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Global metabolome changes induced by cyanobacterial blooms in three representative fish species

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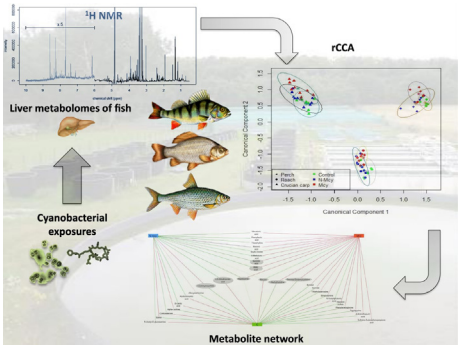
HIGHLIGHTS

- Fish were exposed to cyanobacterial biomasses producing or not microcystins.
- Metabolic changes are revealed following the exposure to cyanobacteria.
- Candidate biomarkers are revealed by relevance network based on rCCA analyses.
- Energy metabolism and antioxidative response are the main pathways involved.

GRAPHICAL ABSTRACT



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ABSTRACT

Cyanobacterial blooms induce important ecological constraints for aquatic organisms and strongly impact the functioning of aquatic ecosystems. In the past decades, the effects of the cyanobacterial secondary metabolites, so called cyanotoxins, have been extensively studied in fish. However, many of these studies have used targeted approaches on specific molecules, which are thought to react to the presence of these specific cyanobacterial compounds. Since a few years, untargeted metabolomic approaches provide a unique opportunity to evaluate the global response of hundreds of metabolites at a glance. In this way, our study provides the first utilization of metabolomic analyses in order to identify the response of fish exposed to bloom-forming cyanobacteria. Three relevant fish species of peri-urban lakes of the European temperate regions were exposed for 96 h either to a microcystin (MC)-producing or to a non-MC-producing strain of *Microcystis aeruginosa* and metabolome changes were characterized in the liver of fish. The results suggest that a short-term exposure to those cyanobacterial biomasses induces metabolome changes without any response specificity linked to the fish species considered. Candidate metabolites are involved in energy metabolism and antioxidative response, which could potentially traduce a stress response of fish submitted to cyanobacteria. These results are in agreement with the already known information and could additionally bring new insights about the molecular interactions between cyanobacteria and fish.

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1. Introduction

During the past decades, the development of high-throughput technologies such as transcriptomics, proteomics and, more recently, metabolomics, has allowed the researchers to study the phenotypic responses of various organisms in different ecological contexts (McLean, 2013; Franzosa et al., 2015; Hultman et al., 2015). As the transcriptome and the proteome changes directly influence the metabolome of an organism, metabolomics has become an especially valuable approach to study the integrated response of the organisms submitted to various and complex ecological constraints (Bundy et al., 2008; Brandão et al., 2015). In ecotoxicology, changes in metabolites concentrations provide information on the health status of organisms and on the physiological processes involved in the homeostatic responses to exposure to contaminants of multiple origins. Thus, metabolomic analyses help i) to better understand the molecular mechanisms implicated in the toxicological responses of organisms and ii) to suggest new research hypotheses concerning the interactions between biocenoses and their biotopes at both individual and population scales (Samuelsson and Larsson, 2008; Cappello et al., 2016a, 2016b; Sardans et al., 2011).

In aquatic ecosystems, cyanobacterial blooms are regularly implicated in ecological disturbances affecting phytoplankton, zooplankton and fish communities (Chorus and Bartram, 1999; Codd et al., 2005; Ibelings and Havens, 2008). The presence of blooms constitutes an indirect constraint for other organisms, by forming large surface scum that decreases the light availability for other phytoplanktonic taxa and by constituting a weak nutritional resource for zooplankton, which hardly ingests them (according to their individual shapes and colonial forms) (Havens, 2008). Nevertheless, the potentially main negative impacts of cyanobacteria are very likely provoked by their capacity to produce a wide range of secondary metabolites, among them the cyanotoxins, which very likely induce toxic effects on various aquatic organisms. Negative impacts of the cyanotoxins, in particular of the microcystins (MCs), the most commonly observed hepatotoxic cyanotoxin in freshwater ecosystems, have been already assessed on the ichthyofauna, which represents one of the most relevant indicators of environmental disturbances (Bols et al., 2001; Malbrouck and Kestemont, 2006). However, actual knowledge concerning fish-cyanobacteria interactions has reported mainly the molecular mechanisms involved in the accumulation-detoxification dynamics and effects of MCs, generally submitted to fish as a single compound at high concentrations, during short-term exposures and under laboratory controlled conditions (Malbrouck and Kestemont, 2006). Thanks to these studies, it is well known that high doses of MCs induce inhibition of the protein phosphatases 1 (PP-1) and 2A (PP-2A) as well as occurrence of a cellular oxidative stress *via* the formation of reactive oxygen species (ROS), with different physiological responses and consequences depending on the species studied (Malbrouck and Kestemont, 2006; Amado and Monserrat, 2010). However, there is still a lack of knowledge concerning the molecular mechanisms involved in these different responses, particularly in environmentally relevant approaches investigating the direct effect of the exposure of fish to cyanobacteria as whole organisms, producing in most of the cases a "cocktail" of secondary metabolites.

