# METHYLMERCURY PRODUCTION BY THE GUT MICROBIOTA OF HERBIVOROUS AND PISCIVOROUS FRESHWATER FISHES FROM CAZAUX-SANGUINET LAKE (SOUTH-WESTERN FRANCE)

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## Introduction

Monomethylmercury (MeHg) is the most toxic and bioaccumulated mercury species with deleterious effects on higher organisms (Allen et al., 2002). Consumption of mercury contaminated food is recognised as the main entry of this contaminant in organisms and biomagnifications vector in food webs (Fitzgerald and Clarkson, 1991), but biotransformation processes of mercury by gut microbiota remains unclear (Pennacchioni *et al*., 1976; Rudd *et al*., 1980). The recent discovery by Parks et al. (2013), of genes necessary to achieve mercury methylation (*hgcA* and *hgcB*), has opened new opportunities to answer this question. To date, only one global survey of *hgcA* and *hgcB* metagenomic data has been performed and did not reveal the presence of these genes in gut microbiota of vertebrates (Podar *et al*., 2015). In this study, we determined the 16S rRNA microbial diversity and the presence of *hgcA* gene and explored *ex vivo* mercury methylation capacity of gut microbiota samples isolated from the intestinal tract of fishes of two different trophic levels: the carnivorous perch (*Perca fluvatilis*) and the herbivorous roach (*Rutilus rutilus*).

## Methods

Perches and roaches specimens were sampled in September 2015 in Cazaux-Sanguinet Lake which is an oligotrophic lake located on the Atlantic coast in south-west of France. The intestinal content was immediately collected and processed under anoxic and dark conditions to assess *ex vivo* mercury methylation capacity by using $^{199}Hg^{2+}$ and $Me^{201}Hg^{+}$ stable isotopic tracers (Rodriguez-Gonzalez *et al*., 2013). We evaluated the relative contribution of methanogenic and sulfate-reducing bacterial (SRB) populations to the mercury methylation activity by supplementing the intestinal content from roach specimens with either 2-bromoethane-sulfonic-acid or molybdate as specific inhibitors, respectively (Compeau and Bartha, 1985). Inversely, we favoured the activity of sulfate-reducers, which are known strong mercury methylators in freshwater sediments (Gilmour *et al*., 1992) by the addition of sulfate and determined the impact on the mercury methylation activity. Finally, we achieved the determination of the 16S rRNAgene diversity and eventually the detection of mercury methylation gene (e.g., *hgcA*) by using primers developed elsewhere (Shaefer *et al*. 2014).

## Results

In untreated intestinal samples of roach and perch, MeHg consisted in 17% and 77% of total natural endogenous mercury, respectively. After four hours of incubation with isotopic tracers, methylation potential was higher in roaches than in perches intestinal samples (c.a., 0.34±0.07% and 0.17±0.02%, respectively). As expected, the addition of specific metabolic inhibitors negatively impacted MeHg production (c.a., 0.01±0.03% with molybdate and 0.21±0.04% with 2-bromoethane-sulfonic-acid). Alternatively, sulfate addition significantly enhanced mercury methylation (0.82±0.02%) that suggests enhanced metabolic activity of mercury-methylating SRB population. These data were used to estimate daily methylation potentials as presented in Table 1. The biological mercury methylation was confirmed by

the lack methylmercury production in autoclaved intestinal controls. More importantly, the metabolic potential necessary for mercury methylation was confirmed by the detection of *HgcA* in intestinal samples from roaches and perches.

**Table 1.** Inorganic mercury $^{199}Hg^{2+}$methylation (M in % $day^{-1}$) determined in *ex vivo* incubations of perches and roaches gut content under anoxic and dark conditions. The relative contribution of sulfate-reducers and methanogens to $Me^{199}Hg^{+}$ formation was assessed by supplementing samples with sulfate to enhance SRB activity or with specific metabolic inhibitors (e.g., molybdate and 2-bromoethane-sulfonic-acid, respectively).

| Matrix<br>Supplement | Roaches gut microbiota<br>(M % $day^{-1}$) | Perches gut microbiota<br>(M % $day^{-1}$) |
|---|---|---|
| None | 2.04 ±0.42 | 1.02 ±0.12 |
| 2-bromoethane-sulfonic-acid | 1.26 ±0.24 | n.d. |
| Molybdate | 0.06 ±0.18 | n.d. |
| Sulfate | 4.92 ±0.12 | n.d. |

n.d. not done due to the very low quantity of sample available

## Conclusion

The gut microbiota from roaches and perches of Cazaux-Sanguinet Lake, harbour *hgcA* gene and have the potential to transform inorganic mercury into MeHg. However, its impact on the host organism should be limited due to (i) the low methylation potential and (ii) greater MeHg inputs from food intakes, especially for piscivorous fishes. Nonetheless, MeHg freshly produced *in vivo* could have a greater mobility -from the intestine to the blood system of fishes - than that of MeHg bio-accumulated in ingested plant and prey tissues. Interestingly, net MeHg production was much higher in roach than in perch which was unexpected in regard to the lower endogeneous (natural) MeHg concentrations in roach gut. These results suggest that roach intestinal microbium harbours mercury-methylating microorganisms that are either in greater density or have a better capacity to methylate mercury in comparison to that in perch, which ingested much of its mercury from preys under the form of MeHg. In the other hand, roach feed much on periphyton where lower percentage of endogenous MeHg (c.a., 1% of total mercury) was measured. Under specific conditions sulfate-reducing microorganisms that harbour mercury methylation genes may be more active and play a major role in the production of MeHg in the intestinal tract of roaches.

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