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Anabaenopeptins and cyanopeptolins induce systemic toxicity effects in a model organism the nematode *Caenorhabditis elegans*

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HIGHLIGHTS

- Anabaenopeptins and cyanopeptolins induce toxicity in *C. elegans*.
- Anabaenopeptins show greater toxicity than microcystin-RR.
- Anabaenopeptins induces severe vulval integrity detects in *C. elegans*.
- Cyanopeptolins show comparable toxicity to microcystin-RR.
- Microginin is the least toxic among all cyanopeptides tested.

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ABSTRACT

Cyanobacterial blooms represent a significant risk to environmental and human health due to their production of toxic secondary metabolites, cyanopeptides. Anabaenopeptins and cyanopeptolins are cyanopeptides increasingly detected in surface waters at concentrations exceeding regulatory toxicity levels for other cyanotoxins such as microcystins. Yet their toxicity to aquatic organisms are not well understood. Here we assessed the toxicological effects of three anabaenopeptins (AP-A, AP-B, and AP-F) and three cyanopeptolins (CYP-1007, CYP-1020, and CYP-1041) to a model organism the nematode *Caenorhabditis elegans*. Examined toxicity endpoints included reproduction, hatching time, growth rate, lifespan, and age-related vulval integrity. Microcystin RR (MC-RR) and microginin 690 were also included in the study for comparisons. At an identical mass concentration (10 μg/L, corresponding to a molar concentration ranging 0.01–0.014 μM depending on the specific peptide), anabaenopeptins (APs) showed the greatest toxicity among all cyanopeptides tested. APs decreased worm reproduction by 23%–34% and shortened worm lifespan by 5 days (a 30% reduction) compared to the controls. APs also induced a remarkable age-related vulval integrity defect (Avid phenotype) in the worm, where over 95% of exposed worms developed the phenotype, compared to a less than 15% in control worms. CYPs showed similar toxicity as MC-RR, and Microginin 690 was the least toxic. These findings suggest that APs and CYPs may pose significant health risks to aquatic organisms. More toxicological studies of these cyanopeptides using different species across different trophic levels are needed to gain a thorough understanding of their potential impact on ecological systems and human health.

1. Introduction

Cyanobacterial blooms have increased in frequency and magnitude across the globe over the past decade (Pick, 2016). These blooms have the potential to wreck havoc on environmental and public health, as well as local economies (Wilkinson et al., 2018) as they can produce highly toxic secondary metabolites (cyanopeptides) known as cyanotoxins. Cyanotoxins have been detected in drinking water and food sources at concentrations up to mg/L (Gkelis et al., 2015; Kurmayer et al., 2016), posing a significant risk to the public's health. In August 2014, an algal bloom contaminated the drinking water of Toledo, Ohio forced nearly half a million people to drink bottled water for three days (MDH, 2017). A bloom of *Pseudo-nitzschia* containing the neurotoxin domoic acid occurred off the west coast, shutting down fisheries from Baja California in Mexico up to Alaska in 2015 (Michalak, 2016). More recently in May

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2018, Salem in Oregon shut down drinking water for kids because of algal toxin contamination caused by algae blooms in Detroit Reservoir (Michalak, 2016). As a result, the maintenance of safe drinking water and recreational aquatic environments may become more challenging with the increased occurrences of cyanobacterial blooms (Murphy et al., 2012; Miller et al., 2017).

Cyanotoxins can be classified as peptides or alkaloids based on their chemical structures. They may also be classified as hepatoxins, neurotoxins, or dermatotoxins based on their mechanisms of toxic action in vertebrates, especially mammals (Ferrao-Filho and Kozlowsky-Suzuki, 2011). Peptides are the largest group of cyanotoxins and there have been more than 600 cyanobacterial peptides described to date (Gkelis et al., 2015). Microcystins (MCs) are among the first discovered and described cyanopeptides, occurring abundantly in waterbodies in temperate climates, and can pose significant health risks to livestock, wildlife, fishes, and humans (Chorus and Fastner, 2001). The WHO has proposed implementation measures for monitoring and control of MCs and determined guideline values for drinking and recreational waters (WHO, 2008). Other commonly found bioactive peptides include microginins, cyanopeptolins, anabaenopeptins, anabaenopeptilides, microvirdins, and nostophycins (Welker and Von Dohren, 2006). Anabaenopetins (APs) and cyanopeptolins (CYPs) are cyclic nonribosomal oligopeptides produced by a broad range of cyanobacterial species. APs are characterized by a ring of five amino acid residues including a conserved lysine. CYPs have a six amino acid residue ring structure, a conserved 3-amino-6-hydroxy-2-piperidone (AHP) residue and a side chain with variable length (Fig. 1). More than 100 different APs and CYPs have been reported (Chilipala et al., 2012; Cerasino et al., 2017), and they are commonly detected along with MCs. A recent study has found that AP concentrations in blooms can exceed 1000 μg/L in freshwater bodies in Greece (Gkelis et al., 2015).

