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The gut bacterium *Extibacter muris* produces secondary bile acids and influences liver physiology in gnotobiotic mice

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ABSTRACT

Extibacter muris is a newly described mouse gut bacterium which metabolizes cholic acid (CA) to deoxycholic acid (DCA) via 7α-dehydroxylation. Although bile acids influence metabolic and inflammatory responses, few *in vivo* models exist for studying their metabolism and impact on the host. Mice were colonized from birth with the simplified community Oligo-MM^{12} with or without *E. muris*. As the metabolism of bile acids is known to affect lipid homeostasis, mice were fed either a low- or high-fat diet for eight weeks before sampling and analyses targeting the gut and liver. Multiple Oligo-MM^{12} strains were capable of deconjugating primary bile acids *in vitro*. *E. muris* produced DCA from CA either as pure compound or in mouse bile. This production was inducible by CA *in vitro*. Ursodeoxycholic, chenodeoxycholic, and β-muricholic acid were not metabolized under the conditions tested. All gnotobiotic mice were stably colonized with *E. muris*, which showed higher relative abundances after HF diet feeding. The presence of *E. muris* had minor, diet-dependent effects on Oligo-MM^{12} communities. The secondary bile acids DCA and surprisingly LCA and their taurine conjugates were detected exclusively in *E. muris*-colonized mice. *E. muris* colonization did not influence body weight, white adipose tissue mass, liver histopathology, hepatic aspartate aminotransferase, or blood levels of cholesterol, insulin, and paralytic peptide (PP). However, proteomics revealed shifts in hepatic pathways involved in amino acid, glucose, lipid, energy, and drug metabolism in *E. muris*-colonized mice. Liver fatty acid composition was substantially altered by dietary fat but not by *E. muris*.In summary, *E. muris* stably colonized the gut of mice harboring a simplified community and produced secondary bile acids, which affected proteomes in the liver. This new gnotobiotic mouse model can now be used to study the pathophysiological role of secondary bile acids *in vivo*.

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Introduction

The gut microbiome, *i.e.* the community of microbes residing in the intestine of humans and other animals, plays an important role in host health and the development of chronic diseases such as metabolic disorders and liver cancer.[1,2] In contrast to the high number of studies focusing on shifts in fecal microbiomes under disease conditions, there is a limited amount of data on how microbe-host interactions function, with few models available to study those interactions.[3,4] Hence, there is an urgent need for experimental studies dissecting mediators of microbe-host interactions, such as the myriad of metabolites produced by

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microbes. Bile acids are among the best examples of such metabolites: they are synthesized in the liver in the form of conjugated primary bile acids that, once secreted in the small intestine, can be metabolized by gut bacteria. This bacterial metabolism substantially impacts the bioavailability and bioactivities of bile acids, which are known to regulate inflammatory and metabolic responses.[5] Thus, it is essential to study the bacteria capable of converting bile acids.

The enzymatic reactions involved in bile acid metabolism by gut bacteria start with deconjugation (the removal of amino acid residues bound to primary bile acids) via bile salt hydrolase (BSH) activity.[6] Free primary bile acids can then be metabolized further via removal of hydroxyl groups (dehydroxylation), oxidation (dehydrogenation), or epimerization.[7] Whereas BSH activity is known to be carried out by a variety of phylogenetically diverse taxa,[8] including species able to colonize the small intestine, the other reactions are thought to be restricted to bile acids escaping re-absorption in the ileum and catalyzed by a more restricted number of bacterial species colonizing distal parts of the gut. Whilst the so far best studied bile acid-dehydroxylating bacterium in the human gut is *Clostridium scindens*, species from mice are either unknown or, if already isolated, have not been taxonomically described and deposited in reference culture collections. One secondary bile acid-producing species that we have recently cultured and characterized from the mouse gut microbiota is *Extibacter muris*, the first representative of a novel genus which is able to metabolize the primary bile acids cholic acid (CA) to deoxycholic acid (DCA) via 7α-dehydroxylation.[9,10]

Considering the influence of bile acids on metabolic and inflammatory responses and the scarcity of experimental studies using bile acid-metabolizing gut bacteria,[11–16] the goal of the present work was to assess the effects of targeted colonization by *E. muris in vivo*. To that end, germ-free mice were associated with the minimal bacterial community Oligo-MM[12,17] with or without *E. muris* and fed one of two experimental diets varying in fat content. The main focus was to study responses in the liver because of its functional relevance for lipid metabolism.

Results

In vitro metabolism of bile acids

As a foundation for later *in vivo* experiments, we aimed to clarify the metabolism of bile acids by the Oligo-MM[12] strains and *Extibacter muris in vitro*.

First, due to the release of bile acids by the host in conjugated form, Oligo-MM[12] strains were tested for BSH activity via incubation with either TDCA or GDCA in both agar- and broth-based assays. Three Oligo-MM[12] strains were consistently positive across the different assays: *Bacteroides caecimuris* DSM 26085, *Bifidobacterium animalis* DSM 26074, and *Enterococcus faecalis* DSM 32036 (Figure 1a). *Clostridium innocuum* DSM 26113 and *Flavonifractor plautii* DSM 26117 deconjugated TDCA but not GDCA, which tended to inhibit the growth of several strains under the conditions tested. *Muribaculum intestinale* DSM 28989 showed inconsistent results depending on the combination of bile acid tested and assay used: it was positive for TDCA on agar and GDCA in broth. *Lactobacillus reuteri* DSM 32035 was positive for GDCA deconjugation both on agar and in broth. The other Oligo-MM[12] members either tested negative for all reactions or their growth was inhibited by addition of the bile acids (*Acutalibacter muris* DSM 26090, *Akkermansia muciniphila* DSM 26127, *Blautia coccoides* DSM 26115, *Enterocloster clostridioformis* DSM 26114, *Turicimonas muris* DSM 26109).

The previously observed ability of *E. muris* to produce DCA[10] was confirmed, albeit at an amount approx. 24-fold lower than the positive control strain *C. scindens* (0.75 *vs.* 18 µM, respectively) (Figure 1b). The fact that the amount of DCA produced did not mirror the decrease in CA concentrations suggests the formation of intermediates (e.g. 3, 7, 12-oxo bile acids) not measured by the quantification method used. *E. muris* did not convert UDCA and CDCA into LCA when provided as single substrates under the conditions tested (Figure 1b). When incubated in the presence of mouse bile (1:300 dilution), *E. muris* produced up to 2.2 µM DCA, which was still ca. 6.5-fold lower than the complex mouse cecal microbiota used as positive control (Figure 1c). Moreover, *E. muris* was not able to catalyze the isomerization of βMCA into ωMCA.