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MINI-REVIEW

Cyanobactins—ribosomal cyclic peptides produced by cyanobacteria

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Abstract Cyanobactins are small cyclic peptides that are produced by a diverse selection of cyanobacteria living in symbioses as well as terrestrial, marine, or freshwater environments. They include compounds with antimalarial, antitumor, and multidrug reversing activities and potential as pharmaceutical leads. Cyanobactins are produced through the proteolytic cleavage and cyclization of precursor peptides coupled with further posttranslational modifications such as heterocyclization, oxidation, or prenylation of amino acids. Cyanobactin gene clusters encode two proteases which cleave and cyclisize the precursor peptide as well as proteins participating in posttranslational modifications. The bioinformatic mining of cyanobacterial genomes has led to the discovery of novel cyanobactins. Heterologous expression of these gene clusters provided insights into the role of the genes participating in the biosynthesis of cyanobactins and facilitated the rational design of novel peptides. Enzymes participating in the biosynthesis of cyanobactins may prove useful as catalysts for producing novel cyclic peptides in the future. The recent discovery of the cyanobactin biosynthetic pathway in cyanobacteria extends our knowledge of their potential as producers of interesting metabolites.

Keywords Cyanobacteria · Cyanobactins · Peptides · Bioactive compounds

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Cyanobactins

Cyanobacteria are one of the most promising microbial groups in the search for novel bioactive compounds. Low molecular weight peptides containing a range of proteinogenic and nonproteinogenic amino acids are a major class of bioactive compounds produced by cyanobacteria (Burja et al. 2001). A plethora of small cyclic or linear peptides with a surprisingly high level of structural variation have been reported from cyanobacteria (Welker and von Döhren 2006; Sivonen and Börner 2008). These peptides are produced by both nonribosomal and ribosomal biosynthetic pathways in cyanobacteria. The first nonribosomal pathways for cyanobacterial peptides were described in 2000 (Tillett et al. 2000; Rouhiainen et al. 2000) whereas the first ribosomal pathway was shown in 2005 for the cyanobactin patellamide (Schmidt et al. 2005).

Cyanobactin was proposed as a collective name for cyclic peptides which contain heterocyclized amino acids or isoprenoid amino acid derivatives (Donia et al. 2008a; Schmidt and Donia 2009). Cyanobactins were initially defined to contain oxazolines, thiazolines, or their oxidized derivatives oxazoles and thiazoles (Fig. 1). This definition was recently broadened to include cyclic peptides which consist solely of proteinogenic amino acids (Leikoski et al. 2010). Isoprenoid amino acid derivatives are rare but found for example in trunkamide, patellin, and anacyclamides (Tables 1 and 3, Fig. 1).

Occurrence and chemical diversity of cyanobactins

More than a hundred cyanobactins have been identified from symbiotic associations formed between cyanobacteria and ascidians (Table 1) or from free-living cyanobacteria

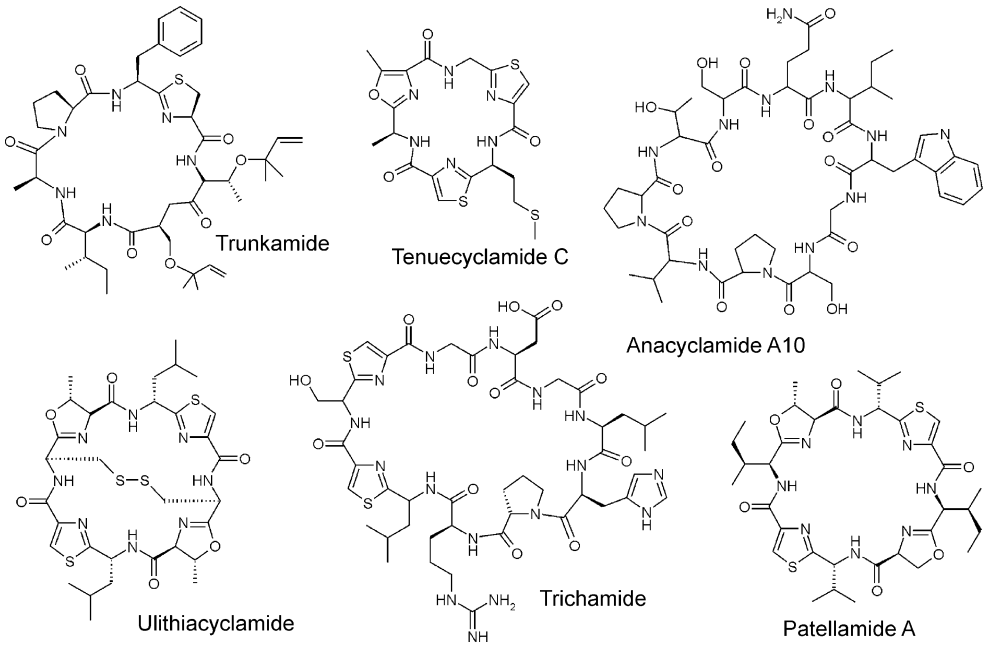

Fig. 1 The chemical structures of a selection of cyanobactins. Trunkamide was isolated from *L. patella*, tenuecyclamide from *N. spongiaeforme*, anacyclamide from *Anabaena*, trichamide from *T. erythraeum*, and ulithiacyclamide and patellamide from *Prochloron* (originally from *L. patella*). The corresponding biosynthetic gene clusters are shown in Fig. 2

(Tables 2 and 3, Fig. 1). This makes cyanobactins one of the largest classes of cyanobacterial peptides (Donia et al. 2006; Schmidt and Donia 2009). Cyanobactins identified from filter-feeding organisms, such as ascidians and sponges, usually contain from six to ten amino acids and varying numbers and combinations of oxazoles, oxazolines, thiazoles, and thiazolines. A few cyanobactins, such as comoramides, contain prenylated amino acids, and ulithiacyclamides have disulfide bridges between two cysteine amino acids (Table 1).

