



Published in final edited form as:

Nat Prod Rep. 2012 March ; 29(3): 372–391. doi:10.1039/c2np00075j.

The chemical ecology of cyanobacteria

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Abstract

This review covers the literature on the chemically mediated ecology of cyanobacteria, including ultraviolet radiation protection, feeding-deterrence, allelopathy, resource competition, and signalling. To highlight the chemical and biological diversity of this group of organisms, evolutionary and chemotaxonomical studies are presented. Several technologically relevant aspects of cyanobacterial chemical ecology are also discussed.

1. Introduction

Cyanobacteria are found in almost every environment on Earth. However, historically, research on these organisms has been, for the most part, carried out by groups that work almost exclusively with either freshwater (and terrestrial) or marine strains. This is reflected in both the primary and secondary literature. A number of recent reviews have been dedicated entirely or partially to cyanobacterial chemical ecology, usually focusing on a specific type of chemo-ecological interaction or on a particular habitat type. For example, recent reviews have appeared on the chemical ecology of marine plankton¹ and the benthos.² Singh *et al.*³ have reviewed chemical and biological aspects of radiation protection in cyanobacteria. Allelopathic interactions, including those perpetrated by cyanobacteria, have been reviewed under slightly different perspectives.⁴⁻⁷ Broader-scope reviews on aquatic chemical ecological processes that have relevance for cyanobacteria were conveyed by Pohnert⁸ and Hay.⁹ Tidgewell *et al.*¹⁰ and Jones *et al.*^{11, 12} provide important insights into cyanobacterial natural products diversity and diversification, a key concept underlying the chemical ecology of these organisms.

Here, we review the current state of knowledge on cyanobacterial chemical ecology, regardless of the environment in which such interactions occur. The divergent treatment of freshwater and marine cyanobacteria is, of course, unavoidable and implicitly present throughout the review. Nevertheless, we find that it is useful to feature examples from both habitats (whenever possible) that result in roughly the same ecological function or purpose. We have tried to emphasize the rationale behind the discovery of the secondary metabolites covered in this review, their structural features, and, for a few more recent examples, provide some detail on their biosynthesis. We then present, in a short section, examples of evolutionary studies of selected cyanobacterial natural products followed by a discussion on the relationship between biodiversity and chemodiversity, emphasizing recent insights into the taxonomy of these organisms. A final section showcases some examples of how fundamental chemoecological findings may find application in biotechnology.

2. Ecological roles played by cyanobacterial metabolites

2.1. UV-protection

Ultra-violet radiation (UVR) is a high-energy fraction of the solar spectrum that causes detrimental effects on living systems. In particular, it can damage DNA and proteins directly,¹³ or indirectly through production of reactive oxygen species (ROS).¹⁴ Deleterious effects of UVR in cyanobacteria include, apart from DNA and protein damage, and production of ROS, photosynthesis inhibition, delayed cell differentiation, motility impairment, lower growth rates and decreased survivability.³ Cyanobacteria were probably the first oxygen-evolving photosynthetic organisms to have appeared on Earth, with contrasting evidence placing such an event from 2.7 to 3.5 billion years ago.^{15, 16} Since these photoautotrophic microorganisms are thought to be responsible for the present oxygenic atmosphere, and the ozone layer,¹⁷ their evolutionary history encompasses dramatic differences in terms of the amount of solar radiation reaching the Earth's surface. It is, therefore, perhaps unsurprising that cyanobacteria are capable of synthesizing a diverse number of metabolites that confer protection from UVR, usually termed as 'cyanobacterial sunscreens'. Other UVR-related defence mechanisms, such as avoidance behaviour, antioxidant response (enzymatic or non-enzymatic) and cellular repair mechanisms are also known to occur among cyanobacteria.³ In the context of the present review, however, cyanobacterial secondary metabolites that confer photoprotection, especially to UVR long-term exposure, by absorbing/screening harmful UVR, deserve special attention. While cyanobacteria produce several UV-absorbing metabolites, "cyanobacterial sunscreen" is usually employed when referring to mycosporines and mycosporine-like amino acids (MAAs) or scytonemin, for which a natural role in photoprotection has been clearly established.³

2.1.1 Mycosporines and MAAs—Mycosporines and MAAs are small (< 400 Da) aminocyclohexenone or aminocyclohexenimine derivatives, respectively, with λ_{max} ranging from 310 to 362 nm. Usually the ring structure is modified by one (mycosporines) or two amino acids (MAAs), which may or may not be of proteinogenic origin. Several organisms, such as bacteria, cyanobacteria, eukaryotic phytoplankton and macroalgae are able to synthesize these compounds.¹⁸ Members from different genera of benthic and planktonic

cyanobacteria, from marine and freshwater habitats are known to produce both mycosporines and MAAs,¹⁹ including mycosporine glycine (**1**), shinorine (**2**),²⁰ porphyra-334 (**3**),²¹ asterina-330 (**4**), palythanol (**5**),²⁰ euhalothece-362 (**6**)²², palythene (**7**),²³ mycosporine-tau (**8**),²⁴ and palythine serine (**9**)²⁵. Sugar-linked MAAs have also been reported from cyanobacteria.²⁶

The biosynthesis of mycosporine and MAAs was thought to proceed via the shikimate pathway intermediate 3-dehydroquinone to produce the gadusols prior to the addition of the amino acid substituents.¹⁸ However, Balskus and Walsh²⁷ have very recently demonstrated that, at least in cyanobacteria, the starting material for mycosporine and MAAs biosynthesis is actually sedoheptulose 7-phosphate (**10**), a pentose-phosphate pathway intermediate. By means of genome-mining, heterologous expression, as well as feeding studies, the same study provided evidence for a four-enzyme biosynthetic pathway responsible for the production of the MAA **2** in the cyanobacterium *Anabaena variabilis* ATCC 29413.²⁷ Two of the enzymes (one dehydroquinone synthase homologue, Ava_3858, and one *O*-methyltransferase, Ava_3857) are involved in the conversion of **10** to 4-deoxygadusol (**11**). Glycine and then serine are added to this intermediate, through the action of two enzymes, Ava_3856 (ATP-grasp homologue) and Ava_3855 (NRPS-like). These enzymes catalyse the installation of the amino acid substituents via imine linkages, but by distinct biochemical mechanisms.²⁷ The genes encoding for Ava_3858 and Ava_3857 had previously been implicated in MAA biosynthesis,²⁸ and were shown to have been horizontally transferred from cyanobacteria to dinoflagellates²⁸, and then to metazoans.^{28, 29} Regarding the ecophysiological properties of this group of sunscreen molecules, they can reach high concentrations in the producing cyanobacterial cells (often above 1% of dry weight),³⁰ and owing to their high molar absorptivities (up to 50,000 M⁻¹ cm⁻¹),¹⁸ cells can be effectively protected from UVR-damage. As an example, Garcia-Pichel *et al.*³¹ have estimated a sunscreen factor of 0.3 for MAAs in the cyanobacterium *Gloeocapsa* sp., indicating that 30% of the photons were prevented from reaching cytoplasmic targets. Other ecologically relevant findings include the increase in biosynthesis of particular MAAs by exposure to UVR,^{21, 24, 31, 32} and the photostability of MAA compounds under different conditions.^{33, 34} Interestingly, recent findings suggest that MAAs and scytonemin (**12**) act in a complementary fashion in the cyanobacterium *Nostoc flagelliforme* to provide UVR protection in both the UV-A and UV-B wavelength ranges (280-400 nm).³⁰ The authors of the study suggest that this complementarity might be responsible for the UV-insensitivity exhibited by this cyanobacterium.³⁰ Aside from UVR-protection, mycosporine and MAAs have also been implicated in other biological functions and considered to be “multipurpose secondary metabolites”.³⁵ There is evidence that MAAs are involved in oxidative stress responses as antioxidants,^{36, 37} osmotic regulation²³ and iron scavenging,²² and their synthesis has been shown to be upregulated by different abiotic stress conditions.^{25, 38}

2.1.2 Scytonemin—While the previously described sunscreens are found inside of cyanobacterial cells, the extracellular pigment scytonemin (**12**) accumulates in the mucilaginous sheath that many filamentous or unicellular cyanobacteria secrete.³⁹ It is a dimeric alkaloid of 544 Da, composed of indolic and phenolic moieties. Its structure was determined in 1993,⁴⁰ despite the existence of such a yellow-brown pigment having been

reported more than 150 years ago.⁴¹ Its role as a sunscreen was established following studies in *Chlorogloeopsis* sp. in which the pigment was directly associated with reduced sensitivity to UVR damage and also shown to passively protect physiologically inactive (dried) cells.⁴² Metabolite **12** confers photoprotection to producing cells by strongly absorbing UVR in the 325-425 nm range (UVA, *in vivo* $\lambda_{\text{max}} = 370$ nm), and, additionally, in the UVB and UVC spectral ranges.⁴⁰ Thus, it serves as an effective measure to prevent harmful UVR from reaching cyanobacterial cells. In fact, it is estimated that approximately 90% of UVA radiation is filtered out by **12**.⁴²

