Role of dietary sulfonates in the stimulation of gut bacteria promoting intestinal inflammation

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,Insanity is doing the same thing over and over again, but expecting different results.'

Rita Mae Brown, 1983

Summary

The interplay between intestinal microbiota and host has increasingly been recognized as a major factor impacting health. Studies indicate that diet is the most influential determinant affecting the gut microbiota. A diet rich in saturated fat was shown to stimulate the growth of the colitogenic bacterium *Bilophila wadsworthia* by enhancing the secretion of the bile acid taurocholate (TC). The sulfonated taurine moiety of TC is utilized as a substrate by *B. wadsworthia*. The resulting overgrowth of *B. wadsworthia* was accompanied by an increased incidence and severity of colitis in interleukin (IL)-10-deficient mice, which are genetically prone to develop inflammation.

Based on these findings, the question arose whether the intake of dietary sulfonates also stimulates the growth of *B. wadsworthia* and thereby promotes intestinal inflammation in genetically susceptible mice. Dietary sources of sulfonates include green vegetables and cyanobacteria, which contain the sulfolipids sulfoquinovosyl diacylglycerols (SQDG) in considerable amounts. Based on literature reports, the gut commensal *Escherichia coli* is able to release sulfoquinovose (SQ) from SQDG and in further steps, convert SQ to 2,3-dihydroxypropane-1-sulfonate (DHPS) and dihydroxyacetone phosphate. DHPS may then be utilized as a growth substrate by *B. wadsworthia*, which results in the formation of sulfide. Both, sulfide formation and a high abundance of *B. wadsworthia* have been associated with intestinal inflammation.

In the present study, conventional IL-10-deficient mice were fed either a diet supplemented with the SQDG-rich cyanobacterium Spirulina (20%, SD) or a control diet. In addition SQ, TC, or water were orally applied to conventional or gnotobiotic IL-10-deficient mice. The gnotobiotic mice harbored a simplified human intestinal microbiota (SIHUMI) either with or without *B. wadsworthia*. During the intervention period, the body weight of the mice was monitored, the colon permeability was assessed and fecal samples were collected. After the three-week intervention, the animals were examined with regard to inflammatory parameters, microbiota composition and sulfonate concentrations in different intestinal sites.

None of the mice treated with the above-mentioned sulfonates showed weight loss or intestinal inflammation. Solely mice fed SD or gavaged with TC displayed a slight immune response. These mice also displayed an altered microbiota composition, which was not observed in mice gavaged with SQ. The abundance of *B. wadsworthia* was strongly reduced in mice fed SD, while that of mice treated with SQ or TC was in part slightly increased. The intestinal SQ-concentration was elevated in mice orally treated with SD or SQ, whereas neither TC nor taurine concentrations were consistently elevated in mice gavaged with TC. Additional colonization of SIHUMI mice with *B. wadsworthia* resulted in a mild inflammatory response, but only in mice treated with TC. In general, TC-mediated effects on the immune system and abundance of *B. wadsworthia* were not as strong as described in the literature.

In summary, neither the tested dietary sulfonates nor TC led to bacteria-induced intestinal inflammation in the IL-10-deficient mouse model, which was consistently observed in both conventional and gnotobiotic mice. For humans, this means that foods containing SQDG, such as spinach or Spirulina, do not increase the risk of intestinal inflammation.

Zusammenfassung

Die mikrobielle Lebensgemeinschaft im Darm des Menschen, die intestinale Mikrobiota, übt einen beträchtlichen Einfluss auf die Gesundheit des Wirts aus. Der Wirt wiederum beeinflusst die intestinale Mikrobiota durch seine Ernährung. Bei Mäusen wurde beobachtet, dass eine Ernährung reich an gesättigten Fettsäuren zu Darmentzündung führen kann, wenn die Tiere Interleukin (IL)-10-defizient sind, was sie empfänglich für Entzündungen macht. Durch die fettreiche Ernährung wurde vermehrt die sulfonierte Gallensäure Taurocholat (TC) sekretiert, welche wiederum das Wachstum des entzündungsfördernden Bakteriums *Bilophila wadsworthia* stimulierte.

Aufgrund dieser Beobachtung stellte sich die Frage, ob auch nahrungsrelevante Sulfonate bei IL-10-defizienten Mäusen zu einer bakteriell induzierten Darmentzündung führen können. Bei den in dieser Arbeit untersuchten Sulfonaten handelt es sich um die Sulfolipide Sulfoquinovosyldiacylglycerole (SQDG), welche in den meisten photosynthetischen Organismen wie Pflanzen, Moosen und Cyanobakterien vorkommen. Aus der Literatur ist bekannt, dass SQDG durch das kommensale Darmbakterium *Escherichia coli* zu Sulfoquinovose (SQ) und in weiteren Schritten zu 2,3-Dihydroxypropan-1-sulfonat (DHPS) und Dihydroxyacetonphosphat gespalten werden kann. DHPS kann von *B. wadsworthia* wiederum als Wachstumssubstrat verwendet und zu Sulfid reduziert werden. Sowohl für *B. wadsworthia* als auch für Sulfid wird angenommen, dass sie zur Entstehung von Darmentzündungen beitragen.

Um diese Hypothese zu untersuchen, wurden konventionelle IL-10-defiziente Mäuse für drei Wochen mit einem Futter gefüttert, welches das SQDG-reiche Cyanobakterium Spirulina (20%, SD) enthielt. Weiterhin wurde IL-10-defizienten Mäusen mit einer komplexen oder minimalen intestinalen Mikrobiota für drei Wochen SQ oder TC oral verabreicht. Die Tiere mit der minimalen Mikrobiota waren mit einer simplifizierten humanen intestinalen Mikrobiota (SIHUMI) mit oder ohne *B. wadsworthia* besiedelt. Während der Versuche wurden die Tiere gewogen, Fäzesproben wurden gesammelt und ein Darm-Permeabilitätstest wurde durchgeführt. Nach der dreiwöchigen Intervention wurden Entzündungsparameter, Mikrobiotazusammensetzung und Sulfonatkonzentrationen in den einzelnen Darmabschnitten der Mäuse untersucht.

Die Ergebnisse dieser Untersuchungen zeigten, dass keines der getesteten Sulfonate zu Gewichtsverlust oder Darmentzündung führte. Lediglich die Mäuse, die mit SD gefüttert oder denen TC appliziert wurde, zeigten Anzeichen einer schwachen Immunantwort. Auch wiesen diese Mäuse Veränderungen in der Zusammensetzung der Darmmikrobiota auf, was bei den mit SQ behandelten Mäusen nicht der Fall war. Die Zellzahl von *B. wadsworthia* war in SD-gefütterten Mäusen deutlich reduziert, während die Zellzahl dieses Bakteriums in den Mäuse, die mit SQ oder TC behandelt wurden, nur teilweise leicht erhöht war. Die SQ-Konzentrationen in den Inhalten einzelner Darmabschnitte waren bei den mit SD oder SQ behandelten Mäusen erhöht. Die Taurin- und TC-Konzentrationen glichen bei mit TC behandelten Mäusen überwiegend denen der Kontrolltiere. Die zusätzliche Besiedlung der SIHUMI-Mäuse mit *B. wadsworthia* führte nur in Tieren, denen TC appliziert wurde, zu leicht erhöhten Entzündungswerten. Allgemein übte die orale Applikation von TC weniger starke Effekte auf das Entzündungsgeschehen und die Mikrobiotazusammensetzung der Mäuse aus als in der Literatur beschrieben.

Die Ergebnisse dieser Studie legen nahe, dass weder das SQDG-reiche Futter noch die orale Applikation von SQ oder TC zu einer bakteriell induzierten Darmentzündung bei IL-10-defizienten Mäusen führt. Für den Menschen bedeutet dies, dass SQDG-haltige Lebensmittel wie Spinat oder Spirulina das Risiko für Darmentzündungen nicht erhöhen.

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16S rRNA	Ribosomal ribonucleic acid of the small subunit of procaryotic ribosomes
18S rRNA	Ribosomal ribonucleic acid of the smal subunit of eukaryotic ribosomes
28S rRNA	Ribosomal ribonucleic acid of the large subunit of eukaryotic ribosomes
A. caccae	*Anaerostipes caccae*
A. platensis	*Arthrospira platensis*
AMP	Anti-microbial peptides/ adenosine-5'-monophosphate
Apr	APS reductase
APS	Adenosine-5'-phosphosulfate
ATP	Adenosine-5'-triphosphate
ATPS	ATP sulfurlyase
B. longum	*Bifidobacterium longum*
B. product	*Blautia producta*
B. thetaiotaomicron	*Bacteroides thetaiotaomicron*
B. wadsworthia/ Bw	*Bilophila wadsworthia*
BEH	Bridged ethyl hybrid
BLAST	Basic Local Alignment Search Tool
BSH	Bile salt hydrolase
C. butyricum	*Clostridium butyricum*
C. ramosum	*Clostridium ramosum*
CA	Cholic acid
CD	Control diet
CDCA	Chenodeoxycholic acid
cDNA	Complementary DNA

Cfu	Colony forming units
CT	Cycle threshold
d_4-CDCA	Deuterium labeled CDCA
d_4-LCA	Deuterium labeled LCA
DCA	Deoxycholic acid
DEPC	Diethyl dicarbonate
DHPS	2,3-dihydroxypropane-1-sulfonate
DNA	Deoxyribonucleic acid
DNS	3,5-dinitrosalicylic acid
dNTP	Deoxynucleotide triphosphate
DS medium	Medium adapted from da Silva et al.
Dsr	Dissimilatory sulphite reductase
DSS	Dextran sodium sulfate
E. coli	*Escherichia coli*
ED	Entner-Doudoroff
EDTA	Ethylenediaminetetraacetic acid disodium salt dehydrate
ELISA	Enzyme-linked immunosorbent assay
EMP	Embden-Meyerhof-Parnas
F	Fecal suspension
Fwd	Forward
FITC	Fluorescein isothiocyanate
FXR	Farnesoid X receptor
g	Acceleration of gravity
GCA	Glycocholic acid
GCDC	Glycochenodeoxycholic acid
GDCA	Glycodeoxycholic acid
GWAS	Genome-wide association studies
H & E	Haematoxylin and eosin

H. hepaticus	*Helicobacter hepaticus*
H_2S	Hydrogen sulphide
HIF-1α (*Hif-1α*)	Hypoxia-inducible factor 1 alpha (gene)
HpfD	3-sulfopropionaldehyde reductase
HpfG	DHPS-dehydratase
HPLC	High pressure liquid chromatography
HPRT (*Hprt*)	Hypoxanthine guanine phosphoribosyl transferase (gene)
HspG	DHPS-sulfolyase
IBD	Inflammatory bowel diseases
IFN-ɣ (*Ifn-ɣ*)	Interferone gamma (gene)
IL (*Il*)	Interleukin (gene)
IL-$10^{-/-}$	IL-10 deficient
IMNGS	Integrated microbial NGS
iNOS	Inducible nitric oxide synthase
ISTD	Internal standard
KDSG	2-keto-3,6-dideoxy-6-sulfogluconate
L. plantarum	*Lactobacillus plantarum*
LADA	Latent autoimmune diabetes in adults
LC	Liquid chromatography
LCA	Lithocholic acid
LCN	Lipocalin
M	Mutant
MCA	Muricholic acid
mLN	Mesenteric lymph nodes
MOPS	3-(N-morpholino)propanesulfonic acid
MRM	Multiple reaction monitoring
MS	Mass spectrometry
mWAT	Mesenteric white adipose tissue

NADH/NAD+	Nicotinamide adenine dinucleotide
n. d.	Not detected
NGS	Next generation sequencing
NMDS	Non-metric multi-dimensional scaling
NOD	Nucleotide-binding oligomerization domain-containing protein
NTA	Nitrile triacetate
OCLN (*Ocln*)	Occludin (gene)
OD	Optical density
Oligo-MM	Oligo-Mouse-Microbiota
PBS	Phosphate-buffered saline
PMN	Polymorph nuclear cells
PRR	Pattern recognition receptor
Q1 – Q3	First quartile (25^{th} percentile) – third quartile (75^{th} percentile)
(q)PCR	(Quantitative) Polymerase chain reaction
Rev	Reverse
$R_{1,2}$	Fatty acids 1 and 2
RNA	Ribonucleic acid
RT	Room temperature
σ	Standard deviation
S. enterica	*Salmonella enterica*
SAA	Serum amyloid A
Sat	Sulfate adenyltransferase
SCFA	Short chain fatty acids
SD	Spirulina diet
SG	6-deoxy-6-sulfogluconate
SGL	6-deoxy-6-sulfogluconolactone
SIHUMI	Simplified human intestinal microbiota
SL	3-sulfolactate

SLA	3-sulfolactaldehyde
Sp.	Species
SPF	Specific pathogen-free
SQ	Sulfoquinovose
SQDG	Sulfoquinovosyl diacylglycerols
SQR (*Sqr*)	Sulfide:quinone oxidoreductase (gene)
SRB	Sulfate-reducing bacteria
STAT	Signal transducer and activator of transcription
TAE	TRIS-acetate EDTA
TC	Taurocholate
TCA	Taurocholic acid
TCDCA	Taurochenodeoxycholic acid
TDCA	Taurodeoxycholic acid
TGR5	G-protein coupled bile acid receptor
Th1	T helper cell 1
TJP (*Tjp*)	Tight junction protein (gene)
TLC	Thin layer chromatography
TNBS	2,4,6-trinitrobenzene sulfonic acid
TNF-α (*Tnf-α*)	Tumor necrosis factor alpha (gene)
TNFRSF	TNF receptor superfamily member
TRIS	Tris(hydroxymethyl)aminomethane
UDCA	Ursodeoxycholic acid
UDP	Uridin-5-phospho
UFZ	Helmholz Centre of Environmental Research
v/v	Volume by volume
w/v	Weight by volume
WT	Wild type
YCFA	Yeast extract-casitone-fatty acid

YH-BHI	Yeast Hemin Brain-Heart-Infusion Broth
YihS	SQ isomerase
YihQ	Sulfoquinovosidase
ZnAc	Zinc acetate

1 Introduction

The microbial community on and in a host is referred to as microbiota. Interactions between host and microbiota are manifold and may have a significant impact on host health. Examples for such interactions will be given in chapter 1.1. One of the most important factors influencing microbiota composition is the diet consumed by the host [1]. A diet rich in saturated milk fat was shown to increase the proportion of the sulfonated bile acid taurocholate (TC), thereby stimulating the growth of the sulfite-reducing bacterium *Bilophila wadsworthia* and causing intestinal inflammation [2]. To investigate whether dietary sulfonates could have the same effect, the bacterial metabolism of diet-derived sulfonates was studied, which will be described in chapter 1.2. Under certain circumstances, acute intestinal inflammation develops into chronic inflammation and subsequently into diseases introduced in chapter 1.3. Investigation of such mechanisms is often challenging because of the complexity of microbe-microbe and microbe-host interactions. Therefore, animal models have been established, allowing to study certain aspects of disease development (see chapter 1.4).

1.1 Interplay between the host, microorganisms and environmental factors

The microbiota largely impacts host physiology, while being affected by the host at the same time. This interplay is a result of the co-evolution of both partners over decades of years [3]. As an example, the intestinal microbiota affects the maturation the immune system of its host, degrades substances that are indigestible for the host, builds up a defense against invading pathogens (colonization resistance) and affects the well-being of the host in a beneficial or detrimental way. Food and drugs ingested by the host impact the composition and activity of the intestinal microbiota [4]. To prevent bacterial translocation from the gut through the epithelial cell layer and into circulation and potentially body tissues, specific defense mechanisms have evolved. These mechanisms include a physical barrier lining the epithelial surface, namely the mucus, a cellular immune response against bacterial antigens and the secretion of immunoglobulins. While the mucus in the small intestine is of low complexity, which allows nutrients to penetrate and get absorbed, the mucus in the colon consists of multiple layers with distinct functions. The outer mucus layer has a relatively lose structure, is colonized by bacteria and serves as a nutrient source for commensals. The inner mucus layer has a dense structure, which is resistant to bacterial penetration. Adjacent to the inner mucus layer is the epithelial layer consisting of enterocytes, mucus-producing goblet cells and Paneth cells, which produce anti-microbial peptides. These peptides represent an additional defense mechanism to prevent the bacteria from entering the inner mucus layer [5]. Invading pathogens are recognized by pattern recognition receptors (PRR) based on their pathogen-associated molecular patterns and elicit a cascade of innate immune reactions including recruitment of macrophages, neutrophils and dendritic cells as well as secretion of cytokines. The family of PRR includes the toll-like receptors and nucleotide-binding oligomerization domain (NOD)-like receptors (NOD1 and NOD2), which recognize different kinds of pathogen-associated molecular patterns [6]. Thus, PRR are able to discriminate between harmless and harmful bacteria and prevent an unnecessary immune response towards commensal bacteria, which could cause inflammation and potentially damage

intestinal tissue. The tolerance of the immune system towards commensal bacteria on the one hand and the recognition and destruction of pathogenic bacteria on the other hand is a crucial mechanism to maintain intestinal homeostasis and health [7].

