HETEROLOGOUS PRODUCTION OF POTENT PROTEIN KINASE C ACTIVATORS FROM AN UNCULTURED MARINE CYANOBACTERIUM IN *ESCHERICHIA COLI*

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Introduction. Heterologous expression of polyketide (PK) and nonribosomal peptide (NRP) biosynthetic pathways for enhanced production of chemically complex bioactive compounds is frequently reported for metabolites produced by actinobacteria and proteobacteria. However, descriptions of heterologous expression, and therefore exploitation and characterization, of the many known cyanobacterial PKs and NRPs are not found in the literature, with the exception of 4-O-demethylbarbamide that was produced in *Streptomyces* at very low levels (<1 µg/L) (1). Cyanobacteria are prolific producers of natural products products that exhibit a diverse range of bioactivities, ensuring they are highly valued as lead pharmaceutical and industrial compounds (2).
Here we develop a framework for the expression of these multifunctional cyanobacterial assembly lines in *Escherichia coli* using the lyngbyatoxin biosynthetic pathway (3), derived from a marine microbial assemblage dominated by the cyanobacterium *Moorea producens*.

Methods. The fosmid containing lyngbyatoxin biosynthetic genes (3) was modified by the introduction of a new regulatory region to achieve inducible expression in the unrelated host, namely *E. coli* harboring a broad-range phosphopantetheinyl transferase (4).

Results. Heterologous expression of this pathway showed that *E. coli* is, by far, superior to *Streptomyces* as a host for the expression of cyanobacterial nonribosomal peptide synthetase pathways with the production of high titers of both lyngbyatoxin A (25.6 mg/L) and its precursor indolactam-V (150 mg/L). Production, isolation and identification of all expected chemical intermediates of lyngbyatoxin biosynthesis in *E. coli* also confirmed the previously proposed biosynthetic route setting a solid chemical foundation for future pathway engineering.

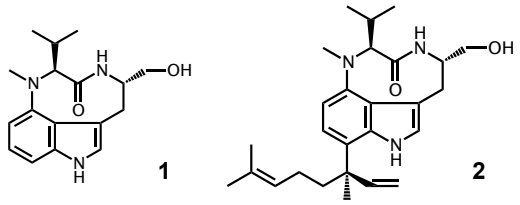

Fig.1 Indolactam-V (1) and lyngbyatoxin A (2)

Conclusions. We have demonstrated the biological synthesis, via a multienzyme thiotemplate mechanism, and overproduction of such molecules in a heterologous expression system. This technological development will allow the exploitation of molecules with commercial significance from sources whereby previously the only feasible route was synthetic chemistry. The next step for cyanobacterial natural products will utilize the amenability of heterologous hosts to genetically engineer pathways for the generation of structural analogs with modified pharmacological properties.

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References.

1. Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC (2001) *Tetrahedron* 57: 9347–9377.
2. Kim EJ, Lee JH, Choi H, Pereira AR, Ban Y-H, Yoo YJ, Kim E, Park JW, Sherman DH, Gerwick WH, Yoon YJ (2012) *Org. Lett.* 14: 5824–5827.
3. Edwards D, Gerwick W (2004) *J Am Chem Soc* 126: 11432–11433.
4. Fu J, Bian X, Hu S, Wang H, Huang F, Seibert PM, Plaza A, Xia L, Müller R, Stewart AF, Zhang Y (2012) *Nature Biotechnol.* 30: 440-446.