The gene cluster responsible for the biosynthesis of **12** was identified in *Nostoc punctiforme* ATCC 29133, following analyses of scytonemin-deficient mutants, as comprising 18 genes all transcribed in the same direction⁴³. Some of these genes had no ascribed function, while others encoded proteins involved in amino acid biosynthesis, in particular that of tryptophan and tyrosine.⁴³ Further studies have laid out the biochemical basis for key biosynthetic steps leading to metabolite **12**.^{44, 45} Tryptophan serves as a building block, being converted to the corresponding pyruvic acid **13** by a leucine dehydrogenase homologue, ScyB.⁴⁴ However, *p*-hydroxyphenylpyruvic acid (**14**) seems to be generated directly from prephenate (rather than tyrosine) by the prephenate dehydrogenase TyrA, also encoded in the biosynthetic pathway.⁴⁴ ScyA, a homologue to thiamine diphosphate-dependent enzymes, is responsible for the acyloin coupling of both pyruvic acids, yielding **15**.⁴⁴ The product of one of the cluster genes with previously unknown function, ScyC, catalyses an elegant cyclization of **15** via an intramolecular reaction, followed by decarboxylation, yielding the monomer **16**, a precursor which should have a propensity for oxidative dimerization to afford **12**.⁴⁵ Feeding studies using isotopically labeled tryptophan and tyrosine in *Tolypothrix distorta*⁴⁶ provided *in vivo* evidence for both amino acids acting as substrates in the biosynthetic pathway, as well as for the decarboxylation of **13** and **14** during the biosynthesis of **12**. Moreover, the data obtained suggested that enzymes involved in aromatic amino acid transformations, which are not present in the gene cluster, may also play a role in the biosynthesis of **12**.⁴⁶ Interestingly, two genes just outside of the initially proposed scytonemin gene cluster encode for a putative histidine kinase and a putative transcriptional regulator with a signal receiver domain.⁴³ It has been proposed^{47, 48} that these configure a regulatory system controlling the transcription of the gene cluster. The 18 genes in the cluster have also been shown to be under transcriptional control from UVR, being upregulated with increased exposure to such radiation.^{47, 48} This is in agreement with the initial observations of the accumulation of **12** following UVR exposure.^{39, 42}

2.2. Feeding deterrence

As primary producers, and key constituents of the phytoplankton and phytobenthos, cyanobacteria serve as food for a plethora of micro- and macro-organisms in both freshwater and marine ecosystems. As such, strategies to cope with foraging are an important theme in cyanobacterial chemical ecology. The ecotoxicity of cyanobacterial metabolites to aquatic organisms is an active field of research, and a variety of such compounds have been found to be highly toxic to potential foragers of cyanobacteria.⁴⁹ Notwithstanding, assigning a natural role of defence against foraging to such compounds is not always straightforward, a classic example being the case of the microcystins. Several studies have reported the antifeedant

and toxic properties of different microcystins to potential grazers.⁵⁰⁻⁵³ However, the finding that microcystin synthase genes evolved before the appearance of zooplankton⁵⁴ has ruled out defence from foragers as the natural role for this group of metabolites, at least initially.

In fact, the overwhelming majority of work that has led to a hypothesized anti-predatory role for cyanobacterial natural products was conducted with tropical cyanobacteria of the genus *Lyngbya*. Different members of this genus display distinctly different palatabilities to predators and some actually deter feeding by natural foragers, a feature that has been frequently associated with secondary metabolite production.^{55, 56} A handful of *Lyngbya* metabolites have been isolated on the basis of such ecologically relevant activities. Ypaoamide (**17**) is one of such compound, isolated from a Guam *L. majuscula* strain.⁵⁷ This cyanobacterium formed a bloom that was associated with mass mortalities of juvenile rabbitfish. Feeding deterrence toward the parrotfish *Scarus schlegeli* guided the isolation of **17**,⁵⁷ which was later found to correspond to the *S*-enantiomer.⁵⁸ The pure metabolite, at ecologically relevant concentrations, deterred feeding by *S. schlegeli*, the urchin *Echinometra mathaei* and the rabbitfish species that motivated the study (*Siganus argenteus* and *Siganus spinus*).⁵⁹ Other metabolites isolated from *L. majuscula* were also shown to influence the feeding behaviour of juvenile *S. schlegeli* and *S. spinus*.⁶⁰ Malyngamides A (**18**) and B (**19**),⁶¹ as well as malyngolide (**20**),⁶² had low palatability to these fishes, under periodic and continuous feeding protocols.⁶⁰ In fact, the low palatability of compounds **18** and **19** had previously been demonstrated experimentally with the pufferfish *Canthigaster solandri* and crabs from the genus *Leptodius*.⁶³ The sea hare *Stylocheilus longicauda* was chemically deterred in its feeding activity by natural concentrations of **17**, **20**, and microcolin B (**21**),⁶⁴ all produced by *L. majuscula* which is curiously a major part of the sea hare's diet.⁶⁵ Pitipeptolides A (**22**) and B (**23**) are cyclic depsipeptides that were isolated as major constituents of another *L. majuscula* strain from Guam.⁶⁶ Metabolite **22** was found to deter feeding by *E. mathaei*, *Menaethius monocerus* (crab), *Parhyale hawaiiensis* and *Cymadusa imbroglia* (amphipods).⁶⁷ A bloom-forming *Lyngbya polychroa* strain from Belize produces the methionine-sulfoxide containing cyclic depsipeptide carriebowmide (**24**).⁶⁸ This metabolite was isolated from a lipophilic extract that had shown feeding deterrence activity toward a natural assemblage of Belizean coral reef fish. However, the low amount of pure **24** isolated was not enough to confirm whether this metabolite was responsible for the activity in the lipophilic extract.⁶⁸

Overall, these studies emphasize, on one hand, the chemical proficiency of tropical *Lyngbya* species, and, on the other, the important role that limiting predation plays for these benthic cyanobacteria. Indeed, feeding deterrence may be a decisive factor allowing *Lyngbya* blooms to develop in tropical coastal waters.^{55, 67}

The fact that several herbivorous insect larvae feed on cyanobacteria⁶⁹ motivated a screening for cyanobacterial strains that exhibit toxicity toward grazing insects (chironomid larvae)⁷⁰. This ultimately led to the identification of four hapalindoles [12-*epi*-hapalindole J isonitrile (**25**),⁷¹ 12-*epi*-hapalindole C isonitrile (**26**),⁷² hapalindole L (**27**),⁷³ and 12-*epi*-hapalindole E isonitrile (**28**)] as quite active insecticides (100% lethality at 48 h for compound **25** at 26 µM).⁷¹

A wealth of information is available concerning the toxicity of pure cyanobacterial secondary metabolites, as well as cellular extracts, toward ecologically relevant predators in freshwater, marine and brackish water environments.^{49, 74-76} Therefore, experimental approaches to investigate whether these substances act as feeding deterrents in their respective ecosystems are warranted.

2.3. Allelopathy

The allelopathic potential of cyanobacteria first became revealed by the work of Keating in the 1970's.^{77, 78} In a freshwater lake, the ability of cell-free filtrates from dominant cyanobacteria, as well as lake water, was demonstrated to induce positive or negative effects on the growth of other cyanobacteria and diatoms from the same lake. Furthermore, these studies showed that these allelopathic interactions were correlated to the succession of algal blooms in this lake. Many studies have since reported allelopathic properties of cyanobacteria, mostly in freshwater environments.^{4, 7, 79} Nonetheless, the majority of such evidence is drawn from co-culturing experiments, or from the exposure of target species to cell-free filtrates. Hence, information on which metabolites mediate such allelopathic interactions (allelochemicals) is scarce. The first cyanobacterial metabolite to be reported as an allelochemical was cyanobacterin (**29**), isolated from a freshwater *Scytonema hofmanni* strain.⁸⁰⁻⁸² This γ -lactone contains a chlorine atom, a feature more commonly observed in marine metabolites where this element is more abundant. Cyanobacterin was isolated following laboratory observations that compounds were being released from *S. hofmanni* that were able to inhibit the growth of other photoautotrophs, both in liquid and solid media.⁸⁰ Compound **29** was shown to target photosynthesis by specifically damaging thylakoid membranes^{83, 84} and inhibiting electron transport in photosystem II (PSII).^{83, 85} Several cyanobacteria, eukaryotic algae and angiosperms have been reported to be susceptible to this allelochemical.⁸³⁻⁸⁶

Based on growth inhibition of *Anabaena* spp. by extracellular compound(s) produced by cyanobacteria of the genus *Hapalosiphon*, Moore and co-workers isolated and characterized the allelochemical hapalindole A (**30**) from a terrestrial *Hapalosiphon fontinalis*.⁸⁷ While several other hapalindoles were reported from this same source,^{73,87} the anti-cyanobacterial (and antimycotic) activities of the cyanobacterium were assigned mostly to the chlorine- and isontitrile-bearing alkaloid **30**. Another member of the hapalindole family of natural products, the at-the-time known compound 12-*epi*-hapalindole E isonitrile (**28**),⁸⁸ was isolated from *Fischerella muscicula* as a result of a screening program for allelopathic activity by cyanobacteria towards green algae and other cyanobacteria.^{89, 90} It was shown to have inhibitory activity towards a wide range of other organisms and cell lines.⁹⁰ Further studies revealed that **28** interacts directly with the RNA polymerase, preventing RNA chain elongation in *Bacillus subtilis*.⁹¹ The related secondary metabolite 12-*epi*-hapalindole F isothiocyanate (**31**)⁷² was shown to inhibit the growth of cyanobacteria of the genera *Microcystis* and *Synechococcus*.⁹²

