

Hunting Down the Eagle Killer: First Insight into the Aetokthonotoxin's Biosynthesis

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Avian vacuolar myelinopathy (AVM) constitutes a prominent example of the risk that cyanobacteria pose to aquatic environments. This fatal neurological disease causes severe motor impairment of bird individuals that have been exposed to *Aetokthonos hydrillicola* (a true-branching cyanobacterium) through the food chain or by direct feeding. Recently, a novel cyanobacterial secondary metabolite (aetokthonotoxin) has been found to be likely responsible for AVM. This toxin is a penta-brominated compound constituted by two indole moieties, whose presence in both natural *A. hydrillicola* colonies growing in the AVM season as well as in unusual cultures supplemented with bromide has been corroborated by mass spectrometry imaging (MSI). Considering the highly brominated nature of the compound, we screened for the toxin's putative gene cluster through bioinformatic analysis of a newly sequenced draft genome. We identified a putative gene cluster (9.2 Kbp) composed of six predicted genes, five with an identifiable function (a tryptophanase, a cytochrome P450, a cyclase/dehydrase, and two halogenases) and a single hypothetical protein. To provide first insights into the aetokthonotoxin biosynthetic pathway, six Golden Gate plasmids containing these candidate genes were designed and propagated to competent *E. coli* cells. This allowed us to induce the production of the cluster's encoded enzymes and to characterize their activity through *in-vitro* enzymatic assays. Here we present our advances on the characterization of enzymatic activities of the proteins encoded in the aetokthonotoxin gene cluster as well as the status of the elucidation of its biosynthesis.