Genome mining and bioactivity assays as tools to discover cyanobacterial natural products

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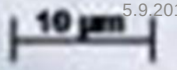
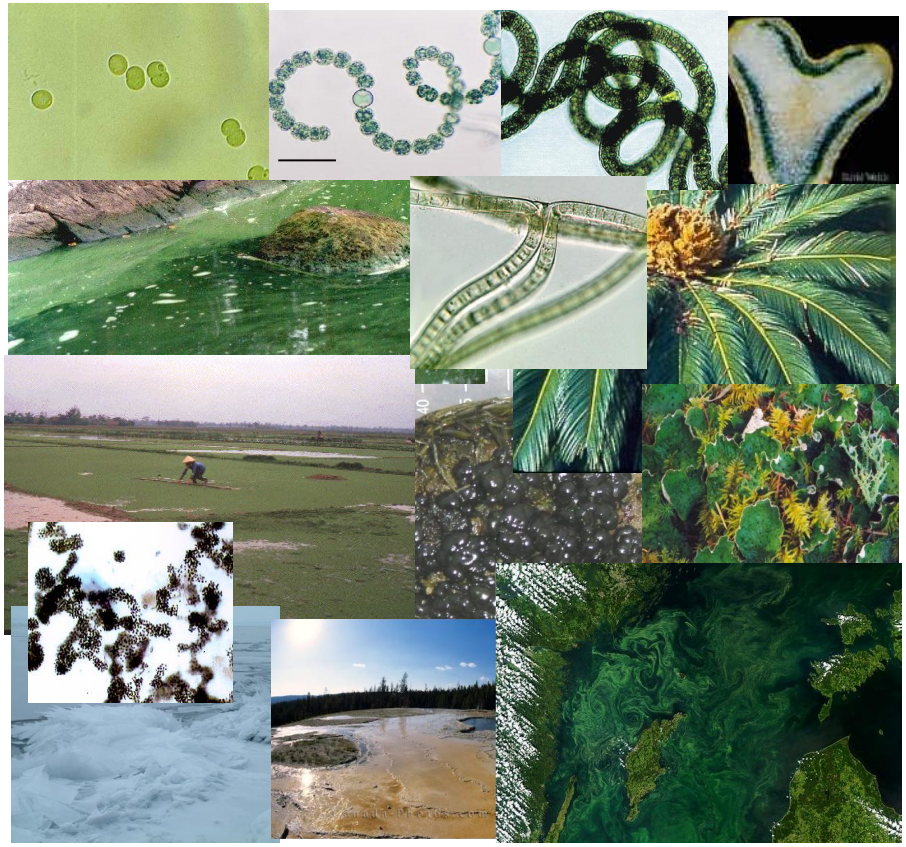


Cyanobacteria occur in various environments



Cyanobacteria are photosynthetic microbes and morphologically diverse (unicellular -> filamentous)

Aquatic environments:

Lakes, brackish water, oceans, hot springs, ice – benthic and planktonic

Terrestrial:

Soils, desert crusts

Symbioses

Plants, lichens, ascidians

How to find new bioactive compounds?

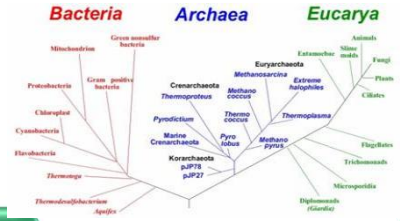
Culture collections

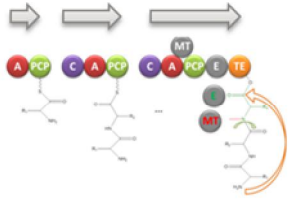
Bioactivity

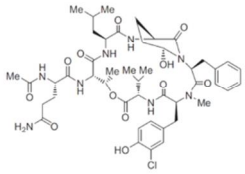
- Preparation of crude extracts
- Bioactivity assays

Genomics & bioinformatics

- Sequencing and annotation
- Identification of gene clusters and their evolution
- Substrate predictions







Chemical analysis

- Purification of compounds
- Stable isotope labelling
- LC-MS, Q-TOF + NMR
- Amino acid analysis

Molecular biology

- Heterologous expression
- Gene knock-outs
- Site-directed mutagenesis

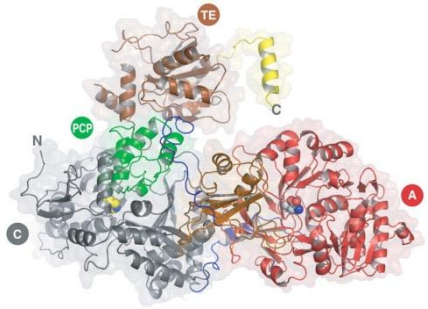
Biochemistry

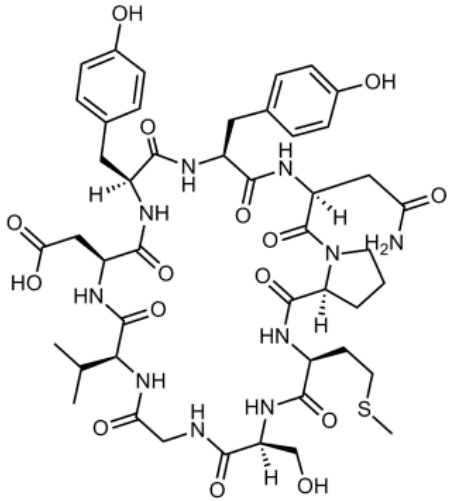
- ATP-pyrophosphate exchange assay
- Characterization of enzymes