Another screening effort⁹³ tested the ability of cyanobacterial cells or cell-free filtrates to clear cyanobacterial lawns (of 30 target strains) on solid media. Strikingly, the cyanobacterium *F. muscicula* UTEX 1829 was found to produce an anticyanobacterial

compound that was active toward all of the target strains.⁹³ Subsequent studies led to the isolation and characterization of the allelochemical fischerellin A (**32**),^{94, 95} containing two heterocyclic rings and an enediyne moiety, and to the establishment of its function as a potent PSII inhibitor thereby affecting photosynthetic redox chemistry and O₂ evolution.⁹⁴⁻⁹⁶ As in the case of **30**, compound **32** is active toward cyanobacteria, green microalgae and higher plants,⁹⁴⁻⁹⁶ but does not inhibit heterotrophic bacterial growth⁹⁴ nor photosynthesis in the purple bacterium *Rhodospirillum rubrum*.⁹⁶

In the above mentioned screening study leading to the discovery of **32**, the cyanobacterium *Nostoc* sp. 31 exhibited activity toward 29 out of the 30 target strains.⁹³ A bioassay-guided chemical exploration of this strain allowed the isolation of the allelochemical responsible for the majority of such growth inhibitory activity, nostocyclamide (**33**), a cyclic peptide bearing two thiazole- and one oxazole moieties.⁹⁷ A series of bioassays confirmed that **33** inhibited the growth of cyanobacteria, diatoms, and chlorophytes, but not that of the fungus *Saccharomyces cerevisiae* nor heterotrophic bacteria.^{97, 98} Interestingly, **33** was also moderately toxic to *Brachionus calyciflorus*. Since this freshwater rotifer feeds on cyanobacteria,⁹⁹ compound **33** may actually serve more than one distinct ecological functions. The related metabolite, nostocyclamide M (**34**), was also present in the biomass of *Nostoc* sp. 31, and differs only from **33** in one amino acid residue (D-Met replacing D-Val).¹⁰⁰ Due to its growth inhibitory activity towards cyanobacteria in agar diffusion assays, **34** was also reported to be an allelochemical.¹⁰⁰ It is interesting to note the structural resemblance of these two metabolites with other natural products,¹⁰⁰ in particular **34** with the epimeric substance tenuocyclamide C (**35**).¹⁰¹ Metabolite **35** was isolated from a soil *Nostoc spongiaeforme* strain, and displays antibacterial and toxic properties, but no clear ecological function was ascribed to this compound.¹⁰¹ However, it has recently been shown that **35** (and the other tenuocyclamides) are synthesized ribosomally, and not by NRPS machinery, and are thus members of the 'cyanobactin' group of cyanobacterial metabolites.¹⁰² It is thus likely that the nostocyclamides **33** and **34** are also of ribosomal origin, and underscores the metabolic plasticity involved in cyanobacterial chemical ecology.

More recently, a new family of compounds with allelopathic properties has been isolated from *Oscillatoria* sp. LEGE 05292 and named the portoamides A-D (**36-39**).¹⁰³ This freshwater strain of *Oscillatoria* was identified as potentially allelopathic in a screening study during which cyanobacterial culture filtrates were used as growth media for eukaryotic microalgae.¹⁰⁴ The extracellular products from *Oscillatoria* sp. LEGE 05292 inhibited the growth of *Chlorella vulgaris*, a ubiquitous green microalga. Moreover, it was shown that this was a cytostatic effect, as the exposed microalgae grew at normal rates when placed in regular growth medium.¹⁰⁴ These findings motivated the bioassay-guided isolation and characterization of the portoamides, large cyclic depsipeptides containing several modified amino acids, including a β -amino acid and an *N*-Ac-*N*-Me-Tyr moiety; the latter is present in compounds **36** and **37** but not in **38** and **39**.¹⁰³ Compounds **36** and **37** were found to be the most abundant portoamides in the cyanobacterial biomass, while **38** and **39** were the major components in the culture medium, suggesting a specific role for the doubly modified Tyr residue in the exudation process. Furthermore, the mixture of depsipeptides **36** and **37** was

found to be more active toward *C. vulgaris* than either a mixture of **38** and **39** or the individual pure compounds **36** or **37**.¹⁰³ As such, understanding the contribution of the portoamides to the allelopathic behaviour of *Oscillatoria* sp. LEGE 05292 requires further investigation. However, the synergistic mixture of **36** and **37** inhibited the growth of other phytoplanktonic organisms with IC₅₀ values below 30 µg·mL⁻¹.¹⁰³ A subsequent study¹⁰⁵ provided evidence for community wide effects of the allelochemicals produced by this cyanobacterium. A natural microbial community was exposed in a laboratory setting to a portoamides-containing fraction of the exudates from *Oscillatoria* sp. LEGE 05292. As a result of this exposure, the relative abundance of several bacteria (including cyanobacteria) and eukaryotes (including algae and zooplankton) was altered from a control situation. Strikingly, the *Oscillatoria* sp. LEGE 05292 exudates also influenced the population structure of a *Microcystis* sp. which dominated the community, and moreover, different *Microcystis* genotypes displayed distinctive sensitivities to the portoamides-containing exudates.¹⁰⁵

The above studies highlight the role that screening approaches have had on the discovery of novel cyanobacterial allelochemicals. It can thus be expected that such experimental strategies will continue to reveal new chemical mediators of allelopathic interactions. At the same time, such molecules become tools that allow researchers to examine the ecological, biochemical and physiological aspects of allelopathy on both the producing and target organisms.

2.4. Resource competition

Cyanobacteria from different environments have developed various strategies by which to competitively acquire or more efficiently use available resources, which may be limiting. In certain cases, this is achieved by making use of secondary metabolites and these examples will be briefly discussed here. One of the most prominent examples of a chemically-mediated resource acquisition strategy is the production of siderophores, high-affinity ligands of metal ions, most notably iron (Fe). This element is scarce in most aquatic environments, in part due to the low solubility of Fe(III). Moreover, Fe is essential for a number of metabolic processes carried out by cyanobacteria, including photosynthesis and nitrogen fixation.¹⁰⁶ The ability of freshwater and marine cyanobacteria to produce siderophores has been known since the 1970's,^{107, 108} however, only a few such compounds have been isolated and chemically characterized from this source.

Schizokinen (**40**), a citrate-derived hydroxamate siderophore, first isolated from the bacterium *Bacillus megaterium*,¹⁰⁹ was found to be produced and released to the culture medium by the freshwater cyanobacterium *Anabaena* sp. under Fe-limiting conditions.¹¹⁰ A regulated uptake system specific for schizokinen-bound Fe was shown to be present in *Anabaena* sp. ATCC 27898,¹¹¹ and proof was provided that other *Anabaena* sp. strains were able to incorporate this ferric complex.¹¹² Recently, an outer membrane protein, SchT, expressed by *Anabaena* sp. PCC 7120, was implicated in ferric **40** transport and incorporation by this cyanobacterium.¹¹³ Interestingly, this siderophore was also shown to bind copper and to considerably reduce its toxicity to *Anabaena* sp. PCC 7120 through lack

of uptake.¹¹⁴ As such, the authors have suggested that this may be an additional ecological role of metabolite **40**.¹¹⁴

Structurally related to **40**, the synechobactins A (**41**), B (**42**) and C (**43**) were isolated from the marine cyanobacterium *Synechococcus* sp. PCC 7002, grown under Fe-limiting conditions.¹¹⁵ The fatty acid chains present in each of these hydroxamate siderophores is thought to anchor them to membranes, and may represent an evolved functionality when compared to **40**. Additionally, **41** was found to be photoreactive, releasing a 46 amu fragment under fluorescent light, probably corresponding to the conversion of the citrate moiety to β -ketoglutarate with concomitant loss of two protons and CO₂, but still binding Fe(III). This photoreactivity may also play a role in the Fe acquisition mechanism by *Synechococcus* sp. PCC 7002.¹¹⁵

The metabolites anachelin-H (**44**),^{116, 117} anachelin-1 (**45**) and anachelin-2 (**46**)^{118, 119} were isolated from two freshwater *Anabaena cylindrica* strains in independent research efforts, based on their Fe-binding properties^{116, 118} (metabolite **45** was reported in both studies, but its structure characterized only by Itou and coworkers¹¹⁸). These catecholate-bearing siderophores are structurally more complex than the citrate-derived hydroxamates presented above. An interesting aspect of anachelins is their hybrid biogenetic characteristics, as they feature peptide, polyketide and alkaloid fragments. It has been suggested that the natural bioactive metabolite is **44** and that **45** and **46** result as artefacts of the isolation process.¹²⁰ Unfortunately, not much is known about the ecophysiological implications of any of the anachelins.