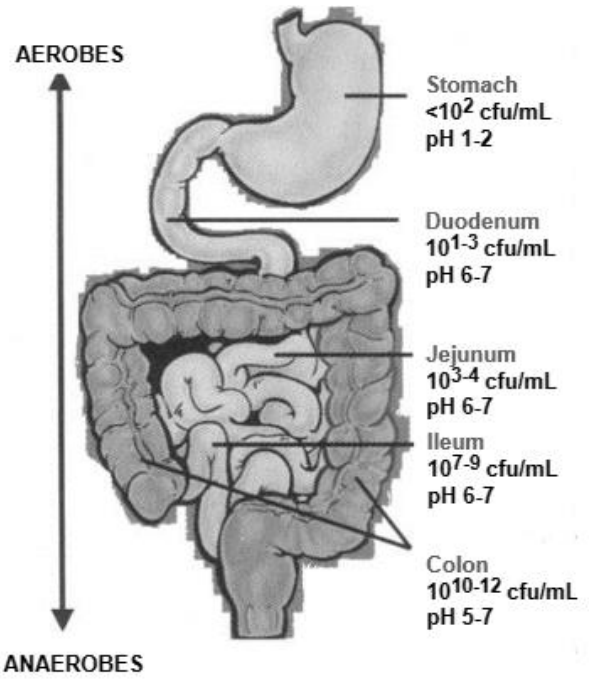


Figure 1: Overview of the gastrointestinal tract with colony forming units (cfu) of bacteria per ml, pH range and distribution of aerobe and anaerobe bacteria (modified from [8]).

One of the reasons for the varying complexity of microbe defense mechanisms alongside the gastrointestinal tract is the difference in the types and density of bacteria in different sites of the intestine. While the low pH in the stomach allows only few acid-resistant bacteria to proliferate, the abundance and complexity of bacterial communities increase together with the pH alongside the intestinal tract. Apart from the pH, further environmental parameters impact the bacterial growth and composition, such as the availability of oxygen (Fig. 1) and nutrients. Highest bacterial cell numbers are reached in the colon, where up to 10^{12} colony forming units per ml of primarily anaerobic bacteria are present [8]. Dominant phyla of the human intestinal microbiota are Bacteroidetes and Firmicutes, followed by Actinobacteria and Proteobacteria, which in sum make up to 98% of total bacteria per individual [9]. The total number of bacteria colonizing a human is approximately 3.8 x 10^{13}, which equals the estimated number of human cells and underlines the numerical significance of the bacteria [10]. In addition to bacteria but in lower abundance, fungi, protozoa, viruses and archaea colonize the gut, which further may impact the bacterial community and host health status [11, 12].

Colonization and subsequent shaping of the intestinal microbial community starts with the day of birth and continues until the end of life, while the first year of life has the strongest impact. Factors such as mode of birth (vaginal or caesarian) and breast or formula feeding can influence the microbiota composition and host health sustainably [13]. Later in life, the core microbial community is relatively stable, but diet and drug intake still have the potential to impact the microbiota. It has been shown that the human microbiota composition and metabolic capacity change within few days, in response to dietary interventions [1].

An example for diet-induced changes in the gut microbiota is the elevated cell number of carbohydrate-fermenting bacteria and higher concentrations of the health-promoting short chain fatty acids (SCFA) acetate and butyrate in the gut of individuals consuming a high-fiber diet as compared to individuals on a low-fiber diet. In contrast to that, an animal-based high-fat diet promotes the growth of bile-resistant bacteria and yields higher concentrations of products from protein degradation by bacteria [1]. An increase of bile-resistant bacteria was also observed, when a high-fat diet based on saturated milk fat was fed to colitis-prone interleukine-10-deficient (IL-$10^{-/-}$) specific pathogen-free (SPF) mice [2]. This diet induced intestinal inflammation, which was linked to a higher proportion of the taurine-conjugated hepatic bile acid TC secreted into the intestinal lumen, which in turn led to elevated cell numbers of *Bilophila wadsworthia*. This bacterium also proliferated,

when TC was orally applied to IL-10$^{-/-}$ mice fed a low-fat diet, which was accompanied by an increased incidence and severity of colitis [2].

1.1.1 *Bilophila wadsworthia*, a potentially pathogenic bacterium

B. wadsworthia was first described in 1989 by Baron et al., who isolated this bacterium from appendicitis specimens and feces from healthy humans [14]. Its presence in healthy and diseased individuals suggests that it is a pathobiont-type of bacterium, meaning that it causes inflammation under certain genetic and environmental conditions only [15]. As implied by its name (bilo-phila = bile-loving), *B. wadsworthia* grows well in media supplemented with bile (20%) and is equipped with genes necessary for taurine degradation [16]. This bacterium is obligatory anaerobic, Gram-negative, non-motile, non-spore forming, strongly catalase-positive, and reduces sulfite to sulfide [14]. *B. wadsworthia* is the third most common anaerobe recovered from gangrenous and perforated appendicitis and was further found in scrotal and hepatic abscess, mandibular osteomyelitis, axillary hidradenitis suppurativa, blood, pleural and joint fluid, saliva and vaginal samples [17, 18]. In children, *B. wadsworthia* was documented to be the second most often isolated anaerobic bacterium from appendicitis specimens [19]. However, it is unclear whether inflammation is caused by *B. wadsworthia* or whether the chemical or microbial milieu associated with inflammation promotes the expansion of *B. wadsworthia*. Pathologic traits of *B. wadsworthia* include cytotoxicity towards cell lines, adhesion to intestinal cell lines, induction of gelation of *Limulus* lysates in a dose-dependent manner and a pro-coagulant activity [17, 20].

The ability of *B. wadsworthia* to cause systemic inflammation even in SPF wild-type mice has been reported previously [21]. The authors described a reduction in body weight and feed intake and higher plasma cytokine concentrations in mice daily gavaged with a *B. wadsworthia* isolate derived from a patient with latent autoimmune diabetes in adults (LADA). Another research group conducted *in vitro* and *in vivo* studies and demonstrated that *B. wadsworthia* induces a T helper cell 1 (Th1)-type immune response, which led to colitis in genetically susceptible IL-10$^{-/-}$ mice [2]. Furthermore, *B. wadsworthia* has been described to worsen high-fat diet-induced metabolic dysfunctions including those of the glucose and bile acid metabolism in conventional and gnotobiotic mice, which was shown to be independent of its pro-inflammatory capacity [22].

The mechanism behind these effects has not been identified so far. One possible explanation for *B. wadsworthia*'s cytotoxicity is the production of hydrogen sulfide from sulfonated substrates. The role of hydrogen sulfide has been discussed controversially in the last decade, ranging from health-promoting to toxic effects (see chapter 1.2.2). Another possible reason for the colitogenic effect of *B. wadsworthia* is the production of secondary bile acids, which have been associated with inflammation and colon cancer [2, 23].

1.1.2 Bile acids and their impact on the composition of bacterial communities

One group of substances that are readily influenced by the diet and have the potential to alter the intestinal microbiota composition are the bile acids. The main function of these amphiphilic

molecules is to increase the absorbability of fat and fat-soluble vitamins by increasing their solubility. Primary bile acids are synthesized from cholesterol in the liver, stored in the gall bladder and upon fat-containing food-intake secreted into the upper small intestine. Primary bile acids commonly found in humans and rodents are cholic acid (CA), chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA), while α-muricholic acid (MCA) and β-MCA are solely found in rodents [24]. All bile acids may also be present in conjugated forms, bound to glycine or taurine. Upon secretion into the small intestine, primary bile acids are deconjugated and secondary bile acids are formed via 7α/β-dehydroxylation by bacterial enzymes. Bacterial dehydroxylation of CA, CDCA and MCAs results in deoxycholic acid (DCA), lithocholic acid (LCA) and ω-MCA, respectively. Secondary bile acids are more hydrophobic, which facilitates their passive absorption in the colon but is also associated with higher toxicity and potential carcinogenic traits [25]. In the enterohepatic cycle, 95% of bile acids are actively transported from the lower small intestine to the liver. The 5% escaping the enterohepatic cycle (about 400 – 800 mg) enter the colon, where they are further deconjugated and converted into secondary bile acids by bacteria [26, 27]. The homeostasis of bile acids is regulated by receptors present in the intestine, liver and other organs. Two major receptors regulating the expression of genes responsible for bile acid synthesis, metabolism and transport are the intracellular farnesoid X receptor (FXR) and the nuclear G-protein coupled bile acid receptor TGR5. This tight regulation is crucial to avoid accumulation of bile acids, which would have detrimental effects due to their detergent characteristics. Contrarily, if the concentration of bile acids becomes too low, bacterial overgrowth would be mediated and inflammation initiated [28]. In addition to the maintenance of bile acid homeostasis, the mentioned receptors are further involved in the regulation of the metabolism of fatty acids, lipoproteins, glucose, energy as well as of cell proliferation and inflammatory processes [24].

1.2 Bacterial sulfonate metabolism

The starting point for the present work was the finding that *B. wadsworthia* is colitogenic upon stimulation of its growth by the endogenous sulfonated bile acid TC [2]. Based on this link, the question arose, whether exogenous dietary sulfonates may also stimulate the growth of *B. wadsworthia* and cause intestinal inflammation in a genetically susceptible host.

1.2.1 Metabolism of sulfonates by environmental bacteria

The exogenous sulfonates studied in this project are the sulfolipids sulfoquinovosyl diacylglycerols (SQDG, structure in Fig. 2). They can be found in most photosynthetic organisms such as green plants, mosses, algae and cyanobacteria [29]. In phototrophic plants, SQDG are present in the thylakoid membrane of chloroplasts, where they enhance the stability of the membrane and play a role in photosynthetic processes, especially under phosphate starvation conditions [29, 30]. SQDG are an integral part of the human diet, as they can be found in spinach, lettuce and other leafy green vegetables. The cyanobacterium *Arthrospira platensis*, commercially known as Spirulina, is especially rich in SQDG compared to other sources, and is promoted as a food supplement. Extensive research

indicates beneficial effects of Spirulina's functional ingredients such as phycocyanin and spirulan [31, 32], but nothing is known about potentially detrimental effects of Spirulina's sulfonated lipids.

The polar head group of SQDG, sulfoquinovose (6-deoxy-6-sulfoglucose, SQ, structure in Fig. 2) represents a glucose derivative with a sulfite moiety at the C6 position of the hexose, which is one of the most abundant organic sulfonates in the biosphere [33]. The liberation of SQ from SQDG is catalyzed by the sulfoquinovosidase YihQ found in *Escherichia coli* and other Gammaproteobacteria [34]. Two pathways have been identified in aerobic bacteria to degrade SQ either to 2,3-dihydroxypropane-1-sulfonate (DHPS) and dihydroxyacetone phosphate through the sulfo-Embden-Meyerhof-Parnas (EMP) way or to 3-sulfolactate (SL) and pyruvate via 6-deoxy-6-sulfogluconate through the sulfo-Entner-Doudoroff (ED) way (Fig. 2). A third pathway employing a novel 6-deoxy-6-sulfofructose transaldolase has been described recently in *Bacillus* strains resulting in the formation of SL and fructose-6-phosphate from SQ [35, 36]. Alternatively, strictly anaerobic clostridia produce DHPS using the transaldolase pathway [35]. This so-called sulfoglycolysis is followed by biomineralization of the sulfite moiety to sulfate, which is then utilized for biosynthesis of SQDG by photosynthetic organisms [29]. The C-S bond between the sulfite moiety and the hexose is stable and only cleavable by specific bacterial enzymes. The degradation and biosynthesis of SQDG hugely contributes to the biogeochemical sulfur cycle with an estimated synthesis of sulfolipids by plants of approximately 10^{13} kg per year [33]. Breakdown of SQ and formation of sulfate under oxic conditions was observed in *Agrobacterium* sp. strain ABR2 and *Klebsiella* sp. strain ABR11; the latter also produced small amounts of SL [37]. Another organism able to degrade SQ to SL via the sulfo-ED pathway under oxic conditions is the environmental isolate *Pseudomonas putida* SQ1. The genes involved in this degradation include an NAD^+-dependent dehydrogenase converting SQ to 6-deoxy-6-sulfogluconolactone (SGL), a lactonase forming 6-deoxy-6-sulfogluconate (SG) from SGL, a dehydratase degrading SG to 2-keto-3,6-dideoxy-6-sulfogluconate (KDSG), an aldolase producing pyruvate and 3-sulfolactaldehyde (SLA) from KDSG and a dehydrogenase for the final conversion of SLA to SL. Homologues of this gene cluster were shown to be distributed among other α-, β- and ɣ-Proteobacteria [38]. Enzymes for the conversion of SQ to DHPS under oxic conditions were identified in *E. coli* K-12 and other *Enterobacteriaceae* [39]. The resulting DHPS was shown to be converted to sulfate by *Cupriavidus pinatubonensis* JMP134 [39]. An anoxic co-culture model in which SQ is converted to DHPS and than further to sulfite was reported for *E. coli* K-12 and *Desulfovibrio* sp. strain DF1, the latter being isolated from anaerobic sewage sludge [40]. Both publications reporting SQ degradation by *E. coli* state a much slower growth rate with SQ as sole carbon and energy source than with glucose [39, 40]. Recently,

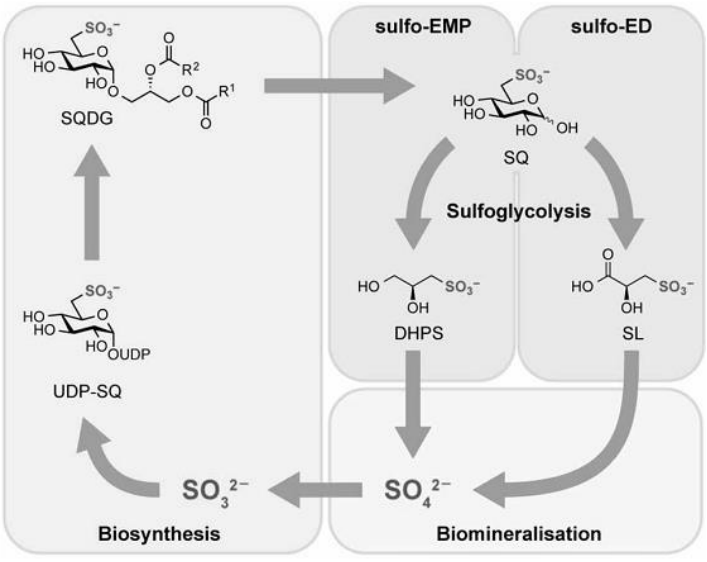


Figure 2: Cycle of degradation and biosynthesis of the sulfolipids sulfoquinovosyl diacylglycerols (SQDG) under oxic conditions via sulfoglycolysis and biomineralization. EMP: Embden-Meyerhof-Parnas, ED: Entner-Doudoroff, SQ: sulfoquinovose, DHPS: 2,3-dihydroxypropane-1-sulfonate, SL: sulfolactate, UDP: uridin-5-phospho, R_1 and R_2: fatty acids (modified from [28]).

the sulfolyase HspG involved in the degradation of DHPS to hydroxyacetone and sulfide was characterized in *B. wadsworthia* and other sulfate- and sulfite-reducing bacteria [41]. The authors further described an oxygen-sensitive dehydratase (HpfG) converting DHPS to 3-sulfopropionaldehyde and a reductase (HpfD) for the subsequent formation of 3-hydroxypropane-1-sulfonate in fermenting bacteria. These enzymes were found in various environmental bacteria and in those present in the human gut (e. g., *Faecalibacterium prausnitzii, Hungatella hathewayi*), which suggests a possible role of these pathways in the fermentation of the human diet [41].