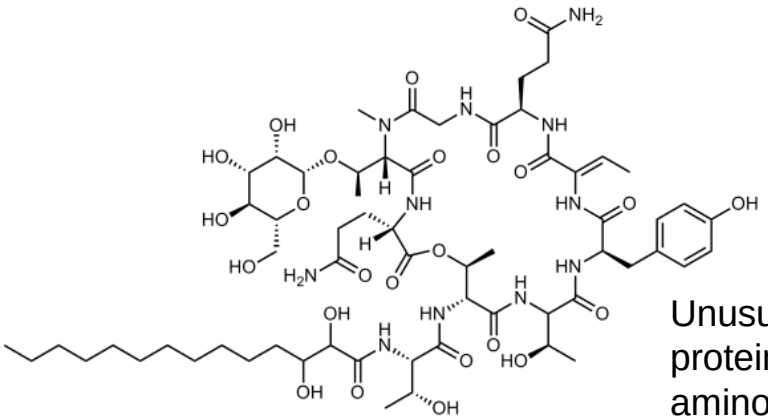
Biosyntheses of bioactive compounds

Ribosomal pathway
+ post-translational modifications

Nonribosomal pathway



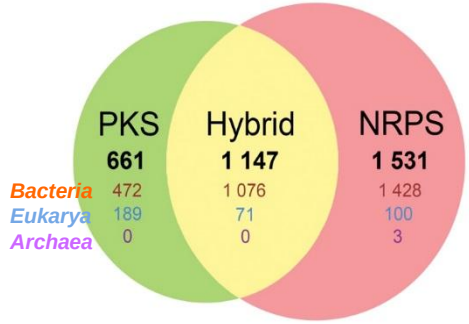


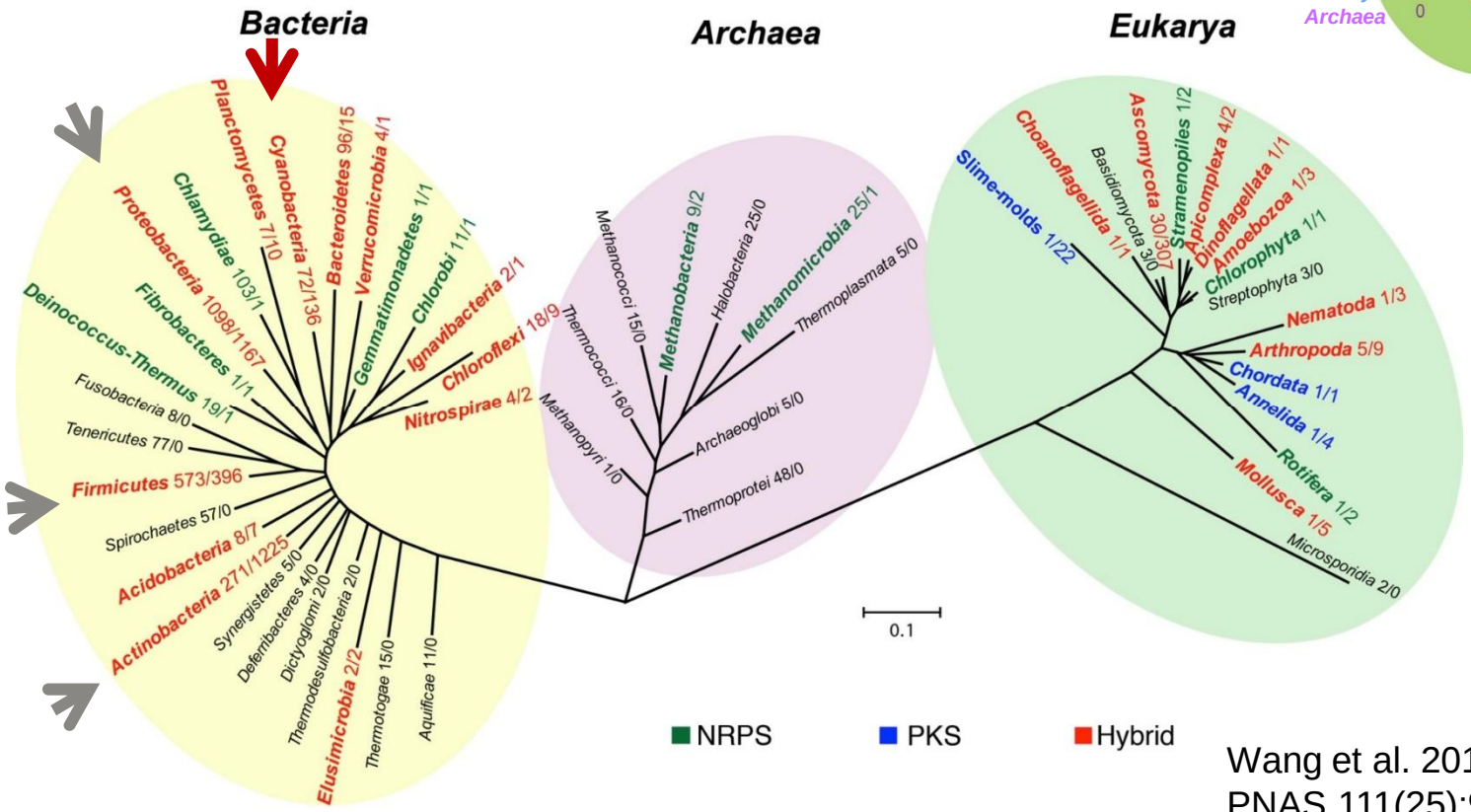


Unusual non-proteinogenic amino acids

Widespread distribution of NRPSs and modular PKSs across three domains of life

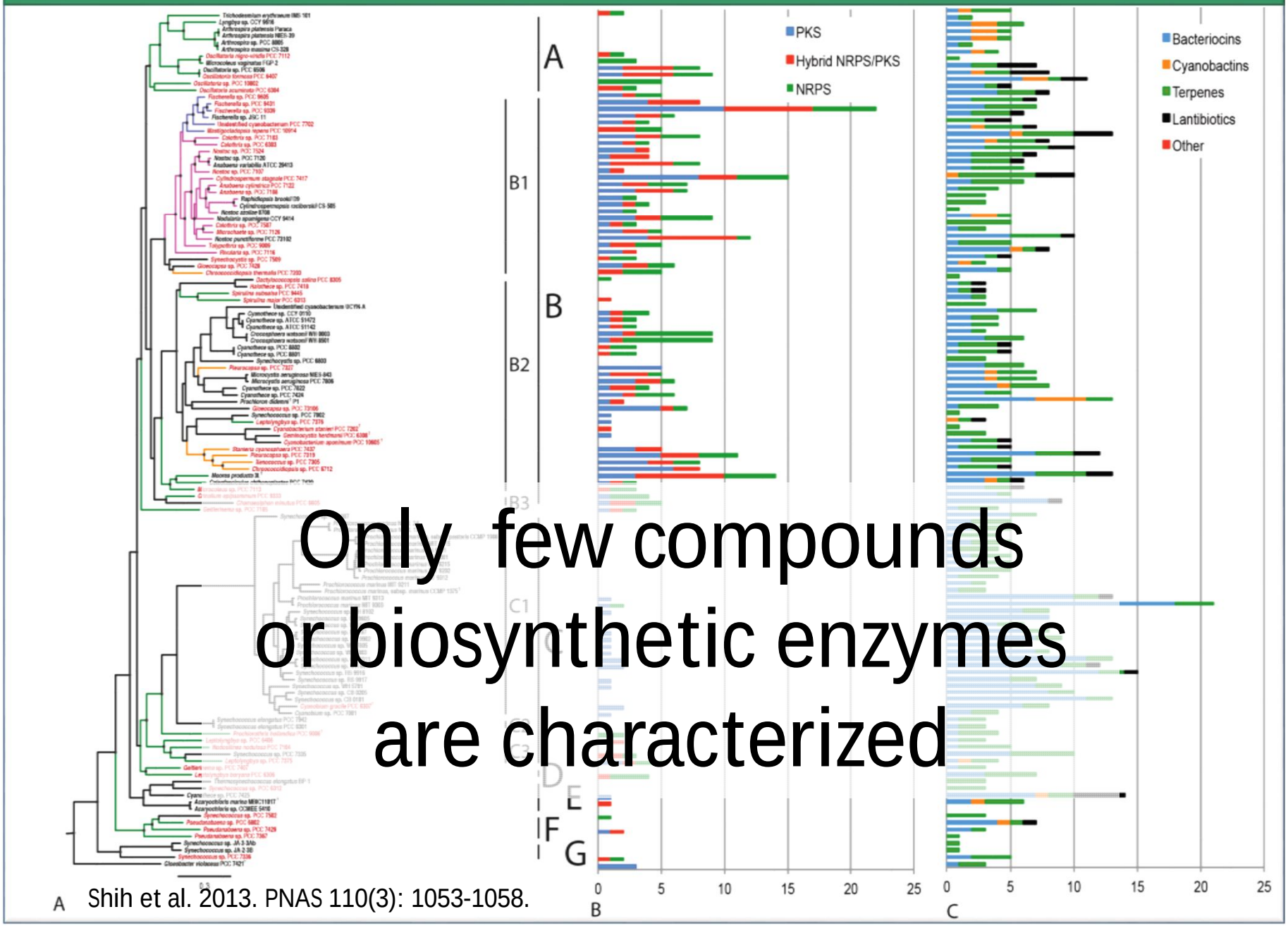
Total of 2478 bacterial, 160 archeal and 61 fugal genomes were analyzed