It is unclear if the cyanobactins are produced by the filter-feeding organisms themselves, heterotrophic bacteria, or cyanobacteria associated with these organisms (Table 1). Some cyanobactins have now been verified to be produced by cyanobacteria (Schmidt et al. 2005; Donia et al. 2006). The biosynthetic origin of most of the analogous cyclic peptides reported from cyanobacteria (Tables 2 and 3) is currently unknown. To date, only ribosomal biosynthetic pathways have been described to produce these cyclic peptides (Schmidt et al. 2005; Donia et al. 2006, 2008a; Sudek et al. 2006; Ziemert et al. 2008b; Leikoski et al. 2010). However, a nonribosomal peptide synthetase pathway could be an alternative route for the biosynthesis of these compounds.

Cyanobacterial strains produce ribosomal cyanobactins which contain heterocyclized amino acids (Table 2) and also cyclic peptides which consist solely of unmodified proteinogenic amino acids, occasionally with prenyl attachments (Table 3). Cyanobactins which are found in by cyanobacteria and contain heterocyclized amino acids range in size from six to eleven amino acids (Table 2). Oxazoles and thiazoles are common while oxazolines or thiazolines occur with a lower frequency (Table 2). The cyanobactins which lack heterocyclized amino acids vary in length from seven to 20 amino acids (Table 2). Interestingly, a feature uniting cyanobactins without heterocyclized amino acids in addition to the occasional prenyl attachment is the conserved presence of a proline residue (Table 3).

The biosynthetic genes for cyanobactin production have been described in distantly related cyanobacteria *Prochloron*, *Trichodesmium*, *Microcystis*, *Nostoc*, *Lyngbya*, and *Anabaena* (Schmidt et al. 2005; Donia et al. 2006, 2008a; Sudek et al. 2006; Ziemert et al. 2008b; Leikoski et al. 2010; Fig. 2). In addition, one of the protease genes responsible for cleavage of the cyanobactin precursor peptide was shown to be common among planktonic freshwater cyanobacteria and present in 48 out of 132 strains studied (Leikoski et al. 2009). These planktonic cyanobacteria included fresh and brackish water strains from filamentous heterocystous (*Anabaena*, *Aphanizomenon*, *Nodularia*), filamentous (*Planktothrix*), as well as colony-forming (*Microcystis* and *Snowella*) cyanobacteria. The biosynthetic pathway appears to be relatively common in these strains (Leikoski et al. 2009), but detailed analysis of the gene clusters should be carried out and the structure of the compounds remains to be identified.

Biosynthesis of cyanobactins

Cyanobactins are produced through the proteolytic cleavage and head-to-tail (N–C) cyclization of precursor peptides coupled with modification of specific amino acids as in many other natural products (Oman and van der Donk 2010). The cyclic structure is formed via an amide linkage of the α-carbonyl of C-terminal amino acid and α-amino

Table 1 Examples of cyclic peptides and their structural features characterized from ascidians and sponges

Compound	Molecular weight	Number of amino acids	Amino acids	Oxazole	Oxazoline	Thiazole	Thiazoline	Prenyl	SS bridge	Bioactivity	Source organism	Reference
Ascidiacyclamide	757.0	8	ITVCITVC		2	2				Cytotoxic	*Lissoclinum patella*	Ishida et al. 1988; Schmitz et al. 1989
Axinastatin 1	752.9	7	FVVPVNP							Cytostatic against cancer cells	*Axinella* sp.	Pettit et al. 1994
Axinastatin 2	766.9	7	FVLPVNP									
Axinastatin 3	781.0	7	FILPVNP									
Axinellin C	938.1	8	FPLTVPWP								*Stylotella aurantium*	Tabudravu et al. 2002a
Bistratamide A	570.7	6	ITACFC		1		2			Cytotoxic	*Lissoclinum bistratum*	Degnan et al. 1989b; Foster et al. 1992; Perez and Faulkner 2003
Bistratamide B	568.7	6	ITACFC		1	1	1					
Bistratamide C	503.6	6	VSACVC	1		2						
Bistratamide D	530.6	6	VTVSVC	1	1	1						
Bistratamide E	543.2	6	VTVCVC		1	2						
Bistratamide F	532.7	6	VTVSVC		2	1						
Bistratamide G	528.6	6	VTVSVC	2		1						
Bistratamide H	544.7	6	VTVCVC	1		2						
Bistratamide I	548.7	6	VTVSVC	1		1						
Bistratamide J	564.7	6	VTVCVC			2						
Comoramide A	668.9	6	ITFTAC		1		1	1		Mild cytotoxicity against tumor cells	*Didemnum molle*	Rudi et al. 1998
Comoramide B	686.9	6	ITFTAC				1	1				
Cyclodidemnamide	693.9	7	FTVPCVC		1	1	1			Weakly cytotoxic	*Didemnum molle*	Toske and Fenical 1995; Arrault et al. 2002
Cyclodidemnamide B	689.9	7	ITVPCLC			2						
Cyclonellin	963.1	8	YTANPRYP								*Axinella carteri*	Milanowski et al. 2004
Cycloxazoline	546.7	6	VTVTVT		3					Cytotoxic	*Lissoclinum bistratum*	Hambley et al. 1992
Didmolamide A	538.6	6	ACFTAC		1	2					*Didemnum molle*	Rudi et al. 2003
Didmolamide B	556.7	6	ACFTAC			2						
Dolastatin I	516.6	6	ITVSAC	1	1	1				Cytotoxic	*Dolabella auricularia*	Sone et al. 1997
Dolastatin E	490.6	6	ASACIC	1		1	1					Ojika et al. 1995
Haliclonamide A	857.0	8	PASYPTIP	1	1			1		Antifouling	*Haliclona* sp.	Guan et al. 2001; Sera et al. 2003
Haliclonamide B	788.9	8	PASYPTIP	1	1							
Haliclonamide D	806.9	8	PASYPTIP	1								
Haliclonamide E	875.0	8	PASYPTIP	1				1				
Hexamollamide	696.9	6	VVCTFP				1	1		Moderate cytotoxicity against HeLa S_3 cells	*Didemnum molle*	Teruya et al. 2008
Hymenamide A	880.1	7	VPFWRPP							Immunomodulating activity	*Hymeniacidon* sp.	Kobayashi et al. 1993
Hymenamide B	830.9	7	NFVEFPP									
Hymenamide C	826.9	7	FGPELWP									Napolitano et al. 2001
Hymenamide D	769.9	7	YDPLAIP									
Hymenamide E	854.0	7	TTPYFFP									
Hymenamide F	765.0	7	AVMLRPP									
Hymenamide G	893.1	8	YVPLILPP									Tsuda et al. 1994