1.2.2 Metabolism of dietary sulfonates by intestinal bacteria

Although much is known about the distribution and degradation of SQ by aerobic bacteria in the environment, its fate in the human or animal gastrointestinal tract and its effects therein have hardly been studied. There is only one study, in which radioactively labeled [^{35}S]SQDG was orally applied to guinea pigs, which were killed after one, two, three or four hours [42]. Small intestine, blood and liver were screened for radioactivity and the degradation products were identified. Only 1 – 5% of intact SQDG was present in the intestinal mucosa, the remaining 95 – 99% of SQDG was converted into water-soluble metabolites, namely sulfoquinovosyl glycerol (SQG, 60%) and sulfate (40%). Blood and liver only contained sulfate with the highest concentrations found in blood. The authors concluded, that SQDG is almost completely metabolized in the small intestine and that the cleavage of the C-S bond is catalyzed by enzymes in the intestine [42].

The sulfur metabolism of intestinal bacteria has primarily been studied in the context of sulfur-containing amino acids. Especially a high-protein diet (e.g., rich in meat) is considered to be a major source of sulfur-containing amino acids such as methionine and cysteine [43]. The latter are essential and conditionally essential, respectively, to the human body and, thus, need to be taken up with the diet. Cysteine is considered conditionally essential because it can be generated from methionine by transsulfuration [44]. Several bacterial taxa contribute to the sulfur metabolism in the intestinal tract, among them the group of sulfate-reducing bacteria (SRB), which include *Desulfovibrio* spp. [45]. SRB belong to the limited number of bacterial taxa able to perform a dissimilatory sulfate reduction with the aim to conserve energy. A more widely distributed pathway for sulfur metabolism is the assimilatory sulfate reduction to fulfill the bacterial cell's need for sulfur [43].

Another dietary amino acid is taurine, which contains a sulfite moiety in addition to an amino group (2-aminoethane-1-sulfonic acid). Taurine has been attributed with a number of health-promoting properties, among them the reduction of blood pressure and cytoprotective activity [46, 47]. Enzymes for the degradation of taurine have been found in both aerobic and anaerobic bacteria; the degradation under oxic conditions seems to be more effective [48]. In the human gut, bacterial taurine degradation involves its conversion by a taurine:pyruvate aminotransferase to sulfoacetaldehyde, which subsequently undergoes reduction to isethionate, from which sulfite is liberated by a sulfo-lyase. The gene cluster for taurine degradation is widely distributed among sulfate- and sulfite-reducing bacteria, including *B. wadsworthia* [16, 49].

SRB are a phylogenetically diverse group of bacteria which have in common the ability to utilize sulfate as terminal electron acceptor through the dissimilatory sulfate reduction pathway which includes the reduction of sulfate (SO_4^{2-}) to sulfite (SO_3^{2-}) and then further on to (hydrogen) sulfide, as depicted in figure 3 [50]. Therefore, SRB are commonly detected when fecal or intestinal DNA samples were screened for the genes encoding sulfate- and sulfite-reducing enzymes [51, 52]. The first reduction from sulfate to sulfite can be further subdivided into two steps, which are the energy (i. e. adenosine triphosphate, ATP)-dependent activation of sulfate and the subsequent reduction to sulfite. The activation of sulfate is necessary, because sulfate is the energetically favorable state of the molecule as it is in the most oxidized level. The activating reaction is catalyzed by the ATP-sulfurlyase and adenosine-5'-phosphosulfate (APS) is formed. The reduction step is catalyzed by the

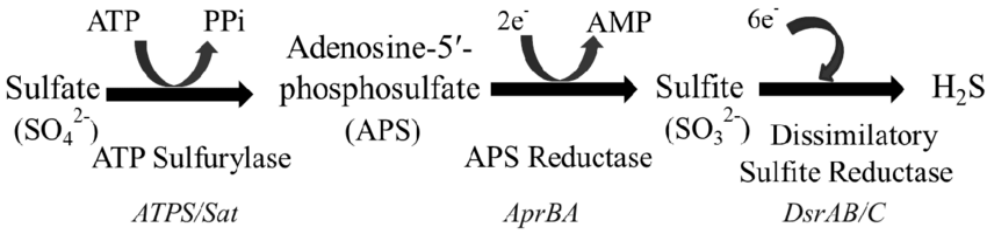


Figure 3: Dissimilatory sulfate reduction by sulfate-reducing bacteria (SRB). ATPS: ATP sulfurlyase, Sat: sulfate adenyltransferase, Apr: APS reductase, Dsr: dissimilatory sulfite reductase. Modified from [50].

APS reductase and sulfite as well as adenosine-5'-monophosphate (AMP) is released. The subsequent conversion of sulfite to sulfide is catalyzed by the dissimilatory sulfite reductase complex DsrAB and its co-substrate DsrC [51, 53]. The energy-dependent activation of sulfate is circumvented by sulfite-reducing bacteria such as *B. wadsworthia*. These bacteria gain energy from the reduction of sulfite to sulfide and, thus, have a growth advantage over SRB utilizing sulfate.

The presence of SRB and formation of sulfide has been associated with inflammatory bowel diseases (IBD) and colorectal cancer [43]. For example, release of sulfide from feces of patients suffering from ulcerative colitis was found to be three to four times higher than that of healthy individuals [54]. A number of studies also report a higher abundance of SRB in ulcerative colitis patients compared to healthy controls [55]. Furthermore, it was shown that enzymes involved in the detoxification of sulfide were lacking at the tumor side in colorectal cancer patients [56]. As a consequence of insufficient sulfide detoxification, sulfide could accumulate and lead to an impairment of butyrate oxidation in colonic epithelial cells [57, 58]. As a consequence, the epithelial cells would starve and die, resulting in intestinal inflammation [56]. However, in the healthy gut a highly effective mechanism enables the detoxification of sulfide by neutralizing increased sulfide concentrations, evoked for instance by feeding rats a high-protein diet [59]. This detoxification is catalyzed by a number of enzymes, including the mitochondrial flavoprotein sulfide:quinone oxidoreductase (SQR), which is a key enzyme. Elevated sulfide concentrations in the rat cecum (lower µM) were associated with an increased *Sqr* expression in colonocytes [59]. *In vitro* studies showed that the detoxification of sulfide was accompanied by a higher relative oxygen consumption of colonocytes and an increase in the production of ATP (Fig. 4A). However, when the sulfide concentration exceeds a certain threshold (middle µM to mM), the respiratory chain in mitochondria of colonocytes is inhibited and the cells enter a hypoxic state (Fig. 4B) [60]. This was assessed by quantifying the relative gene expression of the hypoxia-inducible factor-(*Hif*)-*1α* in colonocytes stimulated with sodium sulfide. The expression of which was higher than that of untreated colonoytes. This sulfide-induced hypoxia was accompanied by a reduced energy (ATP) generation and induction of inflammation-associated genes such as interleukin (*Il)-6* and inducible nitric oxide synthase (*iNos*) [59, 60].

Furthermore, hydrogen sulfide is suspected to disrupt the integrity of the inner mucus layer by reducing the disulfide bonds to thiols and trisulfides, thereby exposing the epithelial layer to bacteria and initiating intestinal inflammation [61].

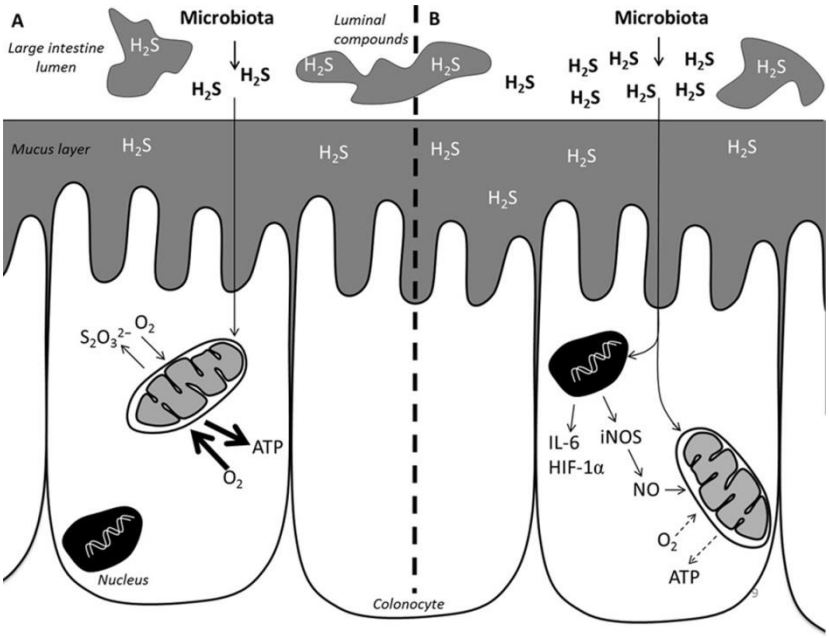


Figure 4: Schematic overview of a low and high luminal hydrogen sulfide (H_2S) concentration and consequences for colonocytes. While low H_2S concentrations support respiratory processes in mitochondria, excessive H_2S leads to hypoxia and inflammation. ATP: adenosine-5'-triphosphate, IL-6: interleukine-6, HIF-1α: hypoxia-inducible factor-1 alpha, iNOS: inducible nitric oxide synthase [58].

1.3 Chronic inflammatory bowel diseases

Approximately 2.2 million people in Europe are affected by IBD [62]. In developed countries, the incidence of IBD is high and relatively stable, whereas in developing countries the former low-incidence is rising constantly, which suggests a possible link between industrialization and disease development [63, 64]. IBD are multifactorial diseases (Fig. 5) with a largely unknown etiology. The group of IBD includes Crohn's disease and ulcerative colitis, which are both characterized by alterations of active and inactive disease episodes. Characteristics of Crohn's disease include the distribution of inflammatory sites along the entire gastrointestinal tract and a transmural penetration. Ulcerative colitis only affects the colon and rectum with a superficial inflammation. IBD patients suffer from persistent diarrhea, abdominal pain, rectal bleeding, fatigue and weight loss, among others [65]. Disease development is believed to be in part mediated by genetic defects of the host, which allow commensal bacteria to penetrate the epithelial barrier and evoke an uncontrolled immune response. An impairment in the regulation of the host immune system leads to chronic inflammation and concomitant tissue damage [66]. Bacteria-induced intestinal inflammation can be mediated by bacterial toxins, (surface) proteins or metabolic products, which harm the intestinal wall and stimulate the immune system. Furthermore, a reduction in health-promoting bacteria accompanied by an increased abundance of harmful bacteria might be another cause of disease progression and exacerbation. As an example, a smaller proportion of butyrate-producing bacteria and higher numbers of adherent invasive *E. coli* strains has been documented in IBD patients [67]. These bacterial changes may further be promoted by antibiotic intake or consumption of an unhealthy diet.

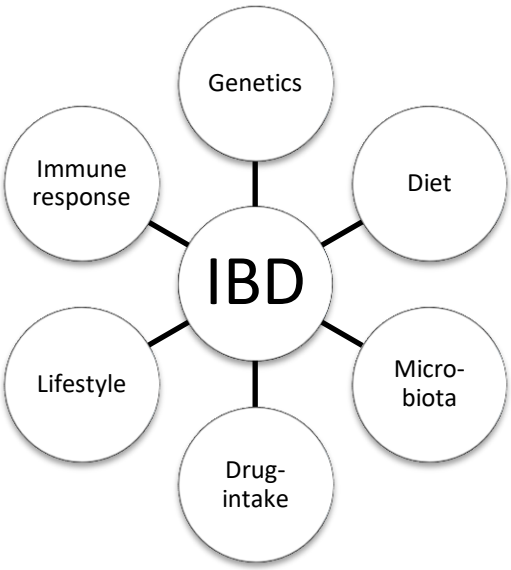


Figure 5: Factors contributing to the pathogenesis of inflammatory bowel diseases (IBD).

To identify genes that are of relevance for IBD initiation and progression, genome-wide association studies (GWAS) have been performed [68]. Among the identified gene variants found in Crohn's disease and ulcerative colitis patients are those coding for IL-23 and IL-10 receptors, the IL-12 subunit IL-12p40 and signal transducer and activator of transcription (STAT) 3, all of which important components of the immune system. Additionally, alterations of disease-specific loci were found, such as the Crohn's disease-specific NOD 2 or the ulcerative colitis-specific tumor necrosis factor receptor superfamily member (TNFRSF) 14 [69]. However, these loci can only explain a small part of the disease's hereditary [70, 71], which further supports the theory of a multifactorial etiology.

1.4 Animal models

The use of animals to investigate scientific research questions is a matter of controversial discussion. On the one hand, ethical reasons argue against animal experiments but on the other hand, they are sometimes necessary to gain new insights and develop treatments for diseases. One of the most commonly used laboratory animals is the mouse. Advantages of the use of mice include a short reproduction time, the opportunity to introduce genetic modifications and to define their gut microbial status. Examples for both will be given in the next chapters.

1.4.1 Animal models of intestinal inflammation

Owing to the unknown etiology of IBD, research on the genetic, microbial and environmental factors contributing to disease initiation and progression continues. As this is a complex field with many contributing factors, cell-culture experiments are often insufficient to answer important research questions. Therefore, animal models mimicking specific aspects of disease have been developed. Methods for the induction of IBD include chemical treatment, colonization with colitogenic bacteria and incorporation of genetic variations, or a combination of two or more procedures. Inflammation-inducing chemicals include dextran sodium sulfate (DSS), which disrupts the epithelial barrier in the gut, and 2,4,6-trinitrobenzene sulfonic acid (TNBS), which is a haptenating agent rendering colonic proteins immunogenic to their host and, thus, initiating mucosal immune responses [72, 73]. Colitis-inducing bacteria such as *Helicobacter hepaticus* and *Salmonella enterica* require special conditions to induce IBD-like symptoms. *H. hepaticus* leads to typhlocolitis only in the absence of the anti-inflammatory cytokine IL-10 [74] and *S. enterica* infection requires pre-treatment with antibiotics to reduce the colonization resistance provided by commensal intestinal bacteria [75]. Genetic variations of susceptibility genes are broadly studied owing to the multitude of gene variants associated with IBD detected by GWAS studies. These variants can be introduced into an animal and consequences for the host can be studied. The most prominent model is the IL-$10^{-/-}$ mouse, which develops chronic enterocolitis under conventional conditions and local colonic inflammation in an SPF environment [76]. The lack of the anti-inflammatory cytokine IL-10 results in uncontrolled immune responses towards commensal bacteria in the gut, including recruitment of CD 4^{+} Th 1 and Th 17 cells as well as secretion of interferon gamma (IFN-ɣ), IL-17, IL-12 and IL-6 [77]. Bacteria were demonstrated to play a crucial role in disease initiation, as germ-free IL-$10^{-/-}$ do not develop colitis [78]. As mentioned above, the susceptibility towards inflammation depends on the genetic background and environmental factors [79]. For example, inflammation in C57BL/6 J IL-$10^{-/-}$ mice is less severe than in C3H/HeJBir IL-$10^{-/-}$ mice, and mice housed in a German animal facility were reported to have lower cecal histopathology scores than those of the same strain housed in an animal facility located in the USA [79].

1.4.2 Gnotobiotic animal models

The field of gnotobiology (from the Greek gnotos = known, bios = life) investigates the impact of a defined bacterial community on specific aspects of microbiota-host and microbe-microbe interactions. Gnotobiology also includes investigation of germ-free animals, for example to decipher microbiota-associated characteristics. According to these studies, germ-free animals have a thinner intestinal wall with a lower cell turnover, a larger cecum, a high tryptic activity in feces, fewer contractions of intestinal smooth muscles and a reduced sensitivity towards biogenic amines. Furthermore, there is no transformation of primary into secondary bile acids or degradation of mucus in germ-free mice and the production of SCFA is reduced in quantity and variety [80]. These are only some of the processes affected by the microbiota, which underlines the important role of microorganisms in the gut.

To decipher the effects of individual bacteria, germ-free animals are mono-associated with a single bacterial strain followed by studying the consequences for the host. However, mono-colonization does not take into account the interactions between bacteria and competition for nutrients, making the transferability of some of the experimental results to the human situation difficult [81]. To get a better understanding of specific bacteria and avoid the complexity of a conventional microbial community, minimal consortia have been established. The first characterized minimal consortium, which is still taken advantage of nowadays, is the so-called Altered Schaedler Flora [82], which consists of eight bacterial species isolated from the intestine of mice and brought into germ-free mice to get a standardized model that builds up a defense against invading pathogens. However, the immune system of Altered Schaedler Flora-colonized mice is only partly maturated and mice develop only some of the microbiota-associated characteristics [83, 84]. Therefore, another minimal consortium named simplified human intestinal microbiota (SIHUMI) was established comprising eight bacterial species that are prominent members of the human gut [83]. In the original publication, the eight-member-consortium was named ‘SIHUMIx’ to separate it from the first established seven-member consortium ‘SIHUMI’ (without *Clostridium butyricum*), but in the following, ‘SIHUMI’ will stand for the eight-member consortium. The eight bacterial species were selected because of their prevalence in the adult human gut, their fermentative abilities and because rodents harboring the consortium showed most of the microbiota-associated characteristics. The SIHUMI consortium is stably transferred from one generation of mice to the next and its composition remains stable over time. The consortium was established in rats as well as in mice and represents a useful tool to investigate the effects of dietary interventions on bacterial composition, production of metabolites and consequences for the host [83, 85]. As an example, SIHUMI mice fed with a fiber-free diet had lower total bacterial cell numbers and lower SCFA concentrations in cecum and colon than SIHUMI mice fed with a fiber-rich diet, which is in accordance with observations made in humans [83, 86].