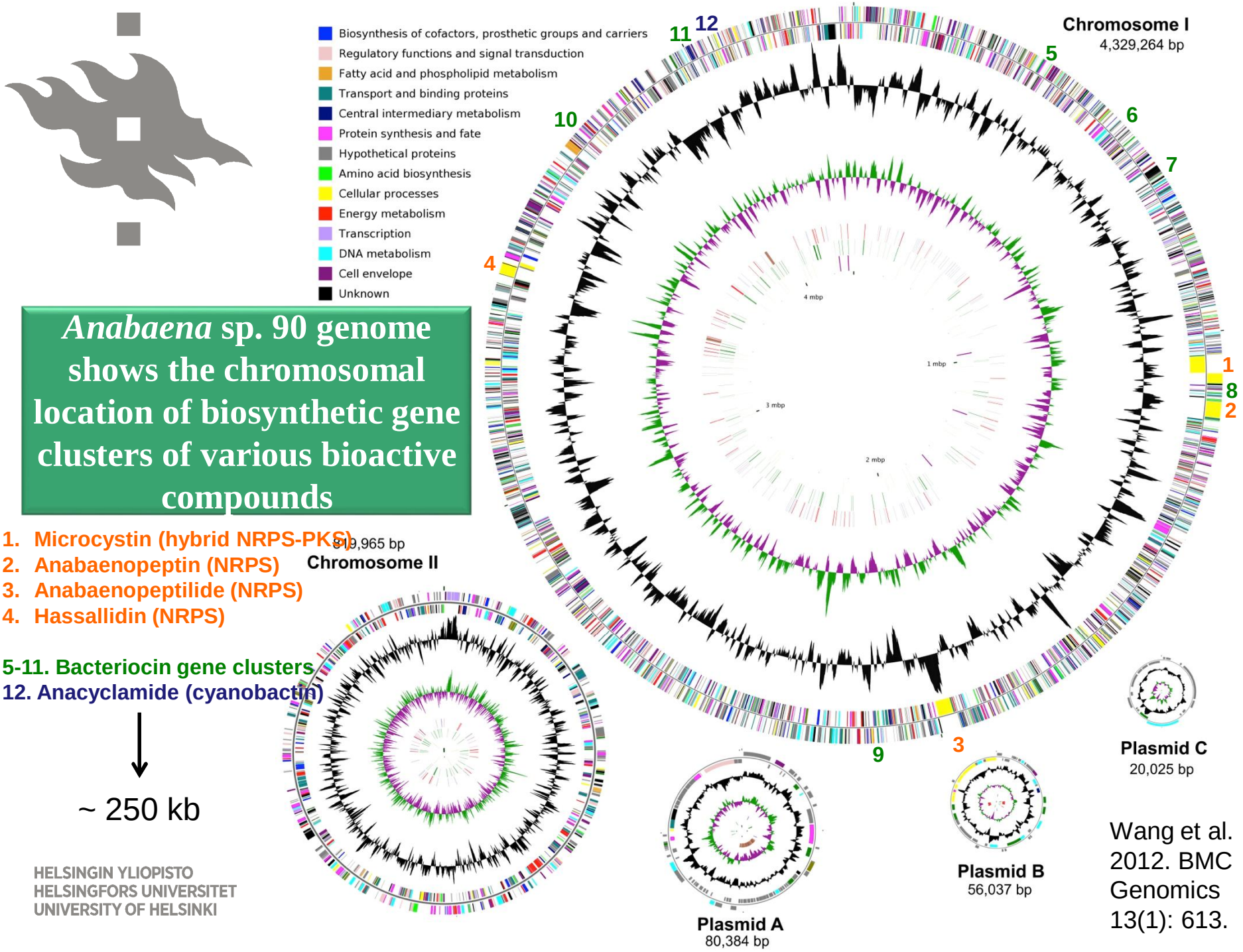
Wang et al. 2014
PNAS 111(25):9259-9264

Cyanobacterial species tree and distribution of peptide biosynthesis



Only few compounds or biosynthetic enzymes are characterized

A Shih et al. 2013. PNAS 110(3): 1053-1058.


Biosynthesis of cofactors, prosthetic groups and carriers
Regulatory functions and signal transduction
Fatty acid and phospholipid metabolism
Transport and binding proteins
Central intermediary metabolism
Protein synthesis and fate
Hypothetical proteins
Amino acid biosynthesis
Cellular processes
Energy metabolism
Transcription
DNA metabolism
Cell envelope
Unknown
Anabaena sp. 90 genome shows the chromosomal location of biosynthetic gene clusters of various bioactive compounds
1. Microcystin (hybrid NRPS-PKS)
2. Anabaenopeptin (NRPS)
3. Anabaenopeptilide (NRPS)
4. Hassallidin (NRPS)
5-11. Bacteriocin gene clusters
12. Anacyclamide (cyanobactin)
~ 250 kb
Chromosome I
4,329,264 bp
Chromosome II
Plasmid A
80,384 bp
Plasmid B
56,037 bp
Plasmid C
20,025 bp
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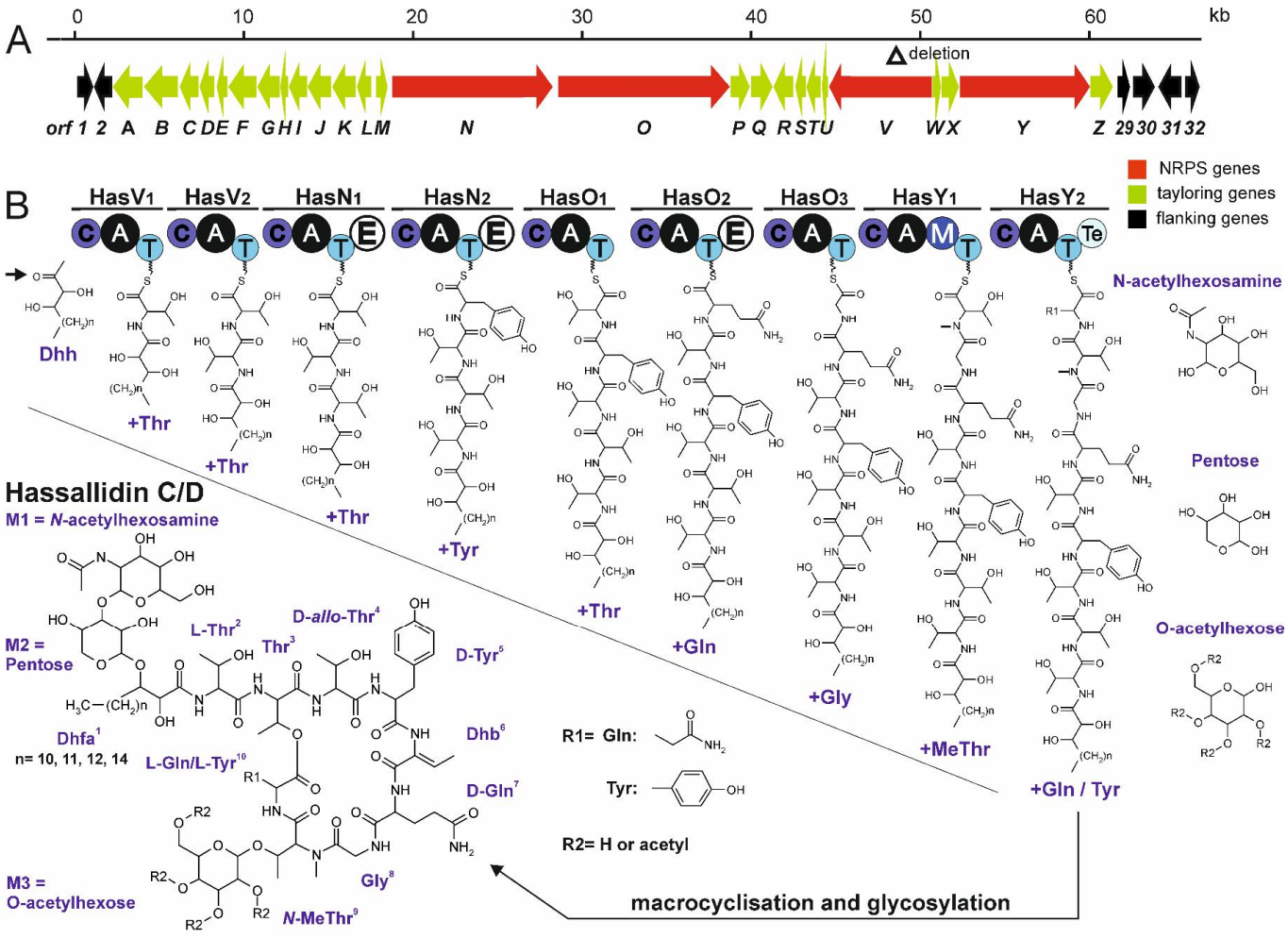
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI
Wang et al. 2012. BMC Genomics 13(1): 613.

Gene cluster of antifungal hassallidin

Hassallidins were found to be produced by
Anabaena
Cylindrospermopsis
Aphanizomenon
Tolypothrix
Planktotrix

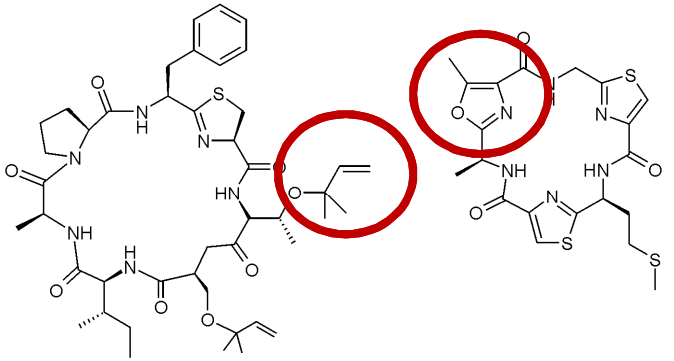


Vestola et al. 2014. PNAS 111(18):E1909-17. Pancrace et al. 2017. ACS Chemical Biology 12 (7):1796–1804.