Table 1 (continued)

Compound	Molecular weight	Number of amino acids	Amino acids	Oxazole	Oxazoline	Thiazole	Thiazoline	Prenyl	SS bridge	Bioactivity	Source organism	Reference
Hymenamide H	904.1	8	LPWVPLTP									
Hymenamide J	1,099.3	8	YDFWKVYP									
Hymenamide K	1,007.2	8	YDFWKAVP									
Lissoclinamide 1	705.9	7	ICFPTVC		1	2				Cytotoxic	*Lissoclinum patella*[a]	Wasylyk et al. 1983; Degnan et al. 1989a; Schmitz et al. 1989; Donia et al. 2006; Hawkins et al. 1990; Morris et al. 2000
Lissoclinamide 2	679.9	7	ACFPTIC		1	1	1					
Lissoclinamide 3	679.9	7	ACFPTIC		1	1	1					
Lissoclinamide 4	741.9	7	FCFPTVC		1	1	1					
Lissoclinamide 5	739.9	7	FCFPTVC		1	2						
Lissoclinamide 6	741.9	7	FCFPTVC		1	1	1					
Lissoclinamide 7	743.9	7	FCFPTVC		1		2					
Lissoclinamide 8	741.9	7	FCFPTVC		1	1	1					
Lissoclinamide 9	707.9	7	VCFPTIC		1	1	1					
Lissoclinamide 10	726.9	7	ICFPTIC		1		2					
Mayotamide A	694.0	7	VPCICMC			1	2				*Didemnum molle*	Rudi et al. 1998
Mayotamide B	679.9	7	VPCVCMC			1	2					
Mollamide	808.0	7	IPISFPC				1	1		Cytotoxic	*Didemnum molle*	McKeever and Pattenden 2003; Donia et al. 2008b
Mollamide B	696.9	6	VFPTVC				1	1		Cytotoxic against several cancer cell lines		
Mollamide C	618.8	6	IPGSLC			1		1				
Nairaiamide A	802.1	7	VTIPIIP					1			*Lissoclinum bistratum*	Foster and Ireland 1993
Nairaiamide B	816.1	7	ITIPIIP					1				
Patellamide A	743.0	8	ISVCITVC		2	2				Cytotoxic antineoplastic; patellamides B and C had multidrug reversing activity	*Lissoclinum patella*[a]	Ireland et al. 1982; Fu et al. 1998; Schmitz et al. 1989; McDonald and Ireland 1992; Rashid et al. 1995; Schmidt et al. 2005; Donia et al. 2006
Patellamide B	777.0	8	LTACITFC		2	2						
Patellamide C	763.0	8	VTACITFC		2	2						
Patellamide D	777.0	8	ITACITFC		2	2						
Patellamide E	791.0	8	VTVCITFC		2	2						
Patellamide F	763.0	8	VTVCITFC		2	2						
Patellamide G	795.0	8	ITACLTFC		1	2						
Patellin 2	733.0	6	TVPTLC				1	2		Moderately cytotoxic	*Lissoclinum patella*[a]	Donia et al. 2008a, Zabriskie et al. 1990
Patellin 3	943.0	8	TLPVPTLC				1	2				
Patellin 6	963.2	8	TFPVPTVC				1	2				
Phakellistatin 1	828.0	7	YPIPIFP							Cancer cell growth inhibitory	*Phakellia costata* sponge and *Stylotella aurantium*	Pettit et al. 1993a, b
Phakellistatin 2	828.0	7	YPFPIIP							Cell growth inhibitory	*Phakellia carteri*	Tabudravu et al. 2002b
Phakellistatin 7	1,109.4	10	YIPPIFALPP							Cancer cell growth inhibitory	*Phakellia costata* sponge	Pettit et al. 1995
Phakellistatin 8	1,137.4	10	YIPPIFVLPP									
Phakellistatin 9	1,123.4	10	YVPPIFVLPP									
Phakellistatin 12	1,139.4	10	IFTLPPYIPP								*Phakellia* sp.	Pettit and Tan 2003; Li et al. 2003
Phakellistatin 13	798.9	7	LWPFGPT								*Phakellia fusca*	
Stylisin 1	828.0	7	LPYPIFP								*Stylissa caribica*	Mohammed et al. 2006

