Cyanobacteria as bio-factories for production of UV-screening compounds

N Browne¹, F Donovan¹, P Murray¹, SK Saha²*

Abstract

Introduction
There is a growing demand for the replacement of chemical sunscreens with bio-sunscreens. Production of bio-sunscreen alone requires an alternative source of ultraviolet-screening compounds than the existing wild source. At present, bio-sunscreen compounds are sourced from marine macroalgae containing mycosporine-like amino acids such as, palythine, porphyra-334 and shinorine. Importantly, cyanobacteria being the most successful prokaryotic photosynthetic organisms in various extreme environments can produce palythine, porphyra-334, shinorine and other types of mycosporine-like amino acids with multiple bio-functions. Some cyanobacteria additionally produce special type of pigments embedded within their extracellular sheaths for their cellular protection from ultraviolet light damage. Cyanobacteria can be cultivated in a sustainable manner for the production of desired ultraviolet-screening compounds using their photosynthetic machinery, meagre amounts of nutrients, sunlight or artificial lights; atmospheric or industrial waste CO₂ and marine water. This alternative bio-factory neither depends on the local weather nor supports un-sustainable harvesting of bio-materials from the wild. The tools required for cyanobacterial genetic manipulations are well developed, and more than 50 cyanobacterial genome sequences are available in the public domain, which allows further genetic improvement of cyanobacteria through comparative gene distribution and synteny analysis. Therefore, cyanobacteria can be considered as novel alternative bio-factories for the production of ultraviolet-screening compounds.

This review briefly discusses the types of ultraviolet-screening compounds of cyanobacteria and their usefulness as bio-factories for the production of alternative source of ultraviolet-screening compounds.

Conclusion
The fact that the ability to cultivate cyanobacteria in a controlled in vitro environment with specific growth and induction requirements makes them ideal bio-factory for the production of ultraviolet-screening compounds. The extent of cyanobacterial potential must yet be manipulated by conventional and genetic manipulations, which require further scrutiny at a molecular level for efficient bio-synthesis of ultraviolet-screening compounds.

Introduction
Cyanobacteria and their habitats
Cyanobacteria are photosynthetic, oxygen-evolving prokaryotic organisms present on the Earth for approximatel 3.5 billion years. They are the original oxygenic photosynthetic organisms and are morphologically diverse ranging from unicellular to multicellular (Figure 1), cocoid to branched filaments, almost colourless to variously pigmented. There are more than 150 genera and over 2000 species described so far. Cyanobacteria are mostly autotrophic in their mode of nutrition, but there are some heterotrophic forms also. They grow as free-floating, colonial as well as endosymbionts with higher plants. Cyanobacteria inhabit diverse ecological conditions from psychrophilic to thermophilic, acidophilic to alkylphilic, epilithic to endolithic and freshwater to halophilic. Cyanobacteria are found in a wide variety of habitats ranging from freshwater to oceans, soil to bare rocks, deserts to ice shelves and hot springs from Arctic and Antarctic lakes. They thrive in harsh environmental conditions, namely ultraviolet (UV) irradiance, photodegradation, drought and desiccation, nitrogen starvation, heat–cold shocks, anaerobiosis, osmotic and salinity stresses due to their unique survival strategies¹².

Ultraviolet irradiation and bio-sunscreen
Cyanobacteria are naturally exposed to copious amounts of UV irradiation from the sun, their primary source of energy. A stratospheric ozone layer shields the Earth from penetration of UV irradiation (UV-A, 315–400 nm; UV-B, 280–315 nm; UV-C, 100–280 nm) and their harmful effects on cellular damaging. Ozone layer is continuously depleting due to increasing use of anthropogenically released environmental hazards such as chlorofluorocarbons, chlorocarbons and organobromides. This is leading to an increase in UV-A and UV-B exposure on the Earth³. UV-A is the most commonly penetrating type of UV light, while UV-B’s penetration is increasing due to reduction of ozone layers. UV-C cannot reach the Earth’s atmosphere.
Figure 1: Morphological and bio-chemical variations of Irish cyanobacteria. Right panel shows corresponding absorption spectra of methanolic extracts of (a) Chlorogloeoa microcystoides, (b) Calothrix crustacea, (c) Lyngbya majuscula (d) and Nostoc commune.

