Identification and characterization of kawaguchipeptin biosynthetic pathway in *Microcystis aeruginosa*

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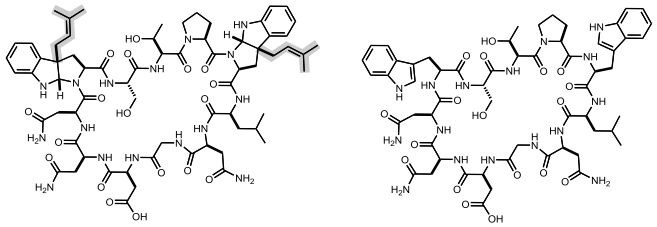
Cyanobactins are a rapidly growing family of linear and cyclic peptides produced by cyanobacteria. Here we show that kawaguchipeptin A and B, two cyclic undecapeptides reported earlier from *Microcystis aeruginosa* NIES-88, are the products of a cyanobactin pathway. We identified the 10-kb kawaguchipeptin (*kgp*) gene cluster in a 5.37 Mb draft genome of *Microcystis aeruginosa* NIES-88. We verified that this gene cluster is responsible for the production of kawaguchipeptins through successful heterologous expression in *Escherichia coli*. We overexpressed the KgpF prenyltransferase and showed that it prenylates *C*3 of Trp residues in both linear and cyclic peptides *in vitro*. Our findings serves to further enhance the structural diversity of cyanobactins to include tryptophan-prenylated cyclic peptides.

Cyanobactins are a family of ribosomally-synthesized and post-translationally modified peptides (RiPPs) produced by cyanobacteria.[1-6] They are biosynthesized as linear precursor peptide which is processed by a set of modifying biosynthetic enzymes. Several modifications have been characterised including *N*-to-*C* macrocyclization, epimerization, heterocyclization to form thiazolines and oxazolines, oxidation of heterocycles to thiazoles and oxazoles, *O*-prenylation on Ser, Thr, and Tyr and *N*-methylation on His. Many products ~~have better target affinity~~, are biologically active, some cross biological membranes and the macrocyclic derivatives are inherently more stabile against metabolic enzymes.[1] Biological activities that have been detected for natural cyanobactins include anticancer, antimalarial, antibacterial, protease inhibitory activity among others [ChemBioChem 2010, 11, 1803 – 1815].[2]
The leader sequences in the patellamide and trunkamide precursor peptides were found essential for the heterocyclase function and is cleaved off by a protease after heterocyclization [Angew. Chem. Int. Ed. 2013, 52, 13991 –13996; Angew. Chem. Int. Ed. 2014, 126, 14395-14398; Our Nat Chem Biol]. It remains an open question whether the leader has other roles in processing. The heterocyclase-processed and N-terminal proteolysed core sequence is the substrate of the macrocyclase which cleaves off its *C*-terminal recognition sequence and macrocyclse the core sequence.
The post translational prenylation of cyanobactins is catalyzed by the prenyltransferase enzyme encoded within the cyanobactin biosynthetic gene cluster using 3-methyl-but-2-en-1-yl group derived from dimethylallyl pyrophosphate (DMAPP).[12] Although a putative prenyltransferase gene is present in all known cyanobactin gene clusters, only a few of the cyanobactins are known to be actually prenylated these include prenylagaramides, aestuaramides, trunkamides and anacyclamides.[4,6,10] The patellamide gene cluster from *Prochloron didemni,* for example, contains a non-functional prenyltransferase, PatF, whose structure has been recently elucidated [Acta Cryst. (2013). F69, 618–623]. The known cyanobactin prenyltransferases are *O*-prenyltransferases that catalyse the *O*-prenylation of tyrosine, threonine and serine in forward or reverse orientation. *C*-prenylated cyanobactins have been shown to be synthesized originally as *O*-prenylated peptides that later undergo a claisen rearrangement to yield *C*-prenylated peptides.[12]
Kawaguchipeptins are macrocyclic undecapeptides produced by the cyanobacterial strain *Microcystis aeruginosa* NIES-88.[13,14] Two variants of kawaguchipeptin have been reported. Kawaguchipeptin A contains two *C*-3 prenylated tryptophans and a D-amino acid.[13] Kawaguchipeptin B consists of solely unmodified amino acids.[14] Kawaguchipeptins bear a strong resemblance to anacyamides, prenylagardmidies and piricyclamides and kawaguchipeptins are predicted to be the product of a cyanobactin pathway.[2] Here we report the genome sequence for *Microcystis aeruginosa* NIES-88, identify the kawaguchipeptin biosynthetic gene cluster and confirm enzymatic prenylation activity.