A variant of a simplified human microbiota consortium was established by another group, who combined seven IBD-related human bacterial strains [87]. The aim of these researchers was to investigate the consortium’s pathogenic effect on two different strains of IL-$10^{-/-}$ and wild-type mice (129S6/SvEv and C57BL/6) and changes in bacterial composition. The authors showed that induction of colitis occurred in genetically susceptible mice only and that inflammation was more severe in 129S6/SvEv than in C57BL/6 mice. The consortium’s composition was not stable over time and differed between mouse strains or among contents from different intestinal sites. Furthermore, the authors pointed out that bacterial strains that showed the highest inflammatory potential towards

mesenteric lymph node cells *ex vivo* were numerically not the most abundant in the colonic mucosa of mice colonized with the consortium [87].

Another prominent example of a defined microbiota community is the Oligo-Mouse-Microbiota (Oligo-MM), which consists of 12 bacterial strains representing major phyla in the murine intestine [88]. The author's objective was to establish a minimal consortium that builds up a colonization resistance against the human pathogen *Salmonella enterica* serovar Typhimurium, similar to the one observed in conventional mice. However, the colonization resistance could only be completely achieved when Oligo-MM-harboring mice were additionally colonized with three facultative anaerobic bacteria, among them an *E. coli* strain closely related to *Salmonella*. The authors speculated that the extended consortium occupies all essential intestinal niches and, thus, prevented colonization of the pathogen [88].

The variety of gnotobiotic animal models underlines the importance of model selection for the investigation of a specific research question. Apart from rodents, other animals may be used to investigate bacteria-related research questions. As an example, pigs mono-associated with various *E. coli* strains were established to investigate the bacterial pathogenesis and the impact of antisera against this infection [89]. Pigs resemble humans in many aspects, including nutrition and intestinal physiology, which makes them a good model for translational medical research. However, maintenance of gnotobiotic animals requires special environmental conditions to avoid contamination with other microorganisms. Therefore, gnotobiotic animals are kept in isolators and all the material brought into these isolators must be sterile. This kind of housing is very laborious especially for pigs, as they require more space and equipment than mice and rats [84]. Furthermore, it is important to keep in mind that this is an artificial model system and insights gained in this setting are needed to be verified in human studies.

1.5 Objectives and research questions

The intestinal microbiota was found to have a major impact on host health. At the same time, the microbiota composition is influenced by a number of host-derived factors. One of these factors is the diet ingested by the host, which supplies substrates to the intestinal bacteria and, thus, substantially affects their growth [1]. A diet rich in saturated milk fat was shown to stimulate the growth of the sulfite-reducing bacterium *B. wadsworthia* by increasing the proportion of the sulfonated bile acid TC. This TC-induced overgrowth of *B. wadsworthia* was accompanied by an increased incidence and severity of colitis in genetically susceptible IL-10$^{-/-}$ mice [2]. Therefore, the question arose whether dietary sulfonates may have the same detrimental effect. Sulfonated compounds abundantly found in the diet are sulfolipids such as SQDG, which are present in leafy green vegetables and cyanobacteria, among others. SQDG was reported to be converted to SQ by the common gut inhabitant *E. coli*, which is also able to degrade SQ further to DHPS through a pathway named sulfoglycolysis. The sulfonate moiety of DHPS could potentially be used by *B. wadsworthia* as terminal electron acceptor for its growth and, thereby, lead to intestinal inflammation in a genetically susceptible host.

This hypothesis was investigated in conventional and gnotobiotic IL-10$^{-/-}$ mice, which were orally treated with the SQDG metabolite SQ. The gnotobiotic mice either harbored the SIHUMI consortium

or SIHUMI plus *B. wadsworthia*, allowing to dissect the effects of this bacterium as a member of a minimal microbial community. To further assess the impact of a diet rich in SQDG, conventional IL-$10^{-/-}$ mice were fed a diet supplemented with the cyanobacterium *A. platensis* (Spirulina). The intestinal distribution and degradation of the ingested sulfonates was monitored and their impact on intestinal microbiota composition and the murine immune response investigated. Furthermore, the capability of the murine fecal bacteria and the SIHUMI consortium with or without *B. wadsworthia* to degrade SQ was assessed *in vitro*. The investigations were performed with the aim to answer the following research questions:

- Are the dietary sulfonates SQDG and SQ degraded by intestinal bacteria?
- Is the growth of *B. wadsworthia* stimulated by these dietary sulfonates?
- Do dietary sulfonates elicit bacteria-induced intestinal inflammation in a murine model of chronic gut inflammation?
- If yes, what causes the inflammatory response?

2 Material and methods

2.1 Bacteria and growth conditions

All bacterial strains used in this project were cultivated anoxically at 37 °C and their purity was verified by Gram-staining. *Bilophila wadsworthia* DSM 11045 was purchased from the German Collection of Microorganisms and Cell Cultures GmbH (Braunschweig, Germany) and grown in a liquid culture medium adapted from da Silva et al. (DS medium, Appendix I) [90]. The medium was adjusted to a pH of 7.4, autoclaved and gas flashed with N_2/CO_2 (80:20, v/v) in gas-tight Hungate tubes [91]. Additionally, 20 ml titan (III)-nitrile triacetate (Ti (III) NTA) solution [92], 1 ml 7-vitamin solution (Appendix I), 30 mmol $NaHCO_3$ and 20 mmol taurine (Roth, Karlsruhe, Germany) per liter of medium were sterile filtered, gas flushed with N_2/CO_2 (80:20, v/v) and added to 16.5-ml Hungate tubes directly before use, resulting in a total volume of 10 ml DS medium.

The SIHUMI consortium consisting of *Anaerostipes caccae, Bacteroides thetaiotaomicron*, *Bifidobacterium longum*, *Blautia producta*, *Clostridium butyricum*, *Clostridium ramosum*, *Escherichia coli* K-12 and *Lactobacillus plantarum* (for strain designation see Table 1) was derived from the strain collection of the German Institute of Human Nutrition Potsdam-Rehbruecke. Each member of the consortium was cultivated individually in Yeast Hemin Brain-Heart-Infusion Broth (YH-BHI, Appendix I), except *C. ramosum*, which was grown in YH-BHI medium mixed 1:1 (v/v) with Yeast extract-casitone-fatty acid (YCFA, Appendix I) medium.

2.2 Incubation experiments

To investigate whether fecal bacteria from conventional mice (chapter 2.2.1) or the SIHUMI consortium with or without *B. wadsworthia* (chapter 2.2.2) are capable of converting SQ and TC to sulfide, fecal incubation experiments were performed under anoxic conditions. Samples were withdrawn from the incubation vessels at different time points and their sulfide (chapter 2.5.1), SQ (chapter 2.5.3) and TC (chapter 2.5.4) concentrations as well as bacterial cell numbers (chapter 2.4.2) were quantified.

2.2.1 Fecal incubation experiments

The DS medium was utilized for the *in vitro* incubation experiments with murine feces. To test the conversion of different growth substrates, taurine was replaced by 4 mM SQ (Mcat, Donaueschingen, Germany) or 20 mM sodium TC (Roth). For the incubation of a complex microbiota with SQ and TC, freshly collected fecal samples from four IL-$10^{-/-}$ mice fed a standard diet (Ssniff standard chow A153 F0300 Am R/M-H; Ssniff, Soest, Germany) were transferred into an anaerobic chamber (Whitley M85 workstation, Don Whitley Scientific Limited, Shipley, UK) with a gas phase composed of $N_2/CO_2/H_2$ (80:10:10, v/v/v) and diluted 1:10 in DS medium. Following the addition of sterile glass beads, the fecal dilution was vortexed and centrifuged at 300 x *g* and 4 °C for 5 min. Under a clean bench, the supernatant was injected at a dilution of 1:10 into sterile Hungate tubes containing DS medium supplemented with SQ or TC. Incubations with fecal suspensions but without sulfonates as well as incubations without feces but with sulfonates were included as negative controls. All incubations were carried out in duplicates and samples were withdrawn at start and after 168 h of anaerobic incubation at 37 °C. Each 1-ml sample was split into 250 µl for the quantification of sulfide and 750 µl for immediate centrifugation (14 000 x g, 4 °C, 10 min). Aliquots of the supernatant and the pellet

were frozen at – 20 °C until further processing. The pellets were subjected to DNA extraction (chapter 2.5.2) for qPCR-based analysis of bacterial cell numbers. The supernatants were utilized for the quantification of SQ and detection of TC.

2.2.2 SIHUMI incubation experiments

SIHUMI bacteria and *B. wadsworthia* were individually cultivated as described above. Cells of overnight cultures were enumerated using a Thomas counting chamber. For each culture, the volume subjected to the incubation corresponded to cell numbers detected in cecal contents of SIHUMI mice fed the semisynthetic control diet (CD, chapter 2.3.2) (Table 1). Purity of each cell culture was verified by microscopic inspection of bacterial cells after Gram-staining. All bacterial strains were combined in a Hungate tube containing DS medium supplemented with SQ or TC resulting in a total volume of 10 ml and cultivated under anoxic conditions at 37 °C. Samples (600 µl) were withdrawn after 0, 1, 3, 20, 24, 48 and 168 h for the quantification of sulfide, SQ, TC, and bacterial cell numbers. Bacterial growth was monitored by measuring the optical density at 600 nm (Infinity M200 Pro, Tecan, Männedorf, Switzerland).

Table 1: Members of the SIHUMI consortium (numbers 1 to 8) and *B. wadsworthia* (number 9) with strain designation and cell number subjected to the 10 ml incubation medium.

Bacterial species	Strain designation (^{T} type strain)	Cell number for incubation experiment
1. *Anaerostipes caccae*	DSM 14662^T	10^7
2. *Bifidobacterium longum*	NCC 2705	10^6
3. *Blautia producta*	DSM 2950^T	10^9
4. *Bacteroides thetaiotaomicron*	DSM 2079^T	10^{10}
5. *Clostridium butyricum*	DSM 10702^T	10^7
6. *Clostridium ramosum*	DSM 1402^T	10^9
7. *Escherichia coli* K-12	MG 1655	10^9
8. *Lactobacillus plantarum*	DSM 20174^T	10^3
9. *Bilophila wadsworthia*	DSM 11045	10^9

2.3 Animal experiments

2.3.1 Animal housing conditions

Animal experiments with 10- to 12-week-old conventional and gnotobiotic C57BL/6.129P2-Il10^{tm1Cgn} (IL-10$^{-/-}$) mice were approved by the State Office for Occupational Safety, Consumer Protection and Health of the State of Brandenburg, Germany (approval number: 2347-10-2016). Conventional male mice were bred under SPF conditions in the animal facility of the German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany. Mice were housed in individually ventilated cages and kept in a 12-hour light-dark cycle at 22 ± 2 °C and 55 ± 5% air humidity. Feed (Ssniff standard chow A153 F0300 Am R/M-H; Ssniff, Soest, Germany) and water were applied *ad libitum*.

Gnotobiotic male and female mice were kept under the same environmental conditions but in sterile positive-pressure isolators (Metall & Plastik, Radolfzell, Germany), in which the drinking water was autoclaved twice. Feed (Altromin fortified type 1310; Altromin, Lage, Germany) and wood chips were

irradiated with 50 kGy (Synergy Health Radeberg GmbH, Radeberg, Germany). Gnotobiotic mice were born harboring the SIHUMI community consisting of eight bacterial species listed in Table 1 (numbers 1 to 8).

2.3.2 Experimental design

Two weeks before the start of the experiments, mice were single-caged and adapted to the semisynthetic CD (Table 2), which was sterilized by irradiation (50 kGy, Synergy Health Radeberg GmbH). At the last day of adaptation, mice were weighed and fecal samples were collected. At the beginning of the intervention, mice were either fed one of the experimental diets (experiment A) or orally treated with one of the experimental substances (experiments B and C).

Experiment A

Conventional mice were split into a control group receiving the CD (n = 12) and a test group fed a diet containing 20% Spirulina (SD, n = 12). The composition of the experimental diets is shown in Table 2.The macronutrient composition of both diets was analyzed using the Weender analysis (Agrolab, Kiel, Germany) and the energy content was assessed using a bomb calorimeter (C5003 calorimeter, IKA, Staufen, Germany). Body weight and feed intake were measured twice per week and fecal samples were collected at day 18 of the experiment.

Table 2: Composition of the semisynthetic control diet (CD) and Spirulina diet (SD).

Ingredient	Supplier	CD (g/kg)	SD (g/kg)
Casein	Altromin	200.0	71.2
Wheat starch	Kröner-Stärke, Ibbenbüren, Germany	564.0	527.0
Sunflower oil	Gut & Günstig, Hamburg, Germany	35.0	24.7
Coconut fat	Palmin, Elmshorn, Germany	9.0	6.4
Line seed oil	Kunella, Cottbus, Germany	6.0	4.2
Cellulose	J. Rettenmeier und Söhne, Rosenberg, Germany	50.0	43.0
Vitamin mix (C-1000)	Altromin	20.0	20.0
Mineral mix (C-1000)	Altromin	60.0	48.0
Choline bitartrate	Altromin	2.5	2.5
L-cystein	Altromin	3.0	3.0
Maltodextrin	Altromin	50.0	50.0
Spirulina PREMIUM+ Powder	Institute for Food and Environmental Research, Nuthetal, Germany	0.0	200.0

Experiment B

With the beginning of the intervention phase, mice were allocated to three groups receiving oral applications of SQ (0.45 g/kg body weight; n = 12), sodium TC (1 g/kg body weight; n = 10) or water (n = 12). SQ and water were applied daily, whereas TC was applied twice per week. Body weight and feed intake were monitored twice weekly and feces were collected at day 18 of the intervention phase.

Experiment C

Following the adaptation phase, half of the SIHUMI mice was transferred to a separate isolator and additionally colonized with 10^8 cells of *B. wadsworthia*. Mice from the respective control groups received a single oral application of sterile DS medium instead. Both groups were split again into three subgroups each: a group gavaged with water (n = 8), a group treated with SQ (0.45 g/kg body weight; n = 8) and a group receiving sodium TC (1 g/kg body weight; n = 8). The substances were orally applied to the mice twice per week. At the same time body weight and feed intake were determined. One day before the end of the experiment (day 20), fecal samples were collected. For the group additionally colonized with *B. wadsworthia*, fecal samples were also collected one day after colonization, to verify the presence of this bacterium and for comparison of the initial and final cell numbers in the course of the experiment. Tests for common bacterial contaminants were routinely performed by incubating fecal suspensions aerobically and anaerobically on Columbia blood agar (Biomérieux, Marcy l´Etoile, France), Kenner fecal *Streptococcus* agar, Rogosa agar (both from Oxoid, Hampshire, UK), ASLA agar (Fluka, Neu-Ulm, Germany) and Mannitol salt agar (Roth). If bacterial growth was detected, bacterial morphology was microscopically inspected following Gram staining. In addition, cells from bacterial colonies were subjected to DNA extraction (see chapter 2.5.2) followed by PCR and partial sequencing of the 16S rRNA gene (see chapter 2.4.3) to verify the bacterium's identity.

After three weeks of intervention, mice were euthanized with isoflurane, and blood was drawn by cardiac puncture into sterile EDTA-coated tubes. The tip of the cecum including its content and the distal part of the colon (SPF experiments) or half of the colon dissected longitudinally and formed to a Swiss roll (gnotobiotic experiments) were fixed in Carnoy's solution and embedded in paraffin for later histological assessment. Liver, gall bladder, spleen, kidney, mesenteric white adipose tissue (mWAT) including mesenteric lymph nodes (mLN) and mLN extracted from the mWAT were weighed, and the size of spleen, kidney and mLN was determined. Parts of the liver, spleen and kidney were fixed in formaldehyde and embedded in paraffin for later assessment of tissue morphology. Contents of small intestine, cecum and colon were collected, and the mucosa of cecum and colon was scraped. Mucosa samples were snap-frozen in liquid nitrogen and stored at - 80 °C, whereas the intestinal contents were kept on ice until storage at - 20 °C. Blood was centrifuged (2 000 x *g*, 4 °C, 10 min) and the plasma stored at - 80 °C.