Stylisin 2	812.0	7	IPYPPFP					*Stylissa caribica*	Schmidt et al. 2007
Stylissamide A	845.0	7	VYPYKPP						
Stylissamide B	812.0	7	IYPFPPP						
Stylissamide C	862.0	7	FIPYPFP						
Stylissamide D	828.0	7	FIPYPLP						
Tawicyclamide A	806.1	8	VCFCICVP	2	1		Weakly cytotoxic	*Lissoclinum patella*	McDonald et al. 1992
Tawicyclamide B	772.0	8	VCLCICVP	2	1				
Trunkamide A	838.0	7	TSIAPFC		1	2	Cytotoxic, multidrug reversing activity	*Lissoclinum patella*[a]	Caba et al. 2001; Salvatella et al. 2003; Donia et al. 2008a
Ulicyclamide	677.8	7	FPTICAC	1	2		Not reported	*Lissoclinum patella*	Ireland and Scheuer 1980; Wasylyk et al. 1983
Ulithiacyclamide	763.0	8	CTLCCTLC	2	2	1	Cytotoxic	*Lissoclinum patella*[a]	Ireland and Scheuer 1980; Ireland et al. 1982; Williams et al. 1989; Fu et al. 1998
Ulithiacyclamide B	797.0	8	CTFCCTLC	2	2	1			
Ulithiacyclamide E	833.0	8	CTFCCTLC		2	1			
Ulithiacyclamide F	815.0	8	CTFCCTLC	1	2	1			
Ulithiacyclamide G	815.0	8	CTFCCTLC	1	2	1			
Wainunamide	745.9	7	GLFPHPP				Weakly cytotoxic	*Stylotella aurantium*	Tabudravu et al. 2001

The peptides are organized alphabetically. The sequence of the peptide is presented in linear form so that the last amino acid is cysteine or proline. The number of each modification is given after the amino acids of the core peptide

[a] Confirmed to be produced by the *Prochloron* spp. symbiotic partner of the ascidian

group of the N-terminal amino acid yielding a homodetic cyclic peptide. In cyanobactin biosynthesis, the precursor peptide directly encodes one or more cyanobactins flanked by the putative recognition sequences at which the precursor peptide is cleaved by two proteases (Schmidt et al. 2005; Lee et al. 2009). Cyanobactin precursor peptides encode more than one cyanobactin, and this could be a means to enhance the levels of peptide production or represent a mechanism for generating chemical diversity. The cyanobactin gene cluster encodes two proteases demonstrated to be involved in the cleavage of the precursor peptide and cyclization of the cyanobactin (Lee et al. 2009). The PatA protease encoded in the patellamide biosynthetic gene cluster was shown to cleave the precursor peptide at the N-terminal recognition sequence while the PatG protease cleaved the precursor peptide at the C-terminal recognition sequence (Lee et al. 2009). However, only the PatG protease was required for N–C cyclization of the cyanobactins (Lee et al. 2009).

The cyanobactin biosynthetic genes encoded in gene clusters are approximately 10 kb in size and contain between 7 and 12 genes (Fig. 2). The gene order is not strictly conserved (Fig. 2). However, most often, the biosynthetic genes are organized as in the patellamide *pat* gene cluster. All of the cyanobactin gene clusters contain two proteases, which work in tandem, a short precursor peptide as well as proteins involved in the maturation of the cyanobactins. The cyanobactin gene clusters in *Prochloron* are highly similar (Schmidt et al. 2005; Donia et al. 2006).

Thiazoles and oxazoles are formed through the heterocyclization and subsequent oxidation of cysteine, serine, and threonine amino acids. Cyanobactin gene clusters typically contain a gene encoding a PatD homolog which is predicted to heterocyclize cysteine, serine, and threonine to thiazolines and oxazolines (Schmidt et al. 2005). A PatF homolog is often encoded in cyanobactin gene clusters and thought to be involved in the heterocyclization and/or prenylation of cyanobactins (Schmidt and Donia 2009). The oxidase domain of the bimodular PatG protein is believed to catalyze the oxidation of thiazolines and oxazolines to thiazoles and oxazoles (Schmidt et al. 2005). Intriguingly, PatB and PatC are encoded in nearly all cyanobactin gene clusters but are nonessential, with patellamides being produced by heterologous expression of the *pat* gene cluster in *Escherichia coli* in the absence of the *patB* and *patC* genes (Donia et al. 2006, 2008a).

