Biotechnological Potential of Mycosporine-like Amino Acids and Phycobiliproteins of Cyanobacterial Origin


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ABSTRACT

Cyanobacteria are considered to be a potential source of novel bioactive compounds that are highly intriguing from biotechnological perspective. A number of bioactive compounds such as cyanotoxins, mycosporine-like amino acids (MAAs), scytonemin, phycobiliproteins (PBPs), phytohormones etc. have been reported from diverse group of cyanobacteria that show assorted biological activities ranging from antioxidants, antibiotics, anticancer, antiviral to antiinflammatory action. The synthesis of UV-absorbing/screening compounds (MAAs) as well as phycobiliproteins (PBPs) from cyanobacteria is of particular interest as promising biomolecules that may be biotechnologically exploited in various pharmaceutical industries with significant biomedical research. The accessory light-harvesting pigments, PBPs are hepatoprotective, neuroprotective and can act as fluorescent neoglycoproteins probe. It can also serve as natural colorants in food and cosmetics industries. MAAs has been shown to play a significant role in skin protection from UV-B-irradiation and hence, can be a suitable candidate in the cosmetic industries for the development of natural UV sunscreens. The present review discusses biotechnological potential of MAAs as well as PBPs from cyanobacteria.

Keywords: bioactive compounds, cyanobacteria, mycosporine-like amino acids, phycobiliproteins

INTRODUCTION

Cyanobacteria are prokaryotic photoautotrophs found in almost every plausible habitat on Earth [1,2]. The existing knowledge on the diversity and physiology of cyanobacteria provides an outstanding base for exploring their applications in biotechnology. In the last few years, cyanobacteria have been proved to be one of the most promising groups of organisms that produce a number of bioactive compounds with anticancer, antibacterial, antifungal, antiplasmodial, algicidal and immuno-suppressive properties [3-6]. Some cyanobacteria have been found to accumulate polyhydroxyalkanoates (PHA) intracellularly that can be used in the production of biodegradable plastics [7]. Cyanobacteria have a lot of biotechnological applications that await potential uses in agriculture, mariculture, biomedicals, food, fuel and colorants [8-12]. Some of the cyanobacteria constitute potential sources for large-scale production of vitamins such as vitamins B and E, phycobiliproteins (PBPs), toxins and pharmaceuticals [13].

Recently, it has been found that cyanobacteria can form ideal consortia with chemotrophic bacteria and can effectively be used to clean up oil contaminated sediments and waste waters [14]. One of the interesting properties of cyanobacteria is their capacity of overcoming the toxicity of ultraviolet radiation (UVR) by means of UV-absorbing/screening compounds such as mycosporine-like amino acids (MAAs) and scytonemin that are considered as natural photoprotectants [15-18]. Due to strong UV absorption maxima (310 to 362 nm) and high molar extinction coefficients (ε=28100-50000), MAAs have received much attention for their putative role in UV photoprotection. Besides MAAs, certain other bioactive compounds such as PBPs, found in
cyanobacteria and in some other organisms [19-21], have quite a lot of commercial values such as
natural food colouring agents, feed additives to improve the health and fertility of cattle, enhancers
of the color of egg yolks, drugs, fluorescent probes and in the cosmetic industry, exhibiting its
immense biotechnological potentials [4,22,23]. The present review summarizes biotechnological
potential of MAAs and PBPs synthesized by cyanobacteria.

MYCOSPORINE-LIKE AMINO ACIDS (MAAS)

Ultraviolet radiation (UVR) is responsible for a wide range of detrimental effects on all sun exposed
living systems from bacteria to human beings [24-27]. However, certain photosynthetic organisms
including cyanobacteria are able to overcome the toxicity of UVR or high photosynthetically active
radiation (PAR) by synthesizing some UV-absorbing/screening compounds such as mycosporine-
like amino acids (MAAs) [6,16,28,29]. MAAs are among those natural photoprotective products
induced by cyanobacteria in response to ultraviolet-B (UV-B; 280-315 nm) radiation [29,30]. They
are synthesized by taxonomically diverse organisms as photoprotectant, except animals, where
MAAs are supposed to be accumulated through the food chain [28]. These are small (< 400 Da),
colorless, water-soluble compounds composed of a cyclohexenone or cyclohexenimine
chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol
[16,31,32]. So far, more than 20 MAAs have been identified from diverse organisms, structures of
some are shown in Table 1 [16,33]. These are highly resistant against abiotic stressors such as
temperature, UV radiation, various solvents and pH [34,35]. Some MAAs also contain sulfate esters
or glycosidic linkages through the imine substituents [36]. The basic chromophores responsible for
the UVR absorbance in MAAs are apparently derived from the early stages of the shikimic pathway
[12], present in bacteria, cyanobacteria, algae, phytoplankton and macroalgae but not in animals,
because they lack the shikimate pathway [17]. Among natural substances isolated from
cyanobacteria that could provide a great variety of biotechnological applications, MAAs are
becoming promising due to their UV screening properties and potential antioxidant activities.