Mycosporine-like amino acids
To date, approximately 21 MAAs have been discovered in marine and freshwater organisms, and/or UV-B region of the spectrum. These compounds play an important role supporting cyanobacterial growth and survival in habitats exposed to strong irradiation. MAAs and scytonemin are able to protect cyanobacterial cells through absorbing the harmful UV irradiation and dissipating the energy in a harmless form of heat radiation. MAAs bio-synthesis is induced when cyanobacteria are exposed to UV-B irradiation. Basic chromophores in MAAs responsible for the UV absorbance are possibly synthesised during the preliminary stages of cyanobacterial shikimate pathway. This pathway connects the carbohydrates metabolism to the bio-synthesis of aromatic compounds. However, both the complete enzymatic pathway of MAAs bio-synthesis and their regulation by environmental conditions are not fully understood. The genes involved in MAA biosynthesis in cyanobacterium Anabaena variabilis PCC 7937 are YP_324358 (predicted 3-dehydroquinate synthase) and/or UV-B region of the spectrum. These compounds play an important role supporting cyanobacterial growth and survival in habitats exposed to strong irradiation. MAAs and scytonemin are able to protect cyanobacterial cells through absorbing the harmful UV irradiation and dissipating the energy in a harmless form of heat radiation. MAAs bio-synthesis is induced when cyanobacteria are exposed to UV-B irradiation. Basic chromophores in MAAs responsible for the UV absorbance are possibly synthesised during the preliminary stages of cyanobacterial shikimate pathway. This pathway connects the carbohydrates metabolism to the bio-synthesis of aromatic compounds. However, both the complete enzymatic pathway of MAAs bio-synthesis and their regulation by environmental conditions are not fully understood. The genes involved in MAA biosynthesis in cyanobacterium Anabaena variabilis PCC 7937 are YP_324358 (predicted 3-dehydroquinate synthase) and/or UV-B region of the spectrum. These compounds play an important role supporting cyanobacterial growth and survival in habitats exposed to strong irradiation. MAAs and scytonemin are able to protect cyanobacterial cells through absorbing the harmful UV irradiation and dissipating the energy in a harmless form of heat radiation. MAAs bio-synthesis is induced when cyanobacteria are exposed to UV-B irradiation. Basic chromophores in MAAs responsible for the UV absorbance are possibly synthesised during the preliminary stages of cyanobacterial shikimate pathway. This pathway connects the carbohydrates metabolism to the bio-synthesis of aromatic compounds. However, both the complete enzymatic pathway of MAAs bio-synthesis and their regulation by environmental conditions are not fully understood. The genes involved in MAA biosynthesis in cyanobacterium Anabaena variabilis PCC 7937 are YP_324358 (predicted 3-dehydroquinate synthase) and
Table 1 List of cyanobacterial MAAs with their specific absorption maxima

<table>
<thead>
<tr>
<th>Cyanobacteria</th>
<th>Type of MAAs</th>
<th>λ-max (nm)</th>
<th>Specific growth conditions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synechocystis sp. PCC 6803</td>
<td>Mycosporine—taurine</td>
<td>309</td>
<td>UV-A and B</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>M-343</td>
<td>343</td>
<td>UV-A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dehydroxylysulirone</td>
<td>356</td>
<td>UV-A</td>
<td></td>
</tr>
<tr>
<td>Anabaena dolium</td>
<td>Mycosporine—glycine</td>
<td>310</td>
<td>PAR</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Porphyra-334</td>
<td>334</td>
<td>UV-B</td>
<td></td>
</tr>
<tr>
<td>Anabaena variabilis PCC 7937</td>
<td>Palythine—serine</td>
<td>320</td>
<td>Sulphur depletion</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Shinorine</td>
<td>334</td>
<td>PAR + UV-A and B</td>
<td></td>
</tr>
<tr>
<td>Nostoc commune</td>
<td>Palythine—threonine</td>
<td>322</td>
<td>Sunlight</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Porphyra-334</td>
<td>334</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euhalothece sp.</td>
<td>Mycosporine-2-glycine</td>
<td>331</td>
<td>UV-A</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Euhalophece-362</td>
<td>362</td>
<td>UV-A</td>
<td></td>
</tr>
<tr>
<td>Trichodesmium sp.</td>
<td>Asterina-330</td>
<td>332</td>
<td>UV-A</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Palythene</td>
<td>360</td>
<td>UV-A</td>
<td></td>
</tr>
</tbody>
</table>

