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Genotoxicity and cytotoxicity of three microcystin-LR containing cyanobacterial samples from Antioquia, Colombia

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ABSTRACT

The presence of cyanobacterial blooms and cyanotoxins in water presents a global problem due to the deterioration of ecosystems and the possibility of poisoning in human and animals. Microcystin LR is the most widely distributed cyanotoxin and liver cells are its main target. In the present study, HepG2 cells were used to determine DNA damage of three crude extracts of cyanobacterial blooms containing MC-LR, through comet assay. The results show that all extracts at a concentration of 500 μg mL^{-1} caused low damage in hepatocytes exposed for 24 h, but produced total mortality even at low concentrations at 48 h. Moreover, balloons corresponding to cell apoptosis were found.

Through HPLC/MS, MC-LR was detected in all samples of cyanobacterial blooms at concentrations of (5,65 μg ml^{-1}) in sample 1, (1,24 μg ml^{-1}) in sample 2 and (57,29 μg ml^{-1}) in sample 3. In addition, in all samples high molecular weights peaks were detected, that may correspond to other microcystins.

Besides, the cytotoxic effect of a cyanobacterial bloom and some of its chromatographic fractions from the crude extracts were evaluated in U-937, J774, Hela and Vero cell lines, using the enzymatic micromethod (MTT). The highest toxicity was detected in U-937 cells (LC_{50} = 29.7 μg mL^{-1}) and Vero cells (LC_{50} = 39.7 μg mL^{-1}).

Based on these results, it is important to remark that genotoxic and cytotoxicity assays are valuable methods to predict potential biological risks in waters contaminated with blooms of cyanobacteria, since chemical analysis can only describe the presence of cyanotoxins, but not their biological effects.

1. Introduction

The increase in human population has brought a growing demand for water resources for human consumption but also a decrease in the volume and quality of water supply because of the environmental degradation of aquatic ecosystems. This situation has encouraged the development of research aimed to identify and prevent the causal factors of deterioration of water sources, as well as improving water purification techniques.

Cyanobacterial blooms are one of the factors with highest incidence in water quality degradation and are produced by numerous genera of Cyanobacteria Phylum. They are characterized by the production of several compounds (Carmichael, 1994), which are known as hepatotoxins and neurotoxins (Reynolds, 2006). Presence of blooms or cyanobacterial blooms and production of cyanotoxins affect water quality, making treatment processes more expensive (Garcia Nieto et al., 2011; Quesada et al., 2004) and restricting recreative water activities (Quesada et al., 2004) including fishing. Several cases of intoxication by these compounds have been reported worldwide in wild and domestic animals and human (Hillebrand, 1999; Reynolds, 2006).

Microcystins are a class of cyanotoxins produced by species of the genera *Microcystis*, *Anabaena*, *Oscillatoria* (*Planktothrix*), *Nostoc*, and *Anabaenopsis* (Sivonen and Jones, 1999); their ingestion by mammals may lead to increased liver weight, hepatic histological damage (Heinze, 1999), liver cancer (Hernández et al., 2009; Hu et al., 2008; Li et al., 2016) and renal damage (Milutinović et al., 2003). These cyanotoxins may affect the cytoskeleton of liver cells, triggering apoptosis, necrosis and internal hemorrhage that can lead to death due to acute hemorrhagic shock (Dawson, 1998). Microcystin LR is the most common toxin and long-term exposure to this compound is mainly

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associated with expression of proteins in the caspase pathway (Huang et al., 2016), increasing oxidative stress, and also affecting mitochondrial DNA, altering cytokine expression, and causing lung deterioration in mice (Li et al., 2016; Ma et al., 2017).

Nevertheless, the molecular mechanism by which MC-LR (microcystin LR) induces toxicity has not been fully elucidated. Therefore, a great diversity of biological models, techniques and experimental designs have been used for the evaluation of their toxicity. In addition, there are few reports on the cytotoxicity and genotoxicity of samples of cyanobacterial blooms with mixtures of MC-LR and other cyanotoxins. Also, these studies have not been well understood (Žegura et al., 2006).

One of the main problems related to the presence of cyanobacteria in water reservoirs is the risk of exposure to complex mixtures of toxins for consumers of contaminated fish and drinking water (Carneiro et al., 2017). This type of risk could be a problem of public environmental health and must be addressed from different points of view.

Studies carried out by our research group have shown this problem in Riogrande II and Porce II reservoirs used as multiproposite and for power generation respectively (Herrera et al., 2015). Additionally, the bioaccumulation results of these microcystins were reported in Cladocerans (Herrera et al., 2014) for this reason in this study, genotoxicity is evaluated as an indicator of possible chronic effects in human cell lines such as HepG2 and the cytotoxicity in several cell lines to show the effect on different target organs and would be an indicator of possible acute effects.

Due to the lack of knowledge about effects of crude toxin mixtures in mammal cells, the aim of this study was to analyze the DNA in HepG2 hepatocytes and cytotoxic effects induced by different samples of blooms in U-937 promonocyte cells, mouse J774 macrophages, human Hela endothelial cervix cells and monkey kidney Vero epithelial cell lines. These bioassays could be tools to predict the risk of exposure to the health of people and animals who are in contact with this type of samples (blooms) composed of mixtures of toxins and other types of compounds.