Phosphate (P_i), is also a scarce resource in many water bodies, particularly in freshwater. As photoautotrophs, cyanobacteria have an essential requirement for this inorganic nutrient. As such, cyanobacteria have developed strategies to cope with phosphorous-limiting conditions, such as intracellular polyphosphate storage, or the expression of alkaline phosphatases (APs) which hydrolyse a variety of phosphorous-containing organic compounds outside of the cellular membrane.^{121, 122} The activity of the APs generate P_i readily available for uptake by the AP-producing cells, but also contribute to the total dissolved P_i pool.¹²¹ Very recently, the well-studied cyanobacterial secondary metabolite cylindrospermopsin (**47**)¹²³ has been associated with a new strategy to compete for P_i as perpetrated by *Aphanizomenon ovalisporum*.¹²⁴

The discovery of **47**, however, had no chemoecological basis. Rather, it was motivated by an outbreak of human hepatoenteritis that occurred in Queensland, Australia, in 1979, which was linked to the occurrence of *Cylindrospermopsis raciborskii* in a local reservoir that supplied water for domestic use.¹²⁵ While this cyanobacterium was not associated with any reported toxicity at the time, the aqueous extract of a *C. raciborskii* strain isolated from the reservoir exhibited severe hepatotoxicity and other organ toxicity when injected intraperitoneally in mice.¹²⁵ The compound responsible for the toxicity was isolated and its structure elucidated as the guanidino-containing alkaloid **47**.^{123, 125} Due to its implications for public health and aquatic ecosystem management, this metabolite has since been extensively studied. Biosynthetic pathways responsible for the production of **47** have been reported for the cyanobacteria *C. raciborskii* strains AWT205¹²⁶ and CS-505,¹²⁷

Aphanizomenon sp. 10E6¹²⁸ and *Oscillatoria* sp. PCC 6506.¹²⁹ In spite of slight differences among the structures of the respective biosynthetic gene clusters, the biosynthesis of **47** involves NRPS/PKS and PKS enzymatic machinery, as well as several unique tailoring reactions. The starter unit for the biosynthesis of **47** is guanidinoacetate (**48**), derived from glycine and arginine through the action of a pathway-encoded amidinotransferase (CyrA).¹³⁰ This is followed by the thiotemplated NRPS/PKS (CyrB) and PKS (CyrC to F) reactions, leading to intermediate **49**. CyrG and CyrH then catalyse the formation of the uracil moiety via a urea or guanidino donor.¹²⁶ Since no thioesterase or cyclization domains are present in the pathway, ring formation is believed to proceed spontaneously which also leads to the release of intermediate **50** from the enzyme.^{126, 131} The sulphate group present in **47** originates from the action of a pathway-encoded sulfotransferase, CyrJ. The substrate for this enzyme is 3-phosphoadenylyl sulfate, which is provided by CyrN, an adenylyl sulfate kinase that is also encoded in the gene cluster.¹²⁶ The hydroxyl group adjacent to the uracil ring is incorporated through the action of CyrI (a 2-oxo-glutarate-dependent iron oxygenase).¹³² The stereoselectivities of the different orthologs of CyrI appear to be responsible for the variable proportions of **47** to its 7-epimer that are found among **47**-producing strains.¹³²

It was precisely the availability of the gene cluster for **47** that allowed the inference regarding the involvement of this alkaloid in P_i metabolism in *A. ovalisporum*.¹²⁴ This cyanobacterium forms an annual bloom in Lake Kinneret, Israel, which is strongly correlated to AP activity in the lake water. Initially, this activity had been thought to originate from the release of APs by *A. ovalisporum*.¹³³ However, subsequent observations suggested otherwise. These included lack of AP activity, a large P_i quota, and a high amount of polyphosphate bodies in *A. ovalisporum* filaments at the beginning of the bloom. Moreover, *cyr* genes were induced under P_i deprivation. These data motivated a series of laboratory- and field-based experiments that exposed the elegant mechanism by which *A. ovalisporum* deals with the low P_i levels. Under such conditions and when cellular reserves of this nutrient are low, this cyanobacterium enhances its production and release of **47** to the surrounding medium. Concomitantly, the transcript levels of an intracellular AP (*phoD*) and those of a high affinity P_i uptake system component (*pstS*) are upregulated. The presence of **47** in the growth medium causes an increase in extracellular AP-activity in *C. reinhardtii* as well as an upregulation of genes that are induced under P_i deprivation in this microalga. Similar effects were observed with other phytoplankton species. As such, Bar-Yosef *et al.*¹²⁴ have concluded that *A. ovalisporum* and other cylindrospermopsin-producing organisms use this metabolite to induce a sort of “enslavement” by which susceptible phytoplankton species increase their production and release of APs. The now-available P_i is efficiently scavenged by the cylindrospermopsin-producing cyanobacterium which has readied its uptake system for this nutrient. The competitive advantage of this novel mechanism may reside in its reduced nitrogen budget and may help explain bloom-formation by cyanobacteria producing **47**.¹¹²

2.5. Signalling

Bacteria communicate inter- or intra-species through chemical signals or cues that are released into the environment. Quorum-sensing (QS) systems are widespread among

bacteria and constitute a mechanism by which such organisms communicate, sense their environment and coordinate their behaviours.¹³⁴ Independently of being inter- or intra-specific, such systems typically comprise emitter cells, which produce a QS signal. When these signal molecules reach above a certain threshold concentration, they lead to a response in sensitive-cells.¹³⁴ Different QS systems are currently known, and these have been thoroughly reviewed elsewhere from a variety of perspectives.^{e.g. 134-136} For the purpose of this review, we will focus on the *N*-acyl homoserine lactones (AHLs)-mediated signalling, an intraspecific QS-system.¹³⁵ AHLs have the same general structure (**51**), with oxygenation possible at C₃ and side chain lengths varying from C₄ to C₁₈. Usually, producing organisms synthesize more than a single AHL via LuxI or homologous proteins. These signals flow freely across cellular membranes and are recognized by cytoplasmic LuxR-type receptors, leading both to increased AHL production and to a signalling cascade that results in population-wide gene expression changes.¹³⁵ AHL-mediated QS has been shown to be involved in a wide range of cellular processes (*e.g.* motility, bioluminescence, biofilm production, virulence, antibiotic production), in different environments.¹³⁶

To date, LuxI or LuxR homologues have not been found in cyanobacteria. This, however, does not rule out AHLs production by cyanobacteria. In fact, the production of *N*-octanoyl homoserine lactone (**52**) by two *Gloeotheca* sp. strains has been reported.¹³⁷ The authors further reported that for one of the strains, the sheathless mutant *Gloeotheca* sp. PCC 6909/1, the accumulation of **52** in the culture medium followed a pattern typical of a QS system. Initially, low accumulation of **52** was observed, despite regular cyanobacterial growth, and then there was an abrupt increase after a certain point in the culture cycle. Moreover, several proteins related to carbohydrate and amino acid metabolism were differentially expressed when this strain was treated with **52**.¹³⁷ This work was the first to report the finding of a QS signal in cyanobacteria. However, Marner and Moore¹³⁸ had communicated the isolation of *N*-butyryl-*L*-homoserine lactone (**53**) in the late 1970's, a few years before AHLs were associated with QS.¹³⁹

While no other studies present evidence for AHL production by cyanobacteria, given the chemoecologically dynamic nature of microbial communities, and the known roles played by AHL-mediated QS, it seems reasonable that cyanobacteria may have developed the ability to sense, or interfere with this kind of widespread QS system. This seems to be the case regarding the production of an AHL-degrading acylase by *Anabaena* sp. PCC 7120,¹⁴⁰ purportedly produced as a defence mechanism against interference of AHLs with nitrogen fixation.¹⁴¹ Cyanobacteria produce several secondary metabolites structurally resembling AHLs and exhibiting AHL-like or QS-interfering activities. The first such metabolites to have been reported from a cyanobacterium were tumonoic acids E-H (**54-57**), isolated from *Blennothrix cantharidosmum*.¹⁴² These compounds were isolated based on their cytotoxic properties, however, given that their structures were somewhat similar to those of AHLs, the authors interrogated their activities in QS-systems. Metabolites **54-57** hampered bioluminescence production in wild-type *Vibrio harveyi* at micromolar concentrations.¹⁴² However, since this phenotype is regulated by different QS-systems, the mechanism of action could not be pinpointed.

Subsequently, the *L. majuscula* metabolite malyngamide C¹⁴³ (**58**) and its epimer 8-*epi*-malyngamide C¹⁴⁴ (**59**) were shown to interfere with AHL-mediated QS in a bioreporter assay using *Escherichia coli*.¹⁴⁴ A large array of malyngamides has been reported from various cyanobacteria, and therefore the role of this family of fatty acyl-containing amides in signalling is yet to be explored.

