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Lactobacillus acidophilus disrupts collaborative multispecies bile acid metabolism

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ABSTRACT

Bile acids are metabolic links between hosts and their gut microbiomes, yet little is known about the roles they play in microbe-to-microbe interactions. Here we present a study designed to investigate the effect that a common probiotic, *Lactobacillus acidophilus*, has on microbial interactions that lead to formation of secondary bile acids. A model microbial consortium was built from three human gut isolates, *Clostridium scindens, Collinsella aerofaciens,* and *Blautia obeum*, and cultured under different bile acid and probiotic treatments. A multi-omics platform that included mass spectrometry-based metabolomics and activity-based proteomic probes was used to produce two major results. The first, was that an uncommon secondary bile acid – ursocholate – was produced by a multi-species chemical synthesis pathway. This result highlights a new microbe-to-microbe interaction mediated by bile acids. The second finding was that the probiotic strain, *L. acidophilus,* quenched the observed interactions and effectively halted consortial synthesis of ursocholate. Little is known about the role that ursocholate plays in human health and development. However, we did discover that a decrease in ursocholate abundance corresponded with successful weight loss in patients after gastric bypass surgery versus those who did not lose weight after surgery. Hence, this study uncovered basic knowledge that may aid future designs of custom probiotic therapies to combat obesity.

INTRODUCTION

The human gastrointestinal (GI) tract is a complex ecosystem that functions in symbiosis with oral and intestinal microbiomes[1,2]. It has long been recognized that the composition of the gut microbiome has a significant effect on host digestion but more recent research has implicated the microbiome in human health and disease states that include cardiovascular disease risk[3], neurological function[4], and autoimmunity[5]. Rapid gains in knowledge of host-microbiome associations will undoubtedly lead to new practical applications[6]. Of these, the use of probiotics to modulate both the function and composition of gut microbiomes is especially promising[7,8]. Probiotic supplementation likely reduces the risk of developing antibiotic associated diarrhea[9] and necrotizing enterocolitis in infants[10]. However, the therapeutic opportunities for probiotics are advancing to more precisely target specific processes carried out by the gut microbiome to impart health benefits beyond enhanced digestion[11-13]. Probiotic therapies are being explored relieve symptoms of autism[14], depression[4], autoimmune diseases[15,16], and irritable bowel syndrome[17] among many other conditions with positive – albeit conflicting – results. The efficacy of probiotic treatments is variable. Differing results can obviously arise from inconsistent study design – e.g., probiotic strain, dose – trial size, but they are also indicative of a large scientific knowledge gap and incomplete understanding of the mechanisms by which probiotics impact the GI-tract microbial ecosystem.

There are many hypotheses about the modes of action by which the gut microbiome and probiotic microbes impact human health. One proposed model is through modulation of the host immune system[18], which has been concluded from studies that showed probiotic treatments affecting host immune function in humans facing pathogenic challenges[19-21] or with autoimmune disorder[15,16,22]. Another hypothesis is that probiotics increase microbial competition within the

68 intestinal ecosystem, thereby making it more difficult for pathogenic bacteria to survive[23,24].
69 Studies have also speculated that observed therapeutic action from probiotics results from their
70 effect on the intestinal physiology by modulating endothelial junctions[25] and the mucosal
71 lining[12,25] through a variety of proposed metabolic pathways. Another possible mode of action
72 for probiotics is through bile acids. It has long been known that bile acids are important linkers
73 between host and gut microbes. Intestinal bacteria produce secondary bile acids by
74 deconjugation, reduction, oxidation, and epimerization of their host's primary bile acids. Many
75 probiotics can alter bile acid pools in humans[26,27].
76 The primary bile acids (cholic acid and chenodeoxycholic acid) are synthesized in human
77 hepatocytes and conjugated to the amino acids glycine and taurine to increase solubility[27,28].
78 They are then released into the duodenum and moved through the small intestine to assist
79 emulsification of dietary lipophilic substances[29]. Approximately 95% of the bile acids are then
80 passively reabsorbed in transit through the small intestine, resulting in approximately 5% (400-
81 800 mg) passed on to the colon[27]. In the colon, these bile acids are rapidly deconjugated by the
82 microbiome and reduced, oxidized and epimerized to a variety of secondary bile acids[27]. These
83 secondary bile acids are known to have diverse effects on human health ranging from direct
84 cytotoxicity[30], to altered probability of cancer[31], to hormonal function as cell messengers[29,32-34].
85 More recently, this list of known host-related effects has grown to include modulating the
86 composition and function of the gut microbiome[35,36] – e.g., by disassembling lipid membranes.
87 The intestinal microbiome represents a major modifier of the human bile acid pool. This
88 is evinced by the fact that bile in the gall bladder is comprised of 70% primary bile acids but
89 only 4% primary bile acids in the feces[27]. Not only does the microbiome determine bile acid
90 composition, but bile acids also direct microbial communities[37]. For example, studies have

91 shown that high fat diets and diets high in resistant starch[38] have an effect on both the bile acid
92 pool[39] and the microbiome[40]. It has also been shown in rats that oral administration of certain
93 bile acids can shift the microbiome composition[35,41]. Collectively, these studies suggest that there
94 is a complex interplay between microbial species and bile acids that is not fully understood.
95 The relationships between the gut microbiome, probiotics and the bile acid pool are
96 particularly relevant due to the current epidemic of obesity and the comorbidities associated with
97 high adiposity (high cholesterol, high blood pressure, diabetes)[42]. Due to high rates of obesity,
98 bariatric surgery has become a more common procedure with the number and types of surgeries
99 increasing with time[43,44]. It is now well established that bariatric surgery results in reduced
100 weight and a reduction in many comorbidities of obesity[45]. Several different advantages (weight
101 loss, reduction of comorbidity) and disadvantages (surgical complications, malnutrition, weight
102 regain, re-surgery, infection, etc.)[45,46] have been have been identified for different bariatric
103 procedures. Yet, there remains an incomplete understanding of the exact mechanisms of many of
104 these outcomes, making it difficult to predict which patients will benefit most from these
105 procedures. Elucidation of the root causes will require consideration of the impacts that the bile
106 acid pool, microbiome composition and probiotic administration can have[47,48]. Deeper
107 mechanistic insight could not only result in increased surgery success, potentially by pre-emptive
108 modulation of the microbiome by bile acid pool[49,50], but could also result in less surgeries
109 necessitated if some of the positive results can indeed be realized via targeted use of probiotics.
110 Here we present a study that was designed to investigate the community dynamics of
111 microbes commonly found in the gut and the impact that both the addition of bile acids and a
112 probiotic has on interspecies interactions. An *in vitro* model was built as a three-species bacterial
113 consortium, *Clostridium scindens, Collinsella aerofaciens,* and *Blautia obeum,* each of which

