# CHARACTERISATION OF THE BIOSYNTHETIC GENE CLUSTER OF THE CYTOTOXIC AERUGINOGUANIDINES FROM *MICROCYSTIS AERUGINOSA* NIES98.

**Douglas Gatte-Picchi [1, 2*], Keishi Ishida [3], Christian Hertweck [3] and Elke Dittmann [2]**

*[1] São Paulo State University, Coastal Campus of São Vicente, Praça Infante Dom Henrique sn, 11330-900, São Vicente, Brazil; [2] Institute of Biochemistry and Biology (University of Potsdam), Karl Liebknecht-Str. 24-25, Building 25, 14476, Potsdam (Golm), Germany; [3] Leibniz Institute for Natural Product Research and Infection Biology (Hans Knöll Institute), Beutenbergstraße 11a, 07745, Jena, Germany; douglaspicchi@gmail.com*

** sponsored by the National Council for Scientific and Technological Development (CNPq), an agency linked to the Brazilian Ministry of Science and Technology (MCT) and the German Academic Exchange Service (DAAD).*

Cyanobacteria stand out as prolific producers of bioactive natural products. However, their chemical potential remains untapped, since they were traditionally studied with focus on the production of toxins due to their importance for human health. Therefore, pharmaceutical companies had not systematically screened these organisms as source of bioactive natural compounds in their drug discovery programmes [1]. Cyanobacteria are an exceptional rich source of nonribosomal and ribosomal synthesised peptides with a surprisingly wide range of structural features and bioactivities. Aeruginoguanidines are cytotoxic peptides, whose biosynthetic gene cluster has been only partially identified in the original producer strain *M. aeruginosa* Nies98. From their chemical structure, a nonribosomal synthesis can be predicted, since it harbours some intriguing features, such as the sulfonation of an aromatic moiety Hphpa trisulfate (1-(4-hydroxy-3-hydroxymethyl)-phenyl-1-hydroxy-2-propylamine 4',3',1'-tri-O-sulfate) and an unprecedented prenylation pattern of the arginine residues [2]. Therefore, the objective of this study was the complete sequencing of the missing *agd* biosynthetic gene cluster and the further *in silico* characterisation of the enzymes encoded in the cluster. We projected (i) rational feeding experiments with diverse isotopically labelled substrates aiming to gain more information about the biosynthetic origin of the aromatic unit, and (2) *in vitro* assays with recombinant Agd- key enzymes for the characterisation of the enzymatic activity of the putative 4-hydroxybenzoate synthetase (AgdH) and prenyltransferase (AgdJ). The results of the feeding experiments in conjunction with the *in vitro* assay of AgdH, concerning the conversion of chorismate, strongly indicate that apart from playing a central role in the formation of the aromatic moiety of aeruginoguanidines, hydroxybenzoate may be originated rather from acetate than the shikimate pathway. Moreover, we discussed the origin of the unusual Hphpa trisulfate unit and how it might be incorporated into the aeruginoguanidine core peptide. Although further empirical results are needed to confirm the hypothetical pathway for aeruginoguanidines, our proposed model already anticipates a fascinating biosynthesis mechanism that strongly deviates from the most canonical assembly lines, for example, the iterative incorporation of arginine, the lacking of an initiation module, the unusual $N^{\omega}$-prenylation of the guanidinium groups and several further tailoring modifications. Considering the results reported here, we believe to have contributed to the expansion of the knowledge on the biosynthesis of cyanobacterial natural products.

**References:**

[1] Kehr, J.-C., Gatte-Picchi, D., and Dittmann, E. 2011. Natural product biosynthesis in cyanobacteria: a treasure trove of unique enzymes. Beilstein Journal of Organic Chemistry, 7: 1622–1635.

[2] Ishida, K., Matsuda, H., Okita, Y., and Murakami, M. 2002. Aeruginoguanidines 98-A – 98-C: cytotoxic unusual peptides from the cyanobacterium *Microcystis aeruginosa*. Tetrahedron, 58: 7645–7652.