Cyanopeptides induce toxicity to animals and humans through diverse mechanisms, ranging from hepatotoxic and cytotoxic effects to the inhibition of protein synthesis. MCs are the most commonly observed cyanobacterial liver toxins globally (Miller et al., 2017). They covalently bind to and inhibit protein phosphatase type 1 or 2A in liver cells and may also inhibit other proteins and enzymes. MCs are among the most extensively studied algal toxins due to their high toxicity. They have been suspected as the culprit of numerous animal and human poisonings across the globe (Backer et al., 2013; Weirich and Miller, 2014; Trevino-Garrison et al., 2015). Adverse effects from ingestions of MCs have been observed in various aquatic organisms. Ingestion of MC-producing cyanobacteria resulted in lethal poisoning in *Daphnia galeata*, at an intake of 10.2 ng of MC per 1 mg body weight (Rohrlack et al., 2005). Long-term low dose exposure to MCs in *Daphnia magna* resulted in accumulation of the cyanotoxin in the organism and the phosphatase enzyme activity was also inhibited (Chen et al., 2005). Accumulation of MC-LR in a gastropod pulmonate *Lymnaea stagnalis* following aqueous exposure, accompanied by a strong decrease in egg production in adult organisms was also reported (Gérard et al., 2005). Toxicity of MCs to several fish species have also been reported, and observed effects ranged from mortality to developmental abnormalities, depending on the cyanotoxin concentrations (Zanchett and Oliveira-Filho, 2013).

In contrast, studies on ecological toxicity of APs and CYPs are scarce. Both APs and CYPs are known inhibitors of serine proteases and protein phosphatases (Spoof et al., 2016; Gademann and Portmann, 2008; Gademann et al., 2010), which are enzymes responsible for the regulation of several vital physiological and

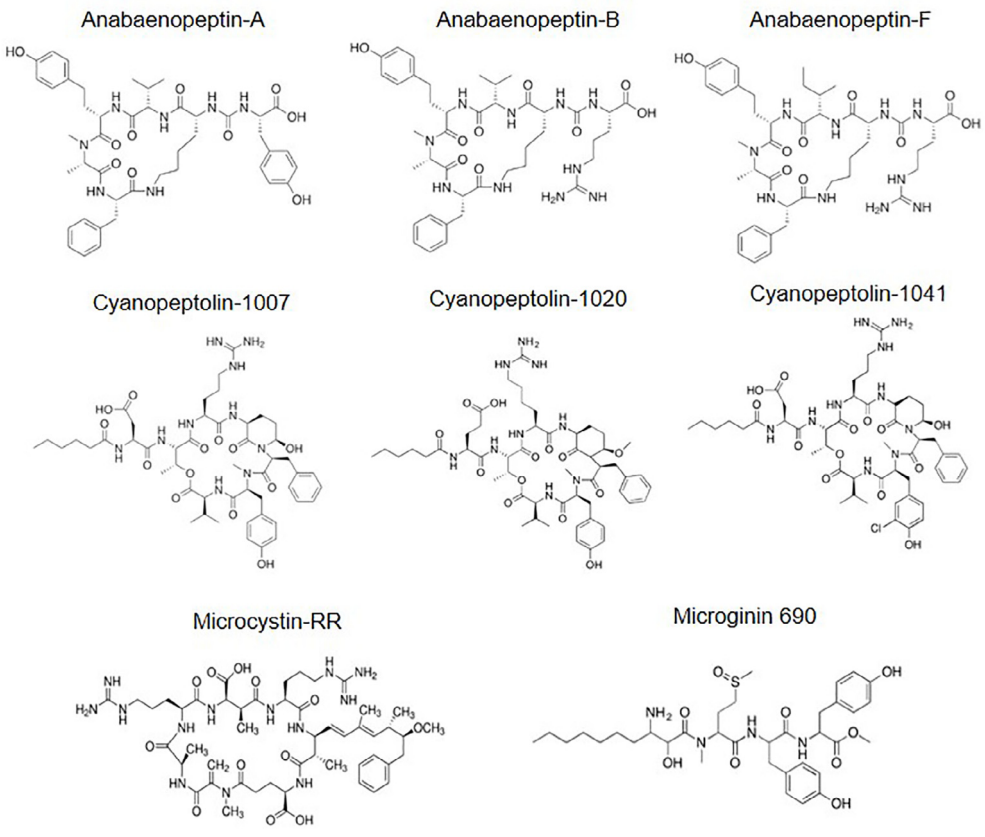

Fig. 1. Structures of the cyanopeptides studied.