Patellamides, lissoclinamides, ulithiacyclamides, and patellins

Biologically active cyclic peptides have been reported from *Lissoclinum patella* ascidians, but the biosynthesis of the compounds is often attributed to the small photosynthetic

Table 2 Examples of cyclic peptides, their structural features reported from a diverse selection of cyanobacteria organized according to the alphabetical order of the compound name

Compound	Molecular weight	Number of amino acids	Amino acids	Oxazole	Oxazoline	Thiazole	Thiazoline	Bioactivity	Producer	References
Aerucyclamide A	534.7	6	ITGCIC		1	1	1	Toxic to fresh water crustacean *Thamnocephalus platyurus*, antimalarial	*Microcystis aeruginosa* (bloom-forming, planktonic)	Ziemert et al. 2008b; revised structures and renaming: Portmann et al. 2008a, b
Aerucyclamide B	532.7	6	ITGCIC		1	2				
Aerucyclamide C	516.6	6	ATVSIC	1	1	1				
Aerucyclamide D (formerly microcyclamide 7806A and B)	586.7	6	FTGCMC		1	1	1			
Banyascyclamide A	538.6	6	ACFTAC		1	2			*Nostoc* sp. TAU strain IL-235	Ploutno and Carmeli 2002
Banyascyclamide B	522.7	6	ACLTAC			2				
Banyascyclamide C	556.7	6	ACFTAC			2				
Dendroamide A	488.6	6	ACATVC	1		2		A - Multidrug-resistance reversing activity	*Stigonema dendroideum* (terrestrial)	Ogino et al. 1996
Dendroamide B	520.6	6	MCATAC	1		2				
Dendroamide C	536.6	6	MCATAC	1		2				
Dolastatin 3	660.8	7	VPLQCGC			2			*Lyngbya majuscula*	Mitchell et al. 2000
Homodolastatin 3	674.8	7	IPLQCGC			2				
Kororamide	969.2	7	LYCNPSLC			1	1		*Lyngbya majuscula*	Mitchell et al. 2000
Microcyclamide	582.7	6	HCATIC	1		2		Moderate cytotoxicity against P388 murine leukemia cells (IC_{50} - 1.2 μg/ml)	*Microcystis aeruginosa* NIES-298	Ishida et al. 2000; Ziemert et al. 2008b
Nostocyclamide	474.6	6	ATGCVC	1		2		Anticyanobacterial and antialgal, toxic also to the rotifer *Brachionus calyciflorus*	*Nostoc* sp. 31	Todorova et al. 1995
Nostocyclamide M	506.6	6	ATGCMC	1		2		Allelopathic anticyanobacterial	*Nostoc* sp. 31	Jüttner et al. 2001
Raocyclamide A	550.6	6	FSASIC	1	1	1		A-moderate toxicity against sea urchin embryos	*Oscillatoria raoi* (soil isolate)	Admi et al. 1996
Raocyclamide B	568.7		FSASIC	1		1				
Tenuecyclamides A	460.5	6	ATACAC	1		2		Inhibited division of sea urchin embryos (B not tested)	*Nostoc spongiaeforme* var. *tenue* (lithophytic)	Banker and Carmeli 1998; Donia et al. 2008a
Tenuecyclamides B	460.5		ATACAC	1		2				
Tenuecyclamides C	506.6		ATGCMC	1		2				
Tenuecyclamides D	522.6		ATGCMC	1		2				
Trichamide	1,099.2	11	GDGLHPRLCSC			2		No effects found (tested for cytotoxicity, antifungal, antibacterial, and antiviral activities)	*Trichodesmium erythraeum* (marine)	Sudek et al. 2006
Venturamide A	488.6	6	ATACVC	1		2		Strong antimalarial activity	*Oscillatoria* (marine)	Linington et al. 2007
Venturamide B	518.6		ATTCVC	1		2				
Westiellamide	546.7	6	VTVTVT		3			Mildly cytotoxic	*Westiellopsis prolifica* (terrestrial)	Prinsep et al. 1992
Wewakazole	1,141.3	11	GVTFSFPISAPP	3					*Lyngbya majuscula* (marine)	Nogle et al. 2003

The sequence of the peptide is presented in linear form so that the last amino acid is cysteine or proline. The number of each modification is given after the amino acids of the core peptide

Table 3 Examples of cyclic homodetic peptides from cyanobacteria

Compound	Molecular weight	Number of amino acids	Amino acids	Prenyl	Bioactivity	Producer	Reference
Agardhipeptin A	834.0	7	WGLHGWP		Plasmin inhibitory	*Oscillatoria agardhii* NIES-204	Shin et al. 1996
Agardhipeptin B	1,036.3	8	WAPWVWLP				
Anacyclamide A10	1,053.2	10	TSQIWGSPVP			*Anabaena*, e.g., 90	Leikoski et al. 2010
Anacyclamide B10	1,010.2	10	SSVIWGSPVP			*Anabaena*, e.g., 1TU31S9	
Anacyclamide C10	1,115.2	10	SAQWQNFGVP			*Anabaena*, e.g., 299A	
Anacyclamide D10	1,151.3	10	NAHWQNFGVP			*Anabaena* 1TU44S9	
Anacyclamide E10	1,087.3	10	YAPLQNFGVP			*Anabaena* PH256	
Anacyclamide E10P	1,155.4	10	YAPLQNFGVP	1		*Anabaena* PH256	
Anacyclamide A11	1,281.4	11	DNWLGEWIGIP			*Anabaena*, e.g., BIR260	
Anacyclamide A15	1,690.9	15	HAFIGYDQDPTGKYP			*Anabaena* 37	
Anacyclamide A15G	1,827.1	15	HAFIGYDQDPTGKYP	1[a]		*Anabaena* 37	
Anacyclamide A7	948.1	7	RERFVYP			*Anabaena* 1TU39S8	
Anacyclamide F10P	1,191.3	10	YSNKPSDFSP	1		*Anabaena* 1TU32S11	
Anacyclamide A9P	1,114.3	9	YDDKLNLSP	1		*Anabaena* 1TU33S10	
Anacyclamide A20P	2,080.4	20	WGNGTGLDWKLLTGGISASP	1		*Anabaena* 202A1	
Anacyclamide A20PP	2,148.5	20	WGNGTGLDWKLLTGGISASP	2		*Anabaena* 202A1	
Anacyclamide A8P	993.1	8	HQPWHAAP	1		*Anabaena* TR232	
Anacyclamide B8	957.1	8	FSPDWRAP			*Anabaena* SYKE816	
Anacyclamide C8	998.2	8	VIQHYLFP			*Anabaena* PH262	
Anacyclamide B7	762.0	7	LIGIMHP			*Anabaena* SYKE844B	
Kawaguchipeptin A	1,421	11	WLNGDNNWSTP	2		*Microcystis aeruginosa* NIES-88	Ishida et al. 1996
Kawaguchipeptin B	1,285	11	WLNGDNNWSTP		Antibacterial	*Microcystis aeruginosa* NIES-88	Ishida et al. 1997
Microphycin AL828	829	8	EIGVYGLP			*Microcystis* sp. TAU strain IL-306	Gesner-Apter and Carmeli 2008
Planktocyclin	801	8	GLVMFGVP		Inhibitor of mammalian trypsin and α-chymotrypsin	*Planktothrix rubescens*	Baumann et al. 2007
Oscillacyclin	995	9	FTTSIAYNP			*Oscillatoria agardhii* 97	Fujii et al. 2000
Prenylagaramide A	1,081	9	YGTGEFFNP	1		*Oscillatoria agardhii* NIES-205	Murakami et al. 1999
Prenylagaramide B	929	7	YLYPINP	1		*Oscillatoria agardhii* NIES-596	