114 occurs naturally in the human gut. This consortium was then treated with the probiotic strain
115 *Lactobacillus acidophilus* to make an altered four-member consortium. Multi-omics assays were
116 used to elucidate the interspecies microbial interactions with and without bile acid treatments
117 (cholic acid and deoxycholic acid). We found that a secondary bile acid, ursocholic acid (7-
118 epicholic acid), was produced from cholic acid through a multispecies chemical synthesis route
119 mediated by an interaction between *B. obeum* and *C. aerofaciens*. This process was quenched by
120 the addition of *L. acidophilus*, which disrupted the coordination between *B. obeum* and *C.*
121 *aerofaciens* and shut down ursocholic acid production. These results were then contextualized by
122 performing targeted metabolite measurements in fecal samples from a human clinical study[44] that
123 investigated secondary bile acid abundances as outcomes of gastric bypass surgery. The
124 abundance of ursocholic acid corresponded with successful post-operative weight loss,
125 highlighting that it may be important to investigate the implications of both patient- and microbe-
126 derived metabolites to help gain a predictive understanding of a patient's response to bariatric
127 surgery. New knowledge in this area will yield opportunities to design custom probiotic
128 therapies. More broadly, this study supports an emerging theme in microbiome sciences that
129 microbial interactions are context dependent and the presence or absence of select species and/or
130 metabolites can have a strong effect on the overall function.
131
132 **RESULTS**
133 **A model microbial consortium responds to probiotic and bile acid treatments.** We
134 constructed an *in vitro* microbial consortium from human gut bacterial isolates. A 3-member
135 consortium (3MC) composed of *Clostridium scindens* ATCC 35704*, Collinsella aerofaciens*
136 ATCC 25986*,* and *Blautia obeum* ATCC 29174 was compared to a 4-member consortium (3MC

137 + L) that included an added a probiotic strain, *Lactobacillus acidophilus* ATCC 4356. The
138 species were chosen for this model based on their capacity to perform one-of-three unique
139 microbial transformations on human bile acids (Table S1): 7α-dehydroxylation, hydroxysteroid
140 dehydrogenatation, or bile salt hydrolsis[27,37,51,52].

141 Anaerobic culturing treatments included supplementation with cholic acid and/or
142 deoxycholic acid and were compared to axenic controls. We found that each member of the
143 consortia grew together under anaerobic culturing conditions but that each consortium (3MC
144 versus 3MC + L) had very different growth and extracellular metabolite profiles (Fig. 1). Despite
145 the anticipated anti-microbial effect of bile acids[26,53,54], all cultures in media containing added
146 cholic acid and deoxycholic acid (0.1 mM) showed faster specific growth rates compared to their
147 corresponding treatments without bile acids (Fig. 1a and b). The axenic controls confirmed that
148 *L. acidophilus* had the fastest specific growth rate, followed by *B. obeum*, *C. aerofaciens* and *C.*
149 *scindens*. The probiotic, *L. acidophilus*, was the least affected by the addition of the bile acids to
150 the growth media, with only a 17% increase in the specific growth rate as compared to 170%,
151 76% and 167% increases for *C. scindens*, *C. aerofaciens* and *B. obeum,* respectively. An adonis
152 test was used to show that the species composition of each consortium – i.e., presence/absence of
153 *L. acidophilus* – was the strongest determiner of variance ($R2 = 0.49$, $p < 0.001$) in the
154 extracellular metabolome. This was in contrast to the effect of treatments that tested for changes
155 in the global metabolome based on bile acid or no bile acid inputs, which were not a statistically
156 significant source of variance (Fig. 1C). Hence, the probiotic *L. acidophilus* was a major
157 modifier of the extracellular chemical environment.
158

