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Hepatotoxicity and metabolic effects of cellular extract of cyanobacterium *Radiocystis fernandoi* containing microcystins RR and YR on neotropical fish (*Hoplias malabaricus*)

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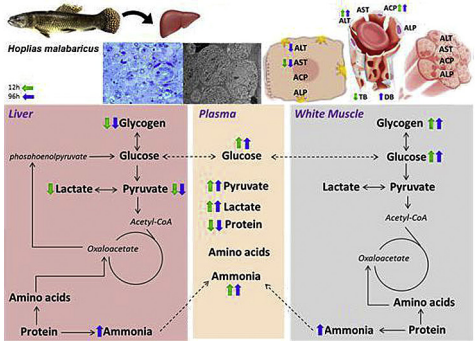
HIGHLIGHTS

- Microcystins RR and YR are majority toxins produced by *R. fernandoi* strain R28.
- Cyanobacterial cellular extract changes the plasma and liver enzyme activity in fish.
- Biliary canaliculus is altered, but biliary duct integrity is maintained.
- Glycogen depletion occurred in liver and increased in white muscles.
- Energetic metabolic compounds are altered in liver, plasma and muscles.

GRAPHICAL ABSTRACT



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ABSTRACT

The toxicological effect of cellular extract of cyanobacterium *Radiocystis fernandoi* strain R28 containing RR and YR microcystins was analyzed in the fish *Hoplias malabaricus* with emphasis on the liver structure and energetic metabolism, after short-term exposure. Fish were intraperitoneally (i.p.) injected with 100 μg of equivalent MC-LR kg^{-1} body mass containing in the cellular extract of *R. fernandoi* strain R28. Twelve and 96 h post-injection, the plasma, liver and white muscle were sampled for biochemical analyses and liver was also sampled for morphological analyses. After i.p. injection, the activity of acid phosphatase (ACP), alanine aminotransferase (ALT) and direct bilirubin increased in the plasma, while ALT and aspartate aminotransferase (AST) decreased in the liver. Glucose, lactate and pyruvate increased while protein decreased in the plasma; glycogen, pyruvate and lactate decreased in the liver; and glycogen and glucose increased in the muscle. Ammonia increased in the plasma, liver and muscle. The hepatocyte cell shape changed from polyhedral to round after cellular extract injection; there was loss of biliary canaliculus organization, but the biliary duct morphology was conserved in the liver parenchyma. In conclusion, microcystins present in the cellular extract of *R. fernandoi* strain R28 affect the liver

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structure of *H. malabaricus*, but the liver was able to continuously produce energy by adjusting its intermediate metabolism; glycogenolysis and gluconeogenesis maintained glucose homeostasis and energy supply.

1. Introduction

The high frequency of algal blooms due to eutrophization of lakes, reservoirs and recreational water is a worldwide problem. *Microcystis*, *Anabaena*, *Oscillatoria, Nostoc* and *Cylindrospermopsis* are toxin-producing cyanobacteria genera that are frequently present in such blooms (Codd, 1995; Sukenik et al., 2015). However, in recent decades, the genus *Radiocystis* Skuja (1948) has been often identified in tropical and neotropical aquatic environments as potentially toxic cyanobacteria (Azevedo et al., 1994; Carvalho et al., 2004; Lombardo et al., 2006; Vieira et al., 2003).

Radiocystis fernandoi is a microcystin (MC) producer cyanobacterium and has been a common species in mesotrophic environments throughout Brazil, reaching up to 70% of the total cyanobacterial biomass in some lakes (Borges et al., 2008; Fonseca et al., 2011; Sant'anna et al., 2008). Microcystins, a group of cyclic heptapeptides, are hepatotoxic and, more than 80 structural variants of MC have been identified. MC-LR is the most common and toxic variant of MC (Gupta et al., 2003; Wang et al., 2010). The action of MC-LR on organisms is known to cause erythrocyte, hepatic and kidney damage (Fischer and Dietrich, 2000; Shi et al., 2015; Sicinska et al., 2006) as well as biochemical and physiological changes in fish (Liu et al., 2015; Shi et al., 2015; Zhou et al., 2012). Few studies have focused on less toxic variants (Gupta et al., 2003; Prieto et al., 2006; Xie et al., 2015) or the effects of whole cellular content of cyanobacteria, which would help extrapolate laboratory findings to the field (Barrios et al., 2015; Okumura et al., 2006).