In a screening program for QS-interfering activity¹⁴⁵, several cyanobacterial extracts were tested for their ability to impair violacein production by the reporter strain *Chromobacterium violaceum* CV017, a process which is mediated by short-chain AHLs. Remarkably, a large fraction of the extracts (19 out of 25) exhibited activity in the bioassay. The QS-inhibiting compound present in one of the most active extracts, a *L. majuscula* strain, was found to be malyngolide (**20**)¹⁴⁵, a metabolite which had previously been described from this species (see Feeding Deterrence section). Further testing with *Pseudomonas aeruginosa* reporter strains suggested a possible detrimental effect of **20** on *lasR* (a *luxR* homologue) expression as the mechanism for QS-inhibition by this metabolite. Malyngolide was also shown to be released to the medium by *L. majuscula* cells.¹⁴⁵

Lyngbyoic acid (**60**) is a simple cyclopropyl-containing metabolite that was isolated as an abundant constituent of a Floridan *L. majuscula* strain.¹⁴⁶ When tested in *E. coli* QS bioassays featuring different LuxR homologues, **60** showed micromolar-level QS-inhibitory activity in three such assays. Conversely, no such activity was found with a *A. tumefaciens* reporter.¹⁴⁶ Gene expression profiling in *P. aeruginosa* allowed inference of an important role for the cyclopropyl moiety in the QS-inhibitory activity of **60** in that the structurally related dodecanoic acid mostly elicited stimulation of QS in this bacterium.¹⁴⁶

As a benthic and sheath-producing cyanobacterium, it is conceivable that *L. majuscula* utilizes secondary metabolites to condition the behaviour of the microbial communities sharing its substrate or associated with its sheath. The production of QS-interfering compounds can also be expected from other, less studied, cyanobacteria.

The natural role of the extensively studied cyanobacterial cyclic peptides microcystins, in particular that of microcystin-LR (**61**),¹⁴⁷ has been a controversial topic^{54, 148} (see also Feeding Deterrence section). Recently, metabolite **61** has been implicated in intraspecific signalling in *Microcystis* sp.¹⁴⁹ Compound **61** is not released to the surrounding medium by live *Microcystis* cells, but rather, upon cell lysis or as a result of grazing.¹⁵⁰ Schatz and co-workers¹⁴⁹ have shown that intact *Microcystis* sp. KLL MG cells greatly increase their production of **61** when exposed to a cell lysate from the same strain or to exogenous **61**. Concomitantly, high-levels of McyB (encoded in the biosynthetic pathway for **61**, see section **3.1.**, and involved in the production of this metabolite) accumulated in the intact cells. The authors propose that these events reflect a defence mechanism by which the *Microcystis* population is able to sense the lysis of nearby individuals (due, for instance, to grazing), and consequently, to increase its toxicity.¹⁴⁹ This implies a level of biological organization close to multicellularity for *Microcystis*.

2.6. Other

Several other ecological features of cyanobacteria reveal an interesting underlying chemistry. Cyanophycin (**62**)¹⁵¹ is a polymer composed of L-arginine and L-aspartate units that is synthesized non-ribosomally by a single protein termed cyanophycin synthetase.¹⁵² The backbone of the polymer consists of poly-L-aspartate, and the β -carboxy group of each of these residues is connected to the α -carboxy group of L-arginine via an isopeptidic bond. Cyanobacteria use **62** as a storage form of nitrogen (N).¹⁵¹ The polymer forms cytoplasmic “cyanophycin granules” when N is available. Under N starvation, **62** is broken down enzymatically and readily used by the cell.¹⁵³ The chemical proficiency of cyanobacteria is also reflected in the way they have evolved to optimize harnessing of energy from sunlight at different wavelengths.¹⁵⁴ Examples of this are the tetrapyrrolic chromophores of several phycobiliproteins (phycobilins), such as phycocyanobilin (**63**)¹⁵⁵ or phycoerythrobilin (**64**),¹⁵⁶ as well as the recently discovered chlorophyll f (**65**). This latter pigment was isolated from Australian stromatolites and absorbs solar radiation further to the red ($\lambda_{\text{max}} = 706 \text{ nm}$) than other chlorophylls.¹⁵⁷

In a recent report, Zilliges *et al.*¹⁵⁸ have described a potential role for microcystins (*e.g.* **61**) in modulating protein status in producer cells. The authors compared the proteomes of microcystin-producing *Microcystis aeruginosa* PCC 7806 and its microcystin deficient mutant *mcyB* under different light and oxidative stress conditions. The abundance of a number of proteins involved in photosynthesis and energy metabolism varied between the two strains. Many of these were shown to be covalently bound to microcystins, which led the authors to hypothesise a physiological regulatory role for this interaction. Further experiments supported such a hypothesis, as the mutant strain showed a higher susceptibility to high-light conditions and oxidative stress.¹⁵⁸ These findings are particularly interesting, as the biosynthesis of microcystins has been found to be under transcriptional regulation by light.^{159, 160}

3. Evolutionary aspects of cyanobacterial chemical ecology

The evolution of secondary metabolite structure and of the underlying biosynthetic machinery is intimately connected to the ecological history of the producing organisms. The molecular mechanisms involved in the synthesis of secondary metabolites by bacteria are among life's most diverse and rapidly evolving genetic elements, evolving over shorter time scales relative to the genes of higher organisms,¹⁶¹ and therefore are fundamental elements to understand the molecular evolution and chemical diversity of natural products. As one of the most ancient and chemically diverse groups of organisms on Earth, cyanobacteria present as a unique subject to study the interplay between chemistry and ecology occurring through time. In particular, the large number of NRPS or hybrid PKS-NRPS derived metabolites has provided researchers with the tools to interrogate their biosynthesis and the intriguing biochemistry that emerges from these pathways. From an evolutionary perspective, it has been possible in a few cases to integrate these genetic and biochemical findings with their putative ecological purpose. Below we highlight several such studies rather than present a comprehensive account of the evolution of biosynthetic pathways or associated enzymes.

The chemical and phylogenetic diversity among cyanobacteria also deserves attention in this review. Cyanobacterial taxonomy is currently under broad revision, and the apparent incoherence between secondary metabolite distribution and morphological diversity is an active topic of research. These subjects are of considerable interest for practical reasons, but they assume exceptional relevance to evolutionary and conservation studies.

3.1. Biosynthetic gene cluster evolution

The microcystins are cyclic heptapeptides (*e.g.* **61**) (see Section 2.4.) and are by far the most studied of the cyanobacterial toxins. They are produced by cyanobacterial strains belonging to quite distant genera, such as *Microcystis*, *Anabaena*, *Anabaenopsis*, *Hapalosiphon*, *Nostoc* and *Planktothrix*.¹⁵⁰ The related cyclic pentapeptide nodularin (**66**) is produced by brackish water strains of the genus *Nodularia*.¹⁵⁰ These two cyclic peptides are end products of phylogenetically related secondary metabolite pathways comprised of PKSs and NRPSs.^{54, 162} The biosynthesis of **61** and analogues is accomplished by a gene cluster spanning ≈ 55 kb composed of nine to ten genes (*mcyA-J*) that encode a giant enzyme complex comprising NRPSs, PKSs, and additional tailoring enzymes.^{163, 164} These peptide products exhibit high structural variation but all share a common cyclic structure (Adda-D-Glu-Mdha-D-Ala-X-D-MeAsp-Z), where X and Z are variable L-aminoacids, Adda is 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid, Mdha is N-methyldehydroalanine, and D-MeAsp is D-erythro- β -isoaspartic acid. Nodularin lacks the 'X' residue and bears an Mdhb (N-methyl-dehydrobutyrine) residue instead of Mdha. A variety of amino acids occupy the X and Z positions whereas all of the other positions remain conserved. This allows for the combinatorial production of an appreciable number of different molecules, with nearly 90 structural variants described to date.¹⁶² Several studies have evaluated genetic variability in the genes of the microcystin gene cluster, especially for those that are involved in the production of the various natural product isoforms,¹⁶⁵⁻¹⁶⁷ and have uncovered a high degree of recombination among the respective adenylation domains. Taken together, these studies make understandable the basis for structural variability of the microcystins, particularly in the hyper-variable residue positions. This hyper-variability likely reflects environmental adaptation and selection of different chemotypes. Interestingly, the cyanobacterial genera with microcystin-producing strains often have related strains that do not produce the toxins, and this appears to be a consequence of an absent or non-functional microcystin gene cluster.^{163, 168} While this distribution of *Microcystis* strains producing **61** and/or its analogues had first been hypothesized to be the result of horizontal gene transfer, recent phylogenetic analyses have indicated a high degree of congruence (co-evolution) between housekeeping genes and microcystin synthetase genes, which argues against the horizontal transfer hypothesis and supports an ancient origin and vertical transmission of microcystin biosynthesis.⁵⁴ One other group of cyanotoxins, saxitoxin (**67**) and some of its derivatives (STXs), are carbamate alkaloids that affect the neuronal impulse generation by selectively blocking the voltage-gated sodium and calcium channels, and by modifying potassium channel gating in excitable cells.¹⁶⁹ Only a few cyanobacterial strains are known to produce STXs, and, as for the microcystins, these are from distantly related genera (*Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Planktothrix* and *Raphidiopsis*).^{169, 170} STXs-producing isolates from these genera exhibit different STXs profiles, even within the same species.^{171, 172} Such varying toxin profiles appear to result

from the presence or absence of genes coding for tailoring enzymes in the saxitoxin gene cluster (*sxt*), recently identified in several cyanobacterial species. The biosynthetic pathway is composed of up to 26 genes whose products are involved in a complex sequence of over 30 steps.^{127, 173-175} Evolutionary analyses of the *sxt* cluster show that it has been preserved by strong stabilizing selection and relatively low recombination rate. This points toward vertical transfer and an ancient evolutionary history, dating back to the divergence of the Nostocales.¹⁷⁶ Taking into account this ancient evolutionary origin, Murray *et al.*¹⁷⁶ have proposed that the STX predecessors original targeted potassium channels, as early organisms such as bacteria had not yet evolved voltage-gated sodium channels. While the *sxt* cluster has been extraordinarily conserved as a whole, during evolutionary history a variety of gene losses, recombination, rearrangement and duplication events have resulted in the present diversity of STXs.¹⁷⁶ Indeed, since the appearance of these metabolites, the aquatic environment that has harboured STX-producing cyanobacteria has undergone profound physical and biological changes. Hence, the natural role of these compounds, while not clearly established today (several roles have been put forward for **67**, including homeostasis,¹⁷⁷ chemical defence and signalling¹⁷⁸), has certainly been of vital importance.¹⁷⁶