1 Kawaguchipeptin A **2** Kawaguchipeptin B

Scheme 1. The cyclic undecapeptides Kawaguchipeptin A **(1)** and kawaguchipeptin B (**2**) reported from *Microcystis aeruginosa* NIES-88. Kawaguchipeptin A contains two *C*-3 prenylated tryptophans (highlighted).

We obtained a 5.37 Mb genome sequence for *Microcystis aeruginosa* NIES-88. The genome was assembled in 23 scaffolds. A total of 7,215 genes were predicted, by Glimmer

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3.02 with a threshold of 110 bp.[15] The 41 tRNA genes were predicted using tRNAscan-SE[16] and rRNA genes were located through homologue searches. We identified the gene clusters for micropeptin, microviridin, microcystin and aeruginosin gene clusters by WHAT METHODS??. The kawaguchipeptin precursor gene was identified through tBLASTn using iterations of the kawaguchipeptin peptide backbone (WLNGDNNWSTP). The precursor gene was located on a short contig and encoded the conserved cyanobactin leader seen previously and the N and C-terminal flanking recognition sequences.[5,17] We used this information to identify the 10-kb kawaguchipeptin gene cluster which was spread over 4 contigs in the draft genome of *Microcystis aeruginosa* NIES-88. Six genes that were homologous to the known cyanobactin genes were identified and found to be organized in a single operon and transcribed in the same direction (Fig. 1). Two genes encoding the cyanobactin proteases KgpA and KgpG were detected on additional contigs. Two genes encoding the KgpB and KgpC proteins, which are homologues of highly conserved proteins having unknown functions reported in all known cynaobactin gene clusters, were present. Interestingly, three KgpE homologs were detected in the biosynthetic gene cluster. One of the homologues, KgpE1 was found to encode three exact copies of the WLNGDNNWSTP core (Fig.1). Two additional kawaguchipeptin precursor proteins KgpE2 and KgpE3 encoded single sequences IYIPGLGGVLQGIP and RVFIVLPP respectively. Macrocyclic peptides corresponding to these core sequences (or prenylated variants) were not detected in standard LC-MS analysis of the *Microcystis aeruginosa* NIES-88 cell extracts suggesting that these two genes are silent under our growth conditions. No homologue of the cyclodehydrase (PatD) was detected in the gene cluster that consistent with the absence of heterocyclized amino acids in the cyclic peptides.

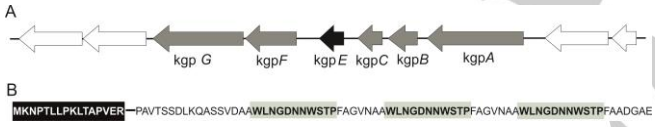

Figure 1. The kawaguchipeptin biosynthetic pathway in *Microcystis aeruginosa* NIES-88. **(A)** The 10 kb biosynthetic gene cluster that consists of six biosynthetic genes (indicated by grey and black) are organized in a single operon and have homology to genes present in other known cyanobactin gene clusters. The open arrows indicate the genes that encode proteins with unknown functions. The *kgpE* precursor gene (black) encodes the 87 amino-acid precursor protein **(B)**. Three identical copies of the undecapeptide core are encoded by the single precursor gene (highlighted in gray).