MAAs are one of the valuable bioactive compounds that may be biotechnologically exploited in
diverse ways. It has been investigated that the MAAs such as shinorine, porphyra-334 (P-334) and
mycosporine-glycine (MG) can protect the human fibroblast cells from UV-induced cell death [37].
Daniel et al. [38] has reported that a mixture of P-334 and shinorine can suppress UV-induced aging
in human skin and thus it may be potentially used in cosmetics and toiletries as UV protectors and
activators of cell proliferation. Recently, Torres et al. [39] has reported porphyra-334 from
Aphanizomenon flos-aquae (AFA), and evaluated its UV-A absorption properties with two
commercial sunscreens, Nivea (Nivea Moisturising Sun Lotion, batch 10932751) and Boots (Boots
Soltan Extra Moisturizing Sun Lotion, batch 1Z) and found that the methanolic extract of porphyra-
334 has significant UV-A screening properties that may be used as a potential natural source for
UV-A protective sunscreens. Several other synthetic analogues of MAAs have also been developed
for commercial purposes [40]. One of the synthetic analogues, tetrahydropyridine (Figure 1),
derivatives of natural MAAs, has been found hydrolytically and oxidatively more stable for
commercial application as suncare products [41,42]. Moreover, a product called Helioguard® 365
that contains MAAs has been commercialized and is now available in the global market. Besides the
role of MAAs in commercial development of suncare products for skin protection, these can be used
as photostabilizing additives in protection of non-biological materials such as plastic, paint and
varnish [43-46]. Interestingly, MAAs can block the formation of most cytotoxic and mutagenic
DNA lesions such as cyclobutane pyrimidine dimer (CPD) and 6-4 photoproduct (6-4PP) [47] and
inhibit the cell to undergo mutation and cell death.

MAAs may also act as antioxidants, preventing cellular damage resulting from UV-
induced production of reactive oxygen species (ROS) [48,49]. Accumulation of highly effective
Table 1. Molecular structure of some important MAAs with their corresponding absorption maxima.

<table>
<thead>
<tr>
<th>Mycosporine-like amino acids</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Molecular structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycosporine-taurine</td>
<td>309</td>
<td><img src="image" alt="Mycosporine-taurine" /></td>
</tr>
<tr>
<td>Mycosporine-glycine</td>
<td>310</td>
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</tr>
<tr>
<td>Palythine</td>
<td>320</td>
<td><img src="image" alt="Palythine" /></td>
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<tr>
<td>Palythine-serine-sulfate</td>
<td>320</td>
<td><img src="image" alt="Palythine-serine-sulfate" /></td>
</tr>
<tr>
<td>Palythine-serine</td>
<td>320</td>
<td><img src="image" alt="Palythine-serine" /></td>
</tr>
<tr>
<td>Mycosporine-methylamine-serine</td>
<td>327</td>
<td><img src="image" alt="Mycosporine-methylamine-serine" /></td>
</tr>
</tbody>
</table>
Mycosporine-methylamine-threonine 327

