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MINI-REVIEW

Cyanobacteria: photosynthetic factories combining biodiversity, radiation resistance, and genetics to facilitate drug discovery

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Abstract Cyanobacteria are ancient, abundant, and widely diverse photosynthetic prokaryotes, which are viewed as promising cell factories for the ecologically responsible production of chemicals. Natural cyanobacteria synthesize a vast array of biologically active (secondary) metabolites with great potential for human health, while a few genetic models can be engineered for the (low level) production of biofuels. Recently, genome sequencing and mining has revealed that natural cyanobacteria have the capacity to produce many more secondary metabolites than have been characterized. The corresponding panoply of enzymes (polyketide synthases and non-ribosomal peptide synthases) of interest for synthetic biology can still be increased through gene manipulations with the tools available for the few genetically manipulable strains. In this review, we propose to exploit the metabolic diversity and radiation resistance of cyanobacteria, and when required the genetics of model strains, for the production and radioactive (^{14}C) labeling of bioactive products, in order to facilitate the screening for new drugs.

Keywords Cyanobacteria · Biodiversity · Secondary metabolites · Toxins · Radioactive labeling

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Introduction

Cyanobacteria, the only prokaryotes performing oxygenic photosynthesis, are regarded as the oldest phylum of Gram-negative bacteria that shaped the atmosphere of our planet (Hamilton et al. 2016). Contemporary cyanobacteria continue to use solar energy to assimilate tremendous quantities of CO2 (Jansson and Northen 2010) and nitrogen (NO_3 and N_2) (Zehr 2011) into a huge biomass that sustains a large part of the food chain. Hence, the edible cyanobacteria of the genus *Spirulina* are being tested as a way to replenish O2, provide food, and recycle wastes (CO2, NO_3, and urea) during long-term space missions (Mazard et al. 2016). Furthermore, cyanobacteria are also viewed as promising microbial factories for the production of chemicals from highly abundant natural resources: solar energy, water, CO_2, and minerals (Savakis and Hellingwerf 2015).

In colonizing most waters (fresh, brackish, and marine) and soils of our planet, where they face environmental challenges (Cassier-Chauvat and Chauvat 2015) and competition (or symbioses) with other biological organisms (Salvador-Reyes and Luesch 2015), cyanobacteria have developed as widely diverse organisms (Narainsamy et al. 2013). They display various cell morphologies (Cassier-Chauvat and Chauvat 2014) and genome sizes (from 1.44 to 12.07 Mb; http://genome.microbedb.jp/cyanobase/ and http://genome.jgi.doe.gov/). In addition, they produce a wealth of natural products, which are often called secondary metabolites because they are not generally biosynthesized by the main metabolic pathways (Dittmann et al. 2015; Micallef et al. 2015). These cyanobacterial secondary metabolites can influence (i) their tolerance to environmental stresses (for example absorb UV rays), (ii) their interactions with their competitors, predators, or symbiotic hosts, and (iii) human health (antioxidants, vitamins, antibacterial, antifungal, antiviral, toxins)

(Kleigrewe et al. 2016; Narainsamy et al. 2016; Salvador-Reyes and Luesch 2015). Altogether, it has been estimated that about 20% of the natural products approved by the American "food and drugs administration," or under clinical trials, are originating from cyanobacteria (Salvador-Reyes and Luesch 2015). For examples, several compounds formerly regarded as being produced by higher organisms, such as (Fig. 1) dolastatin (sea hare), leucamide A (sponge), and westiellamide (tunicate) are actually synthesized by symbiotic cyanobacteria (Mazard et al. 2016).

Some of these cyanobacterial products polluting waters can threaten human health through compromising the quality and/or quantity of drinking water supplies and/or our food chains (Mazard et al. 2016). The best-studied examples of cyanobacterial toxins are the neurotoxins anatoxin A and saxitoxin (Fig. 1) produced by *Anabaena flos aquae* and the hepatotoxin microcystins (inhibitors of protein phosphatase) produced by *Microcystis* species, which colonize fresh and estuarine waters. Interestingly, microcystins can combine to glutathione, the widely conserved anti-oxidant tripeptide (Narainsamy et al. 2013), under the form of microcystin-glutathione complexes that operate in microcystin detoxification (Miles et al. 2016). In truly marine environments, the toxins (such as curacin A and microviridins that inhibit tubulin polymerization and serine protease, respectively) are produced by the cyanobacteria *Lyngbya* (re-named *Moorea*), *Nodularia*, *Oscillatoriales*, and *Trichodesmium* (Mazard et al. 2016).