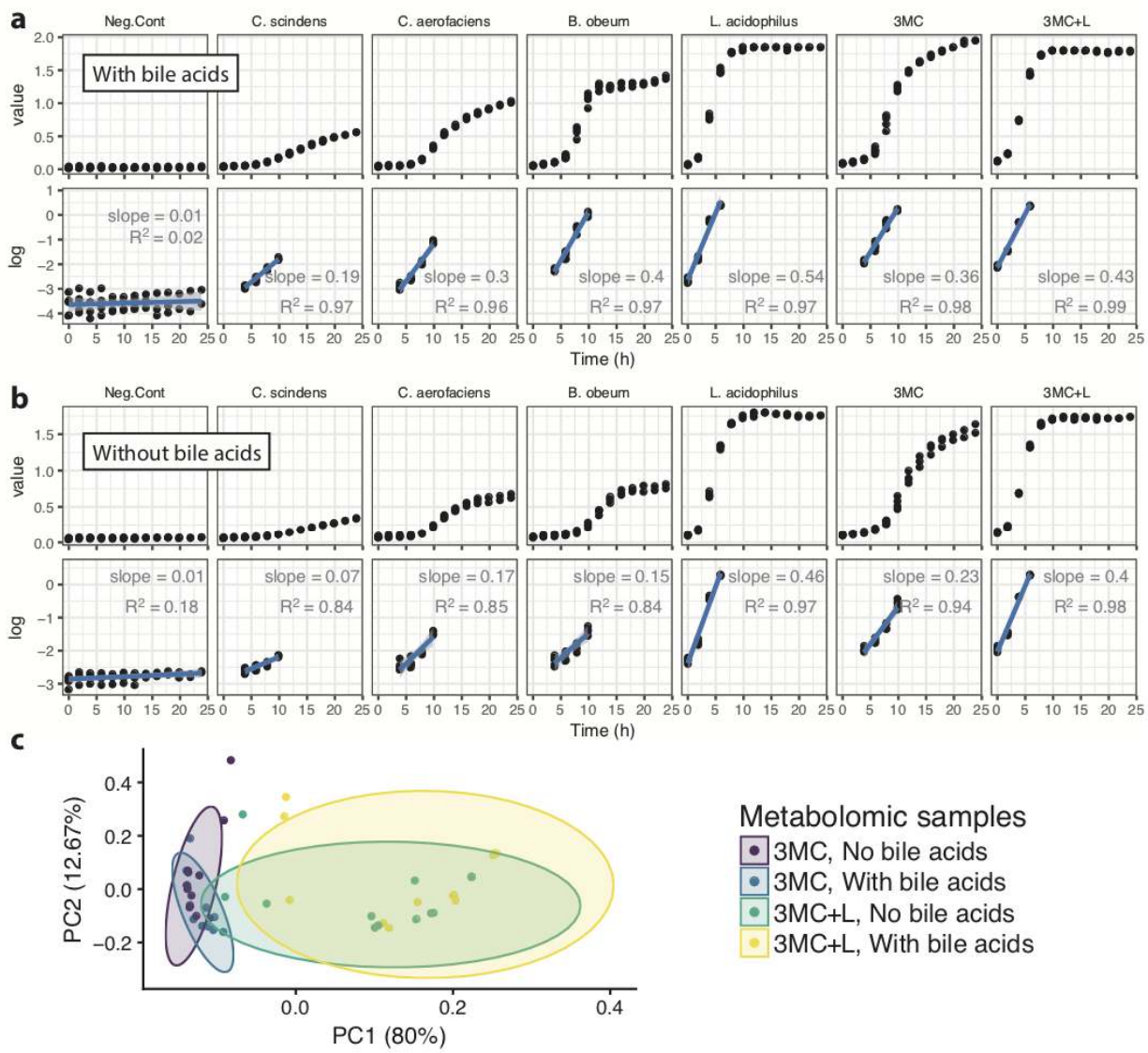

Figure 1. Probiotic influence on consortial growth and the extracellular chemical environment. (A) Growth of the 3-member consortium (3MC) in bile acid supplemented media as compared to (B) the 4-member consortium (3MC + L) that differed by the addition of probiotic, *L. acidophilus.* The y-axis of A and B represent means from triplicate measurements of absolute species abundance defined as the total optical density (OD_{630nm}) at each time point multiplied by each respective measurement of relative abundance obtained from qPCR; error bars represent ± 1 standard deviation. (C) A principle component analysis on the extracellular metabolome ordinated by the Euclidean distance between the metabolic profiles from each treatment. The colored ellipses represent 95% confidence limits assuming a multi-variate t-distribution.

***L. acidophilus* quenches secondary bile acid production.** Ursocholic acid was produced in the 3MC (Fig. 2A), but not by any one species grown under axenic conditions. Hence, ursocholic acid was produced by a multispecies synthesis route that required at least two species from our model consortium. Addition of the probiotic, *L. acidophilus* quenched the production of

ursocholic acid to negligible levels as compared to those measured in the 3MC (Fig. 2b). In addition to ursocholic acid production, *L. acidophilus* also attenuated growth of *B. obeum* and *C. aerofaciens* as observed in the 3MC + L species-specific growth dynamics, which were in stark contrast to the 3MC (Fig.2c). Within the 3MC + L, *L. acidophilus* became a dominant member of the community, but in the absence of the probiotic, the 3MC was dominated by *B. obeum* with *C. scindens* showing the lowest relative abundance in both consortia.

The dynamic profiles of bile acids and bacterial species were correlated for each treatment group, 3MC and 3MC + L (Fig. 2d), respectively. Pearson's correlations were determined between species and for species to bile acids but not between bile acids. The results show that ursocholic acid shared strong positive correlations (Pearson's coefficient; $r > 0.85$) with the abundances of *C. aerofaciens* and *B. obeum* in the 3MC. As expected, cholic acid showed strong negative correlations with *C. aerofaciens* and *B. obeum* within the 3MC, establishing that it was the most likely substrate for ursocholic acid synthesis. Cholic acid, was not correlated with *C. scindens*, implying that this species may not have been involved in production of secondary bile acids directly from cholic acid. The lack of *C. scindens'* participation in secondary bile acid synthesis in the 3MC was also evinced by its strong negative correlation with deoxycholic acid, a known 7α-dehydroxylation product of *C. scindens* that utilizes cholic acid as the substrate[27].

L. acidophilus membership changed the correlations between species and bile acids. Most notably, ursocholic acid was not correlated with any species in the 3MC + L under the probiotic treatment. *L. acidophilus* did not correlate with any of the bile acids and shared strong negative correlations with *C. aerofaciens* and *B. obeum*, indicating that competition and/or antagonism are the likely mechanisms by which the multi-species ursocholic acid synthesis was quenched.