Asterina-330 330

Mycosporine-glutamic acid-glycine 330

Palythinol 332

Mycosporine-2-glycine 334
Shinorine 334

Porphyra-334 334

Mycosporine-glycine-valine 335

Palythenic acid 337

Usujirene 357
antioxidative scavenger complexes can protect cells from damage by free radicals such as superoxide anion and hydroxyl radical. It has been found that the antioxidative potential of *Spirulina platensis* can increase 2.3-fold during oxygen stress. Dunlap and Yamamoto [50] have found that MG has moderate antioxidant activity, providing some protection against photooxidative stress induced by ROS. Furthermore, a precursor of MAAs i.e., 4-deoxygadusol, has strong antioxidant properties and its retrobiosynthesis through bacterial conversion of algal MAAs has been performed for commercial applications [51,52]. Due to the antioxidative components originated from a natural source, their application in cosmetics for preserving and protecting purposes is developing rapidly. However, the antioxidant activities of certain purified MAAs in terms of scavenging of free radicals still remains unknown. Overall, extreme stability at a wide pH and temperatures, excellent UV-absorbing properties as well as their antioxidant activities suggest potential applications of MAAs in the prevention and therapeutic treatment of ailments related to the free radical (such as singlet oxygen, superoxide anions, hydroperoxyl radicals, and hydroxyl radicals) production and UV irradiation of humans for the therapy of oxidation associated diseases, like inflammations [53].

1-(1,1-dimethylethyl)-3octanoyl-4,4-dimethyl-1,4,5,6-tetrahydropyridine
1-(2-methylpropyl)-3-propionyl-4,4-dimethyl-1,4,5,6-tetrahydropyridine

Figure 1. A new class of UVB-absorbing sunscreen compound, 1-alkyl-3-alkanoyl-1,4,5,6-tetrahydropyridines, which are stable analogues of the natural enaminoketone (non-aromatic) chromophore of MAAs [40].

**PHYCObILIPROTEINS**

Phycobiliproteins (PBPs) are brilliantly colored water-soluble pigments, bearing covalently attached open chain tetrapyrroles (Figure 2). PBPs, assembled into macromolecular structures, the phycobilisomes (PBSs), are unique accessory light-harvesting oligomeric proteins, made up of phycoerythrin (PE; \( \lambda_{max} \) 540-570 nm) with a pink/red colour, phycocyanin (PC; \( \lambda_{max} \) 610-620 nm) with a blue colour, and allophycocyanin (APC; \( \lambda_{max} \) 650-655 nm) with a bluish-green colour, play an important role particularly in the photosynthetic processes of cyanobacteria and red algae [23,54]. PBPs are being extensively used as nutrient ingredients and natural dyes for food and cosmetics, potential therapeutic agent in oxidative stress-induced diseases, and as fluorescent markers in biomedical research [55]. Neuroprotective and hepatoprotective effects have also been attributed to phycocyanin [56,57].

There is an increasing demand for natural colors which are of use in food, pharmaceuticals, cosmetics, textiles and as printing dyes. However, their utility is limited, since the natural dyes have low tinctorial values and persistence. PBPs are used as a natural protein dye in the food industry as well as in cosmetic industry. Phycocyanin derived from *Spirulina platensis* is used as a natural pigment in food such as chewing gum, dairy products and jellies. Despite its lower stability to heat and light, phycocyanin is considered more versatile than gardenia and indigo, showing a bright blue color in jelly gum and coated soft candies [58]. They are also used in coloring of many other food products such as fermented milk products, ice creams, soft drinks, desserts, sweet cake decoration, milk shakes. The blue biliprotein, PC, extracted from *Spirulina platensis* has been used in products like chewing gums, candies, dairy products (e.g. yoghurts) and cosmetics such as lipstick and
eyeliners in Japan, Thailand and China [59-62]. However, phycocyanin as food colouring is not yet permitted within Europe or the USA.

Presently, due to inadequate consumption of blue food (PC added) has probably limited the industries interest in PC for food colorings. Only a few studies have addressed the functionality of PC in food with regards to color stability [63,64] and rheological properties [65]. *Spirulina platensis* may be used as human health foodstuff due to major antioxidant properties of PC [66,67].

Phycobiliproteins conjugated to immunologist, protein A and avidin were developed into fluorescent probes [68] and therefore, have wide usage in histochemistry, fluorescence microscopy, flow cytometry, fluorescence-activated cell sorting and fluorescence immunoassays [69-71]. Phycoerythrin is the most studied phycobiliprotein, used as fluorescent probes exclusively in fluorescent immunoassays, fluorescent immunohistochemistry and other methodologies [22] because it is isolated as hexamers (66) [69] and has fluorescence quantum yields of 82-98 %. Phycobilisomes from *Spirulina platensis* combined to streptavidin are being used as fluorescent probes in cytometry [72].