To demonstrate that the *kgp*A-G genes are sufficient to encode for kawaguchipeptin production *in vivo*, we cloned the entire *kgp* operon into a broad host yeast-bacteria shuttle vector pMQ123i (ref) and placed *kgp*A downstream of a pTac promoter to generate an expression plasmid pDK-kgp1. This allows for the regulated expression of *kgp*A-G genes in the heterologous host *E. coli* TOP10. High resolution liquid chromatography mass spectrometry (HR-LC-MS) guided metabolite profiling of *E. coli* cells expressed with pDK-kgp1 revealed the presence of **2**, of which LC retention time and HR-MS profile matched the authentic **2** isolated from *M. aeruginosa* NIES-88 (Figure 2, traces 2-3, Figure SI-xx/xx). *E. coli* cells expressing pDK-kgp1 alone, however did not produce **1** (Figure 2, trace 5), which is potentially derived from **2** by bisprenylation at the *C*-3 of tryptophan by prenyltransferase KgpF. We hypothesized that this observation may be due to the lack of sufficient endogenous prenyl donor dimethylallyl pyrophosphate (DMAPP) in *E. coli* (ref). To overcome this problem, we co-expressed pDK-kgp1 in *E. coli* with plasmid pMBI that harbors four yeast mevalonate-dependent isoprenoid pathway biosynthetic genes that can convert mevalonate to isopentenyl pyrophosphate (IPP) (ref), a precursor to DMAPP. The co-expression of pDK-kgp1 and pMBI in *E. coli* TOP10 supplied with exogenous mevalonolactone **3** (1.0 mM), led to the robust production of both **1** and **2** that matched their authentic standards from *M. aeruginosa* NIES-88 (Figure 2, traces 1-2 & 6-7, Figure SI-xx/xx), as assessed by HR-LC-MS. These experiments establish *kgp*A-G genes confer the biogenesis of **1** and **2** *in vivo*. In addition, the coexistence of **1** and **2** in *E. coli* cells expressed with both pDK-kgp1 and pMBI and the lack of **1** in *E. coli* cells expressed with pDK-kgp1 alone, strongly suggests **2** is the direct biosynthetic precursor to **1** and bis-prenylation by KgpF is likely the last enzymatic step in the biosynthetic maturation of kawaguchipeptins.

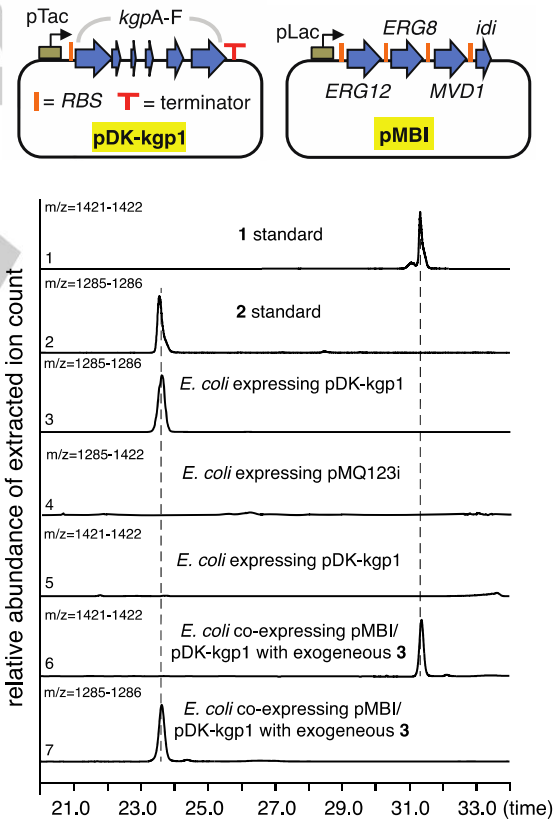

Figure 2. Heterologous expression of *kgp* operon in *E. coli* demonstrates *kgp*A-G genes confer the production of **1** and **2** *in vivo*. Extracted ion chromatographs of HR-LC-MS analysis of: 1-2) Authentic **1** and **2** from *M. aeruginosa* NIES-88; 3) **2** in *E. coli* expressed with pDK-kgp1; 4) **1** and **2** in *E. coli* expressed with pMQ123i; 5) **1** in *E. coli* expressed with pDK-kgp1; 6) **1** in *E. coli* co-expressed with pDK-kgp1 and pMBI and supplied with