Brief genomic view of the production of cyanobacterial bioactive compounds

The progress in spectrometric methods (mass spectrometry and nuclear magnetic resonance) and genomics (comparative analyses of genome sequences) have allowed the identification of cyanobacterial gene clusters that encode the synthesis of several known natural compounds, as well as the discovery of novel (low-abundant) secondary metabolites (anacyclamides, prochlorosins, viridamides, Fig. 1). Most cyanobacterial natural products are non-ribosomal peptides (NRPS, such as lyngbyatoxin), polyketides (PKS, such as anatoxin A), or hybrid peptide–polyketide compounds (for examples apratoxin, curacin A, microcystin, see Fig. 1), which are sequentially synthesized by NRPS and/or PKS enzymes (Dittmann et al. 2015; Kleigrewe et al. 2016; Mazard et al. 2016; Micallef et al. 2015). These assembly lines comprise several modules, each carrying out the addition of a single substrate to the lengthening molecule. Other cyanobacterial secondary metabolites are peptides synthesized by ribosomes and post-translationally modified, such as the N-acetylated tri- and tetra-decapeptides microviridins (serine protease inhibitors) produced by *Anabaena*, *Microcystis*, *Nodularia*, *Nostoc*, and *Planktothrix* (Dittmann et al. 2015; Micallef et al. 2015). These cascades of modular synthesis, assembly and modification activities produce a large array of secondary metabolites, some of which occurring in multiple variants (100 variants in the case of microcystin).

Cyanobacteria use about 5–6% of their genome length to direct the production of secondary metabolites. These gene clusters are often large (Dittmann et al. 2015; Kleigrewe et al. 2016; Micallef et al. 2015; Moss et al. 2016). For examples, the size of the *Moorea producens* 3L (formerly *Lyngbya majuscula* 3L) gene clusters encoding curacin A (an anti-cancer inhibiting tubulin polymerization) and barbamide (Fig. 1, a molluscicidal metabolite) are 64 and a 26 kb, respectively. The synthesis of aeruginosin (a protease inhibitor) is encoded by a 34-kb gene cluster in *Planktothrix* and *Microcystis*, while the synthesis of saxitoxin (a neurotoxic alkaloid) is directed by a 25.7 kb gene cluster in *Raphidiopsis brookii* D9. Many secondary metabolites are encoded by unknown genes, while the majority of the biosynthetic gene clusters code for unknown products (Dittmann et al. 2015; Kleigrewe et al. 2016; Micallef et al. 2015; Moss et al. 2016). Collectively, these findings indicate that the genetic diversity driving the synthesis of cyanobacterial natural products is largely exceeding their known chemical diversity. This large genetic diversity might be attributed to DNA recombinations, mutations, and horizontal gene transfers (Dittmann et al. 2015; Kleigrewe et al. 2016; Micallef et al. 2015; Moss et al. 2016).

Screening of cyanobacterial cell extracts for bioactive products: importance of ^{14}C-labeling

Cyanobacterial secondary metabolites are very interesting in constituting a unique source of widely diverse chemical probes to investigate proteins activity and networks (protein are the most frequent drug targets) and discover novel drugs and their targets. For example, the natural products, trapoxin and trichostatin A (Fig. 1), were pivotal to elucidating the structure function relations of histone deacetylases (Salvador-Reyes and Luesch 2015).

Screenings of secondary metabolites activities can be performed with target-based in vitro systems, eventually combined to in silico analysis (browsing databases in the quest for molecules fitting either an established pharmacophore model or the structure of a macromolecular target). Alternatively, the toxicity of natural products towards various tumor cell lines (brain, lungs, kidneys, etc.) can be evaluated and compared to those of anti-cancer drugs databases (Martins et al. 2014). The compounds with unique differential cytotoxicity profile are of interest because they likely possess a novel mechanism of action, and consequently, their target can be subsequently identified through affinity-chromatography