The peptides are in alphabetical order. The sequence of the peptide is presented in linear form so that the last amino acid is cysteine or proline

[a] Geranyl

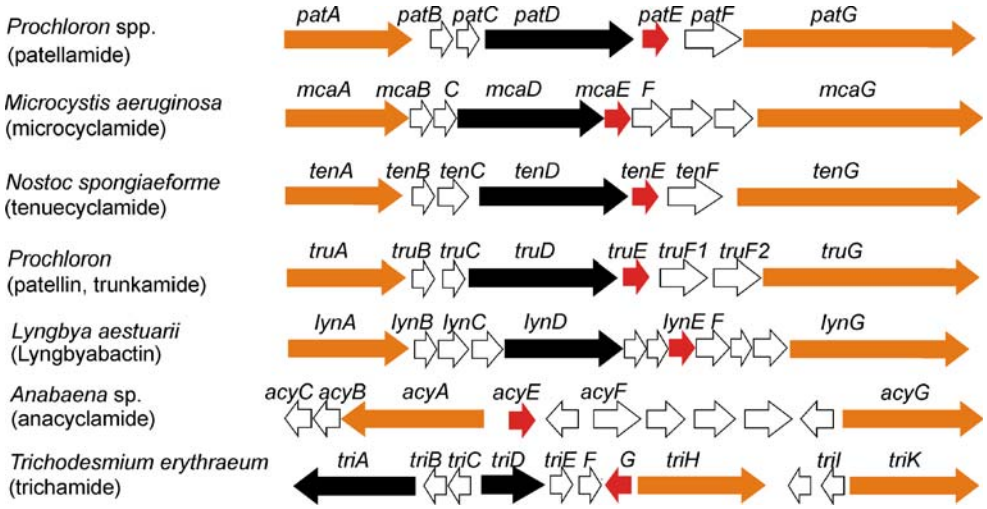

Fig. 2 Cyanobactin gene clusters published from seven distantly related cyanobacteria. These gene clusters are typified by genes encoding proteases (*yellow*), a short precursor peptide (*red*), proteins involved in the maturation of the cyanobactin (*black*), as well as conserved and hypothetical open reading frames (*white*)

obligate symbionts of these ascidians (Sings and Rinehart 1996). The findings of Schmidt and coworkers demonstrated that the cyanobactin pathway was responsible for the production of patellamide in symbiotic uncultured *Prochloron* spp. as well as lissoclinamides, ulithiacyclamides, patellin, and the anticancer drug candidate trunkamide (Schmidt et al. 2005; Donia et al. 2006; Donia et al. 2008a). The cyanobactins which are produced by *Prochloron* spp. are structurally diverse and range in size from six to eight amino acids (Table 1, Schmidt et al. 2005; Donia et al. 2006, 2008a).

Ascidians harbor a mixture of symbiotic *Prochloron* strains. The portion of the precursor peptide gene encoding the core peptide in the cyanobactin gene clusters from *Prochloron* spp. is hypervariable. Hence, a library of cyanobactins is synthesized when multiple uncultivated *Prochloron* strains are present in the tunicate (Donia et al. 2006).

Patellins and trunkamides produced by uncultured symbiotic *Prochloron* spp. contain prenylated amino acids (Donia et al. 2008a). The prenylated cyanobactins do not have oxazoles or thiazoles, only unoxidized forms of these heterocyclized amino acids occur, and the oxidase domain of PatG is missing in the patellin and trunkamide biosynthetic gene cluster (Schmidt and Donia 2009). In addition, the prenylating pathway encodes two PatF proteins whose roles are unclear, and the prenylation is not fully understood at present (Schmidt and Donia 2009). Ulithiacyclamides contain four cysteines, two of which form a disulfide bridge and the other two cysteines are heterocyclized to thiazoles (Table 1). Among the more than hundred cyanobactins, disulfide bridges are only found in ulithiacyclamides (Tables 1, 2, and 3).

