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Project Full Name: Adaptation to cyanobacteria through the symbiotic microbiota in the water flea *Daphnia*

Marie Curie Actions
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1. PUBLISHABLE SUMMARY

**Objectives of the project:** In recent years, it has become clear that the gut microbiota is not just a random set of commensal bacteria, but rather a complex ecosystem that plays a critical role in host physiology, ranging from food digestion and detoxification of foodborne toxins and chemical pollutants, to protection against parasites and pathogens. In addition, due to their short generation time and their high genetic diversity, gut microbiota are responsive communities that can rapidly respond to changes in host diet or health condition, mainly through changes in their relative abundances. Hence, the gut microbiota represents an important source of metabolic flexibility that may allow its host to rapidly acquire phenotypes that are adapted to current environmental conditions. Moreover, recent studies suggest that the gut microbiota has a heritable component, and might thus be submitted to natural selection. Hence, the gut microbiota may be a key, yet understudied, factor driving both acclimatization and adaptation to changing environments, especially in the current context of fast and drastic environmental changes imposed by human activities and global warming.

The ANSWER project aimed at determining the role of gut microbiota in adaptation to changing and stressful environments, using the water flea *Daphnia* and its adaptation to cyanobacterial Harmful Algal Blooms (cyanoHABs) as a model system.

CyanoHABs have strongly increased in frequency and intensity in lakes, ponds, and reservoirs worldwide, because of eutrophication and climate change. Due to the release of powerful toxins (i.e. microcystins) in the water, CyanoHABs pose severe threats on livestock and human health, and disrupt the whole freshwater community. Deciphering the mechanisms underlying resistance to toxic cyanobacteria is thus essential to predict how aquatic communities can respond to such disturbance, and to identify how cyanoHABs can be prevented or controlled. This is especially important for zooplankton grazers that are key players in freshwater food webs and are the main candidates for a top-down control of cyanoHABs. We here hypothesized that gut microbiota may be a key factor in resistance to cyanobacteria.

**Work performed during the project:** During the first period (2014-2015) we optimized the technics to make germ-free *Daphnia* and to perform reciprocal gut microbiota transplants. Through the comparison of germ-free and conventionalized *Daphnia* under different diets, we showed that the microbiota was essential for the fitness of *Daphnia*, and that the strength of the mutualistic interaction was dependent on food availability (1). We also performed reciprocal gut microbiota transplants between genotypes and diets, which revealed that genotype-dependent gut microbiota drive resistance to toxic cyanobacteria in *Daphnia* (2). During the second period (2015-2016), we focused on analyzing the composition of the gut microbial community using next generation sequencing of 16S DNA, and showed that the composition of the gut microbiota changes in response to cyanobacterial exposure, with a strong increase in the proportion of bacteria known to degrade cyanobacterial toxins (2). We also started to optimize the technics (HPLC) to measure the biodegradation of cyanobacterial toxins by the gut microbiota. These measures are currently performed in the laboratory.

**Main results:** Through a multi-factorial assay involving reciprocal gut microbiota transplants between *Daphnia* genotypes and diets, we showed that resistance to cyanobacteria in *Daphnia* is mainly mediated by the gut microbiota (Fig. 1). Resistance to cyanobacteria in recipient *Daphnia* was not affected by the recipient genotype, but was strongly impacted by the donor genotype. Microbiota from resistant donor genotypes conferred a higher resistance to recipient *Daphnia* than microbiota from susceptible donor genotypes. This suggests that gut microbiota is a key component of resistance to toxic cyanobacteria, and that *Daphnia* genotype acts indirectly on resistance to cyanobacteria, by shaping the gut microbiota. In addition, resistance was higher when donors were previously fed cyanobacteria, suggesting that gut microbiota responded to become more efficient in dealing with cyanobacteria after prior exposure. Next generation sequencing of 16S rDNA shows that resistance is associated with changes in microbiota structure: cyanobacterial exposure favored bacterial taxa involved in digestion of cyanobacterial cells and detoxification of cyanobacterial toxins. Together, these results show that resistance to toxic cyanobacteria in *Daphnia* is mediated by the gut microbiota, which may drive both acclimatization and adaptation to cyanoHABs. Gut microbiota might thus be an important mediator of the genetic mosaic of coevolution between toxic cyanobacteria and their grazers, and a key determinant of how freshwater ecosystems respond to climate warming.
Figure 1: Deciphering how genotype, diet and gut microbiota interact to drive resistance to toxic cyanobacteria through gut microbiota transplants. Donor Daphnia populations from either susceptible (red) or resistant (green) genotypes were exposed to either a non-toxic green algal or a toxic cyanobacterial diet for several months, under similar conditions, with an initial seeding of a bacterial inoculum from the field. Their gut microbiota was then extracted and inoculated into germ-free recipient Daphnia juveniles from susceptible (red) and resistant (green) genotypes. Recipient Daphnia were subsequently fed on a cyanobacteria diet to determine their resistance. We found that the level of resistance to cyanobacteria in recipient Daphnia was co-determined by donor genotype and donor diet, and was not influenced by recipient genotype (green face: resistant; red face: susceptible).

Impacts of the project: This project will appeal to a broad audience and impact diverse fields, from ecology and evolution to sustainability and medicine. It will first contribute to unravel the role of gut microbiota in driving adaptation to the environment in natural populations, and to forecast adaptive responses of natural populations to fast and drastic environmental changes imposed by human activities and global warming. Furthermore, the environmental stressor that was studied (i.e. toxic cyanobacteria) is highly relevant, as its represents a major threat on aquatic ecosystems and on human and livestock health. By deciphering the mechanisms underlying resistance to toxic cyanobacteria in zooplankton grazers, this project will help in finding ways to prevent and control cyanoHABs. Besides these ecological implications, these results contribute to improve our understanding of the general mechanisms underlying host-microbiota associations, revealing that host genotype and diet interact to shape not only the structure, but also the functionality of microbiota. Such findings may be applicable to other species, and may contribute to understand diseases linked to gut microbiota in humans. Importantly, these results provide a solid basis for new projects currently developed in the laboratory Aquatic Biology @ KU Leuven to unravel (1) the microbial functions involved in resistance to cyanobacteria, and (2) the functional mechanisms (especially the immune factors) underpinning the establishment of mutualistic gut symbionts in Daphnia.


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