mevalonolactone **3**; 7) **2** in *E. coli* co-expressed with pDK-kgp1 and pMBI and supplied with mevalonolactone **3**.

We purified the recombinant KgpF from *E. coli* and assessed its enzymatic activity to process a range of cyclic and linear peptides (Table 1). With some substrates, we also assessed the ability of the enzyme to use isopentenyl pyrophosphate (IPP), DMAPP and geranyl pyrophospahte (GPP).

Table 1. Substrates and co-factors used for the *in vitro* reactions.

Substrate	Co-factor	Mono-prenylation	Di-prenylation	Unprocessed substrate
Cyclic [WLNGDNNWSTP] (**2**)	DMAPP	+	+	
Cyclic [WLNGDNNWSTP] (**2**)	IPP	+	-	
Cyclic [WLNGDNNWSTP] (**2**)	GPP	-	-	
Cyclic [TSQIWGSPVP] (**3**)	DMAPP	+		
Cyclic [SAQWQNFGVP] (**4**)	DMAPP	+		
Cyclic [HAFIGYDQDPTGKYP] (**5**)	DMAPP	+		
Cyclic [RERFVYP] (**6**)	DMAPP			+
Cyclic [LIGIMHP] (**7**)	DMAPP			+
WLNGDNNWSTP (**8**)	DMAPP	+	-	
WLNGDNNWSTPAYDG (**9**)	DMAPP	+	-	
EDWYFDHPAYDG (**10**)	DMAPP	-		+
VPWPFPAYDG (**11**)	DMAPP	-		+
Boc-Trp (**12**)	DMAPP	-		+
Boc-Trp (**13**)	IPP	-		+
Boc-Tyr (**14**)	DMAPP	-		+
Boc-Tyr (**15**)	IPP	-		+

[+] Product detected, [-] Product not detected.

Our results showed that the enzyme processes a second Trp residue within macocyclic peptide substrate **2** whereas in the linear peptide **8,** despite 40 h of incubation we see only a single modification when DMAPP is the cofactor (Table 1; Figures S-S). The catalytic activity of the enzyme decreases when IPP is used as a cofactor instead of DMAPP, this seen in the processing of one Trp residue out of two in kawaguchipeptin B (**2**) when IPP not DMAPP is used (Table 1; Figures S-S). Apart from Trp, we did not detect any processing of other residues in the linear or macrocyclic peptide substrates. The enzyme did not process Boc-Trp (13) in presence of DMAPP or IPP. Interestingly, the enzyme could not use GPP as a cofactor.

All cyanobactin gene clusters reported to date encode a putative prenyltransferase.[2,21] *O*- or *C*-prenylation of tyrosine, serine and threonine in forward or reverse orientation have been observed for cyclic cyanobactins, while the linear cyanobactins exhibit *N*-prenylation.[4,5,19,20] Whilst cyanobactins such as prenylagaramide and trunkamide exhibit *O*-prenylated tyrosine, threonine and serine,[6,11] *C*-prenylated peptides like the *C*-prenylated tyrosine in aestuaramides has been reported.[12] However, the *C*-prenylated tyrosine in aestuaramides is the result of reverse *O*-prenylation on oxygen atom of tyrosine by DMAPP followed by claisen rearrangement.[12] The biochemical characterization of *C*-3 tryptophan prenylation[13] and the demonstration of the presence of the homologue of prenyltransferase *kgpF* gene in the gene cluster (see table S1 supplementary content) confirms that this is a direct post-translational modification that is rare in cyanobactins. To our knowledge, *C*-3 prenylation of tryptophan as a post-translational modification of a peptide has been demonstrated previously only for ComX peptide, a pheromone produced by *Bacillus subtilis* and related bacilli.[22,23] Here however ComX precursor peptides are geranylated at tryptophan prior to cleavage and subsequent enzymatic modification to prenylated tryptophan.