2.3.3 Colon permeability test

The colonic permeability was assessed by the fluorescein isothiocyanate (FITC)-dextran assay as described before [93] after two weeks of intervention. Therefore, mice were fasted for three hours and subsequently orally treated with 40 kDa FITC-dextran (Experiment A, mice applied with SQ or water) or 4 kDa FITC-dextran (all remaining groups; both Sigma-Aldrich, 600 mg/kg body weight in sterile-filtered phosphate-buffered saline [PBS]) or PBS for blank measurements. Four hours later, blood samples were drawn from the retro bulbar capillary plexus into heparinized tubes and centrifuged at 2 000 x *g* and 4 °C for 5 min. The supernatant (plasma) was frozen at -20 °C, protected

from light, until further processing. For the measurement of the fluorescence intensity, plasma was thawed and diluted 1:5 (v/v) in PBS. FITC-dextran standards were prepared by performing serial 1:2 dilutions (v/v) of 250 µg/ml FITC-dextran in PBS until a minimal concentration of 0.122 µg/ml was reached. The fluorescence intensity of standards and samples was spectrophotometrically determined in 96-well plates using the Tecan Infinite M200 PRO at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Background measurements of only PBS were included and obtained values subtracted from all standard and sample values. The mean of fluorescence values of mice receiving PBS (n = 3 – 4) were subtracted from those of FITC-dextran-treated mice (n = 5 – 8).

2.3.4 Histopathological assessment

Slices of the paraffin-embedded cecal and colonic tissues were cut, stained with haematoxylin and eosin (H & E) and examined by a veterinary pathologist (Prof. Dr. Robert Klopfleisch, Institute of Veterinary Pathology, Freie Universität Berlin, Germany). For each mouse, one cecal and one colonic tissue sample was scored, except for colonic tissue samples from experiment A and B, for which only a subset of samples was scored (n = 3 – 4). The scoring was performed in a blinded fashion using the criteria listed in table 3 [94]. Mean values were calculated for each region, and the sum thereof was calculated to obtain a total value. Fixed tissue slices from liver, spleen and kidney were cut, stained with H & E and examined using a light microscope (AxioCamHRc, Carl Zeiss, Jena, Germany).

Table 3: Details of the histopathology score with regard to region, characteristic and grade [94].

Region	Characteristic	Grade
Lumen	Necrotic epithelial cells	1 = scant (about 10); 2 = moderate; 3 = dense
	Neutrophils	1 = scant (1); 2 = moderate, 3 = dense
Surface epithelium	Villous atrophy and fusion	1 = scant (about 10); 2 = moderate; 3 = dense
	Desquamation	1 = patchy (<30%); 2 = diffuse (> 30%)
	Ulceration	0 = no; 1 = present
	Crypt abscesses	0 = none; 1 = rare; 2 = moderate; 3 = abundant
	Epithelial hyperplasia	0 = none; 1= present
Lamina propria	Lymphocytes	0 = very few; 1 = some (2–4); 2 = numerous (>5) or 1 large
	Infiltration	0 = none; 1 = rare (<15%); 2 = moderate; 3 = abundant (>50%)
Submucosa	Lymphocytes	0 = one small; 1 = some (2–4); 2 = numerous (>5) or 1 large
	Polymorph nuclear cells (PMN)	0 = none; 1 = few extravascular PMNs; 2 = many polymorph nuclear cells (neutrophils)
	Edema	0 = none to mild (< 10 of the mucosa); 1 = moderate; 2 = severe
	Peyer patch hyperplasia	0 = none; 1 = present

2.4 Molecular biological methods

2.4.1 Genotyping of the IL-10 gene

The IL-10-deficient mouse was developed in 1993 by Kühn *et al.* by introducing termination codons into exons one and three of the IL-10 gene and by replacing a 500 bp fragment in exon one by a *neo* (neomycin resistance) expression cassette [76]. A forward primer targeting an unaltered part of the IL-10 gene (IL-10 Fwd), a reverse primer targeting a sequence of the wild type gene that is replaced in the mutant (IL-10 WT-Rev) and a reverse primer targeting a sequence that is only present in the mutant (IL-10 M-Rev) were used to discriminate between wild type and mutant animals. The disruption of the IL-10 gene was confirmed for each mouse by genotyping. For this purpose, the tail tip was incubated in a 1.5-ml plastic tube containing 39.5 µl water supplemented with 5 µl Gitschier buffer (Appendix I), 5 µl proteinase K (20 mg/ml, Roth) and 0.5 µl β-mercaptoethanol (Amersham Bioscience, Piscataway, NJ, USA) at 50 °C on a thermo shaker (at 1 000 rpm) for at least 3 h. After vortexing for 30 s and centrifugation at 14 000 x *g* at room temperature (RT) for 20 s, the supernatant was transferred into a new plastic tube and heated to 95 °C for 10 min to inactivate the proteinase K. Following another step of vortexing and centrifugation, the supernatant was stored overnight at 4 °C. On the next day, a PCR was performed with 1 µM of each primer (IL-10 Fwd: 5'-CTT GCA CTA CCA AAG CCA CA-3' ; IL-10 WT-Rev: 5'-GTT ATT GTC TTC CCG GCT GT-3'; IL-10 M-Rev: 5'-CCA CAC GCG TCA CCT TAA TA-3'; synthesized by Eurofins Genomics, Ebersberg, Germany), 1 x Dream Taq Green Buffer, 2.5 mM $MgCl_2$, 0.25 mM deoxynucleotide triphosphate (dNTP), 10 U Dream Taq DNA polymerase (all Fermentas/Thermo Fisher Scientific, Scherte, Germany) and 2 µl DNA preparation in a total volume of 12 µl. The thermal conditions for the PCR run were: denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 1 min, elongation at 72 °C for 1 min and a final step of elongation at 72 °C for 10 min. The size of the PCR products was analyzed by gel electrophoresis (as described in 2.4.3) with 2% agarose and fluorescent in-gel staining (Midori Green Advance, 5 µl/100 ml gel; Nippon Genetics Europe, Düren, Germany) using standard DNA fragments as a reference (100 bp plus DNA ladder, Invitrogen, Karlsruhe, Germany). A single PCR product of 312 bp indicated a homozygotic IL-10 mutant and a single PCR product of 137 bp the wild type mouse. Bands at both sizes would indicate a heterozygotic status.

2.4.2 Quantification of bacterial cells

DNA was extracted from 70 to 100 mg intestinal contents or feces using the PSP Spin Stool DNA kit (Invitek/ Stratec Molecular, Berlin, Germany) by following the manufacturer's instructions. In the final step, DNA was eluted in 70 µl Elution buffer, quantified with a NanoDrop ND-1000 (Peqlab) and stored at – 20 °C until further processing. For each qPCR reaction, 2.5 µl of QuantiNova SYBR Green PCR master mix and 0.025 µl ROX reference dye (both Qiagen, Hilden, Germany), 0.5 µl of forward and 0.5 µl of reverse primer (3 µM each) and 0.475 µl water were combined with 1 µl extracted DNA. The Viia™ 7 -Realtime PCR System (Applied Biosystems) connected with the QuantStudio™ Real-Time PCR Software version 1.3 (Thermo Fisher Scientific) was employed for performing the qPCR. Thermal conditions of the qPCR reactions were: polymerase activation at 94 °C for 4 min, 40 cycles of denaturation at 94 °C for 15 s and annealing and elongation at 53 to 65 °C for 30 s. The annealing/elongation temperature was adjusted for each target and is given in Table 4. Specificity of the amplification was confirmed by melt-curve analysis performed after each run by gradually

increasing the temperature from 60 to 95 °C. The species-specific primers used for the quantification of *B. wadsworthia* (*16S rRNA* gene) and the SIHUMI consortium members (*GroEL* gene) were synthesized by Eurofins Genomics and are listed in Table 4. For the quantitative analysis of cell numbers, a standard curve based on defined cell numbers was constructed. For this aim, the cell number of a fresh liquid cell culture was determined microscopically using a Thomas counting chamber. The volume containing 10^8 cells (for *B. wadsworthia*) or 10^9 cells (for the SIHUMI bacteria) was calculated and centrifuged (14 000 x *g*, 4 °C, 5 min). Sterile feces from germfree mice were spiked with the pelleted cells and DNA was extracted as described above. Serial dilutions from 10^9 or 10^8 to 10^3 cells were prepared and included in every qPCR run. All samples and standards were analyzed in triplicates and specificity of amplifications was verified by melt-curve analysis.

Table 4: Sequence of primers targeting species-specific regions of the *GroEL* gene of individual SIHUMI members, the *16S rRNA* gene of *B. wadsworthia,* the dissimilatory sulfite reductase A (*dsrA*) gene and the sulfoquinovose (SQ) isomerase (*yihS*) gene and annealing temperatures used in qPCR analyses.

Target bacterium or gene	Forward primer (5' -> 3')	Reverse primer (5' -> 3')	Annealing temperature	Reference
Anaerostipes caccae	AAC CTT GCG GCA GGT GCA AAT CCG	AGC ATC CGC AAC TAA CTC CCC GAC	65.0 °C	[95]
Bacteroides thetaiotaomicron	TGT GAC TGC CGG TGC AAG CC	ACT TTG CGC ATA GCG TCA GCA	60.0 °C	[95]
Bifidobacterium longum	CGG CGT YGT GAC CGT TGA AGA C	TGY TTC GCC RTC GAC GTC CTC A	55.0 °C	[96]
Blautia producta	AAC CTG GCA GCA GGC GCT AAC	TCA TCG CCT GCG GAG ATA GCT G	60.0 °C	[95]
Clostridium butyricum	AGT AGC TGT TGA AAA GGC AGT TGA AGA	TCA GCA GCA GAA ATA GCA GCA ACT C	60.0 °C	[95]
Clostridium ramosum	TGC GAG CAA AGA GGT AGC AAA AAC T	GCC GAA ATC GTT GCA ACA CTA GCA	65.0 °C	[95]
Escherichia coli	GGC TAT CAT CAC TGA AGG TCT G	TTC TTC AAC TGC AGC GGT AAC	60.0 °C	[95]
Lactobacillus plantarum	GCC GTT GTT CGT GTC GGT GC	TTC TTC AAC GGC GGC CCG AG	60.0 °C	[95]
Bilophila wadsworthia	AAG TCC TTC GGG GCG AGT AA	ATC CTC TCA GAC CGG CTAC	53.0 °C	[97]
Dissimilatory sulfite reductase (dsrA)	CCA ACA TGC ACG GYT CCA	CGT CGA ACT TGA ACT TGA ACT TGT AGG	53.5 °C	[2]
SQ isomerase (yihS)	ACG CGG TGG AAG CTT TCT TGA T	CAC GGT GGC GTT AAA CAG ACC TT	57.0 °C	[39]

2.4.3 Partial *16S rRNA* gene sequencing of specific bacteria

DNA was extracted from pure cultures of bacteria or samples from incubation experiments using the RTP Bacteria DNA Mini kit (Stratec Molecular) as described in chapter 2.5.2. A PCR was performed with primers targeting a specific region of the *16S rRNA* gene (27Fwd: 5'-AGA GTT TGA TCC TGG CTC AG-3' and 1492Rev: 5'- TAC CTT GTT ACG ACT T -3', synthesized by Eurofins Genomics) [98]. The master mix (50 µl per assay) contained 2.5 mM 10 x PCR Buffer with $MgCl_2$ (Invitrogen), 0.1 mM of each primer, 0.25 mM of each dNTP (Invitek), 2.5 U recombinant Taq DNA polymerase (Invitrogen),

PCR-grade water and 1 µl DNA preparation. The thermal conditions for the PCR run were: denaturation at 94 °C for 4 min followed by 30 cycles of denaturation by 94 °C for 1 min, annealing at 50 °C for 1 min, elongation at 72 °C for 1 min and a final step of elongation at 72 °C for 10 min. PCR products were analyzed by gel electrophoresis using TRIS-acetate EDTA (TAE, Appendix I) as running buffer. For this purpose, a 1% agarose gel (Serva, Heidelberg, Germany) was prepared with TAE buffer and EZ Vision ONE (Thermo Fisher Scientific) mixed with the PCR product (1:5, v/v) was pipetted into the slots of the gel. A current of 100 V was applied to the gel for about 1 h, resulting bands were visualized with the G:BOX F3 gel doc system using the GeneSys software version 1.5.2.0 (Syngene, Cambridge, UK). The size of the PCR products was estimated from the reference bands of a 1 kb DNA ladder (Invitrogen). The PCR products were purified using the innuPrep DOUBLE pure Kit (Analytik Jena, Jena, Germany) and the DNA content was quantified using the NanoDrop ND-1000 (Peqlab). The PCR product was sequenced using the 27Fwd primer (Eurofins Genomics). The resulting sequences were assigned to bacterial species using the Basic Local Alignment Search Tool (BLAST) algorithm [99] of the genome database (GenBank: www.ncbi.nlm.nih.gov).

2.4.4 Partial *16S rRNA* gene sequencing of bacterial communities

The bacterial community in cecum and feces (days zero and 18 of the intervention) of conventional mice was analyzed by sequencing the V3 and V4 region of the *16S rRNA* gene with the Illumina method [100]. For this purpose, one to three droppings of feces and 250 µl cecal content from five animals per group were individually mixed with 600 µl Stool DNA Stabilizer (Stratec Molecular) and send on dry ice to the ZIEL Core Facility Microbiome/NGS at the Technical University Munich, Germany. There, the DNA was extracted and sequencing was performed. Sequences were quality checked and further processed using the integrated microbial next generation sequencing (IMNGS) platform [101] and the Rhea software [102]. Beta-diversity was depicted in non-metric multi-dimensional scaling (NMDS) plots.

2.4.5 Gene expression analysis in murine mucosa using quantitative real-time PCR (qPCR)

Extraction of total RNA

Total RNA was extracted from mucosa samples using the peqGOLD TriFast™ reagent (Peqlab) according to the manufacturer's instructions and by applying the guanidinium thio cyanate-phenol-chloroform method [103]. Mucosa scraped from cecum or colon tissue was mixed with 1 ml TriFast ™ and 10 sterile zirconia beads (2.4–2.8 mm; Analytik Jena). The mucosa cells were mechanically lysed by shaking them twice for 90 s at 50 Hz by a TissueLyser LT with a pre-cooled adapter (Qiagen). After incubation of the lysate for 5 min at RT, 200 µl of chloroform was added, the samples were vortexed for 15 s and during another 10 min incubation step at RT, the samples were vortexed four times shortly. In the following centrifugation step (18 400 x *g*, 20 min), the centrifuge started at RT and cooled down to 4 °C during the run. Meanwhile, 500 µl isopropanol (Roth) were placed in RNAse-free plastic tubes and the clear supernatant of the centrifuged samples was added and inverted six times. After 10 min of incubation on ice, samples were centrifuged again at 18 400 x *g*, 4 °C for 20 min. The resulting isopropanol supernatant was discarded and the pellet was washed with 1 ml 70% aqueous ethanol, vortexed and centrifuged at 18 400 x *g*, 4 °C for 10 min. This washing step was repeated with 100% ethanol, the resulting pellet air-dried and dissolved in 100 µl DEPC-treated water (Merck). If required, dissolving was promoted by shaking at 60 °C for 5 min. The RNA sample was kept on ice and the concentration and purity were determined photometrically (at 230, 260 and 280 nm) using

the Eon microplate reader combined with the Take 3 micro-volume plate and the Gen5 software (Bio-Tek, Winooski, VT, USA). RNA extracts were stored at – 80 °C until further processing.

Elimination of DNA

For the degradation of genomic DNA, 8 µg RNA was mixed with 1 x Reaction Buffer with $MgCl_2$, 2 U DNAse I, 0.75 U RiboLock (RNAse inhibitor) (Thermo Fisher Scientific) and adjusted to final volume of 30 µl with DEPC-treated water. The mixture was incubated at 37 °C for 30 min (peqSTAR thermocycler, Peqlab) and subsequently, 1 µl EDTA (50 mM) was added. The mixture was subsequently incubated at 65 °C for 10 min. The concentration of the purified RNA was quantified as described above.

