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Phytotoxic effects of cyanobacteria extract on the aquatic plant *Lemna gibba*: Microcystin accumulation, detoxication and oxidative stress induction

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Abstract

The occurrence of toxic cyanobacteria in the aquatic environment constitutes a serious risk for the ecological balance and the functioning of ecosystems. The presence of cyanotoxins in ecosystems could have eventual adverse effects on aquatic plants, which play an important biological role as primary producers. The original aim of this study was to investigate microcystin (MC) accumulation, detoxication and oxidative stress induction in the free-floating aquatic vascular plant *Lemna gibba* (Duckweed, Lemnaceae). Experiments were carried out with a range of MC levels, obtained from toxic *Microcystis* culture extracts (0.075, 0.15, 0.22 and 0.3 μg equiv. MC-LR mL^{-1}). During chronic exposure of the plant to MC, we examined the growth, photosynthetic pigment contents and also the physiological behavior related to toxin accumulation, possible biodegradation and stress oxidative processes of *L. gibba*. For the last reason, changes in peroxidase activity and phenol compound content were determined. This is a first report using phenol compounds as indicators of biotic stress induced by MC contamination in aquatic plants.

Following MC exposure, a significant decrease of plant growth and chlorophyll content was observed. Also, it was demonstrated that *L. gibba* could take up and bio-transform microcystins. A suspected MC degradation metabolite was detected in treated *Lemna* cells. In response to chronic contamination with MCs, changes in the peroxidase activity and qualitative and quantitative changes in phenolic compounds were observed after 24 h of plant exposure.

The physiological effects induced by chronic exposure to microcystins confirm that in aquatic ecosystems plants coexisting with toxic cyanobacterial blooms may suffer an important negative ecological impact. This may represent a sanitary risk due to toxin bioaccumulation and biotransfer through the food chain.

Keywords: Microcystins; Cyanobacteria; Duckweed; *Lemna gibba*; Phytotoxicity; Uptake; Detoxication

1. Introduction

Eutrophication of water bodies may lead to excessive growth of cyanobacterial blooms that are common in many lakes and rivers all over the world (Skulberg et al., 1984; Carmichael, 1992; Codd, 1995). Many of the cyanobacteria forming blooms are known to produce different types of toxins including neurotoxins, hepatotoxins, cytotoxins and lipopolysaccharide (LPS)

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endotoxins, which can cause a variety of human and animal health, ecological and aesthetic concerns (Carmichael, 1997). Cyanotoxins can have adverse effects on animals like humans and other mammals including sheep, cattle and horses, birds (Carmichael, 2001, 1992; Onodera et al., 1997), fish (Liu et al., 2002), invertebrates (Delaney and Wilkins, 1995) including zooplankton (Rohrlack et al., 2001). Whenever cyanobacterial blooms occur, there is evidence that the abundance of submerged plants decreases and that the diversity of aquatic plant communities is reduced (Abe et al., 1996). Recently, some attention has been paid to the natural role of microcystins on terrestrial and aquatic ecosystems (Barbica et al., 2006, a review). The research on the uptake and effects of microcystins on aquatic plants has increased. It has been reported that microcystin-LR can be accumulated by *Ceratophyllum demersum*, *Elodea canadensis*, *Vesicularia dubyana*, and *Phragmites australis*, and that toxin transfer along the aquatic food chain may occur (Pflugmacher et al., 1998, 1999; Pflugmacher, 2004, 2001). The allelopathic effect of cyanobacterial extract on aquatic plants has also been reported (Pietsch et al., 2001; Pflugmacher, 2002). Rowmanowska-Duda and Tarczynska (2002) found that microcystin-LR can inhibit the growth of an aquatic macrophyte (*Spirodela oligorrhiz*); at concentrations of 0.05–0.2 mg/L microcystin-LR inhibited the growth of fronds and decreased the chlorophyll content of the plant by 60–70% compared to controls after 24 h of exposure. At present, there is some evidence suggesting that oxidative stress might be involved in the toxicity of microcystins on plants, such as the aquatic macrophyte *C. demersum* (Pflugmacher et al., 1998). In accordance with the suggestion that oxidative stresses are involved in the toxicity of microcystins to plants, it has been reported that the activity of peroxidase (POD) and superoxide dismutase (SOD), two antioxidant enzymes, were changed in rape (*Brassica napus L.*) and rice (*Oryza sativa L.*) seedlings after exposure to microcystins (Chen et al., 2004). However, there is no report using the phenolic compounds as indicators of biotic stress induced by cyanobacteria on aquatic plants. Phenols are a diverse class of compounds widely distributed in the plant kingdom. They have been shown to play important roles in plant resistance to biotic and/or abiotic stress as constitutive and induced compounds (Smith, 1996).