Multispecies synthesis of ursocholic acid. Based on the metabolomics results, we hypothesized a multi-species cooperative synthesis of ursocholic acid that involved both *B. obeum* and *C. aerofaciens*. The initial inference was derived by comparing the metabolite

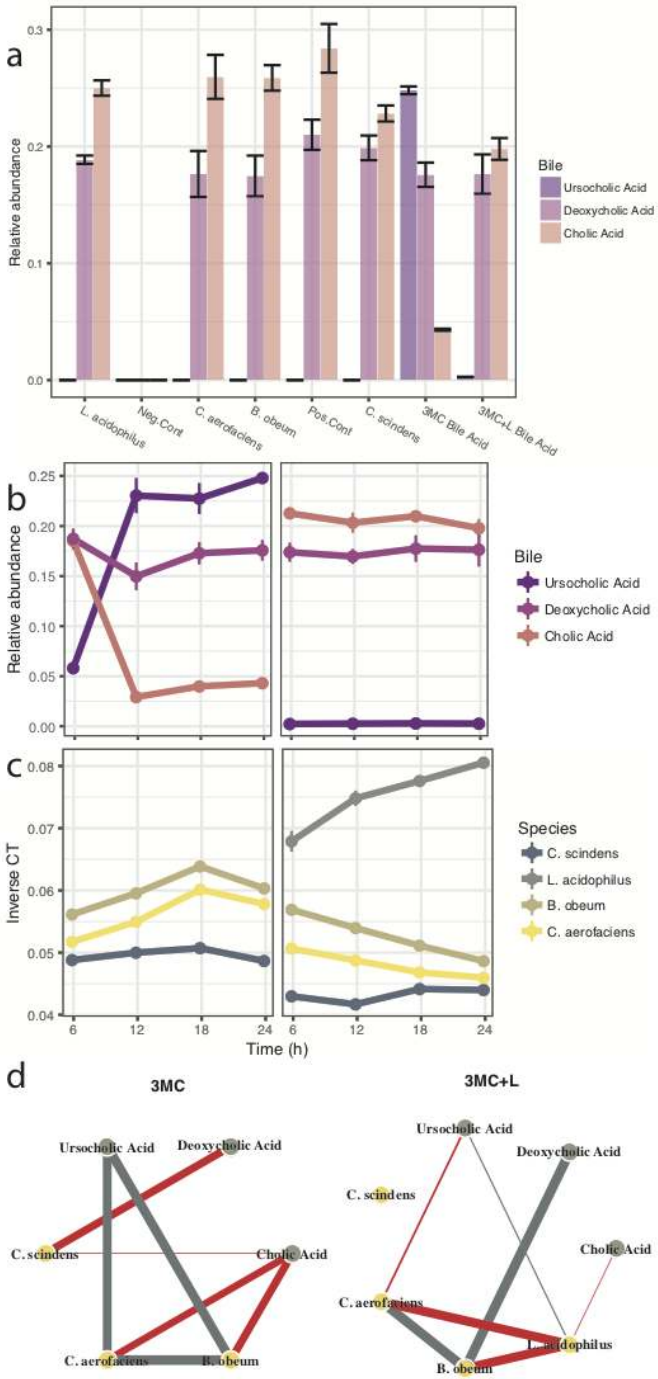

Figure 2. Secondary bile acid – ursocholic acid – was produced by consortia but not axenic cultures; *L. acidophilus* disrupted the consortial synthesis. (A) Comparative abundances of bile acids measured after 24 h incubations (3MC and 3MC + L) and axenic controls. Time course measurements of (B) bile acid abundances and (C) species abundance as shown by the inverse qPCR cycle thresholds (CT). Comparisons are shown between the 3MC and 3MC + L (with *L. acidophilus*) treated with the 0.1 mM bile acid mixture. Each data point shown in panels A-C represent the mean from a minimum of three biological replicates ± 1 standard deviation. (D) Pearson's correlations between bacterial species and bile acid abundances; thicker lines correspond to greater correlation coefficients (cut-off below 0.85); red and grey colors correspond to positive and negative correlations, respectively.

213 profiles between the axenic and consortial treatments (Fig. 2a). The next piece of evidence was
214 obtained from correlations between species and bile acids in the context of *a priori* knowledge of
215 the metabolic reactions that were the basis for choosing each species in the model consortium
216 (Fig. 2d). A possible mechanism for this could start with *B. obeum* conversion of cholic acid into
217 a transient 7-keto intermediate, such as 7-oxodeoxycholic acid. A ketone intermediate was not
218 identified by our bile-acid-targeting LC-MS metabolomics approach but that does not exclude
219 the possibility of its existence. The next step could be achieved by the known genome encoded
220 functions of *C. aerofaciens*, which contains *hdhB* (GenBank accession ZP_01773061)[55]. This
221 gene encodes for a 7β-hydroxysteroid dehydrogenase (7β-HSDH), known to catalyze a reaction
222 that takes a 7-oxodeoxycholic acid to ursocholic acid[56]. However, the *B. obeum* ATCC 29174
223 genome does not contain an oxidoreductase that is clearly annotated to catalyze our hypothesized
224 reaction in this first step. *B. obeum* does contain a *baiA* gene (RUMOBE_03494) that encodes a
225 putative 7α-dehydroxylase, but genes encoding for a 7α-HSDH have yet to be identified. Yet our
226 experiments clearly showed synthesis of ursocholic acid and we confirmed a required
227 participation of *B. obeum*; hence, we concluded that a non-specific or previously unidentified
228 oxidoreductase catalyzed this first step.

229 To test our hypothesis that *B. obeum* expresses enzymes that react with cholic acid (other
230 than the known BaiA protien), we synthesized and employed a custom cholic acid photo affinity
231 probe (CAP; cholic acid probe). This probe is a cholic acid derivative designed to bind and
232 enrich proteins that can then be identified by MS-based proteomics (Fig. 3a). The results were a
233 suite of proteins from each species in the consortium that were significantly enriched by the CAP
234 ($>10^2$ fold-change; p-value < 0.001) (Table S2). Surprisingly, the *B. obeum* BaiA protein
235 (*RUMOBE_03694*) was not enriched by the CAP, indicating the possibility of an incorrect

annotation from homology of UniProt-KB A5ZWVO or lack of expression in these consortial conditions. We identified a suite of *B. obeum* proteins that were significantly enriched by the CAP and specific to the treatments corresponding to ursocholic acid synthesis and the *B. obeum* axenic treatment. Of these, we focused on a pair of oxidoreductases as candidates for the hypothesized two-step, two-species reaction that lead to ursocholic acid in our consortium. While they did not share strong homology with known 7α-HSDH proteins, they were annotated as enzymes that may catalyze the hypothesized alcohol-ketone inter-conversions (Fig. 3b). These CAP-binding proteins were annotated based on homology as an alcohol dehydrogenase (A5ZM66; *RUMOBE_00083*) and an alcohol-aldehyde dehydrogenase (A5ZNA3; *RUMOBE_00470*).