In fish, as in other vertebrates, MC reach the liver through the blood stream and, in the hepatocytes, cause toxicity by inhibiting the serine/threonine phosphatase proteins which is followed by hyperphosphorylation of regulatory proteins and destruction of the hepatocyte cytoskeleton (Fischer and Dietrich, 2000; Gehringer, 2004; Landsberg, 2002). As the liver has multiple functions, such changes may impair fish metabolism (Guzman and Solter, 2002; Marie et al., 2012; Mezhoud et al., 2008) and increase the energetic demand. *R. fernandoi* strain R28 cellular extract, containing MC-RR and MC-YR, has numerous effects on the liver histology and hepatocyte ultrastructure of *Hoplias malabaricus*, an edible fish that is widely distributed throughout Brazilian continental waters (Paulino et al., 2017). Furthermore, the changes in blood variables causing anemia (Paulino et al., 2017) may be aggravated by inducing methemoglobin formation which is unable to transport oxygen (Sedan et al., 2013). These changes may affect aerobic metabolism by reducing the oxygen availability in different tissues.

Therefore, this study was performed to evaluate the changes in the liver structure and energetic metabolism of a neotropical fish, traira *Hoplias malabaricus*, that was intraperitoneally injected with cellular extract of *R. fernandoi* strain R28 which contains the MC-RR and MC-YR. *Hoplias malabaricus* presents high ecologic plasticity (Bialetzki et al., 2002) and occupies a top position in the trophic chain, making it an useful model for evaluating the toxic effects of xenobiotics. Furthermore, this species has a low metabolic rate as well as metabolic adaptation that facilitate energy storage in times of shortage (Barbieri, 1989; Rios et al., 2006, 2009). *R. fernandoi* strain R28 was isolated in the Furnas reservoir on Minas Gerais, Brazil, and did not produce MC-LR; the majority MC produced by this strain are MC-RR and MC-YR (Pereira et al., 2012, 2015; Pereira and Giani, 2014).

2. Material and methods

2.1. Cyanobacterial extract

Lyophilized cyanobacteria of the *R. fernandoi* strain R28, which mostly produces MC-RR and MC-YR, were obtained from the culture collection of the Phycology Laboratory of the Botany Department in Federal University of Minas Gerais. The cyanobacteria were cultivated in 500 mL batch with WC culture medium (Guillard and Lorenzen, 1972) at 25–30 mol m^{-2} s^{-1} irradiance in a 12 h light:12 h dark photoperiod at 20 °C (Pereira et al., 2012). The cell-free cellular extract (CE) was obtained after homogenization in 80% methanol HPLC-grade (Panreac, Spain) in Milli-Q water (18 M Ω cm^{-1}) (Millipore Corporation, UK) using an Ultra Turrax (IKA – T10) and pooling the supernatant. The extract was centrifuged at 12,000 *g* (18 °C) for 20 min, and the supernatant was evaporated at room temperature with a vacuum solvent evaporator (SpeedVac®). Thereafter, the solid phase of extract was diluted with water and total MC quantification in equivalent MC-LR (equiv. MC-LR) was performed using an enzyme-linked immunosorbent assay (ELISA) commercial kit (Beacon Analytical Systems Inc.) in a Molecular Devices Spectra MAX GEMINI X at 450 nm according to the manufacturer's instructions.

Quantitative confirmation of MC-RR and MC-YR was performed using reverse-phase high-performance liquid chromatography (HPLC-UV, Agilent 1200 Series, Agilent Technologies, Santa Clara, USA) equipped with a G1322A degasser, G1311A quaternary pump, G 1367B autosampler, G 1316A thermostated column compartment and G1316A diode array detector according to Aranda-Rodrigues et al. (2005). The MC concentrations were determined by comparing the peak areas of the test samples with those of the standard solutions containing MC-LR, MC-YR and MC-RR (Sigma, USA) as show in Paulino et al. (2017).

2.2. Experimental design

Forty juvenile *H. malabaricus* (body mass: 237.6 ± 5.09 g; total length: 26.2 ± 0.20 cm) were obtained from Santa Candida fish farm (Santa Cruz da Conceição, São Paulo State, Brazil). Fish were kept for 30 days in 1000-L tanks with dechlorinated water flow and constant aeration at 25 ± 1 °C. They were fed with fish pieces every 72 h according to fish feeding habits; food waste in the aquarium was discarded. Food was stopped 24 h before the experimental design.

Fish were randomly divided into the following four groups (n = 10 in each group): control groups (C12 and C96) received an intraperitoneal (i.p.) injection of 0.5 mL of sterile saline solution (0.9% NaCl), and the cellular extract exposed groups (CE12 and CE96) received an i.p. injection of cellular extract of *R. fernandoi* strain R28 containing 100 μg equiv. MC-LR body mass-^{1} diluted in 0.5 mL sterile saline solution. After 12 and 96 h, blood samples were collected from the caudal vein into a heparinized syringe, centrifuged, and plasma was removed. Thereafter, fish were anesthetized