both CO2 and nitrogen fixation rates in *Anabaena cylindrica* was recorded [51]. This cyanobacterium released larger amounts of polysaccharide, roughly corresponding, in terms of carbon balance, to the amount of the organic compound added. It is worth stressing that the possibility of increasing the amount of polysaccharide released without affecting growth, as it occurs in C. capsulata cultures carried out with the addition of glyoxylate, is highly promising owing to the actual enhancement of the final yield of the polymer achieved by this way.

Biotic stress

Microcystins (MCYSTs) are water-soluble toxic polypeptide substances and different aquatic organisms may be confronted with the risk of intoxications from water containing these toxins. Moreover, the transfer of these toxins along food chain may occur and reach humans. Mohamed [52] mentioned that Microcystins (MCYSTs) induced the production of intracellular polysaccharides of Chlorella vulgaris, and their amounts increased with increasing toxin concentration (P\0.05).. Crude MCYSTs induce greater polysaccharide production than pure MCYST-LR. Extracellular polysaccharides released into the culture medium of the algae were also induced by MCYSTs with values varying with toxin concentrations (P\0.05).

Although these polysaccharides are produced within the cells, extracellular polysaccharides were also detected in the algal medium with concentrations increased with the increase of toxin concentrations during this study. It has been suggested that polysaccharides were produced inside the cells during oxidative stress to scavenge the free radicals and remove them from the cells to the medium [53]. However, a variety of other mechanisms such as extracellular detoxification, reduced uptake, efflux, sequestration by polysaccharides have been proposed to explain algal tolerance to oxidative stress.

Antiviral activity of Polysaccharides

Most of the seaweed polysaccharides with antiviral activity carry only sulfate as their anionic group. Nevertheless, in the case of fucoidans and fucans, both sulfate groups and uronic acids are present .As happens with many species of seaweeds, the interest in marine microalgae is growing increasingly, especially because of the compounds they produce. An advantage working with microalgae is the fact that they are easy to grow and culturing, and harvesting does not depend on the climate or season. Being easily controlled, it enables the production of polysaccharides, or whichever other compounds with similar properties, either chemical or physical, throughout the year. Polysaccharides in general and sulphated exopolysaccharides in particular are released by many species of microalgae. They serve as antiviral agents, health foods, antioxidants, they have anti-inflammatory properties and have a role in the immunomodulatory system, and they may also be used as lubricants for bone joints, or even as drag-reducing substances for ships [12].

A number of natural and synthetic sulphated polyanions from microalgae, such as heparin, inhibited the replication of various mammalian viruses [54-55]. It has been suggested that these negatively charged molecules, including the sulphated algal polysaccharides, exert their inhibitory effect by interacting with the positive charges on the virus or on the cell surface and thereby prevent the penetration of the virus into the host cells [56-57]. However, polyanions like heparin prevent viral infection only if they are added during the early stages of infection [58-59].In contrast, Gonzalez et al.[60] found that carrageenan which is an algal polysaccharide, has no effect on virus attachment or penetration into host cells, but the synthesis of viral proteins inside the cell was inhibited. Similarly, other sulfated algal polysaccharides selectivity inhibits reverse transcriptase (RT) enzyme of human immunodeficiency virus (HIV) and its replication in vitro [61]. The internal effect of natural and synthetic polyanions is also manifested by their induction of interferon production, both in vitro and in vivo. Different workers confirmed the antiviral activities of Cyanobacteria species. Belay [62] presented the limited published information on Spirulina and focused the attention of researchers to the particular areas of immune enhancement and cancer. Numerous studies have been published since then and the evidence for immune

modulation of Spirulina in various animal models is so striking that structure function claims have already been applied to some Spirulina products. Also, Hayashi et al. [63] found that calcium spirulina (Ca-SP), A sulphate polysaccharide isolated from *Spirulina platensis* exhibited antiviral activity against both anti-human immunodeficiency virus type 1 (HIV-1) and anti-herpes simplex virus type 1 (HSV-1). Furthermore, Ca-SP is quite promising as an anti-HIV agent because even at low concentrations of Ca-SP an enhancement of virus-induced syncytium formation was not observed, as was observed in dextran sulphate (DS)-treated cultures. Ca-SP had very low anticoagulation activity, and showed a much longer half-life in the blood of mice when compared with that of DS. Thus, Ca-SP can be a candidate agent for an anti-HIV therapeutic drug that might overcome the disadvantages observed in many sulfated polysaccharides. Furthermore, Rechter et al. [64] have analyzed polysaccharide fractions isolated from *Arthrospira platensis*. These fractions containing spirulan-like molecules showed a pronounced antiviral activity against human cytomegalovirus, herpes simplex virus type 1. Also, Ayehunie et al. [65] found that the aqueous extract of *Arthrospira platensis* (*Spirulina platensis*) inhibited HIV-1 replication in human T-cell lines, peripheral blood mononuclear cells (PBMC) and langerhance cells (LC). Extract concentrations ranging between 0.3 and 1.2 g/ml reduced viral production by approximately 50% in PBMCs. Whereas IC_{50} for PBMC growth ranged between 0.8 and 3.1 mg/ml. Depending on cell type used, fractionation of the extract revealed antiviral activity in the polysaccharide fraction and also in a fraction depleted of polysaccharides and tannins.