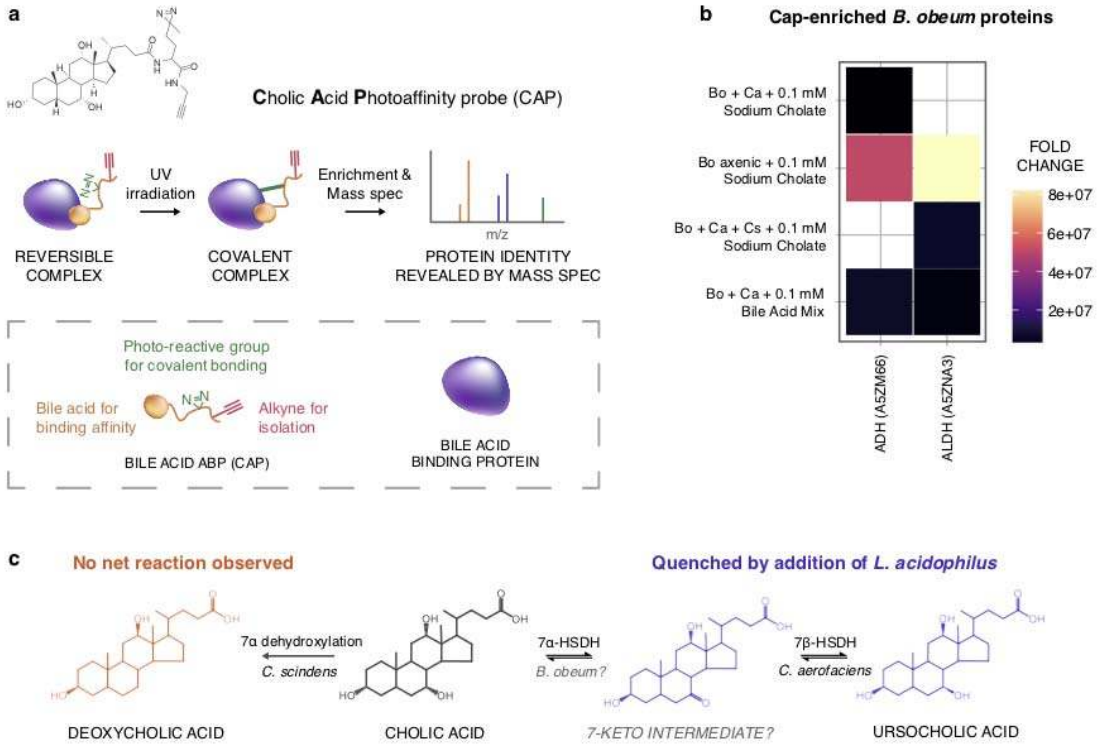

Figure 3. **The multispecies synthesis hypothesis and cholic acid binding oxidoreductases.** (A) The cholic acid photoaffinity probe (CAP) was synthesized and used to enrich proteins for mass spectrometry-based proteomics. (B) Of the proteins enriched by CAP (> 100 fold-change and $p < 0.001$), we identified two oxidoreductases annotated alcohol or alcohol-ketone dehydrogenases (ADH and ALDH, respectively). These proteins are B. obeum candidates for the hypothesized two-step, two-species mechanism (C) leading to the synthesis of ursocholic acid. The abbreviations used to describe experimental treatments are as follows: Bo (*B. obeum*); Ca (*C. aerofaciens*); and Cs (*C. scindens*).

Implications toward obesity and bariatric surgery. The proposed mechanism for physiological effect for Roux-en-Y gastric bypass surgery (RYGB) are BRAVE "Bile flow alteration, Reduction of gastric size Anatomical gut re-arrangement, Vagal manipulation, Enteric gut hormone modulation[57]. Hence, the GI tract of patients that have undergone bariatric surgery is a potential model system to study bile acid metabolism/alterations given that bile acid profiles change by increasing abundance of secondary bile acids[49]. To investigate the clinical relevance of ursocholic acid, we leveraged access to a cohort of patients that had undergone gastric bypass surgery. Targeted measurements of ursocholic acid were performed and compared in fecal samples collected from 24 patients that underwent RYGB surgery: 10 patients with normal weight and 14 morbidly obese controls, which included those scheduled for surgery[44]. We found that the abundance of ursocholic acid corresponded with obesity and the success of gastric bypass surgical procedures (Fig. 4). Success was defined when patients exhibited at least 50% excess weight loss and less than 20% regain. Ursocholic acid levels were significantly higher in the morbidly obese controls (pre-surgery) compared to patients that had experienced successful gastric bypass surgery. There was no statistically

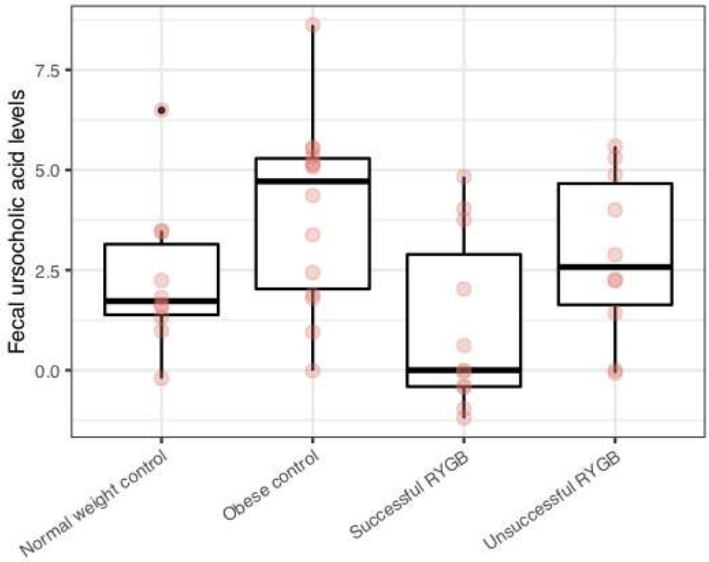

Figure 4. Normalized abundance of ursocholic acid in feces of patients who had undergone gastric bypass surgery – Roux-en-Y gastric bypass (RYGB) – as compared to normal weight and obese controls. Unpaired two-tailed t-tests were used to infer a statistical difference between the means of the obese controls – a group that contained pre-operative patients – and those that experience successful RYGB ($p < 0.005$). Different ursocholic acid abundances were also observed – albeit statistically less conclusive – between successful and unsuccessful RYGB patients ($p < 0.076$).