RNA quality controls

The absence of DNA was checked by performing a qPCR with primers targeting the *18S rRNA* gene (18S-Fwd: 5'-ACC ACA TCC AAG GAA GGC AG-3'; 18S-Rev: 5'-TTT TCG TCA CTA CCT CCC C-3') [104]. For the qPCR reaction, 5 ng purified RNA was combined with 0.5 µl of each primer (both 3 µM), 0.5 µl of an *18S rRNA* gene specific probe (2 µM; 5'-6-FAM-AGG CGC GCA AAT TAC CCA CTC CC-TAMRA-3') [104] and 2.5 µl of a TAQ Man Probe master mix (Thermo Fisher Scientific). Thermal cycling conditions for the qPCR run were: uracil-*N*-glycosylase incubation at 50 °C for 2 min, DNA polymerase activation at 95 °C for 10 min and 40 cycles switching between denaturation at 95 °C for 15 s and annealing/elongation at 60 °C for 1 min. Negative controls with DEPC-water instead of RNA sample were included (n = 6) and their mean CT-values (number of cycles at which the amplification signal reaches above the background noise) calculated. This mean CT-value was subtracted by six and considered as the threshold for all RNA samples. If an RNA sample had a smaller CT-value than the threshold, degradation of DNA was considered not successful and had to be repeated.

RNA integrity was assessed by performing a horizontal gel electrophoresis. For this purpose, a gel was prepared using 10 ml 10 x MOPS buffer (Appendix I), 1 g agarose (Biozym, Hessisch Oldendorf, Germany), 87 ml autoclaved water and 3 ml 37% formaldehyde (Roth). Samples containing 500 ng RNA were mixed 1:1 (v/v) with 2 x RNA loading dye containing ethidium bromide (Thermo Fisher Scientific), denatured at 70 °C for 10 min and placed on ice for 5 min. The gel was loaded with the denatured RNA and 1 x MOPS was used as running buffer. A current of 100 V was applied for 30 min (compact L/XL horizontal gel electrophoresis apparatus, Biometra) and the resulting bands were visualized using the UV prochemie system (Biometra). The integrity of RNA was confirmed when the *18S* and the *28S rRNA* bands were clearly visible.

Reverse transcription of RNA into DNA

Subsequent synthesis of complementary DNA (cDNA) was done using 1 µg of RNA and the RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Fisher Scientific). Briefly, 10 µl purified RNA in DEPC-water was mixed with 1 µl random hexamer primers (100 µM) and incubated at 70 °C for 5 min (peqSTAR thermocycler, Peqlab). The mixture was cooled on ice, shortly centrifuged and 8 µl reaction mix (4 µl 5 x buffer, 1 µl 20 U RNAse inhibitor, 2 µl 10 mM dNTPs and 1 µl DEPC-water) was added. After incubation at 25 °C for 5 min, 1 µl reverse transcriptase solution (200 U) was added. The mixture was further incubated at 25 °C for 10 min, then at 42 °C for 60 min and finally at 60 °C for 10 min. The liquid was spun down using a table centrifuge (for 3 s) and the cDNA sample was diluted 1:9 (v/v) with DEPC-water and stored at – 80 °C until qPCR analysis.

Quantification of gene expression

Each qPCR reaction mix contained 2.5 µl Power SYBR Green master mix (Thermo Fisher Scientific), 0.5 µl forward and 0.5 µl reverse primer (3 µM each), 0.5 µl DEPC-treated water and 1 µl cDNA corresponding to 5 ng of RNA. For the qPCR run the same system as described in chapter 2.4.2 was used and the thermal conditions of the qPCR reactions were as follows: polymerase activation at 94 °C for 4 min, 40 cycles of denaturation at 94 °C for 15 s and annealing and elongation at 60 °C for 30 s. Specificity of the amplification was confirmed by melt curve analysis. Gene expression of tumor necrosis factor alpha (*Tnf-α)*, interferon gamma (*Ifn-γ),* interleukin (*Il*)-6, *Il-1 β*, *Il-12*, sulfide:quinone oxidoreductase (*Sqr*), hypoxia-inducible factor-1α (*Hif-1α*), tight junction protein (*Tjp*) *1* and occludin (*Ocln*) (Table 5) was calculated by the ΔΔCT method (Eq. 1) [105] with hypoxanthine guanine phosphoribosyl transferase (*Hprt*) as the reference housekeeping gene and relative to the respective control group normalized to the value 1. The calculation was based on the assumption that the primer efficiency was 2, meaning that the number of target gene sequences doubled with every qPCR cycle.

$$\text{Relative gene expression} = 2^{\Delta CT \text{ target (control – sample)}} / 2^{\Delta CT \text{ reference (control – sample)}} \quad \text{(Eq.1)}$$

Table 5: Genes included in expression analysis, their accession numbers, encoded proteins and sequences of primer pairs utilized for qPCR analysis.

Protein	Gene	Accession number	Forward primer (5’ -> 3’)	Reverse primer (5’ -> 3’)
Interferon gamma	*Ifn-γ*	NM_008337.4	CAG GCC ATC AGC AAC AAC ATA AGC G	TGG ACC TGT GGG TTG TTG ACC TCA
Tumor necrosis factor alpha	*Tnf-α*	NM_013693.3	GAC CCT CAC ACT CAG ATC ATC TTC T	CCA CTT GGT GGT TTG CTA CGA
Interleukin-6	*Il-6*	NM_031168.2	TAG TCC TTC CTA CCC CAA TTT CC	TTG GTC CTT AGC CAC TCC TTC
Interleukin-1 beta	*Il-1 β*	NM_008361.4	ACC TGT GGC CTT GGG CCT CAA A	TGC TTG GGA TCC ACA CTC TCC AGC
Interleukin-12 alpha	*Il-12α*	NM_001159424.2	GCT GCT GAA ATC TTC TCA CCG TGC A	GTG GCC AAA AAG AGG AGG TAG CGT
Sulfide:quinone oxidoreductase	*Sqr*	NM_021507.5	ACA GAC AAT GGC AAG GAG A	GAG TGT TTG GGA AGG TGA A
Hypoxia-inducible factor-1 alpha	*Hif-1α*	NM_001313919.1	GAA ATG GCC CAG TGA GAA AA	TAT CGA GGC TGT GTC GAC TG
Tight junction protein 1	*Tjp 1*	NM_009386.2	ACC CGA AAC TGA TGC TGT GGA TAG	AAA TGG CCG GGC AGA
Occludin	*Ocln*	NM_008756.2	ATG TCC GGC CGA TGC TCT C	TTTGGC TGC TCT TGG GTC TGT AT
Hypoxanthine guanine phosphoribosyl transferase	*Hprt*	NM_013556.2	CAG TCC CAG CGT CGT GAT TA	AGC AAG TCT TTC AGT CCT GTC

2.5 Biochemical and analytical methods

2.5.1 Sulfide quantification

For the quantification of sulfide the methylene blue method was applied [106]. Solutions of 100 mM NaOH, 327 mM ZnAc, 3,5-dinitrosalicylic acid detection reagent (Appendix I) and 500 µM Na_2S were prepared. A sample aliquot (250 µl) was placed in 1.5-ml plastic tubes containing 25 µl NaOH and 5 µl ZnAc solutions, to trap the volatile hydrogen sulfide, and vortexed. If necessary, dilutions with distilled water were prepared. Twenty microliters of the detection reagent was added and incubated for 20 min at RT. Subsequently, mixtures were centrifuged (12 000 x *g*, RT, 3 min) and 200 µl of the supernatants was pipetted into 96-well plates and the absorbance measured at 670 nm (Infinite M200 PRO, Tecan). Concentrations were calculated using standard solutions of Na_2S (with NaOH and ZnAc) in water ranging from 40 to 200 mM.

2.5.2 DNA extraction from bacterial pellets

Cell pellets obtained from bacterial cultures or suspensions were subjected to DNA extraction using the RTP Bacteria DNA Mini Kit (Stratec Molecular, Berlin, Germany) according to the manufacturer's instructions. In the final step of the protocol, the DNA was eluted using 60 µl Elution Buffer, its purity and quantity were determined photometrically at 230, 260 and 280 nm wavelength (NanoDrop ND-1000, Peqlab, Erlangen, Germany) and DNA preparations were stored at – 20 °C. Cell numbers in bacterial incubation cultures were determined by quantitative polymerase chain reaction (qPCR) (see chapter 2.4.2) using standard solutions of DNA extracted from pure bacterial cultures. These cultures were grown over night as described in chapter 2.1 and cells counted using a Thomas counting chamber. Culture volumes equivalent to 10^9 cells were pelleted and subjected to DNA extractions as described above.

2.5.3 Quantification of SQ

The concentration of SQ was assessed using a colorimetric method described previously [107]. Each sample was mixed 1:1 (v/v) with a reagent containing 3,5-dinitrosalicylic acid (Appendix I) and heated to 100 °C for 15 min. After cooling to RT, 50 µl of the mixture was transferred in duplicate into a 96-well plate and the absorbance measured at 540 nm (Infinite M200 PRO, Tecan). Glucose samples were serially diluted (0.1 to 10 mM) and included as standards to calculate the concentrations of the samples.

2.5.4 Detection of TC

The concentration of TC and cholate was assessed semi-quantitatively by thin layer chromatography (TLC) as described previously [108, 109] followed by calculation of the relative concentration with the help of Image J version 1.52 (MD, USA). Stock solutions of sodium TC and cholate (Sigma-Aldrich, Munich, Germany) were prepared (1 M each) and individually diluted with DS medium to a final concentration of 20 mM as standards. Standard solutions and samples (each 50 µl) were freeze-dried (Alpha 1–4 LSCplus, Christ, Osterode, Germany), the residues dissolved in 50 µl methanol followed by centrifugation (14 000 x *g*, RT, 10 min). Subsequently, 3 µl of standard or sample was applied onto a silica gel 60 plate (20 x 20 cm, Merck, Darmstadt, Germany). The plate was placed into a TLC chamber and developed in a solvent system of 1-butanol/acetic acid/water (100:10:10, v/v/v) for

approximately 50 min. Finally, the plate was dried, treated with 5% concentrated sulfuric acid diluted in ethanol and heated at 110 °C for 10 min. TLC plate imaging was done using the G:BOX F3 documentation system with the GeneSys software version 1.5.2.0 (Syngene, Cambridge, UK). The intensity of each spot was quantified using ImageJ, the background subtracted and the ratio of sample to standard calculated.

2.5.5 Cytokine quantification in plasma

Mouse-specific TNF-α (BMS607-3), IFN-γ (KMC4021), serum amyloid A (SAA, KMA0021) (all from Thermo Fisher Scientific) and lipocalin (LCN) 2 (MLCN20; R & D Systems, Minneapolis, MN, USA) were quantified in a subset of plasma samples (n = 3 – 4 per mouse group) by performing enzyme-linked immunosorbent assays (ELISA) in accordance to the manufacturer's instructions.

2.5.6 Quantification of sulfonates in intestinal contents and feces

For the extraction of sulfonates from feces, small intestinal, cecal and colonic contents, samples (n = 6 per group and region) were diluted 1:20 (w/v) in 50% aqueous acetonitrile, homogenized for 5 min (Vortex Genie 2, Scientific Industries, NY, USA) and centrifuged at 14 000 x *g*, 4 °C for 5 min. Subsequently, 10 µl of supernatant was combined with 490 µl 50% acetonitrile and centrifuged at 18 000 x *g*, RT for 2 min. Finally, 50 µl supernatant was added to 450 µl 50% acetonitrile to obtain a total dilution of 1:10 000. Diluted samples were stored at - 80 °C in glass vials and shipped on dry ice to the Helmholtz Centre of Environmental Research (UFZ, Leipzig, Germany), where the concentrations of SQ, DHPS, taurine and isethionate were determined by a multiplexed targeted multiple reaction monitoring (MRM) approach using high performance liquid chromatography (Ultimate 3000 HPLC, Dionex, Sunnyvale, CA, USA) coupled to tandem mass spectrometry (LC-MS/MS-MRM) [110]. Separation of the analytical substances was achieved by hydrophilic interactions using a stationary phase of the HPLC column coated with bridged ethyl hybrid (BEH)-amides (2.1 x 100 mm, 1.7 µm; Waters, Milford, MA, USA). The mobile phase consisted of solvent A (50% aqueous acetonitrile with 0.1% formic acid) and solvent B (0.1% formic acid in water). Each run started with 80% A for 2 min, followed by a linear gradient for 3 min to 50% A. After 2 min at 50% A, the solvent composition was rapidly increased to 80% A and stayed at this level for 3 min to equilibrate the column. The mobile phase ran with a flow rate of 0.5 ml/min and the injection volume was 15 µl. The column temperature was set to 60 °C. The sulfonates were then identified and quantified by an MS (QTRAP 5500, AB Sciex, Framingham, MA, USA), which was operated at 500 °C in negative ion mode with an ion spray voltage of – 4.5 kV and a dwell time of 20 ms. The quantifier transition mass to charge values (and collision energy) were 125.3 to 95 Da for isethionate (- 20 V), 155 to 95 Da for DHPS (- 24 V), 243 to 123 Da for SQ (- 31 V) and 124 to 81 Da for taurine (- 30 V). Concentrations of the sulfonates were calculated using the Analyst Software (Version 1.6.2, AB Sciex) and by including reference standards of each substance at concentrations ranging from 0.1 to 600 ng/ml.

2.5.7 Quantification of bile acids in cecal contents

Bile acids were extracted from 50 mg cecal contents from six mice per group by homogenization in 450 µl acetonitrile and vortexing for 1 min. The resulting suspensions were centrifuged (13 000 x *g*, RT, 60 min), 400 µl of each supernatant mixed with 100 µl internal deuterium-labeled standards (10 µM d_4-CDCA and 10 µM d_4-LCA ISTD) and dried in a vacuum centrifuge (RCT 90 SpeedVac, Jouan Robotics, St Herblain, France) for 1 h. The residues were dissolved in 500 µl 50% aqueous methanol and transferred into a new plastic tube. Sample aliquots (5 µl) were injected into a 1260 Infinity HPLC (Agilent Technologies, Waldbronn, Germany) and separated using a YMC Triart C18 Plus column (3.0 × 100 mm, 3 µm; YMC, Kyoto, Japan) connected to a guard column (3.0 × 10 mm, 3 µm) of the same material. The mobile phase consisted of 10 mM ammonium acetate/acetonitrile (80:20, v/v, solvent A) and water/acetonitrile (20:80, v/v, solvent B). Each run started with 85% A for 6 min, followed by successive reduction of A to 70, 40 and 20% within 20, 30 and 40 min of total run-time, respectively. Six minutes later, the mobile phase was increased to 85% A again. The temperature of the column was set to 30 °C and the flow rate of the solvent was 0.2 ml/min. Simultaneously, the separated analytes were quantified using a 6490 triple-quadrupole MS (Agilent Technologies, Santa Clara, CA, USA) in negative electrospray ionization mode. The MS was operated with the following parameters: sheath gas temperature, 400 °C; sheath gas flow, 12 l/min of nitrogen; nebulizer pressure, 40 psi; drying gas temperature, 120 °C; drying gas flow, 15 l/min of nitrogen; capillary voltage, 4500 V; nozzle voltage, 0 V; iFunnel high pressure RF voltage, 90 V and iFunnel low pressure RF voltage, 60 V. Retention time, mass transition, collision energy and dwell time are listed for each compound in Table 6. Each bile acid was externally calibrated using standards ranging in their concentrations from 0.09 to 90 µM, while the internal standards were set at 10 µM. Resulting quantities were acquired (MassHunter Software, Agilent Technologies) and calculated relative to the wet weight of cecal content subjected to the extraction. These HPLC-MS/MS analyses were carried out by Dr. Fabian Schumacher at the Department of Toxicology, University of Potsdam, Germany.

Table 6: Parameters for HPLC-MS/MS measurements for the quantification of bile acids. The quantifier mass transition is underlined, [a] d_4-CDCA used as ISTD, [b] d_4-LCA used as ISTD. MCA: muricholic acid, GCA: glycocholic acid, TCA: taurocholic acid, CA: cholic acid, UDCA: ursodeoxycholic acid, GCDCA: glycochenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, TDCA: taurodeoxycholic acid, CDCA: chenodeoxycholic acid, DCA: deoxycholic acid, LCA: lithocholic acid.