The antioxidant and radical scavenging behavior of PC due to the attached phycocyanobilin to PC group, from various cyanobacteria are well known [67,73-76]. The recombinant apo-APC with no attached phycocyanobilins has also been found to be antioxidative [77]. Recently, the clinical potential of *Spirulina* as a source of phycocyanobilin has been investigated. It was found that the phycocyanorubin, which is a reduced form of phycocyanobilin, is an important antioxidant and may be a potential therapeutic agent in oxidative stress-induced diseases [78]. Phycocyanorubin can inhibit the formation of superoxide radicals by NADPH oxidase and play additional protective roles by reducing the generation of reactive oxygen species (ROS) in the body [79,80]. Allophycocyanin also has various biological and pharmacological properties. Allophycocyanin from *Spirulina* sp. has many potential applications in antioxidant and anticancer drug preparation [77]. The radical scavenging property of phycocyanin was established by studying its reactivity with peroxyl and hydroxyl radicals and also by competition kinetics of crocin bleaching. These studies have demonstrated that phycocyanin is a potent peroxyl radical scavenger with an IC<sub>50</sub> of 5.0 µM [73]. The antioxidant activity was also assayed *in vivo* in glucose oxidase (GO)-induced inflammation in mouse paw. The results showed that phycocyanin is able to scavenge OH. (IC<sub>50</sub> = 0.91 mg/mL) and RO. (IC<sub>50</sub> = 76 µg/mL) radicals, with activity equivalent to 0.125 mg/mL of dimethyl sulphoxide (DMSO) and 0.038 µg/mL of trolox, specific scavengers of those radicals respectively [56]. Anti-inflammatory effect of phycocyanin extract was also studied in acetic acid-induced colitis in rats and it was found that phycocyanin show inhibition in inflammatory cell infiltration and reduction to some extent in colonic damage [81]. Phycocyanin from *Spirulina platensis* and its chromophore, phycocyanobilin (PCB), efficiently scavenge ONOO·, a potent physiological inorganic toxin and may also inhibits the ONOO·-mediated single-strand breaks in supercoiled plasmid DNA in a dose-dependent manner. Due to oxygen radical scavenging properties of phycocyanin, it may also be used to treat oxidative stress-induced neuronal injury in neurodegenerative diseases, such as Alzheimer's and Parkinson's [82].

APC can undergo both photo-excitation and photo-ionization and act as both a type I (free radical mechanism) and type II (singlet oxygen mechanism) photosensitizer [83]. Phycobiliproteins are considered to be photochemically quite stable, irradiation of Langmuir-Blodgett (LB) films of phycobiliproteins caused the generation of photocurrents and photovoltages in the electrochemical cell; these results showed that the chromophores of phycobiliproteins, especially those chromophores near the surface, exhibit photoinduced charge transfer phenomena in the LB films [84,85]. Interestingly, preliminary data have shown that phycobiliproteins exert much stronger photodynamic action on tumor cells compared with Photofrin-II and that they might be used as a new type of photodynamic therapeutic agent [86]. phycobiliproteins (PBPs) exert much stronger
photodynamic action on tumor cells than hematoporphyrin derivative (HpD) and might be used as one of promising candidates for new type of photodynamic reagent [87,88].

Recently, potent apoptosis-inducing compounds associated with human health have been recorded that prevent tumor promotion, progression, and the occurrence of cellular inflammatory responses [89]. It has been found that PC extracted from *Spirulina platensis* is a selective inhibitor of cyclooxygenase-2 (COX-2), an inducible isoform that is upregulated during inflammation and cancer. The extent of inhibition depends on the period of preincubation of PC with COX-2. The IC50 value obtained for the inhibition of COX-2 by phycocyanin is much lower (180 nM) as compared to those of celecoxib (255 nM) and rofecoxib (401 nM), the well-known selective COX-2 inhibitors [90]. The PC induced apoptosis mediated by the release of cytochrome c from mitochondria and independent of Bcl-2 expression was also observed in RAW 264.7 mouse macrophage cell line [91].