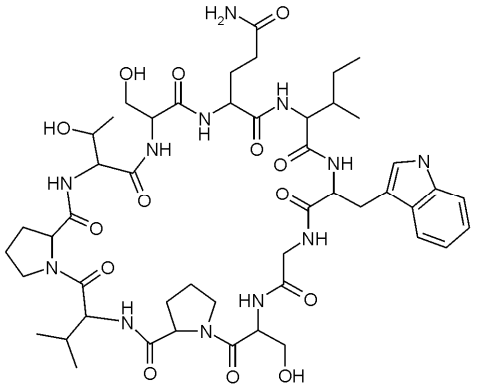
Ribosomal peptides – cyanobactins from cyanobacteria:

Cyanobactins contain oxazoles, thizoles, disulfide bridges, prenyl and geranyl groups

Microcyclamide
(Ziemert et al. AEM 2008)

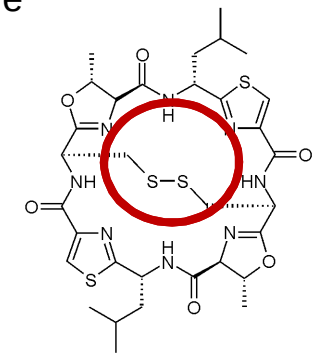


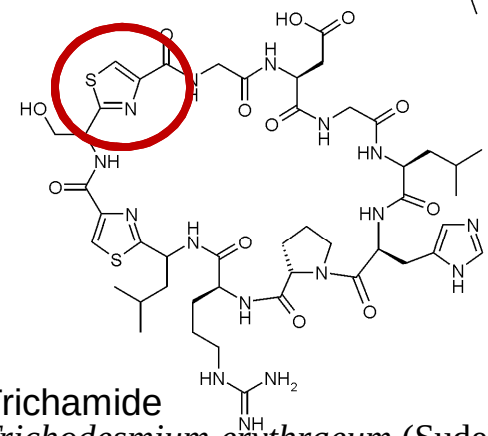
Trunkamide



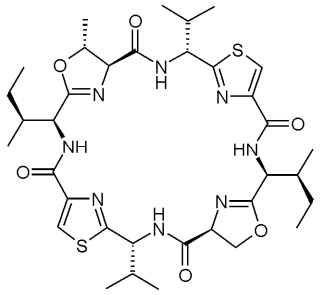
Bioactivities; cytotoxic, multi-drug resistance reversing properties, anti-malarial, antimicrobial, anti-viral and allelopathic

Ulithiacyclamide





Trichamide
Trichodesmium erythraeum (Sudek et al. 2006, AEM)



Patellamide A, *Prochloron didemni* (Schmidt et al. 2005, PNAS)

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Review: Sivonen et al. 2010. Appl. Microbiol. Biotechnol. 86(5): 1213-1225.

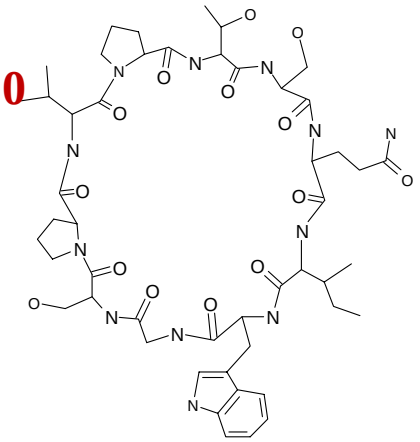
Cyanobactins include peptides without heterocycles

We sequenced precursor genes from ~45 *Anabaena* strains and identified 17 peptides. *Anabaena* 90 gene cluster expressed in *E. coli*

MW	RT	Sequence	
1052,5	23,8	TSQIWGSPVP	(6)
1009,5	28,7	SSVIWGSPVP	(2)
1150,5	19,9	NAHWQNFGVP	(1)
1045,5	30,6	YDDKLNLSP	(1)
1086,6	25,2	YAPLQNFGVP	(1)
1689,8	27,0	HAFIGYDQDPTGKYP	(~1)
1114,5	26,6	SAQWQNFGVP	(2)
1122,5	32,1	YSNKPSDFSP	(1)
761,3	23,4	LIGIMHP	(1)
948,3	14,0	RERFVYP	(1)

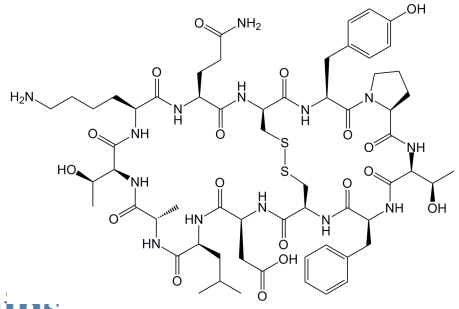
Leikoski et al. 2010. AEM 76:701-709

Anacyclamide A10



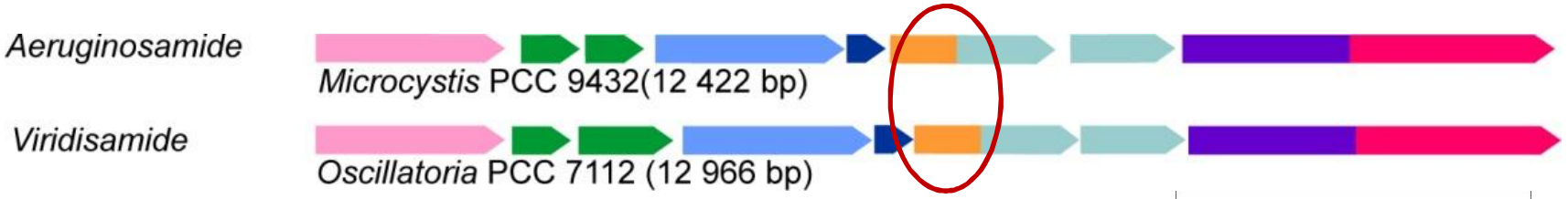
We screened 74 *Microcystis* strains with PCR and LC-MS and identified 10 cyanobactin variants in 6 *Microcystis* strains.

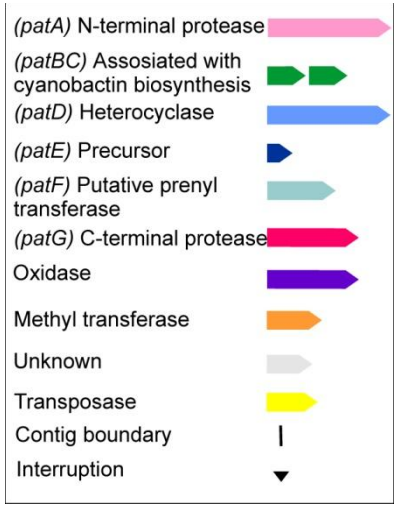
Leikoski et al. 2012 PLoS ONE 7:e43002



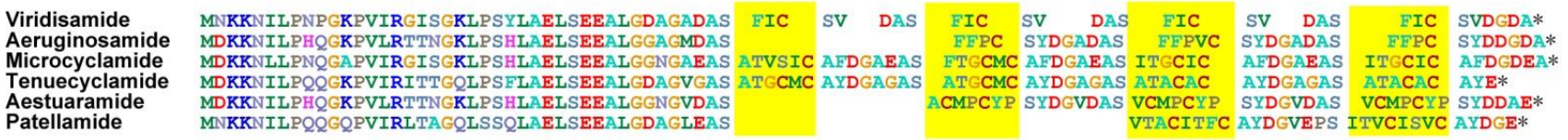
Piricyclamides

Novel cyanobactin pathways





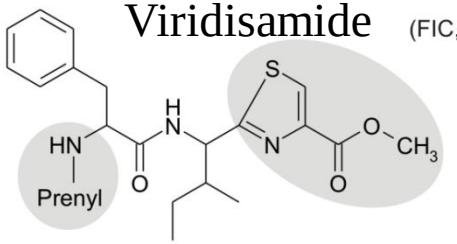
Some of the cyanobactin pathways contained methyl transferases



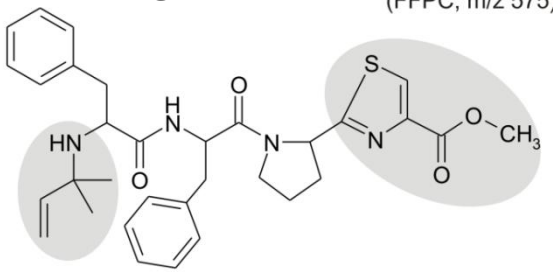
Leikoski et al., 2013. Chemistry & Biology 20:1033–1043.

