PCR-based tools to assess presence of anatoxin-a biosynthesis genes and *Microcoleus anatoxicus* in blooms

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Toxins produced by benthic cyanobacteria have been linked to the death of domestic animals (mainly dogs). An alarming incident in which six dogs were poisoned in a single day - at the Areuse River in Switzerland - heightened concern among scientists and local authorities. This event was followed by other death events throughout Switzerland, with all deaths linked to anatoxin-a and dihydroanatoxin-a. This has led to research efforts aimed at detecting dominating cyanobacteria within these cyanobacterial mats and developing tools to monitor their potential toxicity. We collected floating cyanobacterial mats from various Swiss lakes and rivers related to dogs' death, extracted total environmental DNA, and sequenced the microbial communities to identify dominant cyanobacteria. In parallel, we investigated potential anatoxin-a production using an Oscillatoriales-specific PCR targeting the *anaC* gene. Additionally, we developed primers to target *anaB* and *anaK,* two genes associated with anatoxin and dihydroanatoxin-a synthesis, respectively. Finally, we designed primers targeting *Microcoleus anatoxicus*, the cyanobacterial species identified as responsible for producing these toxins in Switzerland. Our results showed that cyanobacterial mats associated with the death of dogs were usually dominated by *Microcoleus* spp. In some samples, other cyanobacterial genera were highly abundant, such as *Potamolinea* (a non-toxic species) and *Phormidium* (a potentially toxic species). PCR analysis revealed that the *anaBCK* genes and the *M. anatoxicus* specific marker were found in *Microcoleus*-dominated samples. Downstream applications of these primers would be tested to assess the presence of the cyanobacterium and the gene cluster for toxin production in the stomach contents of dogs. This research provided a method to rapidly and confidently detect potential toxic mats in environmental samples.