Also, phenolic compounds, especially phenolic acids and flavonoids are ubiquitously present in vegetables, fruits, seeds, tea, wines and juices. Thus they are an integral part of the human diet. Recently, they have received much attention since many epidemiological studies suggest that consumption of polyphenol-rich foods and beverages is associated with antioxidant properties of phenols. The protective effects of these plants have recently been confirmed to stem from their antioxidant properties (Prior and Cao, 2000a,b; Kaur and Kapoor, 2001; Bravo et al., 2007; Klimczak et al., 2007).

Many reports have demonstrated the effects of cyanotoxins on some biological pathways (growth, photosynthesis, toxin accumulation, oxidative stress etc) of *Lemna gibba*, *Lemna minor* and *Lemna japonica* plants (Weiss et al., 2000; Weiss and Liebert, 1998; Mitrovic et al., 2004; LeBlanc et al., 2005; Jang et al., 2007). The above authors used *Lemna* plants in experiments in order to prove the hypothesis that microcystins (MC), produced by some toxic Cyanobacteria, can act as an allelochemicals – compounds released into the environment by an organism that may affect another organism (Rice, 1984) – in the aquatic environment. Three experimental methods were used to examine the allelopathic effects of microcystin-LR on *L. gibba*: conducting a series of toxicity bioassays, investigating direct and indirect experimental exposure, and inhibiting oxygen evolution in photosynthesis (LeBlanc et al., 2005). It was recently also reported that, when toxic *Microcystis* coexist with duckweed (*Lemna japonica*) reciprocal allelopathic interactions occur (Jang et al., 2007). The present study used *L. gibba* as the test plant for two main objectives: firstly, we investigated if a chronic exposure of *L. gibba* (Lemnaceae) to MC-producing cyanobacteria could affect plant growth, chlorophyll ($a+b$) content and induce an oxidative stress, estimated by the change of phenol compounds and peroxidase activity; secondly, we studied the possible accumulation and biodegradation of microcystins (MC) by *L. gibba*.

2. Material and methods

2.1. Biomass production of the Microcystis isolated strain

A cyanobacterial bloom dominated by *Microcystis aeruginosa* was collected from Lalla Takerkoust reservoir in July 2003. The toxicity, total quantification and variant characterization of MCs from Takerkoust cyanobacterial blooms were previously reported (Oudra et al., 2001, 2002a). A strain of *M. aeruginosa* was isolated and cultured in Z8 medium (Kotai, 1972) under controlled laboratory conditions (26 °C and 82 $\mu Em^{-2}\,s^{-1}$ fluorescent continuous light). The cyanobacterial biomass produced on 2 L Erlenmeyer's batch cultures was harvested at the end of the exponential growth phase (5 days of incubation) by centrifugation and/or flotation. The concentrated material was freeze-dried and stored at −20 °C prior to toxin extraction and analysis.

2.2. L. gibba culture

Specimens of the flowering aquatic plant *L. gibba* were collected from a natural freshwater pond. A stock culture of *L. gibba* was maintained by growing it in a ground-water artificial pond at the department of Biology (Univ. Cadi Ayyad, Marrakesh, Morocco). Prior to the test, sufficient plants were removed and grown in Z8 for 5 days (acclimation time) under standard conditions (26 °C and 82 $\mu Em^{-2}\,s^{-1}$ fluorescent continuous light). *L. gibba* is used extensively in toxicity testing. It reproduces rapidly, is small and easy to culture, can be found in many types of aquatic environments, and is considered a competitor to algae in an aquatic environment (Wang, 1990).

2.3. Toxin extraction, purification and analysis of microcystins by HPLC-PDA

The toxin extraction and pre-purification were done according to Lawton et al. (1994) using methanol which was shown to be the most suitable solvent for microcystin extraction. Briefly,