Expanding the cyanobactins to include linear peptides and novel modifications

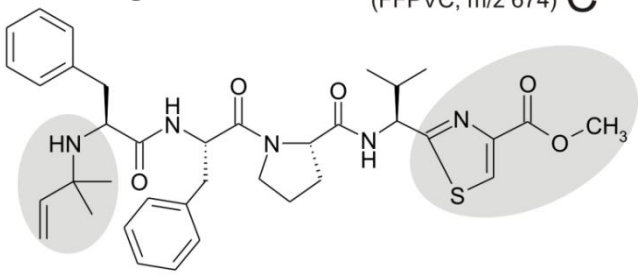
Viridisamide (FIC, m/z 444) A



Aeruginosamide (FFPC, m/z 575) B



Aeruginosamide (FFPVC, m/z 674) C



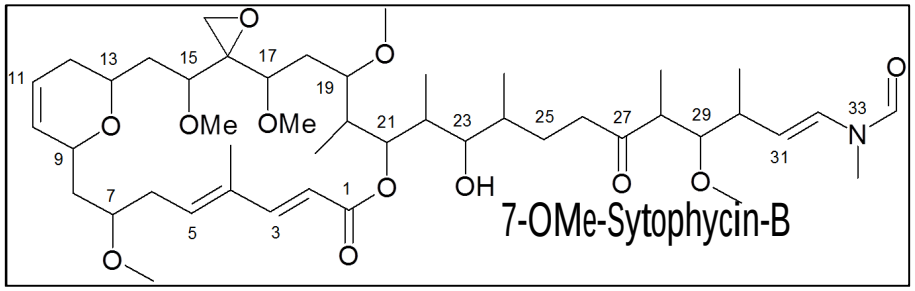
Cyanobacteria produce short linear cyanobactins with a chain length ranging from 3-5 amino acids.

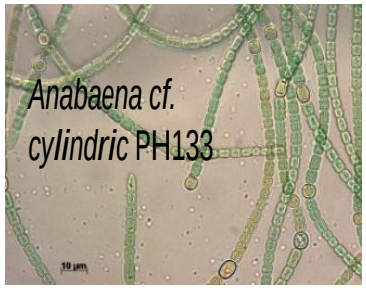
The linear peptides were *N*-prenylated and *O*-methylated on the N and C terminus, respectively, and named aeruginosamide and viridisamide.

Most recently, we showed that the enzyme PirF catalyzes Tyr *O*-geranylation, which is an unprecedented post-translational modification.

Leikoski et al. 2013. Chemistry & Biology 20:1033–1043.
Morita et al. 2018. Journal of the American Chemical Society 140(19): 6044-6048.

Screening of antifungal compounds: Scytophycins were identified in 4 strains





- Scytophycins were fist identified from *Scytonema* (Ishibashi et al 1986 J. Org. Chem 51:5300-5306) and later found also from *Nostoc* and *Cylindrospermum*
- We identified total of 33 structural variants of scytophycins and found *Anabaena* as a new producer and revealed their biosynthesis.

Shishido et al. 2015, Marine Drugs 13(4): 2124-2140. Humisto et al. 2018. Appl. Environ. Microbiol. 84 (3): e02321-17.

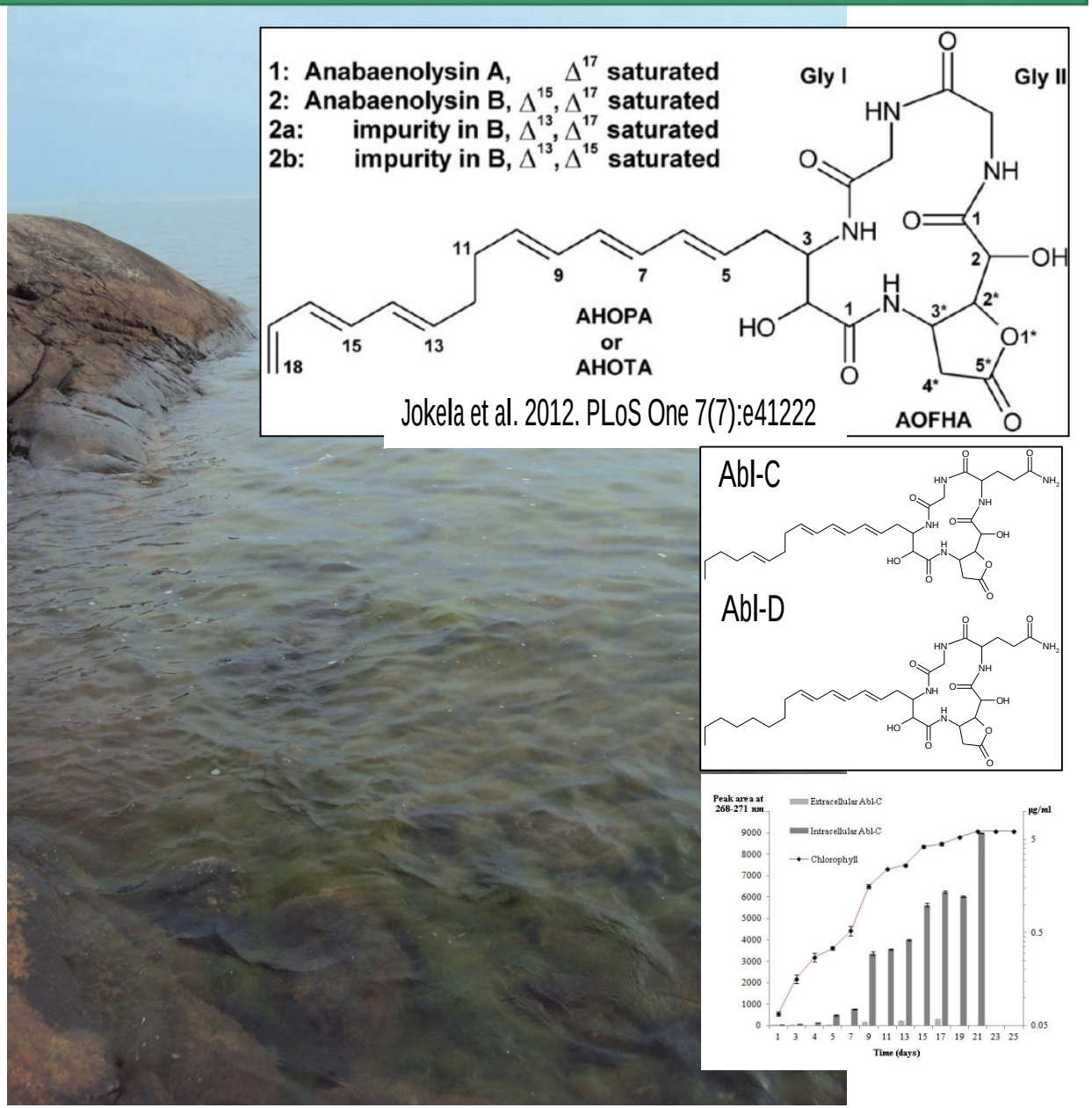
Anabaenolysins produced by benthic *Anabaena*