MAA, mycosporine like-amino acids; PAR, photosynthetically active radiation; UV, ultraviolet.

YP_324357 (α-methyltransferase). The products of the above two genes are involved in the bio-synthesis of the common core (deoxygadusol) of all MAAs. Another study with the gene NpF5557 of Nostoc punctiforme suggested that cyanobacteria possess at least two distinct pathways for the bio-synthesis of bi-substituted mycosporines.

Scytonemin

Scytonemin represents a yellow-brown, low-molecular-weight (544 Da), lipid-soluble pigment of the cyanobacterial sheath with an absorption maximum of 384 nm. Production of scytonemin in certain cyanobacteria is believed to be the earliest developed mechanism of UV protection, more ancient than the flavonoids or melanins. Purified scytonemin has a maximum UV absorption at 384 ± 2 nm, although it can also absorb at 252, 278 and 300 nm. Scytonemin was first reported by Nägeli in 1849 in some terrestrial cyanobacteria and later termed scytonemin. Its structure constituting indolic and phenolic subunits was determined in 1993. Scytonemin is redox sensitive by changing from a greenish brown (when oxidised) to a red (reduced) form. It duced form is usually found in duced form. It is usually found in the oxidised form; however, the redox form depends on the acid–base conditions during the process of extraction. Scytonemin is thought to be synthesised from the metabolites of aromatic amino acid bio-synthesis and can be induced by high photon flux rate. Scytonemin bio-synthesis was also reported to induce in response to UV-A irradiation; high temperature; photo-oxidative stress; deficiency of specific elements such as Fe, Mg or N; high light intensity and periodic desiccation stress. This pigment is excreted and deposited in the extracellular sheaths of some cyanobacteria. Scytonemin is thought to carry out screening activity without any further metabolic investment even after prolonged physiological inactivity. Scytonemin-producing cyanobacteria are typically found in the upper layers of microbial mat communities, which are exposed to high levels of solar irradiance. Thus, the extracellular pigment scytonemin is thought to have a protective role against harmful UV irradiation, allowing organisms to adapt to harsh habitats. Scytonemin also possesses anti-inflammatory and anti-proliferative properties in addition to its UV-screening properties.

A milestone study with cyanobacteria N. punctiforme ATCC 29133 (PCC 73102) led to the understanding of scytonemin bio-synthesis at a molecular level. In this study, a transposon mutagenesis using Tn5-1063a (transposon with antibiotic resistance marker) yielded a scytonemin-less Nostoc mutant strain that did not produce scytonemin under UV irradiation. This was the only phenotypic difference of this mutant strain compared with the wild type. The above mutation was traced to open reading frame (ORF) NpR1273 (now known as scyD), which is a part of a gene cluster of 18 contiguous ORFs (NpR1276 to NpR1259). These
Cyanobacteria have higher growth rates compared with macroalgae and can be cultivated photoautotrophically in outdoor raceway or circular ponds and in in-door photobioreactors under an optimised controlled environment. The cyanobacterial production system can be based on non-arable land because of their ability to thrive in areas that cannot support agriculture; moreover, cyanobacteria can be cultivated throughout the year without depending on local weather adopting in-door cultivation systems. Photoautotrophic cultivation of cyanobacteria requires only marginal amounts of nutrients supplied as inorganic chemicals, organic matters or wastewater; sunlight or artificial lights; atmospheric or industrial waste CO₂ and non-potable water (brackish or marine water). Further, the genetic tools for cyanobacterial strain improvement are well established and more than 50 cyanobacteria genome sequences are available online. The available genome sequences allow comparisons of gene distribution and synteny among various cyanobacteria strains as well as close relatives for their genetic optimisations. Therefore, selected cyanobacteria can be further optimised to produce commercially important UV-screening compounds at industrial scales by developing cheaper and sustainable cultivation systems using available raw materials.