2. Materials and methods

2.1. Study area

Porce II and Riogrande II are two water reservoirs located in the department of Antioquia, Colombia. Porce II is used for power generation and artisanal fishing while Riogrande II has a multipurpose use. In the tributary basins of both reservoirs, take place intensive agricultural and industrial activities.

One sample was taken from the most eutrophic area of Riogrande II, the arm of Rio Chico located 15 km away from the water intake tower (sample 1). Other two samples were taken from a nearby point located in the main body water (sample 2) and the last one in the dam (sample 3) of Porce II reservoir (Fig. 1).

2.2. Sample processing

2.2.1. Lyophilization

The three cyanobacterial bloom samples were collected with a 20-μm mesh nylon net, stored in plastic bottles of five liters and maintained in darkness and refrigerated at 4 °C until their lyophilization in the laboratory, previously reported (Herrera et al., 2015). After that they were processed for the HPLC analyses. Fig. 2 shows the origin of each of the analyzed samples, fractions and subfractions.

For the genotoxicity and cytotoxicity test, only lyophilized samples were used without any further treatment, then, 20 mg were taken and resuspended in 1 ml of DMSO (dimethyl sulfoxide).

2.2.2. Subfractionation

In addition, the sample from the Riogrande II reservoir was fractionated by chromatography and tested for cytotoxicity. Thus, 40 g of the lyophilized sample was resuspended in methanol 90% and sonicated for 15 min with a Branson Ultrasonic (model 2510); afterwards, it was filtered through a glass fiber membrane of 47 mm (Advantec). The extract obtained (8.0 g) was separated by liquid chromatography in column with a mix of dichloromethane: methanol (4:1, v/v) until obtaining 24 fractions of 20 mL each. The last two fractions (***a*** and ***b***) were chosen to continue purifying them, because the presence of microcystins was detected by the ELISA test. The last fractions of this new purification process were eluted with 100% methanol, were selected and named (***a-1***, ***b-1*** **and** ***b-2***). For cytotoxicity assays, each sample was diluted in DMSO to determine the cytotoxic effect on four cell lines.

2.2.3. Cyanobacteria detection

To determine the presence of cyanobacteria, 250 mL of water sample was fixed with 1 mL of Lugol's solution (1%). For the analysis of *Microcystis* mucilage Chinese ink was used. For quantitative analysis, the samples were shaken 30 times and pelleted, following the method of Utermöhl (Rzóska and Margalef, 1979). One milliliter of the precipitate was placed in a counting chamber Sedgwick-Rafter and microscopic observations were performed (Herrera et al., 2015).

2.3. MC-LR detection by HPLC/MS

Two hundred mg of lyophilized material were resuspended in 80% methanol; then the sample was sonicated for 15 min in a Branson 2510 equipment and centrifuged at 3000 rpm for 5 min. Then, samples were dried on a rotary evaporator (Heidolph) at 35 °C and the obtained extract was filtered through C18 cartridges (CNWBOND HC-C18) eluted with methanol and analyzed by HPLC-MS.

Detection and confirmation of MC-LR was carried out by (HPLC-MS/MS) (Agilent HPLC 1200, AB SCIEX 3200 QTRAP), following the methodology by Herrera et al. (2015): Column, Kinetex 2.6 μm C18 100 Å, 50 × 2.1 mm; Mobile phase A, 5 mM ammonium acetate in

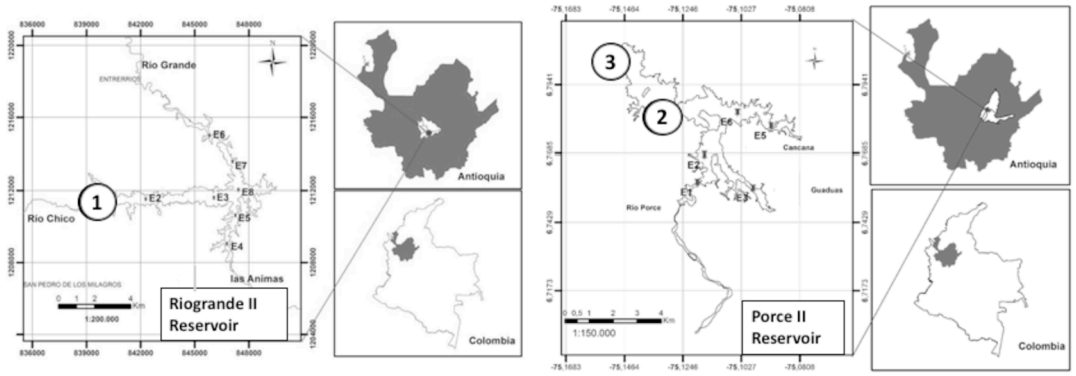

Fig. 1. Localization of the three cyanobacterial bloom samples collected. Three samples of cyanobacterial blooms were taken: two in Porce II dam (3), one in the main body (2), and the last one in the most eutrophic area of Riogrande II, the arm of Rio Chico (1).