Yakoot and Salem [66] have conducted first human trial to address the effect of Spirulinaplatensisdried extract on virus load, liver function, health related quality of life and sexual functions in patients with chronic hepatitis C virus (HCV) infection. They found the therapeutic potential of S. platensis in chronic HCV patients, and in some cases (13 %) the viral infection is complexly nullified. Mansour et al. [67] have found that the polysaccharides isolated from Gloeocapsaturgidus and Synechococcuscedrorum showed higher antiviral activity against rabies virus than that against herpes-1 virus. The exopolysaccharide from Aphanothecehalo phytica has shown anantiviral activity against influenza virus A (H1N1), which shows a 30 % inhibition of pneumonia in infected mice [68].

The mode of antiviral action of these sulfated polysaccharides was suggested to be attributed to the inhibition of very early step of viral replication, i.e., virus attachment and/or penetration to the host cells [69-72]. Researchers showed that several glycoproteins on the viral envelop are involved in the adsorption of virus to host cell. Enveloped viruses have been shown to interact with cell surface heparan sulfate as the initial binding site [73]. Sulfated polysaccharides can block this interaction effectively, herby block the HSV adsorption and infection. It is assumed that sulfated polysaccharides interact with positively charged domains on the glycoproteins of virus or host cell thus the adsorption of virus to the cells is inhibited by the sulfated polysaccharides.

Marine polysaccharides can either inhibit the replication of virus through interfering viral life cycle or improve the host antiviral immune responses to accelerate the process of viral clearance. The life cycle of viruses differs greatly with species. However, there are six basic stages in the life cycle of viruses: viral adsorption, viral penetration, uncoating of capsids, biosynthesis, viral assembly and viral release. Marine polysaccharides can inhibit viral life cycle at different stages or directly inactivate virions before virus infection. Specific antiviral mechanism of marine polysaccharides is commonly related to specific structure features of the polysaccharides and specific viral serotypes [74].

The relation between anticoagulating activity and polysaccharides in cyanobacteria

There are few reports of anticoagulant activity

for SPs isolated from blue-green algae. Table (3) shows the anticoagulant activity of *Spirulina maxima* hot water extracts cultivated under various nitrogen concentrations by clotting time assay as reported by Shalaby et al. [75]. The results illustrated that all the aqueous extracts exhibited anticoagulant activity when compared with heparin (sulphateglucouronic acid) which was used as standard anticoagulant. Among all extracts, the aqueous extract of Spirulina cells grown at low nitrogen level induced anticoagulant activity either similar or nearly to that of heparin. Whereas, anticoagulant of other extracts was less than heparin. For example, clotting time of treated plasma with water extract of Spirulina grown at 410, 205 and 102.5 ppm was 11, 12 and 13 min, comparing with 16 min. that in plasma treated with heparin or Spirulina water extract grown at 51 ppm N. These results suggested that aqueous extract of *Spirulina maxima* was able to be developed as anticoagulant agent and it could be used as a chemopreventive agent or as a model for the same effect. It appeared that there is a relationship between the anticoagulant efficiency and the chemical composition of Spirulina aqueous extract. The water extracts containing sulphated polysaccharides, tannins and some phenolic compounds. There is an evidence that this compound plays an important role as anticoagulants as documented by Shanmugam and Mody, [76].

Regarding the anticoagulation activity of the hot water extract of salt stressed *S. platensis*, the results presented (Fig.5) by Shalaby et al . [32] showed that significant anticoagulating efficiency (expressed by clotting time assay) compared with that of the standard anticoagulatant heparin (sulfate glucouronic acid) and these results were related to antiviral extract (as shown in Table 4).

Furthermore, algal water extract (50 µg/ml) of low salt concentration (0.02 M) exhibited relatively higher (60.0%) antihepatitis A virus-type MBB more than phosphate buffer extract (9.0%) of the same concentration and the activity of the latter extract increased (56.0-58.0%) at moderate salt concentration (0.04 M) using 20 and 50 µg/ml extract concentration respectively. On the other hand, the antiviral activity against herpes simplex virus –type 1 showed a comparable activity by both water and phosphate buffer extracts of both concentrations at all salinity levels with maximum antiviral activity (98.0%) at 50 µg/ml extract concentration. The antiviral activity against HSV-1 (DNA virus) was markedly pronounced (98.0%) than that against HAV-MBB (60.0%) which is an RNA virus. These activities were shown to be controlled by both type and concentration of algal extract (water or phosphate buffer, at 20 and 50 µg/ml) and may be induced by the sulphated polysaccharide and tannins in *S. platensis* extracts.