Since the last decade, Nuclear magnetic resonance (NMR)-based metabolomics has been proved to be a powerful approach for investigating hypotheses relating to fish physiology and development or pollutant-induced toxicity or diseases (Viant, 2008; Brandão et al., 2015; Cappello et al., 2016a). Recently, a NMR-based study has successfully investigated the molecular effects of MC-LR in fish exposed to relevant concentrations (Chen et al., 2017). However, despite its high potential to reveal new research hypotheses and support already known information, such investigations are still rare, especially those concerning the toxicological effects of cyanobacterial biomasses on fish, submitted to relevant environmental conditions.

In this way, three representative fish species (the roach, the crucian carp and the perch) of freshwater ponds from the European temperate regions, which are frequently submitted to cyanobacterial bloom episodes, were exposed during 96 h to environmental concentrations of cyanobacteria in the context of an experimental approach in mesocosms designed to mimic natural conditions. At the end of the experiments, ^{1}H NMR metabolomic analyses were performed on the fish liver in order to investigate the global molecular responses of the different fish species and potentially identify new lines of investigation concerning fish-cyanobacteria interactions.

2. Materials and methods

2.1. Fish and cyanobacteria cultivation

The study was conducted using juvenile of perch (*Perca fluviatilis* (L.), PER), roach (*Rutilus rutilus* (L.), RUT) and crucian carp (*Carassius carassius* (L.), CAR), with an average length of 5.9 ± 0.3 cm, 8.4 ± 0.3 cm and 8.1 ± 0.4 cm, respectively, and an average weight of 2.9 ± 0.5 g, 10.2 ± 1.5 g and 15.4 ± 2.9 g, respectively. Juveniles fish were chosen instead of mature fish because their recruitment rates contributes substantially more to population stability than variation in adult mortality (Shelton and Mangel, 2011). The animals were handled and experiments were performed in accordance with European Union regulations concerning the protection of experimental animals and the experimental procedures were approved (N°68-040 for 2013-18) by the "Cuvier's ethical committee" of the Muséum national d'Histoire naturelle (French national number C2EA - 68). Immature fish were provided by fish farms of the Île-de-France and Bresse regions and acclimatized in 1500-L aerated tanks for 2 weeks before experiments. Fish were fed twice daily with commercial food.

Monoclonal cultures of *Microcystis aeruginosa* Kützing maintained in the PMC (Paris Museum Collection, http://www.mnhn.fr/fr/collections/ensembles-collections/ressources-biologiques-cellules-vivantes-cryoconservees/microalgues-cyanobacteries) were used for the experimental exposure. The PCC 7820 strain was selected as a MC-producer (Mcy) and the PMC 570.08 strain as a non-MC-producer (*N*-mcy), as investigated by both mass spectrometry and ELISA methods. Large volumes of both dense cyanobacterial strains were cultivated in polytetrafluoroethylene (PTFE) plastic bags (20 L) at 25 °C using a BG-11 medium with a 16 h/8 h light/dark cycle (60 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and a constant filtrated air bubbling (0.2 μm, Sartorius Minisart). Prior to the experimental fish exposure, chlorophyll-*a* equivalent concentrations ($\mu g \cdot L^{-1}$ eq. Chl *a*) were estimated for each strain using a bench-top fluorometer (Fluoroprobe II, Bbe-Moldenke, Germany).

2.2. Metabolite characterization of cyanobacterial strains by mass spectrometry

The biomasses of the two *Microcystis* strains were filtered, and freeze-dried. The lyophilized cells were then sonicated in 80% methanol, centrifuged at 4 °C (4000*g*; 10 min). The supernatant was transferred and acidified with formic acid (FA) and 5 μL were analyzed with high-performance liquid chromatography (HPLC) (Ultimate 3000, ThermoFisher Scientific) coupled with a mass spectrometer (ESI-Qq-TOF QSAR Pulsar, Sciex).

HPLC of 5 μL of each of the metabolite extracts was performed on a capillary 1 mm-diameter C_{18} column (Discovery® Bio wide pore 5 μm, Sigma) at a 50 $\mu L \cdot min^{-1}$ flow rate with a gradient of acetonitrile in 0.1% formic acid (10 to 80% for 60 min). The metabolite contents were analyzed with an electrospray ionization hybrid quadrupole time-of-flight (ESI-QqTOF) hybrid mass spectrometer (QStar® Pulsar i, Applied Biosystems®, France) on positive mode using information dependent acquisition (IDA), which allows switching between mass spectrometry (MS) and tandem mass spectrometry (MS/MS) experiments, as previously described (Marie et al., 2012). The data was acquired

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