

Limosilactobacillus reuteri Creates Novel Microbially Conjugated Bile Acids Which Impacts Bile Acid Signaling

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Introduction: Multiple microbes in the gastrointestinal tract can transform primary bile acids into secondary bile acids and other derivatives. Microbes can also conjugate bile acids with diverse amino acids and bioactive amines, but the responsible organisms and physiological consequences of this conjugation remain poorly understood. We investigated bile acid conjugation by *Limosilactobacillus reuteri* ATCC 6475 and the functional effects of its conjugates.

Methods and Results: Using targeted LC-MS/MS, we found that *L. reuteri* conjugated bile acids to several amino acids, including aspartate, glutamine, glutamate, histidine, lysine, and threonine. The abundance of individual conjugates varied according to the bile acid substrate, with UDCA yielding the greatest levels of aspartate- and glutamate-conjugated products and CDCA producing the highest levels of histidine conjugation. To investigate possible receptor-level effects, we modeled interactions between the identified conjugates and FXR or TGR5. Surprisingly, we found that many of the identified *L. reuteri* conjugated CDCA bound the receptors more strongly than unconjugated CDCA. However, predicted receptor-bound poses of amino acid-conjugated CDCA exhibited high RMSD values relative to unconjugated CDCA. These data suggest that although *L. reuteri* conjugated bile acid have a predicted stronger affinity, their orientation and/or conformation in the FXR and TGR5 binding pockets are substantially altered, and these compounds may not activate the receptors. Consistent with these predictions, experiments in transfected HEK293FT cells showed that *L. reuteri*-derived CDCA conjugates reduced FXR and TGR5 activation relative to unconjugated CDCA. FXR competition assays further suggested that this reduction may be attributable, at least in part, to competitive antagonism, as conjugated bile acids diminished activation elicited by the potent FXR agonist XL335. RNA sequencing of human colonic epithelial cells revealed that *L. reuteri*-conjugated CDCA produced a distinct transcriptional response compared with unconjugated CDCA. Microbial conjugation attenuated CDCA-induced activation of the unfolded protein response, reactive oxygen species signaling, and xenobiotic metabolism pathways. **Conclusions:** These findings demonstrate that a single microbe can produce a diverse range of amino acid-conjugated bile acids and that these microbial metabolites can modulate bile acid receptor signaling and epithelial responses.