Tryptophan prenylation is common in some plants, bacteria but mostly in fungi and the respective prenyltransferases catalyze the addition of dimethylallyl group in tryptophan by DMAPP during the synthesis of secondary metabolites.[24,25,26] Those compounds, particularly the indole alkaloids that contain prenylated tryptophan at their core have been subjects of considerable synthetic and biosynthetic interests.[27] The finding in this study expands the chemical diversity of cyanobactins and confirms the existence of a tryptophan prenyltransferase. The prenyltransferase of the cyanobactin family are now known to catalyze the O, C and N prenylation of amino acids in the cyclic peptide.

Experimental Section

Experimental detail can be found in the supplementary online information.

Cultivation of *Microcystis aeruginosa* NIES-88, DNA extraction and genome sequencing

Microcystis aeruginosa NIES-88 was grown in Z8 medium[28]at 22–25˚C at a photon irradiance of 20–27 mmol m^{-2} s^{-1} for 21 days. High molecular weight DNA was extracted from this strain using lysozyme lysis and a phenol-chloroform extraction as previously described. [29] The genome of *Microcystis aeruginosa* NIES-88 was sequenced using 454 platforms with a very high depth of 40.51X coverage, constituting of equal amount of Titanium standard data and the 3kb paired-end data. All reads were assembled using Newbler versions 2.7. Genomic analysis and manual functional annotation was performed using Artemis,[30] and translated into amino acid sequences for homologue searches with the National Center for Biotechnology Information non-redundant database, the InterPro,[31] Cluster of Orthologous Groups[32] for annotation.

Annotation of the *kgp* gene cluster

From the genome sequence of *Microcystis aeruginosa* NIES-88, open reading frame (ORF) were predicted using Glimmer[31] and the *kgp* gene cluster was annotated using Artemis (Sanger institute). [30] The precursor peptide was located in the genome using the amino acid sequence of kawaguchipeptin B WLNGDNNWSTP as a query and subsequently confirmed by BLASTp searches for the homology of the N-terminal sequence of the precursor peptide amino acid with the precursor peptide of patellamide, anacyclamide, trichamide and microcyclamide.[4,9,11,33] The proteins encoded by the biosynthetic gene cluster were analyzed for the function using BLASTp and start sites of the genes were checked manually.

Cloning and heterologous expression of the kawaguchipeptin biosynthetic gene cluster

Kawaguchipeptin A and kawaguchipeptin B were produced by direct heterologous expression of the 10-kb *kgp* gene cluster in *E. coli.*