Compound	Retention time (min)	Mass transition (*m/z*)	Collision energy (eV)	Dwell time (ms)
ω-MCA[a]	7.1	407.3 → 407.3	0	75
Tauro-β-MCA[a]	7.6	514.3 → 124.0 514.3 → 79.9	62 76	75
α-MCA[a]	7.9	407.3 → 407.3	0	75
β-MCA[a]	8.9	407.3 → 407.3	0	75
GCA[a]	11.9	464.3 → 402.2 464.3 → 73.9	40 44	75
TCA[a]	13.4	514.3 → 124.0 514.3 → 79.9	62 76	75
CA[a]	13.7	407.3 → 407.3	0	75
UDCA[a]	15.1	391.3 → 391.3	15	75
GCDCA[a]	18.8	448.3 → 386.3 448.3 → 74.1	40 42	75
GDCA[a]	20.2	448.3 → 404.3 448.3 → 74.1	36 42	75
TCDCA[a]	20.5	498.3 → 124.0 498.3 → 80.0	60 78	75
TDCA[a]	21.8	498.3 → 124.0 498.3 → 80.0	60 78	75
d_4-CDCA	23.2	395.3 → 395.3	15	75
CDCA[a]	23.3	391.3 → 391.3	15	75
DCA[a]	24.1	391.3 → 391.3	15	75
d_4-LCA	33.9	379.3 → 379.3	0	75
LCA[b]	33.9	375.3 → 375.3	0	75

2.6 Statistical analysis

The distribution of data was tested for normality using the Kolmogorov-Smirnov test. If a data set passed the normality test, it was presented as mean ± standard deviation (σ) and analyzed for statistically significant differences using the Student's *t*-test. If the data were not normally distributed, they are shown as median with lower and upper quartile (Q1 and Q3) and the Mann-Whitney U test was applied to identify statistically significant differences. Data presented in graphs are shown as values of individual mice and mean or median. Outliers were identified using the ROUT method (Q = 0.1%) and excluded. Data were considered significantly different at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. The software for statistical analyses was GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA, USA). For the analysis of β-diversity based on the *16S rRNA* gene sequencing data, generalized UniFrac was applied with *P*-values from the PERMANOVA test; the Bonferroni-Hochberg test was utilized to correct for multiple testing. Differences in the relative abundances of bacterial families were identified by performing pairwise Wilcoxon Rank Sum tests.

3 Results

3.1 *In vitro* testing of intestinal bacteria for their capacity to metabolize sulfonates

Intestinal murine bacterial communities and the SIHUMI consortium with or without *B. wadsworthia* were tested *in vitro* for their ability to convert SQ and TC to sulfide. These experiments aimed to clarify whether the dietary sulfonate SQ could serve as a substrate for potentially colitogenic bacteria, as previously demonstrated for TC [2].

3.1.1 The murine fecal microbiota converted SQ and TC to sulfide and the growth of sulfite-reducing bacteria was thereby stimulated

The utilization of the sulfite moiety of SQ and TC as terminal electron acceptor by murine fecal bacteria was investigated *in vitro*. Fecal suspensions from four IL-$10^{-/-}$ mice were incubated anoxically in duplicates for seven days in DS medium. Within this period of time, sulfide concentrations formed from SQ or TC by the fecal suspension were higher than those in fecal suspensions without SQ or TC addition (Fig. 6A). SQ concentrations remained stable when incubated without fecal suspension but declined in the presence of the bacteria. Fecal suspension without SQ or TC addition contained low concentrations of SQ, which were not altered during the incubation (Fig. 6B). The SQ detected in the fecal suspensions could either be due to methodological inaccuracies or result from the uptake of SQ or SQDG with the diet of the donor mice. TC was deconjugated to cholate by the fecal suspension, whereas cholate was not detected in incubations without feces. The level of TC remained stable when fecal material was omitted from the suspension (Table 7).

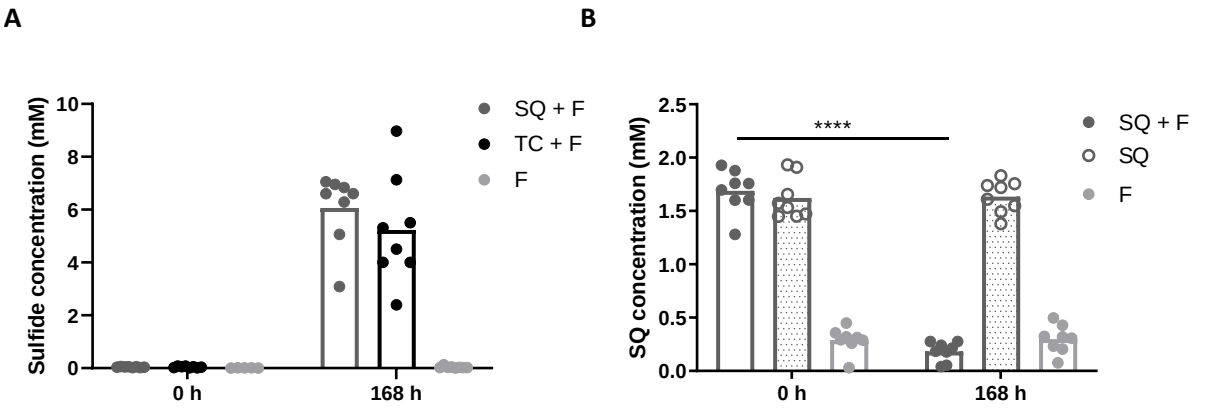


Figure 6: Quantification of sulfide and sulfoquinovose (SQ) in anoxic fecal incubations (F, 1%) with sulfonates at the beginning (0 h) and at the end (168 h) of the experiment. (A) Sulfide concentrations measured by the methylene blue method in fecal incubations with SQ (4 mM), taurocholate (TC, 20 mM) or without sulfonate. Sulfide was not detected in incubations with SQ or TC alone (data not shown). (B) SQ concentrations were determined using the DNS method. Data presented as individual values with mean, ** *P* < 0.0001, n = 4 in duplicate.**

Table 7: Estimation of taurocholate (TC) and cholate levels in incubations with TC (20 mM) and fecal suspension (F, 1%), TC without fecal suspension or in fecal incubations without TC after 0 and 168 h. TC and cholate were separated by thin layer chromatography and quantified relative to their respective 20 mM standards. N. d.: not detected. Data shown as mean ± σ, n = 4 in duplicate.

Group	Time (h)	Taurocholate (%)	Cholate (%)
TC + F	0	70 ± 30	n. d.
	168	n. d.	82 ± 26
TC	0	76 ± 28	n. d.
	168	71 ± 22	n. d.
F	0	4 ± 4	n. d.
	168	3 ± 4	n. d.

The cell numbers of *B. wadsworthia* and of sulfite-reducing bacteria increased to a larger extent when SQ or TC had been added to the fecal suspension compared to the fecal suspension alone (Fig. 7). This suggested that SQ and TC stimulated the growth of bacteria in the suspension. Since the abundance of *dsrA*-carrying bacteria was equal to that of *B. wadsworthia*, it can be assumed that this bacterium was the most prominent sulfite reducer in the murine fecal suspension. Cell numbers of *E. coli* were below the detection limit (data not shown), even though it was reported that *E. coli* is able to utilize SQ as a growth substrate [40].

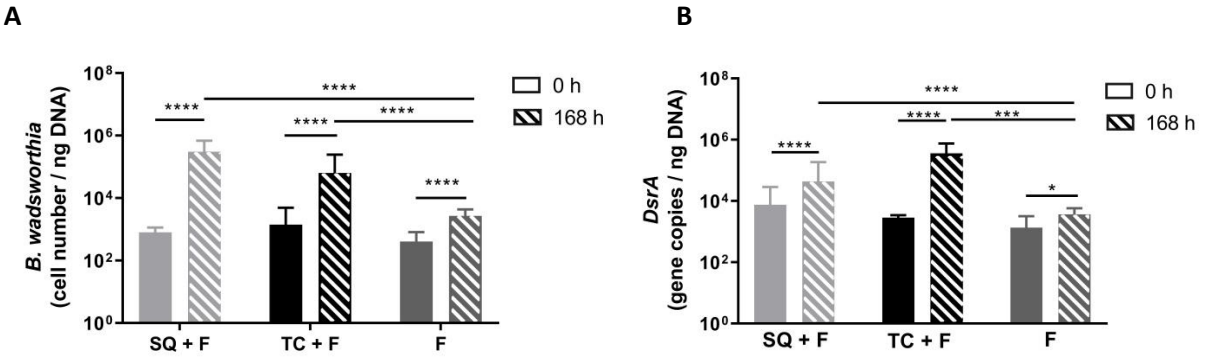


Figure 7: Bacterial abundance in fecal suspensions (F, 1%) at the beginning of the incubation (0 h, plain bars) and after 168 h of incubation (dashed bar) following the addition of sulfoquinovose (SQ, 4 mM) or taurocholate (TC, 20 mM). Fecal suspensions without SQ or TC and medium containing SQ or TC without the addition of feces served as controls. Abundance of (A) *B. wadsworthia* and (B) bacteria equipped with the gene for the dissimilatory sulfite reductase A (*dsrA*) were quantified by qPCR. Data shown as mean + σ;P* < 0.05, ****P* < 0.001, *****P* < 0.0001, n = 4 in duplicate.**

3.1.2 SIHUMI bacteria converted SQ and TC to sulfide only in the presence of *B. wadsworthia*

Incubation experiments with the SIHUMI consortium in the presence or absence of *B. wadsworthia* were conducted on the one hand to find out whether the bacterial communities are able to reduce SQ and TC to sulfide and on the other hand to analyze the rates of conversion. Therefore, SIHUMI bacteria with or without *B. wadsworthia* (Bw) were incubated anoxically with SQ or TC in duplicates. Bacterial growth as quantified by optical density measurements at 600 nm (OD_{600}) was independent of the sulfonates, since growth was detected in SIHUMI and SIHUMI + Bw incubations without SQ or

TC addition (Fig. 8). However, the increase in the OD_{600} was larger in the presence of SQ and partly of TC; and highest values were reached in the presence of *B. wadsworthia*.

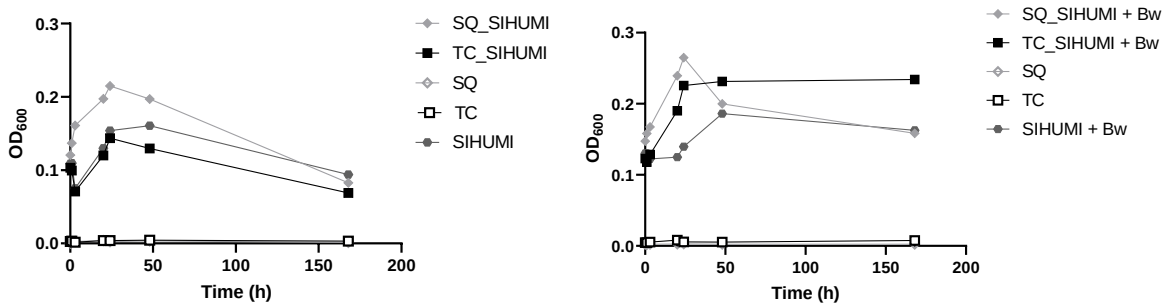


Figure 8: Cell growth determined by OD_{600} measurements in incubations of SIHUMI bacteria and SIHUMI + *B. wadsworthia* (Bw) with sulfoquinovose (SQ, 4 mM) or taurocholate (TC, 20 mM), incubations without sulfonates and incubations without bacteria. Data shown as mean, n = 2.

Sulfide formation would indicate reduction of sulfite released from SQ and TC to sulfide. However, sulfide formation was not observed in the SIHUMI incubations without *B. wadsworthia* indicating that the SIHUMI members alone were not able to liberate sulfide from SQ or TC. In SIHUMI + Bw incubations with TC or SQ added, on average 69% of TC (after 48 h) and 46% of SQ (after 168 h) were reduced to sulfide (Fig. 9).

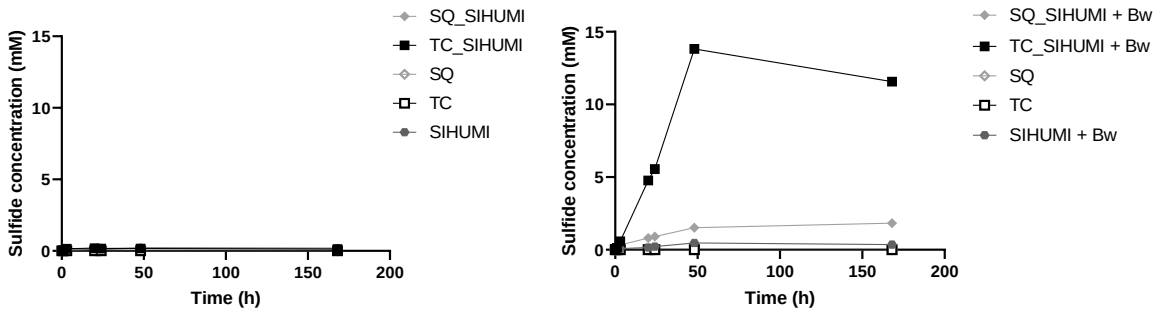


Figure 9: Sulfide formation in incubations of SIHUMI with *B. wadsworthia* (SIHUMI + Bw) added or not following the addition of sulfoquinovose (SQ, 4 mM) or taurocholate (TC, 20 mM) measured by methylene blue method. Data shown as mean, n = 2.

3.1.3 SIHUMI bacteria degraded SQ and TC independently of the presence of *B. wadsworthia*

Even though no sulfide was formed in SIHUMI incubations with SQ or TC, both sulfonates were degraded. SQ concentrations declined in SIHUMI and SIHUMI + Bw incubations with a similar rate, while the SQ concentration in bacteria-free incubations remained stable (Fig. 10). The intermediate DHPS was exclusively detected in SIHUMI incubations with SQ after 24 h (0.72 mM) and after 48 h (1.24 mM), indicating that at least one member of the SIHUMI consortium, most probably *E. coli*, converted SQ to DHPS but not further to sulfide. DHPS was not quantified at 168 h, leaving the possibility that even more DHPS was formed until the end of the experiment.

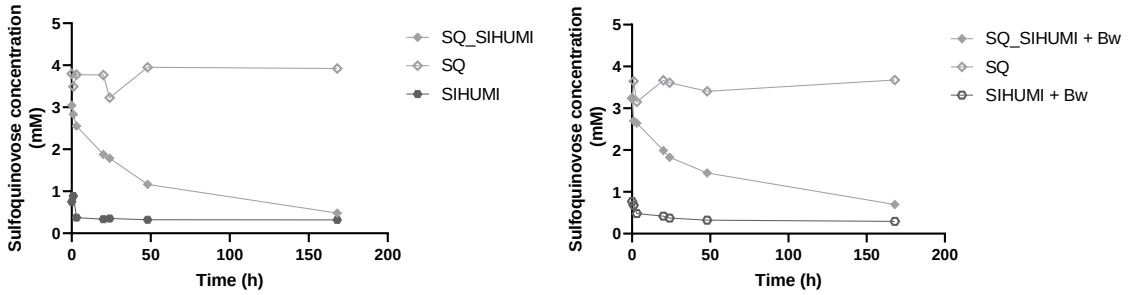


Figure 10: Concentrations of sulfoquinovose (SQ) in SIHUMI suspensions in the presence of *B. wadsworthia* (SIHUMI + Bw) or its absence following the addition of SQ (4 mM). Incubations with SQ but without bacteria served as control. The SQ concentrations were determined by the DNS method. Data shown as mean, n = 2.

The results presented in Table 8 show that TC was partly cleaved and cholate formed in incubations with the SIHUMI consortium containing in addition *B. wadsworthia* or not. Complete disappearance of TC within 168 h was solely detected in the presence of *B. wadsworthia*. Meanwhile, bacteria-free incubations with TC showed no decline in its concentration.

Table 8: Estimation of taurocholate (TC) levels in incubations of SIHUMI bacteria (B) in the absence or presence of *B. wadsworthia* (SIHUMI + Bw) with 20 mM TC added, incubations only with TC and incubations without TC. TC and cholate were separated by thin layer chromatography and quantified as percentage of 20 mM standards. Data shown as mean, n = 2, n. d.: not detected.

Group		SIHUMI		SIHUMI + Bw	
	Time (h)	TC (%)	Cholate (%)	TC (%)	Cholate (%)
TC + B	0	111	n. d.	71	n. d.
	1	103	n. d.	81	n. d.
	3	107	5	61	n. d.
	20	121	3	71	34
	24	94	n. d.	35	35
	48	82	41	n. d.	58
	168	51	33	n. d.	60
TC	0	112	n. d.	81	n. d.
	1	89	n. d.	72	n. d.
	3	124	n. d.	90	n. d.
	20	114	n. d.	69	n. d.
	24	85	n. d.	51	n. d.
	48	90	n. d.	81	n. d.
	168	98	n. d.	79	n. d.
B	0	n. d.	n. d.	n. d.	n. d.
	1	n. d.	n. d.	n. d.	n. d.
	3	n. d.	n. d.	n. d.	n. d.
	20	n. d.	n. d.	n. d.	n. d.
	24	n. d.	n. d.	n. d.	n. d.
	48	n. d.	n. d.	n. d.	n. d.
	168	n. d.	n. d.	n. d.	n. d.