Table 3: Anticoagulation activity of water extracts of *Spirulina maxima* cultivated under different nitrogen conc. [75]

Concentration of nitrogen (ppm)	Clotting time (min)	Ratio to heparin
Blank	-	-
Heparin	17	1
410	11	0.65
205	11	0.65
102.5	13	0.76
51	13	0.76
0	12	0.71

Table 4: Antiviral activity (%) of phosphate buffer and water extracts of the salt stressed S. platensis (at conc.20 and 50µg/ml) using Hepatitis A virus type MBB (HAV-MBB) and Herpes Simplex virus type 1 (HSV-1).

NaCl (M)	HAV-MBB virus (RNA virus)				HSV-1 virus (DNA virus)			
	20 µg/ml		50 µg/ml		20 µg/ml		50 µg/ml	
	Phosphate buffer	Water extract	Phosphate buffer	Water extract	Phosphate buffer	Water extract	Phosphate buffer	Water extract
0.02 (Control)	9.0[c]	40.0[a]	9.0[c]	60.0[a]	90.0	96.0[a]	93.0[b]	98.0[a]
0.04	56.0[a]	32.0[b]	58.0[a]	34.0[b]	88.0	90.0[b]	90.0[c]	94.0[b]
0.08	32.0[b]	25.0[c]	37.0[b]	25.0[c]	90.0	96.0[a]	98.0[a]	98.0[a]
LSD	2.0318	1.1006	2.465	2.465	NS	1.123	2.250	2.250

Each value is presented as mean of triplet treatments, means within each row with different letters (a-c) differ significantly at P #0.05 according to Duncan's multiple range test,

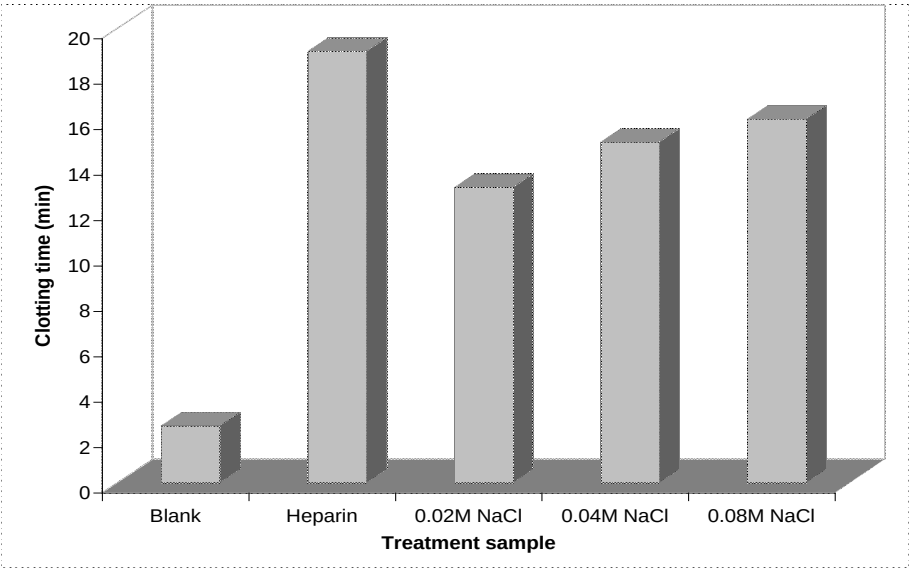

Figure 5: Anticoagulation activity (clotting time) of the hot water extracts of the salt stressed *Spirulina platensis* [32]

Anticoagulant activity of algal SPS has been assigned to the common pathway, primarily HC-II-mediated anticoagulant action. Investigations were conducted employing chromohenic substrates for the major coagulation enzymes, factor Xa and thrombin. No direct activity was demonstrated against the active sites of these enzymes, as color formation was not impaired by the presence of the algal anticoagulants at various concentrations. These enzymes were inhibited indirectly by the algal anticoagulants, however, via their potentiating of the activity of the serine protease inhibitors AT-III and HC-II. The inhibition of thrombin and factor Xa by AT-III was potentiated by the proteoglycan, but not by the SPS. The inhibition of thrombin by HC-II, however, was potentiated by both proteoglycan and SPS. Furthermore, Shanmugam and Mody [76] have extensively studied the anticoagulatant properties of marine algae. They reported that sulphatedgalactans and fucoidansulphates from red and brown algae, and different sugar sulphates like arabinose, rhamnansulphates, etc. from green algae are the identified active molecular species. Such activity is related to the molecular size, type of sugar and sulphate content of the active component. Sulphate position, type of linkage and molecular

geometry are also known to have a role in anticoagulant activity. The proposed mechanisms of action are predominantly on HC-II mediate antithrombin activities, direct antithrombin action (thrombin-fibrinogen complex) and minor AT-III involvements. In future, algal SPS can be developed as anticoagulant/antithrombitic agents.

Conclusion

Cyanobacteria offer a great diversity of polysaccharides showing interesting biological properties. Among different sources of algal polysaccharides, as Ca-Spirulina and especially their LMW derivatives could play an important role in future development of potential antiviral agents is positive correlation between light intensity, salt stresses on algal culture media and polysaccharides production. However, negative correlation may be observed when alga is cultivated under nitrogen deficiency conditions.

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