The complete kawaguchipeptin (*kgp*) biosynthetic gene cluster from *Microcystis aeruginosa* NIES-88 was cloned by PCR and assembled via the *Saccharomyces cerevisiae* yeast recombineering strain VL6-48N. Primer sequences were designed to amplify three DNA fragments of sizes 2420bp, 2952bp, and 2410bp (Supplementary table 2), each of which contained homologous flanking regions amenable for yeast recombineering between the SpeI and HindIII restriction sites of plasmid pMQ123i.[34] PCR was performed using a high fidelity DNA polymerase kit (KAPA Biosystems, Wilmington, Massachusetts). Each PCR reaction proceeded using an annealing temperature of 45°C with an extension time of 4:00 minutes for a total of 38 cycles, and each 10µL-scale PCR reaction contained 100ng of purified genomic DNA as template. The complete kawaguchipeptin biosynthetic gene cluster in plasmid pMQ123i was co-introduced with plasmid pMBI (Martin 2003) into *E. coli* strain TOP10 by electroporation (Bio-Rad, MicroPulserTM) and selected on LB agar plates supplemented with gentamicin (10µg/mL) and ampicillin (100µg/mL). A seed culture of E. coli TOP10 containing the kawaguchipeptin biosynthetic gene cluster was grown overnight in LB media with appropriate antibiotics at 37°C and then diluted 1000-fold to inoculate a 50mL-scale culture grown at 37°C in LB media with appropriate antibiotics. Once the optical density (600nm) of the expression culture reached approximately 0.6, the culture was cooled (25°C) and then induced with IPTG (1mM) for 48hr. The cultures were centrifuged (4696g, 20 minutes) to clarity and the supernatants were extracted with ethyl acetate (2×50mL), the organic portions were combined, and then the combined portions were dried over sodium sulfate. The dried organic layers were evaporated under reduced pressure and gentle heating (37°C). The crude extract was dissolved in methanol to a concentration of approximately 10 mg/mL and centrifuged (21.1×103 g, 5min). The organic supernatant was then used for subsequent liquid chromatography-high resolution mass spectrometric (LC-HRMS) analysis. 10µL of MeOH dissolved supernatant extract was separated by reverse phase high performance liquid chromatography, (Phenomenex Jupiter® C18, 370Å, 3µm, 2×150mm) using a Dionex® Ultimate3000 HPLC and analyzed on a ThermoScientific Q-Exactive HRMS instrument using electrospray ionization. LC method used for analysis (0.2 mL/min flow rate): 5% (v/v) MeCN in H_2O (including 0.1% (v/v) formic acid) for 3 min, ramp to 95% (v/v) MeCN in H_2O (including 0.1% (v/v) formic acid) over 45 min, and then a final wash with 95% MeCN in H_2O (including 0.1% (v/v) formic acid) for 15 min.

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Keywords: kawaguchipeptins • prenyltransferase • tryptophan prenylation • cyanobactins • *C*-prenylation