The huge diversity of cyanobacteria species and related synthesized secondary metabolites provides a wealth of opportunities for genomics research. Currently, more than 60 cyanobacterial genome sequencing projects are underway and publicly available. Genome analysis is a very recent tool in cyanobacterial research but has been used quite successfully to infer important ecological, physiological and biotechnologically relevant characteristics of cyanobacteria.¹⁷⁹ Genomic data for a few chemically prolific cyanobacteria, particularly from the orders Nostocales and Chroococcales has been made available in the past decade.¹² Very recently, a genome project¹⁸⁰ aimed precisely at gaining insight into the secondary metabolism of *L. majuscula* 3L, a Caribbean strain producing the lipopeptides barbamide (**68**),¹⁸¹ curacin A (**69**)¹⁸² and carmabin A (**70**)¹⁸³ revealed several unexpected findings. Jones *et al.*¹⁸⁰ observed that only ~293,000 nucleotides of the draft genome were attributable to secondary metabolism, a genomic portion unable to be responsible for the large number (over 200) of known *Lyngbya* metabolites, and suggesting that these are produced by different strains or chemotypes. In this study, the authors also found evidence for a complex gene regulatory network, which may be related to environmental adaptation, but also to microbial associations. Of note, it is conceivable that at least some of the metabolites reported from *Lyngbya* may in fact derive from metabolic capacities of these other bacteria.¹⁸⁴ These first genome data for a marine *Lyngbya* species¹⁸⁰ clearly calls for further genomic studies of other *Lyngbya* strains as these data will be invaluable to understanding the diversification of secondary metabolism in this genus. However, the chemical prolificacy of cyanobacteria identified as “*Lyngbya*” will be further discussed in the following section of this review (3.2.).

3.2. Morphological, ecological and chemical diversity

As one of the most ancient organisms on the planet, cyanobacteria have, over the last three billion years, adapted to almost all conceivable habitats and are among the most abundant and geographically widespread group of prokaryotes known.^{185, 186} A consequence of these

adaptations over this long evolutionary period is a rather extensive degree of biological and chemical diversity. Unfortunately, the taxonomic systems used to traditionally classify cyanobacteria have not recognized the true extent of this biodiversity. Moreover, the taxonomic distribution of secondary metabolites in cyanobacteria has been described as highly uneven with certain taxa greatly overrepresented in their chemical richness.¹⁰ For example, the cyanobacterial genus *Lyngbya* has been attributed with a total of 326 secondary metabolites or over 60% of all marine cyanobacterial metabolites reported to date.¹⁸⁷ However, a major reason for this skewed taxonomic perception is that the taxonomy of cyanobacteria has been oversimplified and this has resulted in lumping together organisms with very different evolutionary histories.

Historically, the taxonomic classification of cyanobacteria has been based primarily upon morphological characterizations.¹⁸⁸ Moreover, the first comprehensive classification system was constructed based on morphology of European field-collected specimens, and this then formed the basis of relatively modern cyanobacterial systematics.¹⁸⁹ However, many of the traditional phenotypic traits used for taxonomic characterization have been shown to be variable and influenced by environmental factors.¹⁹⁰ More recently, cyanobacteria have largely been accepted to represent a bacterial phylum, and a more comprehensive Bacteriological Code of systematics that incorporates biochemical, genetic, and physiological data has consequently been proposed.¹⁹¹⁻¹⁹³ More importantly, both the Bacteriological Code and the traditional Botanical Code have begun incorporating the evolutionary histories and phylogenetic relationships as a fundamental framework to establish biologically informative classification systems.¹⁹⁴⁻¹⁹⁶ Therefore, to uncover and understand true cyanobacterial biodiversity, new specimens must be placed in a phylogenetic perspective with the type-strains that were used to establish our current taxonomic system.

In regards to the chemically prolific organism *Lyngbya*, this genus has been shown to be a polyphyletic group composed of multiple morphologically similar but phylogenetically distant groups.^{197, 198} Moreover, natural products attributed to *Lyngbya* have been isolated from the various unrelated evolutionary lineages and the polyphyly of this group has consequently been shown to be a major reason for some of the chemical prolificacy of *Lyngbya*.¹⁹⁹

In attempts to better understand biological diversity of chemically rich cyanobacteria, one of the tropical marine *Lyngbya* lineages was recently described as the new genus *Moorea* gen. nov. by polyphasic approaches.²⁰⁰ Several important *Lyngbya*-attributed natural products, such as curacins A (**69**) and D (**71**),²⁰¹ barbamide (**68**), dechlorobarbamide (**72**),²⁰² carmabins A (**70**) and B (**73**),¹⁸³ dragonamides C (**74**) and D (**75**)²⁰³ have been shown to be produced by *Moorea*.^{197, 199} At the same time, other *Lyngbya*-attributed natural products were shown to be produced by other yet undescribed cyanobacteria with morphological similarities to *Lyngbya*. For example, microcolins A (**76**) and B (**21**),⁶⁴ tumonoic acids A-C (**77-79**), and methyl tumonates A (**80**) and B (**81**)²⁰⁴ have all been re-isolated from specimens most closely related to the genus *Trichodesmium*,^{197, 199} but which, in all likelihood, need to be described as novel generic entities.²⁰⁵ While further studies will need to determine how many *Lyngbya*-attributed natural products are actually being produced by

Lyngbya, it is interesting to note that to date, no secondary metabolites have been shown to be produced by *Lyngbya* reference-strain PCC 7419 or related specimens.

In fact, most natural product-rich cyanobacterial groups appear to be polyphyletic, which suggests a great extent of novel biodiversity among these prokaryotes. Moreover, the current lack of description of these groups has skewed our taxonomic understanding of the distribution and evolution of bioactive secondary metabolites in cyanobacteria.

4. Making use of chemoecological interactions

The potential applications of cyanobacterial metabolites, in particular for biotechnological purposes, are based on a substantial body of research which has been previously reviewed.^{206, 207} In this section, we emphasize the contribution of chemical ecology to helping to realize the biotechnological potential of these organisms. This contribution is most evident when the natural role played by a particular metabolite is closely related to the proposed technological application. For example, compounds that are discovered based on their ecologically-relevant antibacterial or antifungal properties, may find direct use in the clinic, should they act on features shared by clinically relevant targets. However, studies describing antibacterial or antifungal activity from cyanobacterial extracts or purified metabolites have most often employed clinical isolates or other biotechnologically important bacteria or fungi as test strains.²⁰⁸ It is, therefore, difficult to ascertain whether such biotechnologically-relevant antimicrobial agents have a similar function in the natural setting. A large fraction of the reported biologically active cyanobacterial metabolites have been isolated precisely on the basis of application-related activities (*e.g.* cancer cell cytotoxicity,²⁰⁹ enzyme inhibition,²¹⁰ ion channel activation²¹¹). Consequently, the natural function for the majority of these compounds is not known, and therefore, they are not covered in the present review.

Naturally occurring cyanobacterial sunscreens may find a homologous application in human photoprotection. In fact, the incorporation of particular mycosporines/MAAs or scytonemin in cosmetic sunscreens has been proposed,²⁰⁶ and evidence supports the photoprotective role of compounds **1**, **2** and **3** in human fibroblasts.²¹² A formulation containing a mixture of MAAs **2** and **3** is currently commercialized under the brand name Helioguard 365®. Additionally, the antioxidant properties (see Section 2.1.) of mycosporines and MAAs may increase the attractiveness of these metabolites as components of cosmetic formulations.

