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Comparison of saxitoxin-genes expression and production profiles between *Aphanizomenon gracile* and *Cuspidothrix issastchenkoi* strains, isolated from freshwater reservoirs

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Saxitoxins (STX) are a group of carbamate alkaloids known to inhibit the axons sodium ion channels, thus affecting the nervous system in vertebrates. These neurotoxins cause paralysis and respiratory failure, ultimately ending in death. They are produced by organisms belonging to two different kingdoms: marine eukaryotic dinoflagellates and freshwater prokaryotic cyanobacteria. The unique biosynthetic pathway, which is responsible for encoding proteins that allow synthesizing and exporting STX, is the cluster *sxt*, already described in several cyanobacterial species. It has been observed that diverse environmental factors affect differently STX production in cyanobacteria. Among those, temperature is the one that aroused greater interest, since it directly affects cyanobacterial growth rates, however controversial results have been reported. Furthermore, studies on the environmental regulation of STX synthesis and transport are quite rare. In this work, we tested the influence of temperature in two STX producers, *Aphanizomenon gracile* LMECYA40 and *Cuspidothrix issatschenkoi* LMECYA31, isolated from Portuguese freshwaters and maintained in the ESSACC. Total STX concentration, the expression of genes linked to STX biosynthesis (*sxtA*) and transport (*sxtM* and *sxtPer*) and the percentage of heterocytes per trichome were determined, in four different phases of cyanobacterial growth (lag, early exponential, late exponential and stationary phases). At 20°C, a higher expression of the *sxt* genes and higher production of STX in the late exponential and stationary phase was observed in both species. Also, *C. issatschenkoi* produced more STX throughout the growth cycle than *A. gracile*. In *A. gracile* the percentage of heterocytes per trichome was higher in the lag phase, decreasing along the cell cycle. *C. issatschenkoi* did not form heterocytes throughout the growth cycle. Further experiments are being performed at 10°C and 30°C in order to understand the effect of temperatures environmentally relevant in STX producers.

Keywords: Saxitoxin, temperature, *Aphanizomenon gracile*, *Cuspidothrix issatschenkoi*, heterocytes, gene expression

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