Tenuecyclamides

Tenuecyclamides are hexapeptides containing two thiazoles and an oxazole (Banker and Carmeli 1998, Table 2). A cyanobactin pathway for the biosynthesis of tenuecyclamide was characterized in an epilithic *Nostoc spongiaeforme* strain (Fig. 2, Donia et al. 2008a). The tenuecyclamide *ten* gene cluster has an identical gene organization to *pat* gene cluster as the genes are transcribed to the same direction, and the gene order is conserved. The TenE precursor peptide encodes four copies of the core peptide amino acid sequences which form tenuecyclamides A and C (Donia et al. 2008a). Tenuecyclamides A and B differ only in stereochemistry (Table 2).

Trichamide

Trichamide is cyclic 11 amino acid cyanobactin which contains two thiazoles (Sudek et al. 2006). This cyanobactin was discovered through genome mining of *Trichodesmium erythraeum* IMS101, a free-living, nitrogen-fixing filamentous marine cyanobacterium (Schmidt et al. 2005; Sudek et al. 2006). The *tri* gene cluster is 12.5 kb and encodes 11 open reading frames (ORFs) with a bidirectional gene order for which only six genes have an assigned function (Fig. 2, Sudek et al. 2006). The trichamide precursor peptide encodes a single copy of the trichamide core peptide flanked by the putative recognition sequences. In contrast to Pat proteins, the oxidase and the protease domains in *Trichodesmium* are encoded by separate genes (Sudek et al. 2006).

Lyngbyabactins

A cyanobactin gene cluster has been also identified from the genome of the marine *Lyngbya aestuarii* CCY9616 (Fig. 2, Donia et al. 2008a). The products of this gene cluster, lyngbyabactins A and B, were predicted from the precursor peptide LynE (Donia et al. 2008a). The peptides

are predicted to include isoprenoid amino acid derivatives (Donia et al. 2008a). However, lyngbyabactins A and B have not yet been detected from *L. aestuarii* CCY9616. The lyngbyabactin biosynthetic gene cluster has homologs of all genes present in the *pat* gene cluster but it also contains five other open reading frames which have no function assigned (Donia et al. 2008a).

Microcyclamides and aerucyclamides

Microcyclamide is a cytotoxic cyclic hexapeptide reported from the freshwater bloom-forming cyanobacterium *Microcystis aeruginosa* NIES-298 (Ishida et al. 2000). The microcyclamides 7806A and B of *M. aeruginosa* PCC 7806 were later renamed as aerucyclamides, and the structures were revised (Portmann et al. 2008a, b). Microcyclamides and aerucyclamides are assembled via the cyanobactin biosynthetic pathway in two strains of *M. aeruginosa* NIES 298 and PCC 7806 (Ziemert et al. 2008b; Portmann et al. 2008a, b). The *mca* gene clusters have the same gene order as the *pat* genes except for two additional open reading frames for which no functions could be assigned (Fig. 2, Ziemert et al. 2008b). The McaE precursor peptide encodes two identical copies of the microcyclamide core peptides in *M. aeruginosa* NIES-298 (Ziemert et al. 2008b).

Anacyclamides

A variety of anacyclamides have been identified from strains of the genus *Anabaena* (Leikoski et al. 2010, Table 3). Anacyclamides consist of proteinogenic amino acids, and some contain prenyl or geranyl groups through the posttranslational modifications of specific amino acids. The *acy* gene cluster in *Anabaena* sp. 90 encodes 11 ORFs, and it is arranged in an eleven kb operon which is bidirectionally transcribed as the trichamide gene cluster (Fig. 2). The gene cluster differs from *pat* gene cluster in that there were no homologs for all *pat* genes. Additionally, there were hypothetical ORFs present in the *acy* gene cluster that were absent in the *pat* gene cluster (Leikoski et al. 2010). The precursor peptide AcyE encodes single copy of the anacyclamide A10 (Fig. 1) flanked by putative recognition sequences which differ substantially from other cyanobactin precursors (Leikoski et al. 2010). The anacyclamide gene cluster lacks a PatD homolog which agrees well with the anacyclamide structure which has no posttranslationally heterocyclized amino acids (Leikoski et al. 2010). The AcyG protein lacks an oxidase domain which is consistent with the absence of heterocyclized amino acids (Leikoski et al. 2010). In anacyclamides, the length of the peptides varies greatly which is achieved by expansion of the AcyE precursor protein. Anacyclamides showed great amino acid variation since only one proline was conserved (Table 3).

Bioactivities

The cyanobactins and cyclic peptides with analogous structures have various reported bioactivities (in detail, see Tables 1, 2, and 3). The diverse bioactivities are derived from versatile structures, but all cyanobactins do not exhibit bioactivities in the tests used or those have not been studied yet (Tables 1, 2, and 3). Several of the cyanobacterial metabolites have been found to be anticancer compounds, e.g., trunkamide (Salvatella et al. 2003); some have multidrug reversing activities (Ogino et al. 1996) as well as activities against tropical parasites such as malaria-causing *Plasmodium falciparum* (Linington et al. 2007; Portmann et al. 2008b). In bacteria, many of the ribosomally produced peptides are antibiotics or bactericides produced to kill or inhibit growth of competing microbes (Nolan and Walsh 2009). Cyanobacteria are photosynthetic autotrophic organisms grazed by eukaryotic organisms. They are believed to form mutualistic association with heterotrophic bacteria, but this may not be always the case (Manage et al. 2000; Berg et al. 2009). Nostocyclamides for example have been shown to contain anticyanobacterial activity (Todorova et al. 1995; Jüttner et al. 2001). There are compounds isolated from cyanobacteria with antibiotic (Ishida et al. 1997) or antiviral (Boyd et al. 1997; Bokesch et al. 2003) effects, leaving open the option that some of the cyanobactins may prove to be antibiotic or antiviral compounds.