A recent study²¹³ revealed that siderophores may be used to bring into culture previously uncultured microorganisms. Of special relevance, the authors have demonstrated that the presence of siderophores produced by microorganisms isolated from the same environment are needed in the culture of other isolates.²¹³ Because the overwhelming majority of marine bacteria have not yet been cultured,²¹⁴ it is expected that an immense biological and chemical diversity will arise from the successful culture of these microorganisms.²¹⁵ The siderophore-based strategy presented by D'Onofrio *et al.*²¹³ to reach untapped biological diversity is therefore promising, and, in that context, cyanobacterial siderophores (known or not) may assume an important role in future drug discovery efforts. These iron chelators may also hold direct pharmaceutical potential, for example, in the treatment of Fe-related

pathologies. The bacterial siderophore desferrioxamine B (**82**) is widely used to treat Fe overload occurring as a result of diverse aetiologies.²¹⁶ Chemical synthesis efforts may also benefit from chemoecological information. The metal-binding properties of siderophore **44** were exploited to anchor poly(ethylene glycol) (PEG) to a metal oxide surface, resulting in a stable, fouling-resistant coating.²¹⁷

Insecticidal activity has not been cited often as the natural role of known cyanobacterial secondary metabolites. However, the toxicity of several hapalindoles towards grazing insects (see section 2.2), together with the insecticidal activity of *N*-methylwelwitindolinone C isothiocyanate (**83**) to blow-fly larvae⁷² suggest that this kind of interaction may be a common and useful feature of cyanobacteria. As such, cyanobacterial secondary metabolites may find more broad application as insect repellents and control agents of disease vectors in the future. Analogously, novel algal bloom mitigation or antifouling agents and herbicides may draw inspiration from cyanobacterial allelochemicals (see section 2.3.), as recently reviewed.^{79, 218}

One particularly promising application of cyanobacterial natural products concerns QS-inhibition. Since many bacteria coordinate virulence and biofilm-forming phenotypes using QS,²¹⁹ accessing the chemotypes that cyanobacteria have evolved to interfere with this process may help develop effective treatments for QS-associated pathologies. Alternatively, some cyanobacterial metabolites may be useful tool compounds for defining the role of QS in human disease. For example, the QS-inhibiting fatty acid **60** elicits a response in *P. aeruginosa* which is similar to the phenotype this bacterium exhibits in cystic fibrosis patients.¹⁴⁶ The authors argue that fatty acid-related signalling could be involved in modulating the changes in genetic composition that occur during chronic infections of *P. aeruginosa* in these patients, and, as such, **60** may provide useful as a research tool.¹⁴⁶

5. Conclusions

Cyanobacteria continue to constitute an extremely productive source of novel secondary metabolites. The underlying chemical ecology is gradually being harnessed, and the field continues to grow into a truly interdisciplinary area of research.

It becomes clear, from this review and others, that screening studies and field observations of cyanobacteria have been the most productive approach for uncovering and characterizing chemoecological interactions. In particular, field studies followed by laboratory investigations (both biological and chemical) have allowed a clear identification of the natural roles of several cyanobacterial metabolites.

However, emerging methodologies in genomics and analytical chemistry will certainly strongly impact this field in coming years. The steeply-decreasing price of DNA sequencing is expected to modify the paradigm of natural product discovery from marine microorganisms. Genes from environmental samples will be used to predict interesting chemical scaffolds with subsequent functional expression in heterologous hosts, even for gene clusters that are silent in the originating wild type organism.²²⁰ While this may be more difficult to accomplish for cyanobacteria than for other microorganisms,¹² we can still

expect this to become a mainstream approach in the future. As a result, large chemical and biological diversity datasets are expected to be available, which in turn will allow for better chemotaxonomic trends to be determined. Further, these data will enable a better understanding of the geographical patterns of secondary metabolite distribution, and this can easily be linked with quantitative data from the field and to the ecology of the producing cyanobacteria.

Mass spectrometric (MS) imaging methodologies²²¹ will certainly have an increasingly important role in chemoecological studies, as hinted by pioneering work from the past few years.^{222, 223} These techniques should facilitate the study of metabolite distribution at the organism level as well as give insight into information exchange between microorganisms or even some macro-organisms, and thus become instrumental for the discipline of chemical ecology. Furthermore, when combined with chemotaxonomy, MS imaging techniques will allow scrutinizing the biological origin of a natural product of interest¹⁸⁴ and allow development of a better chemical picture of the ecological relationships between cyanobacteria, various eukaryotic hosts, and heterotrophic bacteria.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

PNL acknowledges a postdoctoral fellowship (SFRH/BPD/70233/2010) from Fundação para a Ciência e a Tecnologia (FCT); AA was funded in part by the PTDC/AAC-AMB/104983/2008 (FCOMP-01-0124-FEDER-008610) and PTDC/AAC-CLI/116122/2009 (FCOMP-01-0124-FEDER-014029) projects from FCT. VV acknowledges financial support from Interreg Programs, STC-CP2008-1-555612 Atlantox, 2009-1/117 Pharamatlantic. Work in the Gerwick laboratory is supported by NIH grants CA100851, TW006634, CA108874, and NS053398.

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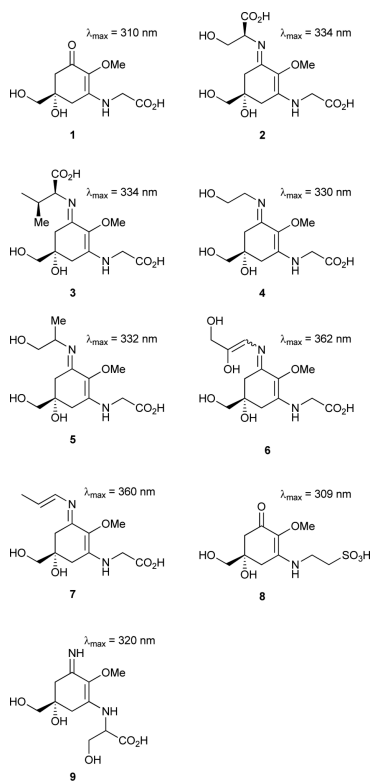
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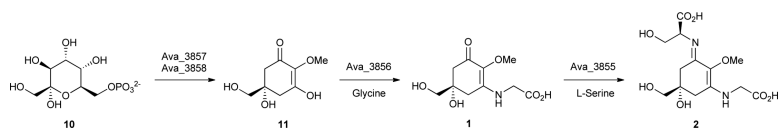
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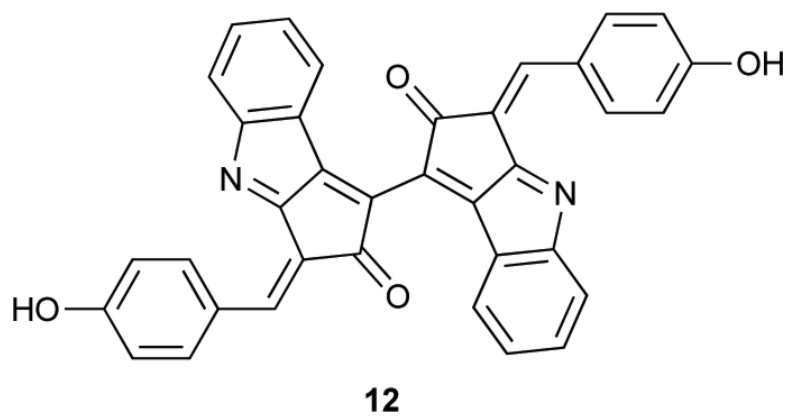
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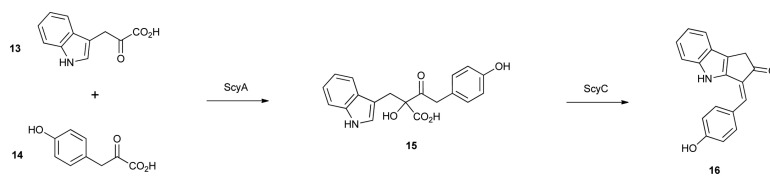
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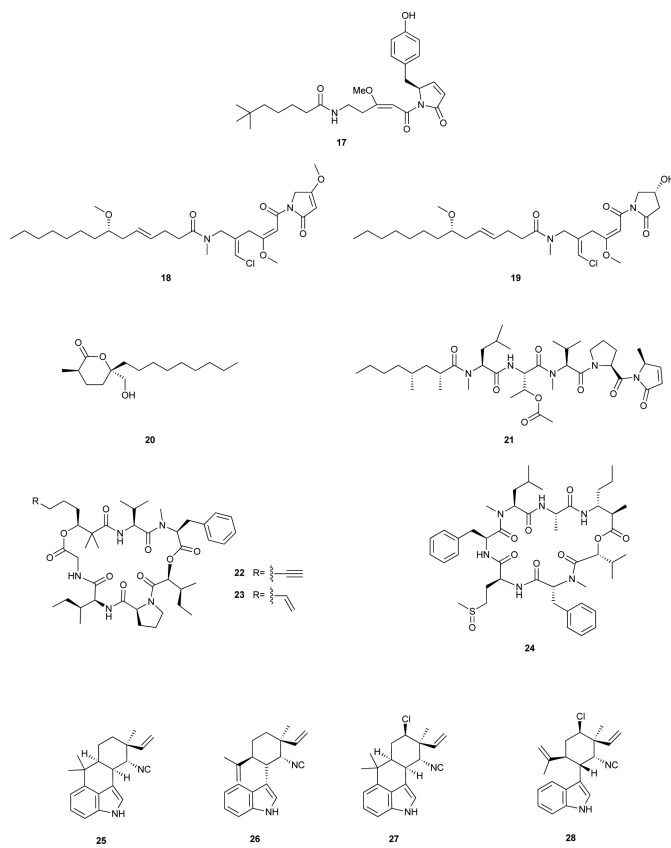
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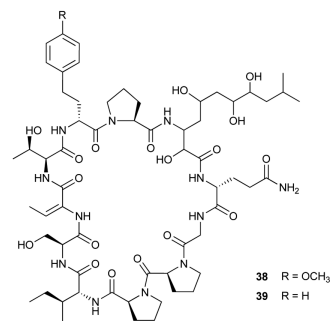
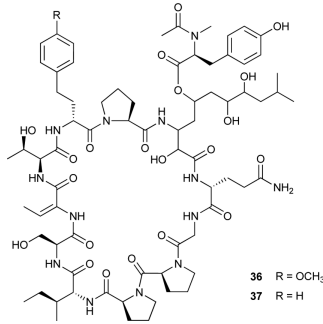
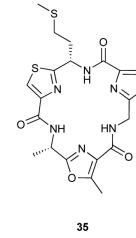
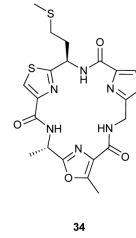
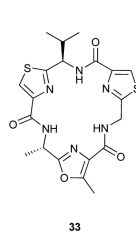
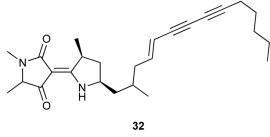
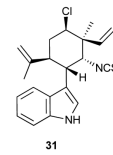
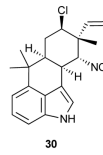
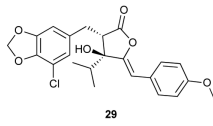
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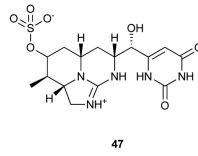
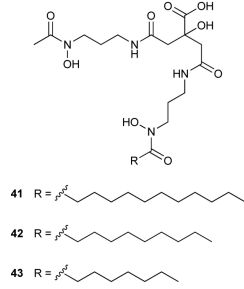
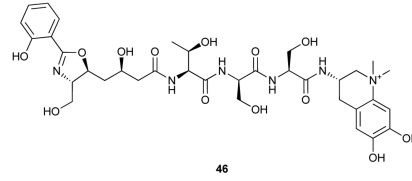
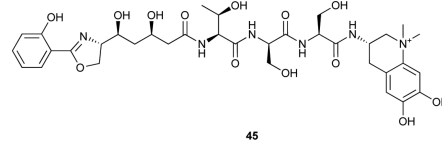
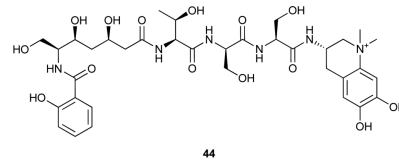
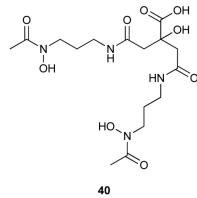
Structure 3.