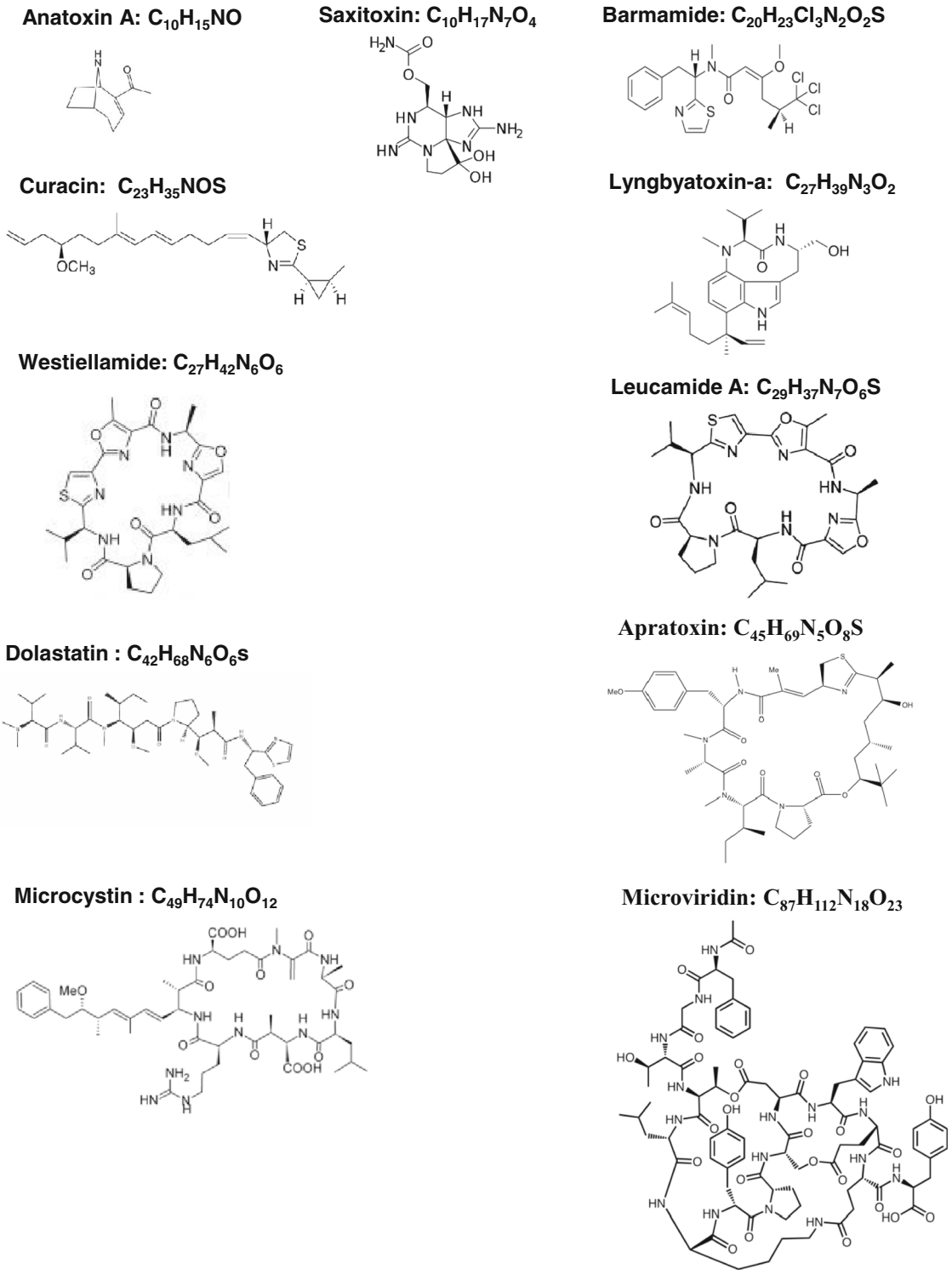

Fig. 1 Structure of the cyanobacterial secondary metabolites mentioned in this review

purification and mass spectrometry analyses. The action mechanisms of secondary metabolites can also be studied via transcriptome, proteome, and metabolome analyses of cells exposed to these small molecules (Salvador-Reyes and Luesch 2015).

However, the in vitro binding of a given ligand to isolated proteins or cells does not necessarily predict its bioactivity in vivo, which depends on its pharmacokinetic properties, i.e., delivery to the target tissue and specific versus non-specific binding and action (Eriksson et al. 2016). Hence,

rigorous studies at the level of the whole animal of ADME properties (absorption, distribution, metabolism, and excretion) of (low) doses of bioactive products are required to thoroughly assess their benefit-risk (Loser and Pietzsch 2015). For this purpose, ^{14}C-labeling of bioactive compounds is of great importance. Although the specific activity of ^{14}C-tracers is much lower than those labeled with short-lived nuclides, this limitation can be partly counterbalanced by employing tracers that contain multiple ^{14}C atoms per molecule (Loser and Pietzsch 2015). Furthermore, powerful (last generation) radio-imagers can be used for digital autoradiography detection in animal tissue sections of weakly labeled and very-low-abundant (few femtomoles) compounds (Czarny et al. 2014). Consequently, we propose to take advantage of the photosynthesis, and radiation resistance (caused by unknown processes) (Cassier-Chauvat et al. 2016), of cyanobacteria for the production of natural products uniformly labeled with ^{14}C.