It has been found that phycocyanin regulates the production of white blood cells, even when bone marrow stem cells are damaged by toxic chemicals or radiation [92]. Several workers has investigated that PC from *spirulina* enhance the immune functions including mucosal or innate immunity through macrophage [93,94] and secretions of the related cytokines [95]. The spleen cells, especially those stimulated with phycocyanin, increased colony formation of bone marrow cells. Shinohara et al. [96] has reported that both phycocyanin and allophycocyanin from *Synechococcus elongatus*, a thermophilic cyanobacterium, promoted the growth of a human myeloid cell line, RPMI 8226 cells. It has been suggested that phycocyanin extracted from *Spirulina platensis* inhibited growth of human leukemia K562 cells in a dose-dependent manner, arresting them at the G1 phase with increased level of cmyc expression and hence, it was suggested that phycocyanin can affect enhancing proliferation or differentiation of immune competent cells including bone marrow cell, which may cause normally sustaining or enhancing immune functions [97].

Thus it has been found that PBP have hepatoprotective, anti-inflammatory, and anti-arthritic properties, although its ability to efficiently scavenge free radicals and effectively inhibit lipid peroxidation may also be involved [90]. Besides above all activities, PC has also been found to show antiviral properties against herpes simplex virus type 2 [98]. Allophycocyanin was found to inhibit enterovirus 71-induced cytopathic effects, viral plaque formation, and viral-induced apoptosis [99]. Phycocyanin derived from *Spirulina platensis* powerfully influenced serum cholesterol concentrations and imparted a stronger hypocholesterolemic activity [100].

**CONCLUSION AND FUTURE PERSPECTIVES**

The biotechnological potentials of cyanobacteria as a source of several bioactive compounds such as MAAs and phycobiliproteins have proved to be a promising area of research. These compounds seem to be very valuable and show commercial potential for food, cosmetics and biomedical research as well as in the design of very specific and potent new pharmaceuticals against variety of diseases. PBPs may be used extensively in the novelty items designed for entertainment, recreation and enjoyments, including play materials, paints, toys, textiles mainly clothing, bubbles in bubble making, personal items such as cosmetics, bath powders, body lotions, toothpastes and other dentifrices, soaps, foods such as gelatins, icings and frostings, beverages such as beer, wine, champagne, soft drinks, and ice fountains, including liquid fireworks and other such jets or sprays [71]. The availability of bioactive compounds derived from cyanobacteria and their advantages in production of commercially valuable products can be optimized to produce sustainable yields on an industrial scale. Continued technical improvement and market demand will result in further major advances and an expansion of the commercially available cyanobacterial species and their products.
Table 2. Application related patents on PBPs [71].

<table>
<thead>
<tr>
<th>PBPs activity</th>
<th>Patent number*</th>
<th>References</th>
</tr>
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<tr>
<td>Cosmetics</td>
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<td>[101,102]</td>
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<tr>
<td>Drink/beverage compositions</td>
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<td>[103-105]</td>
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<td>Anti-oxidant</td>
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<td>Liver protecting</td>
<td>CN 1633889</td>
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<tr>
<td>Treatment of atherosclerosis</td>
<td>US 4886831</td>
<td>[86]</td>
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<tr>
<td>Light protection in packaging</td>
<td>US 6686004</td>
<td>[112]</td>
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<td>Fabric dye</td>
<td>JP 166480</td>
<td>[113]</td>
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<tr>
<td>Quantification of UVR</td>
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<td>Ingredient in tissue culture medium</td>
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<td>Molecular weight marker</td>
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<tr>
<td>Skin function activation factor</td>
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<td>Serum lipid reducing agent</td>
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<td>Construction of recombinant for diverse end-uses</td>
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<td>Signal generators and image contrast agents</td>
<td>US 7014839</td>
<td>[121]</td>
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<tr>
<td>Fluorescent labels, tags and markers</td>
<td>US 7049151, US 7067258</td>
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Genetic engineering methods are also establishing to be used for cyanobacterial strain improvement. In future, a multidisciplinary and supportive effort with the use of more sensitive and fast methods in the analysis of the cyanobacterial bioactive compounds will expedite significantly the discovery of novel compounds from cyanobacteria. Several patents dealing with a number of applications of PBPs have been patented (Table 2). Research on microorganisms, cyanobacteria in particular, is hampered by the low level of knowledge concerning the basic biology and culture techniques and hence, many more biological and ecological investigations are needed before the full biochemical potential of these organisms is realized. In comparison to the occurrence of diverse metabolites in cyanobacteria, few studies have been reported on their biotechnological values. More attention is needed to explore the cyanobacteria and their metabolites to lead the discovery of useful compounds for a range of biotechnological applications.

REFERENCES