significant difference between the fecal abundance of ursocholic acid in the obese controls and the unsuccessful surgical patients.

DISCUSSION

The human gut microbiome is integral to human health and development[58,59]. However, the function and composition of the human GI-tract ecosystem is complex[60], which often makes detailed studies of individual processes difficult. The use of model systems is a valuable approach to dissect complex biological functions. In particular, simplified model consortia gaining interest in microbiome research because they enable experimentalists to manage complexity by controlling multiple system components under defined treatments. The utility of simplified consortia, or bench-top microbiomes, has been demonstrated for a variety of human-associated communities[61,62] and other complex microbial ecosystems related to plants[63], sediment/biofilms[64-66] and marine habitats[67,68]. Here, we developed a simple model microbial consortium that was specifically designed to investigate how the addition of a common probiotic (*L. acidophilus*) effects microbial interactions mediated through bile acid transformations. This study was not designed to directly inform microbial physiologies that should occur in the human GI-tract *in situ*. Rather, it was designed and successfully implemented for controlling the membership of microbial species and presence/absence of bile acids that are associated with human digestive systems. Our model bile acid consortium helped produce two major results. The first was that ursocholic acid was synthesized by the coordinated metabolism of a consortium and not by any single species included within this study. The second important finding was that probiotic, *L. acidophilus* quenched the observed multi-species interactions that resulted in secondary bile acid synthesis.

298 Ursocholic acid is the 7 beta-hydroxyepimer of cholic acid. It is rarely mentioned in the
299 scientific literature and has been termed an "unusual secondary bile acid" as compared to more
300 commonly studied metabolites such as deoxycholic and ursodeoxycholic acid[69]. Previous studies
301 have investigated ursocholic acid as a potential therapeutic to modulate the host's synthesis of
302 primary bile acids[70], or to improve the liver function of patients with primary biliary cirrhosis[71]
303 and reduction of bile cholesterol saturation[72]. In these previous studies, ursocholic acid was
304 typically tested along with ursodeoxycholic acid and found to be notably less effective as a
305 treatment for biliary cirrhosis[71]. Despite these therapeutic studies, little is known about the role
306 that ursocholic acid plays in modulating human-microbe and/or microbe-microbe interactions.
307 The data derived from our clinical study showed that ursocholic acid is in fact present
308 and abundant within the human GI-tract and its relative abundances change when drastic changes
309 to microbiome occur (pre- and post- bariatric surgery)[44]. Our limited understanding of the role
310 that ursocholic acid plays in human health and specifically the host-microbiome interactions that
311 lead to its production represent a major knowledge gap. This is punctuated by the fact that our
312 current study – and a previous study[73] – have observed microbial synthesis of ursocholic acid
313 and/or 7-oxodeoxycholic acid but not conclusively identified the genes and 7α-HSDH proteins
314 responsible.
315 Cataloguing the bacterial genes from the "sterolbiome" is an active area of research[74] that
316 can yield new biological insight and help to improve human health by understanding how
317 microbes modify chemical environments within the human GI tract. In this study, we
318 hypothesized a multispecies chemical synthesis route in which *B. obeum* converts cholic acid
319 into an intermediate ketone via a 7α-HSDH-like reaction which is then proceeded by the known
320 7β-HSDH reaction catalyzed by *C. aerofaciens*. Intraspecies 7-epimerization has been

demonstrated in *Clostridium limosum*[75] and *Clostridium absonum*[76], which express both the required enzymes, 7α-HSDH and 7β-HSDH. However, genes encoding for 7α-HSDH, have yet to be identified in *B. obeum* and other bacteria such as an *Eggerthella* sp. known to express this protien[73]. Yet, we conclusively found that a collaborative reaction between *B. obeum* and *C. aerofaciens* does occur, which highlights an increase in our understanding of bile acid metabolism of bacteria.

We hypothesized a 7-keto intermediate that was transferred between *B. obeum* and *C. aerofaciens* in the observed multispecies chemical synthesis route. We did not identify an intermediate such as 7-oxodeoxycholic acid in the supernatant of the samples and therefore cannot categorically confirm its existence. However, 7α-HSDH mediated synthesis of 7-oxodeoxycholic acid has been previously observed in similar multi-step bile acid transformation processes[56]. It is possible that *C. aerofaciens* has a high affinity uptake mechanism for the hypothesized 7-keto intermediate such that extracellular concentrations were below the detection limits of our LC- and GC-MS metabolite identification methods. Another point of uncertainty is whether the CAP-enriched alcohol dehydrogenase (A5ZM66; *RUMOBE_00083*) and alcohol-aldehyde dehydrogenase (A5ZNA3; *RUMOBE_00470*) were responsible for the hypothesized reactions. Certainly, other proteins were enriched by CAP (Table S2), yet these were the only *B. obeum* proteins annotated with an enzymatic function capable of alcohol-aldehyde interconversion. We cannot rule out the possibility that enriched proteins of unknown function participated in the observed reaction. However, there is some precedent for associating secondary bile acid synthesis genes with alcohol dehydrogenases. BaiA proteins from *C. scindens*, encoding for 3α-HSDH proteins, have previously been shown to align well to short chain alcohol dehydrogenases in *Eubacterium* sp. Strain VPI 12708 and to alcohol/polyol

dehydrogenase genes[77,78]. We chose to use *C. scindens* in the 3MC because it has genes that encode for Bai proteins and express HSDH proteins. In fact, the *C. scindens* reference proteome (VPI 12708) does contain a putative 7α-HSDH (UniProtKB – Q03906); however, *C. scindens* abundances and correlation-based inferences from this study did not provide evidence of *C. scindens'* participation in the transformation of cholic acid to secondary acids. Our conclusion was that *C. scindens* played a minor role in the system and was largely outcompeted by other members.