Genetically manipulable model cyanobacteria can serve as cell factories for the production of ^{14}C-labeled bioactive products

Previous studies showed that cyanobacteria produce secondary metabolites only in low amounts, and they possess various "orphan" gene clusters encoding products of unknown function (Dittmann et al. 2015; Micallef et al. 2015). Since most cyanobacteria have no genetics yet, it is important to use or develop heterologous systems for efficient and stable expression of gene clusters encoding natural products. This strategy will allow to (i) increase the production of low-abundant secondary metabolites, (ii) elucidate their synthesis pathways, (iii) discover the function of "orphan" biosynthetic genes, and (iv) develop combinatorial biosynthesis to generate novel secondary metabolites and improve product diversification.

Well-known bacteria such as *Escherichia coli* have been used for the production of cyanobacterial secondary metabolites such as cyanobactin, lyngbyatoxin (Dittmann et al. 2015), and patellamide (Micallef et al. 2015). However, *E. coli* in being non-photosynthetic and radiation-sensitive (Domain et al. 2004) is not well suited for the synthesis of ^{14}C uniformly labeled bioactive products. Alternatively, the (few) genetically manipulable cyanobacteria can be used for (i) high-level production of bioactive products, (ii) uniform ^{14}C labeling, and (iii) analysis of their ADME properties in small animals. To reach this objective, it is necessary to (i) introduce and express in cyanobacteria the heterologous genes directing the synthesis of the natural products of interest (Videau et al. 2016), (ii) redirect the photosynthetically fixed carbon (^{14}C) towards the production of the intended chemicals (not merely the cyanobacterial biomass), and (iii) if necessary, increase the tolerance of the engineered cyanobacteria to the intended product. So far, most biotechnologically oriented works are performed with the unicellular model cyanobacterium *Synechocystis* PCC6803 (Savakis and Hellingwerf 2015).

Like other cyanobacteria (Chen et al. 1987; Lehmann and Wober 1977; Pelroy et al. 1976; Tovey et al. 1974), *Synechocystis* PCC6803 is well suited for the production of uniformly ^{14}C-labeled products (Benwakrim et al. 1998) because it is radio-resistant (Domain et al. 2004) and it grows well on pure $^{14}CO_2$ as the sole carbon source (Benwakrim et al. 1998). In addition, *Synechocystis* PCC6803 can take up extracellular DNA and integrate it into its own chromosome (Grigorieva and Shestakov 1982). Furthermore, it can also be manipulated with replicative plasmid vectors derived from its smallest endogenous plasmid (Chauvat et al. 1986; Ferino and Chauvat 1989) or the non-cyanobacterial (promiscuous) plasmid RSF1010 (Mermet-Bouvier et al. 1993). Such RSF1010-derived vectors proved useful tools for high-level production of proteins (Mermet-Bouvier and Chauvat 1994) and studies of various biological processes such as gene expression (Dutheil et al. 2012) (and references therein), cell division (Cassier-Chauvat and Chauvat 2014), and stress responses (Cassier-Chauvat and Chauvat 2015). As we seek strong productions of bioactive products that may be toxic to *Synechocystis* PCC6803, it is also advantageous to have the possibility to decouple cell growth (on $^{14}CO_2$ for uniform ^{14}C-labeling purposes) before triggering the synthesis of the natural product. For such purpose, the temperature-controlled system is attractive in affording a tight control of expression of the studied genes proportional to the growth temperature. With this system, the studied genes are (i) not expressed at temperature ≤30 °C (the standard growth temperature of *Synechocystis* PCC6803), (ii) moderately expressed at 34–36 °C, and (iii) strongly expressed at 39 °C (a temperature at which wild type cells *Synechocystis* PCC6803 keep growing well). The value of this system was first verified while producing the heterologous reporter enzymes chloramphenicol-acetyl-transferase and beta-galactosidase, which possess an easily quantified activity, and the values were respectively ≤3 units (30 °C); 700–1000 units (34–36 °C), and 2000–4000 units (39 °C) (Ferino and Chauvat 1989; Mermet-Bouvier and Chauvat 1994). Later, this system was used for the conditional expression of various proteins (Marteyn et al. 2013), some of which being toxic when produced in high amounts (Dutheil et al. 2012).

Conclusions

Cyanobacteria are attractive organisms that combine a vast biodiversity with powerful photosynthesis and radiation resistance and effective synthetic biology tools for several model strains. Hence, they are well suited for future cheap production of a vast array of uniformly ^{14}C-labeled bioactive compounds

to screen for new drugs and analyze their absorption, distribution, metabolism, and excretion properties.

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Compliance with ethical standards

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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