[1] P.G. Arnison, M.J. Bibb, G. Bierbaum, A.A. Bowers, T.S. Bugni, G. Bulaj, J.A. Camarero, D.J. Campopiano, G.L. Challis, J. Clardy, *Nat. Prod. Rep.* **2013**, 30, 108-160
[2] K. Sivonen, N. Leikoski, D.P. Fewer, J. Jokela, *Appl. Microbiol. Biotechnol.* **2010**, 86, 1213-1225
[3] N. Leikoski, D.P. Fewer, K. Sivonen, *Appl. Environ. Microbiol.* **2009**, 75, 853-857
[4] N. Leikoski, D.P. Fewer, J. Jokela, M. Wahlsten, L. Rouhiainen, K. Sivonen, *Appl. Environ. Microbiol.* **2010**, 76, 701-709.
[5] N. Leikoski, L. Liu, J. Jokela, M. Wahlsten, M. Gugger, A. Calteau, P. Permi, C.A. Kerfeld, K. Sivonen, D.P. Fewer, *Chem. Biol.* **2013**, 20, 1033-1043.
[6] M.S. Donia, E.W. Schmidt, *Chem. Biol.* **2011**, 18, 508-519.
[7] J. Lee, J. McIntosh, B.J. Hathaway, E.W. Schmidt, *J. Am. Chem. Soc.* **2009**, 131, 2122-2124.
[8] J.A. McIntosh, M.S. Donia, E.W. Schmidt, *J. Am. Chem. Soc.* **2010**, 132, 4089-4091.
[9] E.W. Schmidt, J.T. Nelson, D.A. Rasko, S. Sudek, J.A. Eisen, M.G. Haygood, J. Ravel, *Proc. Natl. Acad. Sci. U. S. A.* **2005**, 102, 7315-7320.
[10] M.S. Donia, B.J. Hathaway, S. Sudek, M.G. Haygood, M. Rosovitz, J. Ravel, E.W. Schmidt, *Nature chemical biology* **2006**, 2, 729-735.
[11] M.S. Donia, J. Ravel, E.W. Schmidt, *Nature chemical biology* **2008**, 4, 341-343.
[12] J.A. McIntosh, M.S. Donia, S.K. Nair, E.W. Schmidt, *J. Am. Chem. Soc.* **2011**, 133, 13698-13705.
[13] K. Ishida, H. Matsuda, M. Murakami, K. Yamaguchi, *Tetrahedron* **1996**, 52, 9025-9030
[14] K. Ishida, H. Matsuda, M. Murakami, K. Yamaguchi, *J. Nat. Prod.* **1997**, 60, 724-726.
[15] A.L. Delcher, K.A. Bratke, E.C. Powers, S.L. Salzberg, *Bioinformatics* **2007**, 23, 673-679.
[16] T.M. Lowe, S.R. Eddy, *Nucleic Acids Res.* **1997**, 25, 0955-964.
[17] P.J. Casey, M.C. Seabra, *J. Biol. Chem.* **1996**, 271, 5289-5292.
[18] J.B. Gibbs, A. Oliff, *Annu. Rev. Pharmacol. Toxicol.* **1997**, 37, 143-166.
[19] J.A. McIntosh, M.S. Donia, E.W. Schmidt, *Nat. Prod. Rep.* **2009**, 26, 537-559.
[20] J.A. McIntosh, Z. Lin, M.D.B. Tianero, E.W. Schmidt, *ACS chemical biology* **2013**, 8, 877-883.
[21] E.W. Schmidt, M.S. Donia, *Meth. Enzymol.* **2009**, 458, 575-596.
[22] R. Magnuson, J. Solomon, A.D. Grossman, *Cell* **1994**, 77, 207-216.
[23] F. Tsuji, K. Kobayashi, M. Okada, H. Yamaguchi, M. Ojika, Y. Sakagami, *Bioorg. Med. Chem. Lett.* **2011**, 21, 4041-4044.
[24] S. Li, *Nat. Prod. Rep.* **2010**, 27, 57-78.
[25] C. Wallwey, S. Li, *Nat. Prod. Rep.* **2011**, 28, 496-510.
[26] H. Hulvová, P. Galuszka, J. Frébortová, I. Frébort, *Biotechnol. Adv.* **2013**, 31, 79-89.
[27] R. M. Williams, E. M. Stocking, J. F. Sanz-Cervera, *Top. Curr. Chem.***2000**, *209*, 97-173.
[28] J. Kotai, *Norwegian Institute for Water Research, Oslo* **1972**, 11, 5.
[29] D.P. Fewer, J. Osterholm, L. Rouhiainen, J. Jokela, M. Wahlsten, K. Sivonen, *Appl. Environ. Microbiol.* **2011**, 77, 8034-8040.
[30] K. Rutherford, J. Parkhill, J. Crook, T. Horsnell, P. Rice, M.A. Rajandream, B. Barrell, *Bioinformatics* **2000**, 16, 944-945.
[31] Zdobnov and Apweile 2001
[32] R.L. Tatusov, N.D. Fedorova, J.D. Jackson, A.R. Jacobs, B. Kiryutin, E.V. Koonin, D.M. Krylov, R. Mazumder, S.L. Mekhedov, A.N. Nikolskaya, B.S. Rao, S. Smirnov, A.V. Sverdlov, S. Vasudevan, Y.I. Wolf, J.J. Yin, D.A. Natale,*BMC Bioinformatics* **2003**, 4, 41**.**
[33] N. Ziemert, K. Ishida, P. Quillardet, C. Bouchier, C. Hertweck, N.T. de Marsac, E. Dittmann, *Appl. Environ. Microbiol.* **2008**, 74, 1791-1797
[34] R.M. Shanks, D.E. Kadouri, D.P. MacEachran, G.A. O'Toole, *Plasmid* **2009**, 62, 88-97.

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