Towards precision probiotics to complement bariatric surgery outcomes. The effect of probiotics on the human gut microbiome and the bile acid pools are particularly relevant due to the current epidemic of obesity and the comorbidities associated with high adiposity (high cholesterol, high blood pressure, diabetes)[42]. Hence, bariatric surgery is becoming more common as a treatment strategy. Yet knowledge gaps still exist with respect to how metabolites and microbes could play a role in successful outcomes. Here, we show that the abundance of ursocholic acid corresponds with the efficacy of gastric bypass surgery. This new knowledge about the role of specific probiotic strains and/or their metabolic products, are therefore leading towards promising novel treatments for patients undergoing bariatric surgery. It has already been shown that post-operative administration of *Lactobacillus* sp. improves weight loss and vitamin B absorption in RYGB patients[48]. It is possible that the cessation of ursocholic acid production or increased abundances of *Lactobacillus* sp. could result in better control over weight loss. We also note that there is some precedent derived from mouse models for the idea that probiotics or introduction of non-adapted microbial taxa can modulate a hosts' microbiome[7] and microbe-associated bile acid pool[79].

Conclusion. This current study was a fundamental investigation of microbial interactions and the role that a probiotic bacterium plays in modulating the synthesis of secondary bile acids. It was not intended to inform clinical practice. However, the results and conclusions presented establish an important idea related to broader microbiome sciences; microbial interactions are context dependent[64,65] and the presence or absence of select species and/or metabolites can have a strong effect on the overall community-level function.

METHODS

Bacterial strains and cultivation. *Clostridium scindens* ATCC 35704, *Colllineslla aerofaciens* ATCC 25986, *Blautia obeum* ATCC 29174 (formerly *Ruminococcus obeum*) and *Lactobacillus acidophilus* ATCC 4356 were grown under axenic conditions and in consortia on Lactobacilli MRS Broth (BD Difco, Houston, TX, USA). The primary treatment the addition of bile salts, equivalent mixtures of cholic and deoxycholic acid (Sigma-Aldrich 48305, St. Louis, MO, USA), supplemented to 0.1 mM. The secondary treatment was the presence and absence of probiotic, *Lactobacillus acidophilus* ATCC 4356 rendering either a 3-member (*L. acidophilus* negative) or 4-member (*L. acidophilus* positive) consortia. Anaerobic growth conditions were prepared by boiling the media and subsequently sparging with an 80% N_2, 10% H_2, 10% CO_2 gas mixture and transferring to 30 ml sealed Balch tubes under the same gas headspace prior to autoclaving. Each culture was inoculated to a starting $OD_{630nm} = 0.077 \pm 0.033$ by each axenic cell suspension resulting in 1 serial passage of log phase cells; consortia were inoculated with an equivalent volume ratio mL. The optical density (OD_{630nm}) was measured over a 24 h period

389 using a Spectronic 20D+ spectrophotometer (ThermoSpectronic, Madison, WI, USA); each time
390 point was sampled in triplicate via destructive sampling.
391
392 **PCR quantification of gene target.** Bacterial DNA was extracted from consortia and axenic
393 culture using the MoBio (Carlsbad, CA, USA) PowerSoil DNA Isolation Kit following the
394 manufacturer's protocol. A total volume of 5 µL of undiluted consortia DNA or standard curve
395 DNA was analyzed in triplicate with an Applied Biosystems 7500 fast instrument (Foster City,
396 CA). Samples were analyzed in triplicate with the primers shown in Table S3 targeting *rpoB*.
397 PCR reactions were run using the FAST cycling conditions: initial denaturation was done for 20
398 seconds at 95 °C followed by 40 cycles of denaturation (95 °C for 3 seconds), annealing (60 °C
399 for 30 seconds). The output from the real-time PCR assays were C_T values that represent the
400 PCR cycle at which the amplification crosses a given threshold (0.1). All C_T values in Table S4;
401 data are plotted and analyzed as inverse C_T representing the relevant abundance[66,80] assuming
402 equal between species in the consortia.
403
404 **Bile acid identification and quantification.** Stock solutions of the cholic and deoxycholic acids
405 (Steraloids Inc., Newport, RI, USA) were made in methanol (1 mg mL^{-1}) and were then pooled
406 together and diluted in a series in 0.1% formic acid to generate a 7 pt of the calibration curve
407 (0.0062, 0.025, 0.050, 0.1, 0.5 and 1 µg mL^{-1}). An internal standard (23-nor-5β-cholanic acid-3α,
408 12α-diol) was added to the filtered media (0.2 µm) collected from each microbial growth sample.
409 Cold methanol (-20˚C) was added at a ratio of 1:4 (filtrate:MeOH). The samples were mixed via
410 vortexing, chilled at -20˚C for 30 minutes and separated via centrifugation (1725 rpm, 10
411 minutes). The supernatant was removed and dried and then re-suspended in 0.1% formic acid in