Analogous pathways

In addition to cyanobactins, another cyanobacterial peptide class, microviridins, was recently shown to be ribosomally produced in *M. aeruginosa* and *Planktothrix agardhii*, but their biosynthetic machinery differs from that of cyanobactins (Ziemert et al. 2008a; Philmus et al. 2008). These compounds were originally thought to be products of nonribosomal peptide biosynthesis. However, microviridins are synthesized from precursor peptides that are converted into tricyclic depsipeptides through the action of ATP grasp ligases and a transporter peptidase (Ziemert et al. 2008a; Philmus et al. 2008). The work of Philmus et al. (2008) reported similar gene clusters in the genomes of *Anabaena variabilis*, *Nostoc punctiforme*, and *Nodularia spumigena* as well as in genomes of other bacteria.

The biosynthetic gene clusters encoding the production pathway of ribosomal peptides with oxazoles and thiazoles are present in a broad range of bacteria (Lee et al. 2008). Bacteria distantly related to cyanobacteria are known to produce bacteriocins by the posttranslational modification

of gene-encoded precursor peptides, including microcins and the lanthionine-containing lantibiotics of Gram-positive bacteria (Jack et al. 1995; Jack and Jung 2000; Nolan and Walsh 2009). Cyanobactin biosynthesis is analogous in many ways to the biosynthesis of bacteriocins (Franz et al. 2007; Nolan and Walsh 2009). The leader-peptide-guided biosynthesis is common in many ribosomally synthesized natural products where the precursor peptide is synthesized and cleaved, and in some cases the core peptide is posttranslationally modified (Oman and van der Donk 2010). Bacteriocins can be also circularized as cyanobactins, and some bacteriocins have similar posttranslational modifications as cyanobactins for example thiazoles and oxazoles (Jack and Jung 2000; Maqueda et al. 2008; Martin-Visscher et al. 2009).

Many of cyanobacterial bioactive compound classes are synthesized on nonribosomal peptide synthetases (NRPS) or combined NRPS and polyketide synthases (Welker and von Döhren 2006; Sivonen and Börner 2008). However, the cyanobactins have been shown to be produced by the posttranslational modification of the gene-encoded precursor peptides (e.g., Schmidt et al. 2005; Donia et al. 2008a). The gene clusters responsible for ribosomal peptide production are small compared to the large nonribosomal peptide synthetase gene clusters. In NRPS, variation in the chemical structure of the peptide is achieved by utilization of more than 200 nonproteinogenic amino acids (Nolan and Walsh 2009) whereas ribosomal peptides are restricted to 20 proteinogenic amino acids which may be posttranslationally modified. In NRPS, the enzymes seem to have relaxed substrate specificity and thus allow simultaneous production of a number of structural variants in the same strain of a cyanobacterium (Welker and von Döhren 2006).

Biotechnological aspects

The biotechnological exploitation of cyanobactins will require detailed studies on the enzymes involved in the biosynthesis as well as mechanisms of action of these peptides. It is possible to express the cyanobactin gene clusters in heterologous hosts (Schmidt et al. 2005; Donia et al. 2006, 2008a; Leikoski et al. 2010). The small size of the cyanobactin gene clusters and the expression of the entire clusters in heterologous hosts will provide new possibilities to create compound libraries and novel compounds (Donia et al. 2006, 2008a). In the cyanobactin pathway, heterologous expression gives options to study the role of individual genes in biosynthesis as well as produce novel peptides. The cyanobactin pathway was utilized in *E. coli* to synthesize an engineered peptide eptidemnamide, a cyclic peptide similar to an anticoagulant in clinical use (Donia et al. 2006). This approach demonstrates a means to exploit cyanobacterial pathways and produce novel compounds by the rational design of peptides. The peptide-precursor-directed synthesis allows manipulations directly to the precursor gene and enables production of engineered peptides in heterologous hosts (Oman and van der Donk 2010). In addition, the enzymes in the cyanobactin pathways could be used as catalysts in aiding chemical synthesis of the desired compounds. The work by Lee et al. (2009) not only clarified the role of proteases in the cyanobactin biosynthesis but demonstrated the potential of the enzyme as general catalysts for cyclization of peptides. The technological advantages of the PatG protease were that no energy is required for the cleavage and cyclization, and also the protease was proven to be tolerant of different substrate lengths and sequences as long as the C-terminal recognition sequence was present (Lee et al. 2009). This is important as in synthetic peptide manufacture the head-to-tail cyclization step restricts peptide production in bulk amounts.

It should be noted that whole-genome information has already led to the discovery of cyanobactin biosynthesis as well as several new compounds and compound classes, e.g., patellamides (Schmidt et al. 2005), trichamide (Sudek et al. 2006), and anacyclamides (Leikoski et al. 2010). The increasing number of genome projects on cyanobacteria and metagenomic studies (Schmidt and Donia 2009) applied to various environments are likely to yield new discoveries including diverse ribosomal pathways and novel cyanobactins in the future.

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