Approaches for exploration of cyanobacteria
Cyanobacterial cultivation can be based on utilisation of free solar energy (open cultivation systems) or utilisation of artificial (photosynthetically active radiation) lights (closed photobioreactors) for sustainable production of UV-screening compounds. Minimal nutrients requirement for their growth can be supplied as chemical or organic nutrients and to enhance their dense bio-mass yield, use of industrial waste CO₂ within the bio-refinery concept is an approach that may further be improved to achieve sustainable production of UV-screening compounds from cyanobacteria.

Figure 2: Chemical structures of various mycosporine-like–amino acids found in cyanobacteria.
ideal situation. Once, active growth is obtained at desired cell density, cyanobacterial cells can be starved for specific nutrients and/or UV irradiation for induction of specific UV-screening compounds. Then, the biomass would be ready for harvesting and extraction of UV-screening compounds. Depending on the nature of compounds, specific extraction protocol has to be adopted, for example, MAAs can be extracted by aqueous methanol and scytonemin with organic solvent or supercritical CO₂. The spent biomass thus obtained can have applications as bio-fertiliser or in bio-energy generation within the bio-refinery concept (Figure 3).

Challenges for exploration of cyanobacteria
Few cyanobacteria namely *Spirulina platensis*, *Arthrospira maxima* and *Aphanizomenon flos-aquae* have been cultivating photoautotrophically as outdoor cultures for health supplement products, however mostly not based on the bio-refinery concept. Thus, their complete potential is not explored, which needs integrated research and development projects to reduce production cost. Cyanobacteria cultivation in open race-way ponds is effective for free solar energy harvest, but there are several biological threats of contamination with other algae, algae grazers, fungi and amoeba, rotifers, etc. Further, local weather fluctuations including light, temperature and rain may influence cultivation efficiency. Therefore, cultivation in closed photobioreactors could be the best alternative for nutritional quality bio-mass or value-added bio-molecule production throughout the year irrespective of local weather. Large-scale cultivation in tubular, flat plate or other designs of closed photobioreactors are expensive. Overheating and fouling in closed photobioreactors are also major challenges. The present harvesting technologies such as filtration, centrifugation or rolling belt are expensive for large-scale cyanobacterial bio-mass harvesting, which needs optimisation and cheaper technology. Bio-flocculation is the process used for several-folds concentration of cultured bio-mass prior to downstream processing. This process could be the simplest and cost-effective method. However, bio-flocculation for all cyanobacteria is not universal and needs optimisation possibly by minimum use of chemical flocculants.

Conclusion
Sunscreen use has noticeably increased due to growing concern that sun exposure is a primary factor of skin cancer and photoageing. Cyanobacteria can step up the mark to fulfil the global demand for bio-sunscreens by replacing the use of chemical-based sunscreens. Cyanobacteria bio-synthesise UV-screening compounds such as MAAs and scytonemin, which protect them in harsh habitats. Cyanobacteria offer several advantages over the current macroalgae (alternative source

Figure 3: Schematic diagram showing cyanobacterial bio-factory for ultraviolet-screening compounds production within bio-refinery concept. UV, ultraviolet; MAAs, mycosporine-like amino acids.
for bio-sunscreen compounds) such as eliminating the need to harvest from the wild and their ability to be cultivated sustainably. The fact that the ability to cultivate cyanobacteria in a controlled in vitro environment with specific growth and induction requirements makes them ideal bio-factory for the production of UV-screening compounds. The extent of cyanobacterial potential must yet be manipulated by conventional and genetic manipulations, which require further scrutiny at a molecular level for efficient bio-synthesis of UV-screening compounds.

Abbreviations list
MAAs, mycosporine-like amino acids; ORF, open reading frame; UV, ultraviolet.

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References