412 deionized water solution. These samples were analyzed on a Waters nano-Acquity UPLC system
413 (Milford, MA, USA) configured for direct 5 µL sample injections onto an in-house packed fused
414 silica column (360 µm o.d. x 150µm i.d. x 30 cm long; Polymicro Technologies Inc., Phoenix,
415 AZ, USA) containing Waters HSS T3 media (1.8 µm particle size). A flow of 600 nL min^{-1} was
416 maintained using mobile phases consisting of (A) 0.1% formic acid in water and (B) 0.12 %
417 formic acid and 5 mM ammonium acetate in methanol with the following gradient profile (min,
418 %B): 0, 1; 5, 1; 10, 65; 59, 99; 60, 1. Total run time including column re-equilibration was 75
419 min. Mass spectrometry (MS) analysis was performed using an Agilent model 6490 triple
420 quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) outfitted with a
421 custom nano-electrospray ionization interface built using 150 um o.d. x 20 um i.d. chemically
422 etched fused silica [81]. The hexabore ion transfer tube temperature and spray voltage were held at
423 200˚C and -4.0 kV, respectively. Data were acquired in negative ion mode for 75 min from
424 sample injection using a dwell time of 200 µs, fragmentation of 380 volts, and collision energy
425 of 10 volts. Selected reaction monitoring (SRM) transitions were acquired as shown in
426 supplementary Table S5. Ursocholic acid was identified as an unknown in the initial LC-MS
427 trials. After fractionation and purification, we isolated the unknown and verified that it was
428 ursocholic acid via NMR and ion mobility mass spectrometry analyses. Authentic ursocholic
429 acid was purchased from Toronto Research Chemicals (N. York, Ontario, Canada).
430 Comprehensive details of these procedures are provided in the supplementary materials.
431
432 **Untargeted Metabolomics.** The spent media was dried, chemically derivated and analyzed by
433 GC-MS as previously reported[82]. GC-MS raw data files collected by GC-MS were processed
434 using the Metabolite Detector software, version 2.5 beta[83]. Agilent .D files were converted to

435 netCDF format using Agilent Chemstation (Agilent, Santa Clara, CA, USA) and then converted
436 to binary files using Metabolite Detector. Samples were aligned chromatographically across all
437 analyses after deconvolution. Metabolites were identified by matching experimental spectra to a
438 Pacific Northwest National Laboratory (PNNL) augmented version of FiehnLib[84]. This library
439 has spectra and validated retention indices for over 850 metabolites. In order to minimize errors
440 in deconvolution and identification, all metabolite identifications were manually validated after
441 automated data-processing.
442
443 **Bile acid photoaffinity probes and proteomics.** Custom photoaffinity probes were synthesized
444 as derivatives of cholic and deoxycholic acid for this study as described in detail within the
445 supplementary materials. Bacterial lysate samples were normalized to 500 µL 1.8 mg/mL
446 proteome in PBS buffer. Cholic acid photoaffinity probe (CAP) or an equal volume of DMSO
447 control was incubated with proteome for 60 min at 37 °C. Final DMSO concentration was 1%.
448 Samples were exposed to UV light (wavelength: 365nm; 115V, 15W) using a Fisher UVP95
449 lamp (Fisher Scientific, Hampton, NH, USA) for 7 minutes on ice. Subsequent to UV irradiation,
450 the samples were subjected to click chemistry, with final concentrations of reagents being:
451 biotin-azide (60 µM) in DMSO, sodium ascorbate (10 mM), THPTA (4 mM), and $CuSO_4$ (8
452 mM). Each reagent was added individually in that sequence, vortexed, centrifuged, and
453 incubated at room temperature in the dark for 90 min. 800 µL of pre-chilled MeOH was then
454 added to each sample and incubated at -80 °C freezer for 30 min to induce protein precipitation.
455 Samples were centrifuged at 14,000 x g at 4°C for 5 min. The supernatant was discarded, and the
456 pellet was allowed to air-dry for 5 min. Samples were reconstituted and sonicated in 520 µl SDS
457 (1.2%) in PBS; followed by incubation at 95 °C for 2 min. Samples were centrifuged at 14,000 x

458 g for 4 min at room temperature. Protein concentrations were determined via BCA assay and
459 samples were normalized to a volume of 500 µL at 1.2 mg/mL. Trypsin digestion was performed
460 on protein bound to 100 µL Streptavidin-agarose beads. Peptides were reconstituted by adding
461 40 µl of 25 mM NH_4HCO_3 and heating the samples at 37°C for 5 min. Samples were transferred
462 to ultracentrifuge tubes and were centrifuged at 100,000 x g to remove debris. 25 µL was added
463 to glass vials for storage at -20 °C until analysis. All proteomics samples prepared for LC-MS
464 were analyzed using a Velos Orbitrap MS as previously described [85,86].
465
466 **Clinical data and experimentation.** Fecal samples were collected from patients at the Mayo
467 Clinic, Scottsdale, AZ, USA. The metabolomics assays were performed at the Pacific Northwest
468 National Laboratory by the methods described above. The experimental design has been
469 previously described[44] and was approved by The Institutional Review Boards of Mayo Clinic
470 and Arizona State University (IRB# 10-008725).
471
472 **Statistics.** Analysis and graphing was performed in R[87] making use of the 'vegan'[88] and
473 'igraph'[89] packages, along with many packages in the Tidyverse[90]. The adonis test (permutation
474 MANOVA) was used to partition a matrix of Euclidean distances between global metabolite
475 samples based on bile acid and probiotic treatments. An unpaired two-tailed Student's t-test was
476 used to compare fecal ursocholic acid measurements between treatment groups. Linear models
477 were fit to the log of the exponential growth phase of microbial consortia, and for each
478 regression, the goodness of fit and the probability of observing a similar slope if the true slope
479 coefficient was zero was reported. Global proteomics was used to identify proteins enriched by
480 the CAP probes, by selecting proteins with a fold-change increase of >100 and a t-test p-value of

481 < 0.001. The methods for all hypothesis testing and descriptive statistical procedures are
482 included in the R markdown supplied for this study.
483
484 **Data Repositories and Reproducibility.** The raw data sets for this study along with the R
485 scripts used for analysis and graphing are available from the Open Science Framework (OSF)
486 under the name "Bile Acids Consortia" at https://osf.io/5meyd/. The mass spectrometry
487 proteomics data have been deposited to ProteomeXchange with the dataset identifier
488 PXD008617.
489
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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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