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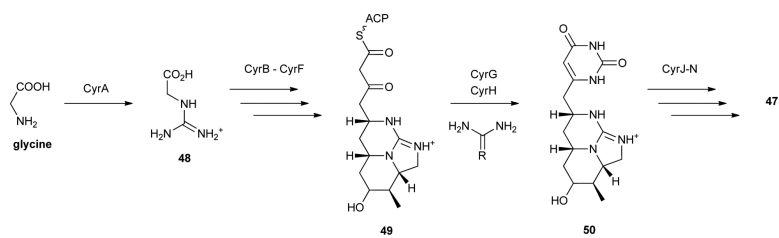
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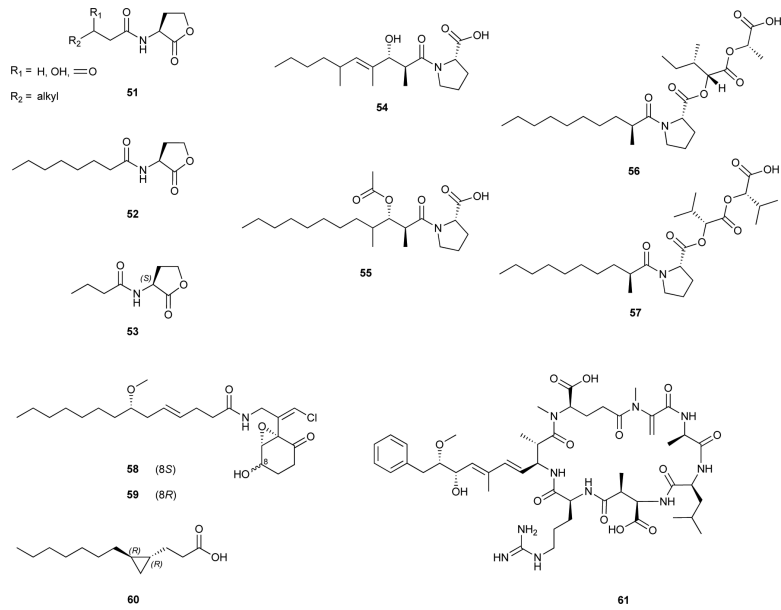


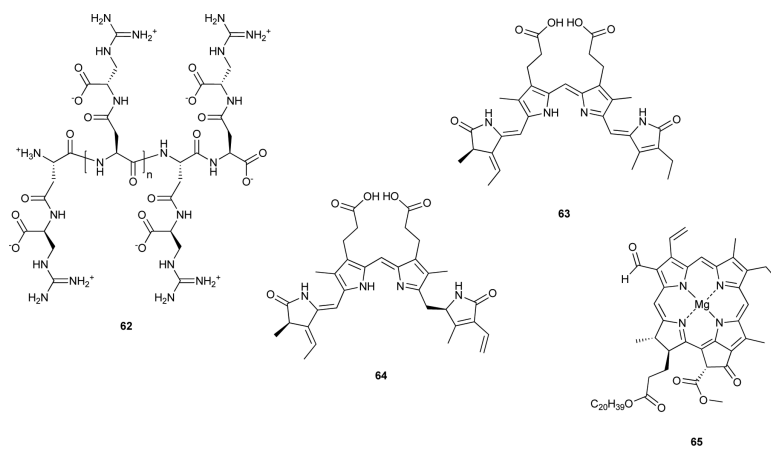
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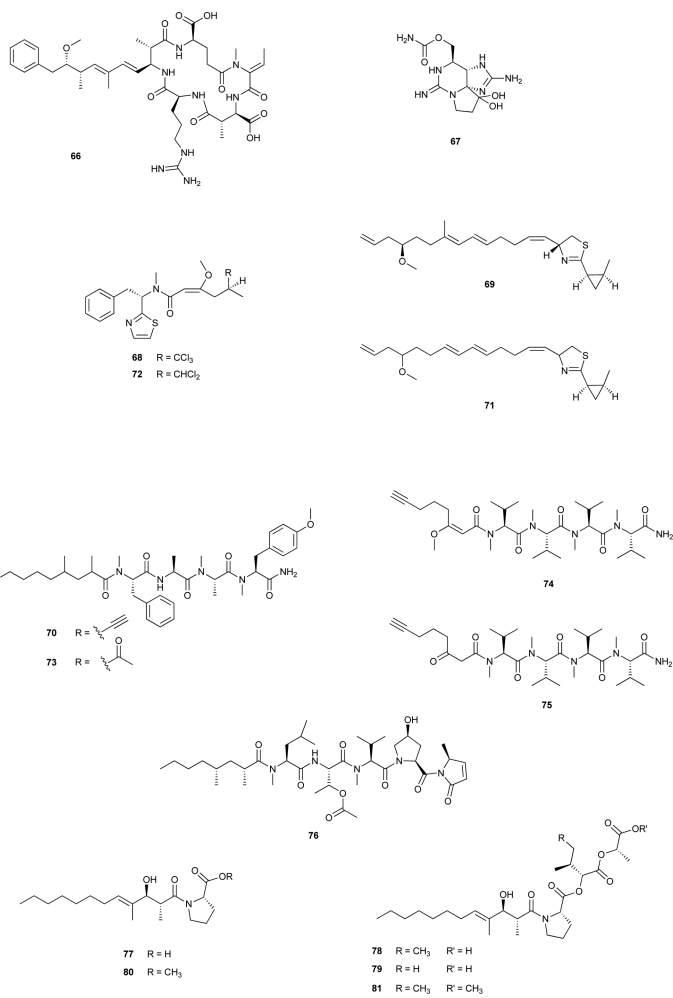


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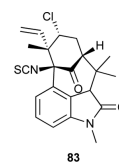
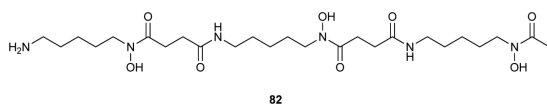
**Structure 8.**

**Structure 9.**

**Structure 10.**



Structure 11.

**Structure 12.**