Anabaenolysins induce

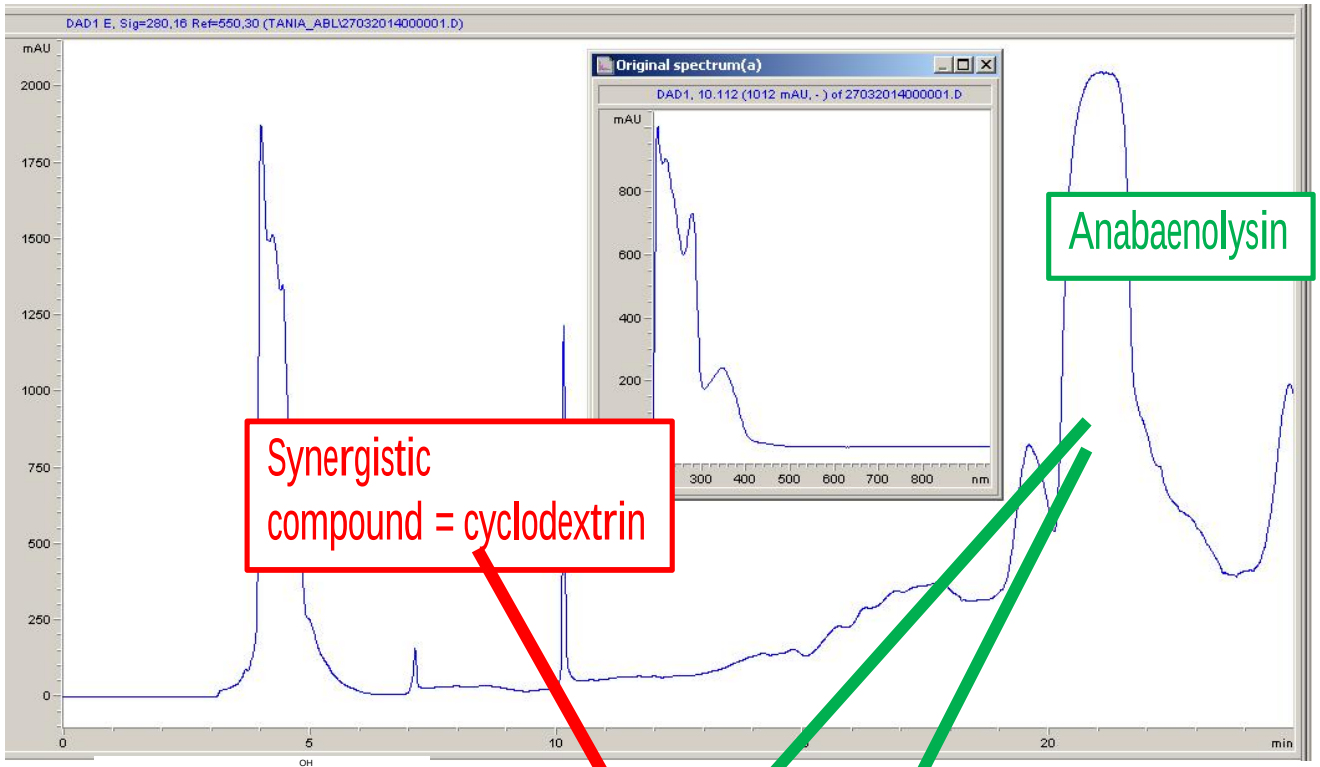
- cytolytic cell death of nucleated cells
- hemolysis of red blood cells
- echinocyte transformation

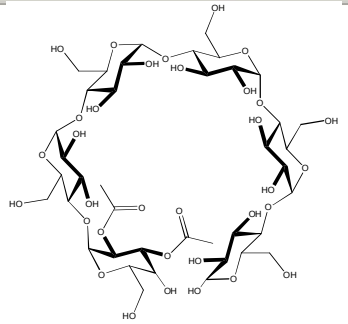
and are potent detergents for membranes containing cholesterol

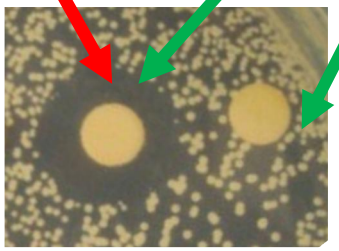
Oftendal et al. 2012. Biochim. Biophys. Acta, 1818: 3000–3009.



Bioactivity of anabaenolysin and synergistic compound







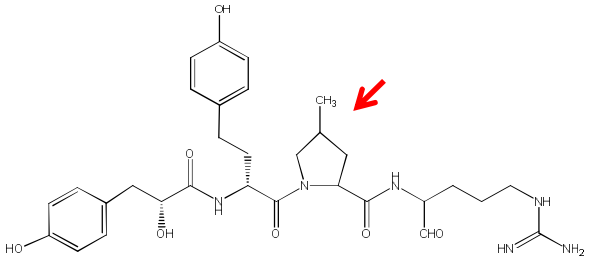
Cyclodextrins form inclusion complexes with many molecules

Shishido et al. 2015. PNAS 112:13669-13674

Compounds containing 4-methylproline

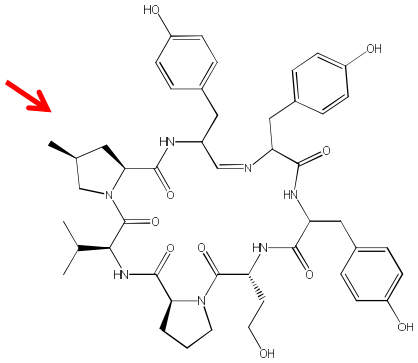
- Bioactivities:
  - anti-bacterial
  - anti-fungal
  - anti-inflammatory
  - antitoxin (microcystins and nodularin)
  - cytotoxic
  - protease inhibition

- Diverse chemical structures



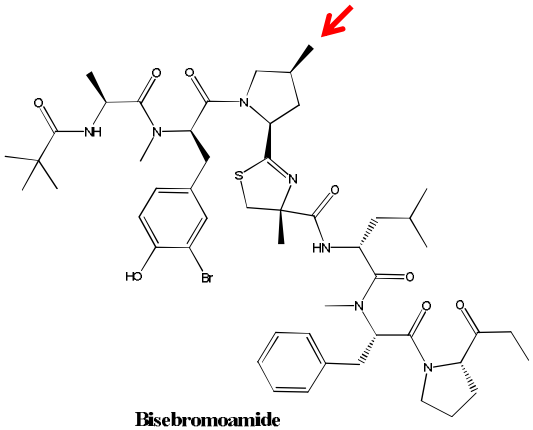
Spumigin E

Fewer et al 2009. Mol. Microbiol. **73**:924-937.



Nostocyclopeptide M1

Jokela et al., 2010. ChemBioChem **11**:1594-1599.



Bisebromoamide

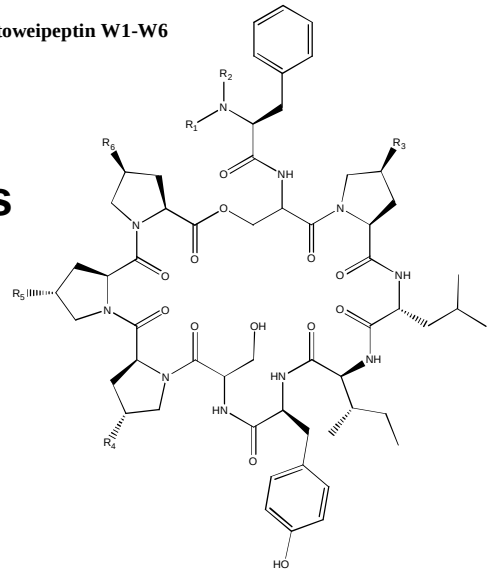
Teruya et al. 2009. Org Lett 11:5062-5065

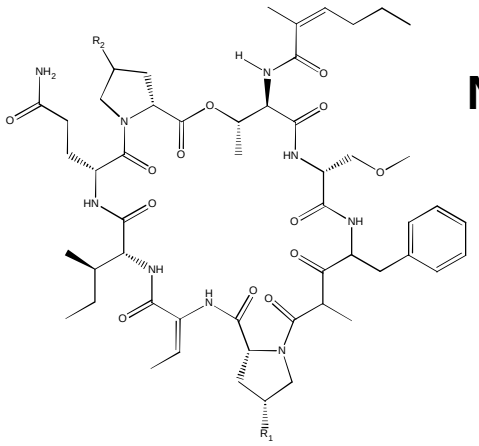
4-methylproline containing compounds in cyanobacterial strains

- 30 cyanobacterial genomes out of 116 strains studied had gene cluster to code for 4-methylproline
- 116 cyanobacteria strains screened by PCR and LC-MS found *Nostoc as* the main producer
- Two new groups of peptides were identified.

