Bile salt hydrolase substrate preference directs C. difficile infection

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Bile acids (BAs) are an essential group of cholesterol-derived molecules that mediate homeostasis in the human gut by binding to key human receptors and by influencing the gut microbiota through their detergent-like properties. Modulation of BA pool composition has been linked with C. difficile infection, one of the most common and dangerous nosocomial infections in the United States. A key enzyme family capable of shaping the BA pool are the gut microbially-encoded bile salt hydrolases (BSH). These proteins catalyze the deconjugation of glycine- and taurine-conjugated BAs, creating free BAs and paving the way for further biotransformations through other microbial pathways. Although it is well-established that BSHs have substrate specificity for glycine or taurine-conjugated BAs, the basis of this specificity and the mechanisms by which their activities impact diseases like C. difficile infection are not yet understood. Here, we present six novel crystal structures of BSHs from Lactobacillus, a human gut commensal genus that ubiquitously encodes BSH enzymes. These structures reveal that BSH substrate specificity is governed by a loop that extends from one monomer into the active site of an adjacent monomer. We then demonstrate that taurine-conjugated BAs are less effective at inhibiting C. difficile germination and growth than glycine-conjugated or deconjugated BAs. In two ex vivo models, we show that Lactobacillus BSH activity effectively inhibits C. difficile by producing these deconjugated BAs, suggesting the utility of these BSH-encoding microbes as therapeutic interventions for C. difficile infection. Finally, we identify that a panel of non-canonical BAs from these ex vivo models are both BSH substrates and inhibitors of C. difficile germination. Our findings establish the structural basis of BSH substrate specificity and demonstrate that glycine- and taurine-preferring BSHs can modulate both the BA pool and C. difficile infection.