Nostoweipeptins
6 variants

Nostoweipeptin W1-W6



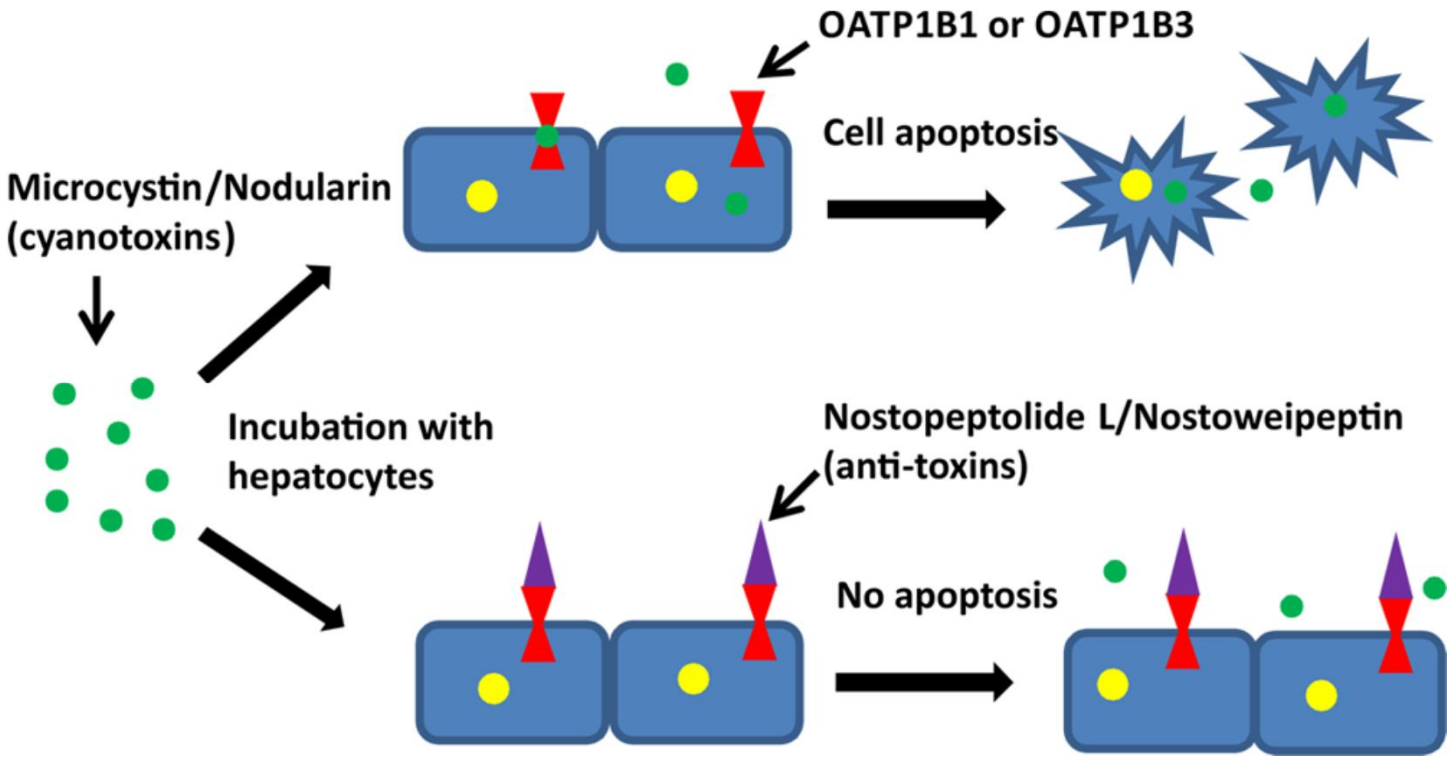


Nostopeptolide L1-L4

Nostopeptolides
4 variants

Liu et al. 2014. ACS Chemical Biology 9(11): 2646-2655.

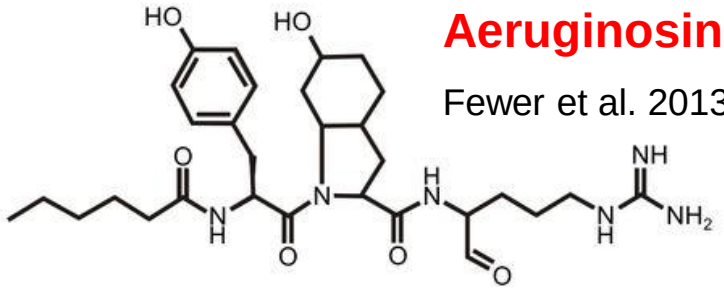
4-methylproline containing compounds prevent apoptosis caused by cyanobacterial hepatotoxins



Liu et al. 2014. ACS Chemical Biology 9(11): 2646-2655.

Pseudoaeruginosins from *Nodularia spumigena* are most likely produced by co-operation of two peptide synthetases

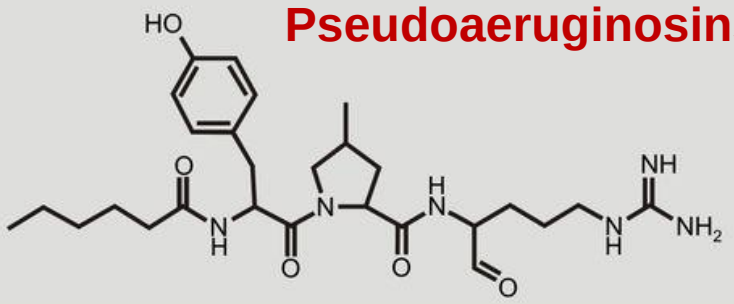
A



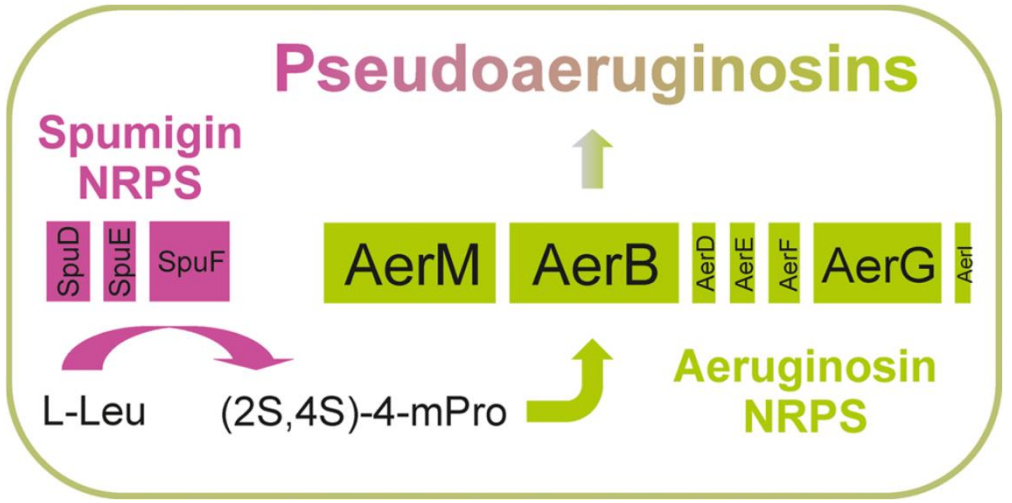
Aeruginosins

Fewer et al. 2013. PLoS One. 8(9):e73618.

B



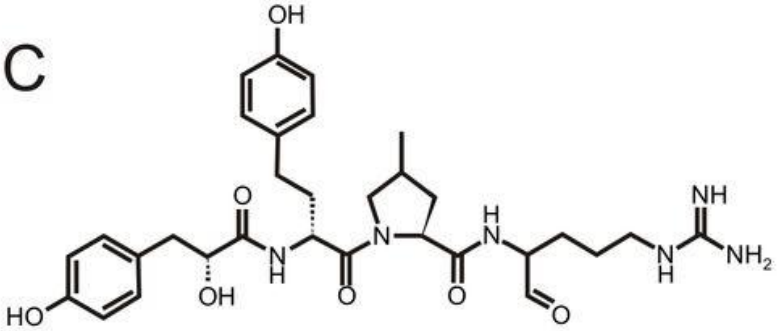
Pseudoaeruginosins



Pseudoaeruginosins were found from 33 *N. spumigena* strains isolated from the Baltic Sea

Liu et al. 2015. ACS Chemical Biology 10(3): 725-733.

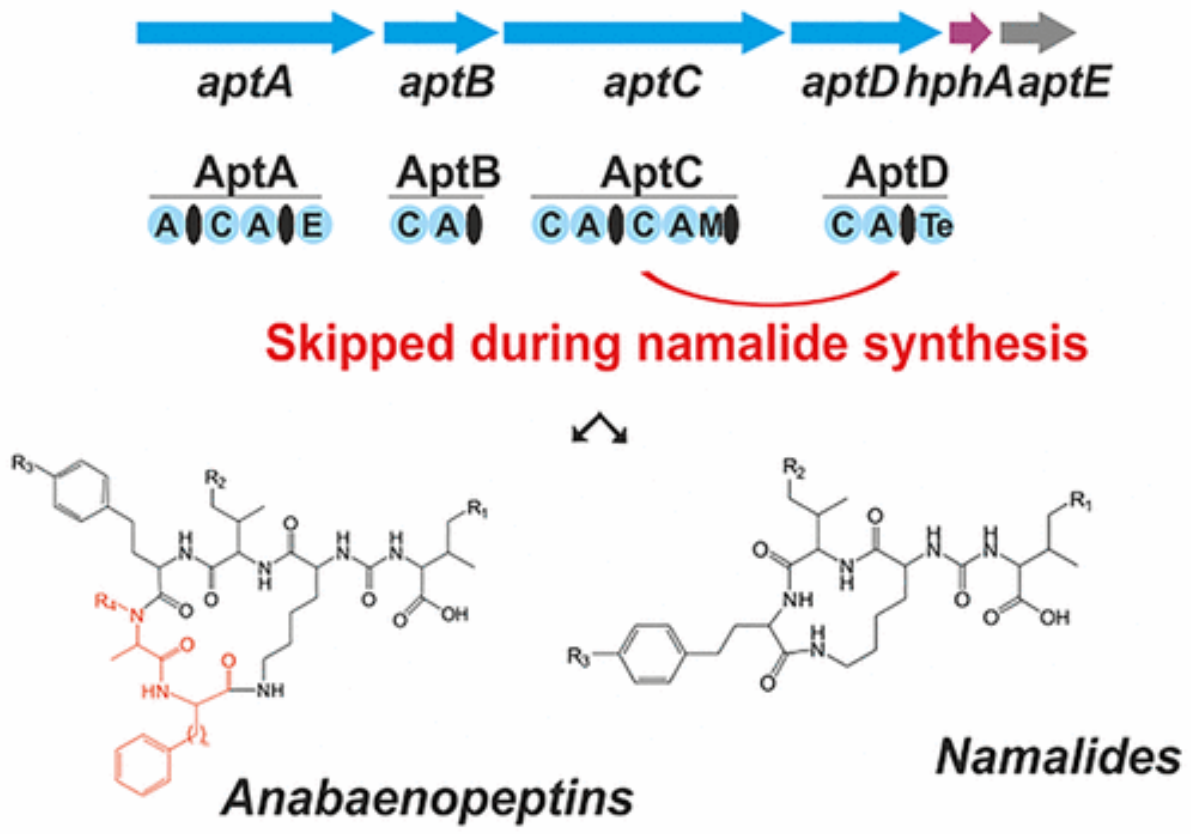
C



Spumigins

Fewer et al. 2009. Mol. Microbiol. 73: 924-937

Anabaenopeptins and namalides originate from same gene cluster

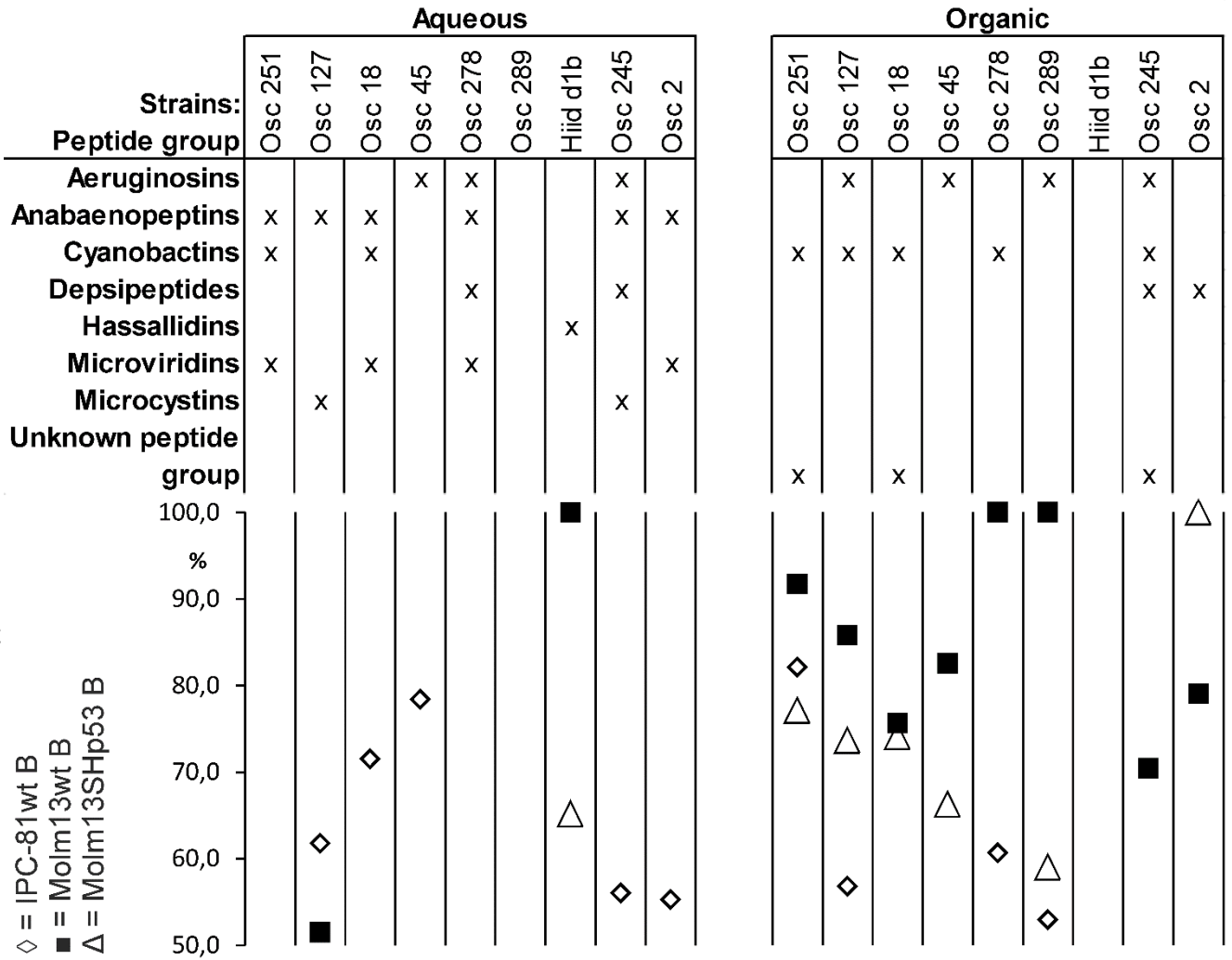


Shishido et al. 2017. ACS Chemical Biology 12(11):2746-2755.

Screening of antileukemic activity of cyanobacterial extracts - MS library helps us to exclude the known compounds

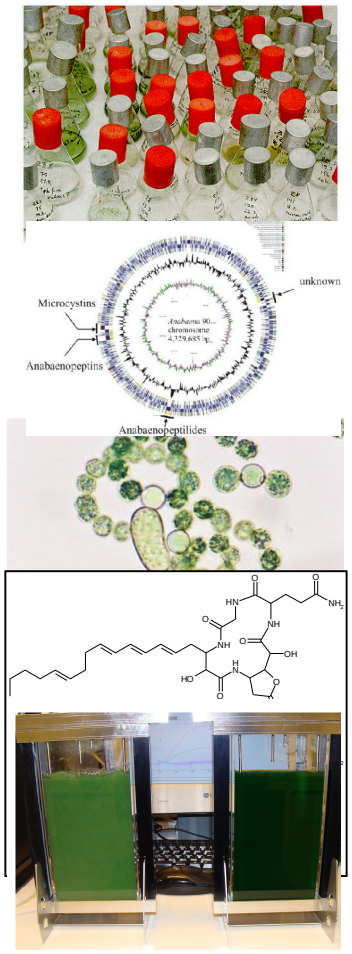
Compounds identified

Antileukemic activity



Humisto et al. 2015.Current Pharmaceutical Biotechnology 17(1):78-91.

Concluding remarks



- Culture collections of cyanobacteria are valuable resources
- Number of new cyanobacterial bioactive compounds as well as their biosynthesis were discovered
- Combination of genomics, bioinformatics, biochemistry, bioactivity screening and chemical identification of compounds are likely to yield number of new discoveries
- Cyanobacteria continue to be important source for novel bioactive compounds and biosynthetic pathways

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