Taurine was formed after 24 h (0.29 mM) in one SIHUMI incubation containing TC and in both replicates after 48 h with a mean value of 0.28 mM. In SIHUMI + Bw incubations containing TC, taurine was detected in only one replicate (3.88 mM after 48 h). This further underlines the ability of the SIHUMI consortium to cleave TC to taurine and cholate no matter whether *B. wadsworthia* is present or not. Bacterial degradation of taurine under anoxic conditions is rare [48] and only few

mechanisms of sulfur assimilation from taurine have been described. *B. wadsworthia* and a member of the SIHUMI consortium, namely *C. butyricum*, were identified to possess the gene cluster necessary for taurine degradation [49]. This degradation results in the formation of sulfite and acetaldehyde, with sulfoacetaldehyde and isethionate as intermediates. Therefore, the formation of isethionate was monitored, but it was solely detected in incubations with TC but without bacteria (0 h: 0.43 mM and 3 h: 0.21 mM) and in one SIHUMI + Bw incubation with TC after 48 h (0.70 mM). The first two values can only be explained by impurities, even though the impurity was not detected in any other set-up. Isethionate detected in SIHUMI + Bw incubations could be an intermediate of the conversion of taurine to sulfide by *B. wadsworthia* [111].

3.1.4 Growth of the SIHUMI bacteria and *B. wadsworthia* was partly affected by SQ and TC

SQ slightly promoted the growth of *B. wadsworthia* in incubations of SIHUMI + Bw (Fig. 11A) but did not affect the cell number of *E. coli* (Fig. 11B) other than observed in the SIHUMI + Bw incubations without the sulfonate. Addition of TC to SIHUMI + Bw stimulated the growth of *B. wadsworthia* which was not the case when the sulfonate was omitted (Fig. 11A), but reduced the abundance of *E. coli* within 168 h (Fig. 11B). The cell numbers of the remaining SIHUMI bacteria were not affected by SQ or TC in incubations with or without *B. wadsworthia* (Appendix II).

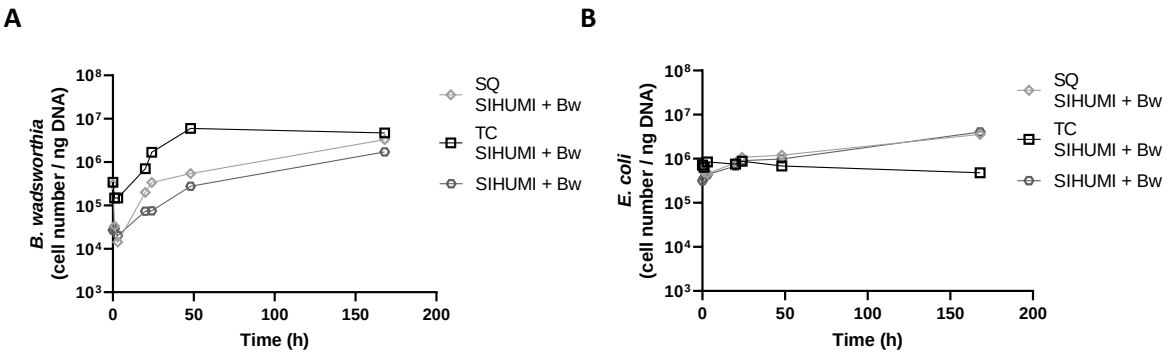


Figure 11: Bacterial abundance in incubations of SIHUMI with *B. wadsworthia* (SIHUMI + Bw) and sulfoquinovose (SQ, 4 mM) or taurocholate (TC, 20 mM) and incubations without sulfonates after 168 h. Cell numbers of (A) *B. wadsworthia* and (B) *E. coli* were determined by q PCR and presented as mean, n = 2.

3.2 Spirulina feeding experiments in conventional IL-10$^{-/-}$ mice

To assess the effects of the SQDG-rich cyanobacterium Spirulina (*A. platensis*) on host health status and intestinal microbiota, experiments in conventional IL-10$^{-/-}$ mice fed a SD or a CD were carried over three weeks. Both diets had a similar macronutrient composition as assessed by the Weender analysis (Appendix II) and the energy content of the SD (17.3 kJ/g) was similar to that of the CD (16.9 kJ/g).

3.2.1 Spirulina feeding initially increased the body weight and feed intake of the mice

The murine body weight development was monitored to clarify whether inflammation-associated weight loss occurs [112]. However, consumption of this diet supplemented with 20% Spirulina increased the body weight and feed intake of the mice within the first three days of the intervention (Fig. 12). However, thereafter the body weight did not increase any further in the SD mice and did

not differ from that of the CD mice at the end of the intervention (Fig. 12A). Feed intake of the SD mice declined in the following days and was not different from that of the CD mice from day seven until the end of the intervention (Fig. 12B).

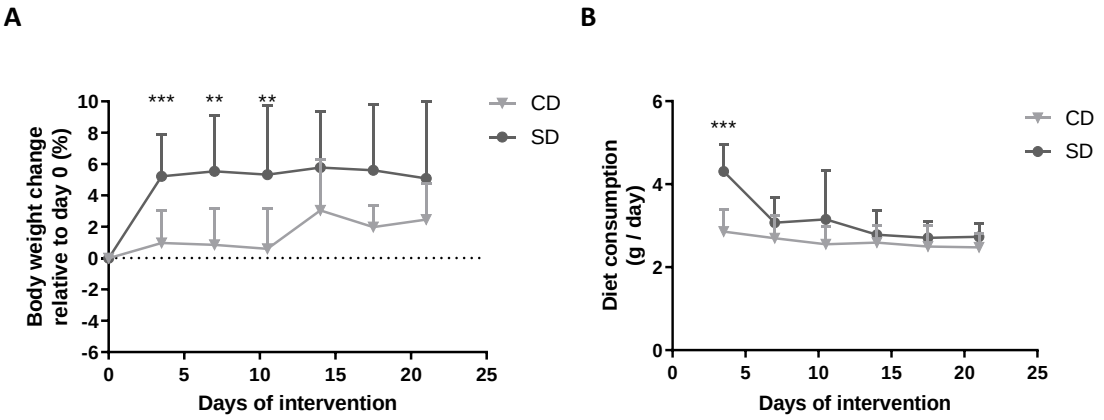


Figure 12: Effects of Spirulina diet (SD) feeding on body weight and diet consumption of conventional IL-10$^{-/-}$ mice in comparison to control diet (CD)-fed mice. (A) Body weight development relative to day zero of the intervention. (B) Diet consumption calculated as gram per mouse and day. Data are presented as mean + σ, ** *P* < 0.01, * *P* < 0.001, n = 12.**

3.2.2 Spirulina diet feeding evoked a mild immune response in IL-10$^{-/-}$ mice

The colon permeability was investigated by performing a FITC-dextran test and by quantifying the relative expression of the *Tjp 1* gene and the *Ocln* gene in cecal and colonic mucosa. Both proteins are integral members of tight-junctions sealing the epithelial mono-layer to prevent invasion of bacteria and dietary components. Downregulation of these proteins or their respective genes would indicate an increased epithelial permeability [113]. An increased colonic permeability would allow bacteria to penetrate the epithelial layer and enter the blood stream, thereby evoking an inflammatory response [114]. Evaluation of the FITC-dextran test was not indicative of a higher permeability caused by SD feeding (Fig. 13). However, the expression of *Ocln* was lower in cecum mucosa of SD compared to CD fed mice (Table 9).

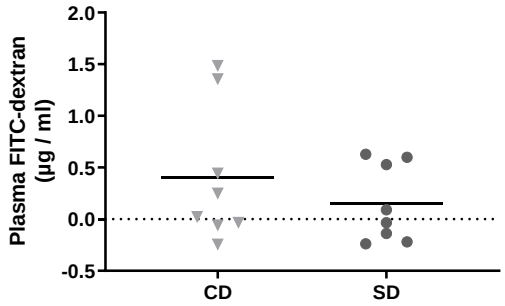


Figure 13: Plasma concentration of FITC-dextran following oral FITC-dextran treatment in conventional IL-10$^{-/-}$ mice fed a Spirulina diet (SD) or a control diet (CD) for three weeks. Data are shown as values of individual animals with mean (n = 8 per group treated with FITC-dextran).

Table 9: Relative gene expression of tight junction protein (*Tjp) 1* and occludin (*Ocln)* in cecal and colonic mucosa of conventional IL-10$^{-/-}$ mice fed a Spirulina diet (SD) or a control diet (CD) for three weeks. Data are presented as median [Q1 – Q3], **P* < 0.05, n = 12.

	CD		SD	
	Cecum	**Colon**	**Cecum**	**Colon**
Tjp 1	1.00 [0.94 – 1.18]	1.00 [0.80 – 1.13]	0.95 [0.76 – 1.14]	1.05 [0.86 – 1.30]
Ocln	1.00 [0.87 – 1.05]	1.00 [0.88 – 1.11]	0.63 * [0.58 – 0.76]	0.93 [0.69 – 1.10]

The assessment of inflammation-associated alterations in cecal and colonic tissue revealed higher cecal histopathology scores in SD mice than in control mice (Fig. 14A), which was mostly due to immune cell infiltration into the lamina propria of SD-fed mice. However, the colonic histopathology scores did not differ between the groups (Fig. 14B).

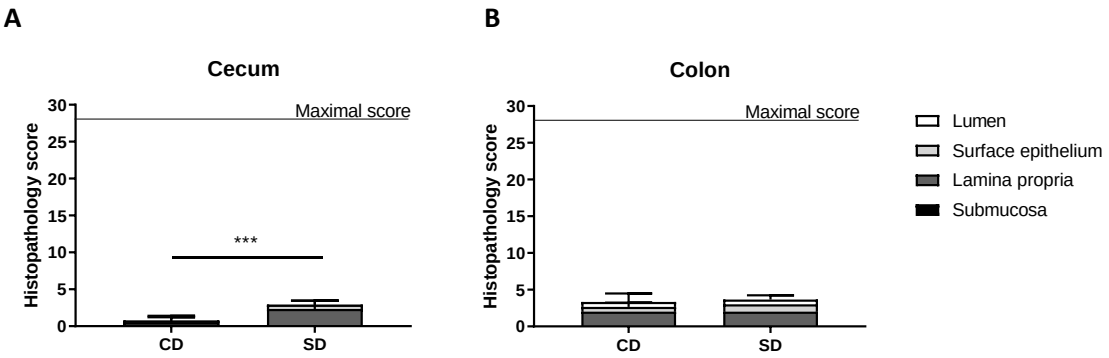


Figure 14: Intestinal histopathology scores of conventional IL-10$^{-/-}$ mice fed the Spirulina diet (SD) and control diet (CD). Lumen, surface epithelium, lamina propria and submucosa were separately scored and their mean values added up to a single value presented as one column per group. Assessment of (A) cecal tissue preparations (n = 12) and (B) of colonic tissue preparations (n = 3). Data shown as mean + σ, ****P* < 0.001.

The degree of local inflammation was assessed by gene expression analysis of cytokines in intestinal mucosa. Alterations of cytokine concentrations in response to Spirulina feeding have been reported previously [31]. In the present study, the expression of *Ifn-γ, Il-1β, Il-6* and *Il-12α* was not altered in cecal or colonic mucosa of conventional SD mice in comparison to CD mice. Solely the expression of *Tnf-α* was 2.38-fold higher in cecum and 3.26-fold higher in colon mucosa of SD mice relative to CD mice (Table 10).

Table 10: Gene expression analysis by q PCR of cytokines in cecal and colonic mucosa of conventional IL-10$^{-/-}$ mice fed the Spirulina diet (SD) or control diet (CD) for three weeks. *Ifn-γ*: interferon-gamma gene, *Tnf-α*: tumor necrosis factor-alpha gene, *Il*: interleukin genes. Data are shown as median [Q1 – Q3], **P* < 0.05, *****P* < 0.0001, n = 12.

	CD		SD	
Gene	**cecum**	**colon**	**cecum**	**colon**
Ifn-γ	1.00 [0.63 – 2.59]	1.00 [0.52 – 1.50]	1.85 [0.86 – 3.49]	0.48 [0.31 – 0.68]
Tnf-α	1.00 [0.66 – 1.31]	1.00 [0.82 – 1.03]	2.38 * [1.86 – 6.05]	3.26 **** [2.22 – 3.87]
Il-1β	1.00 [0.69 – 4.16]	1.00 [0.65 – 1.79]	0.97 [0.82 – 2.82]	0.51 [0.30 – 1.03]
Il-6	1.00 [0.58 – 1.65]	1.00 [0.37 – 3.31]	0.83 [0.79 – 1.20]	0.60 [0.33 – 0.85]
Il-12α	1.00 [0.69 – 1.44]	1.00 [0.44 – 2.38]	1.20 [0.74 – 1.56]	1.14 [0.53 – 2.17]

Gut inflammation has been described to cause macroscopic alterations of intestinal organs. As an example, the colon was found to be shorter in mice treated with DSS and TNBS compared to untreated mice [115]. However, no differences in small intestinal or colonic length were detected, but the cecum was lighter in SD mice compared to CD mice (Fig. 15).

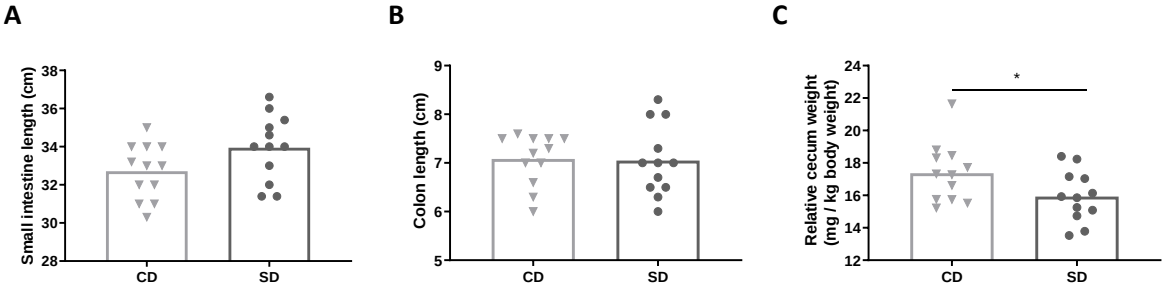


Figure 15: Characteristics of intestinal organs from IL-10$^{-/-}$ mice after three weeks of feeding the Spirulina diet (SD) or the control diet (CD). Absolute length of (A) the small intestine, (B) the colon and (C) weight of the cecum relative to the body weight are displayed. Data shown as values of individual mice with median, **P* < 0.05, n = 12.

The hypothesized growth stimulation of *B. wadsworthia* by the dietary sulfonate SQDG (which is a prominent sulfolipid in Spirulina) could have substantial effects on visceral organs and fat mass. It has previously been shown that the accumulation of *B. wadsworthia* in the gut increased the liver and spleen weights and reduced the fat mass of SPF wild type mice within seven days [21]. However, the relative weight of spleen, kidney and mWAT as well as the area of the mLN did not differ between the groups in the present study. The relative gall bladder weight was higher and the relative liver weight lower in SD-fed mice compared to CD-fed mice (Table 11). The microscopic examination of liver, spleen, kidney and small intestinal tissue slices did not indicate any abnormal tissue alterations (data not shown).

Table 11: Measures of organs and tissues of conventional IL-10$^{-/-}$ mice fed the control diet (CD) or the Spirulina diet (SD) for three weeks. Liver, spleen, kidney, mesenteric white adipose tissue (mWAT) and gall bladder weights are given relative to body weight. Mesenteric lymph node (mLN) area is based on length and width measurements and given per mouse. Data presented as median [Q1 – Q3], *$P < 0.05$, **$P < 0.01$, n = 12.

Mouse group	Liver (mg/g)	Spleen (mg/g)	Kidney (mg/g)	mWAT (mg/g)	Gall bladder (mg/g)	mLN (mm²)
CD	59.30 [54.76 – 63.50]	2.88 [1.63 – 4.03]	6.68 [6.54 – 7.45]	10.96 [8.46 – 13.12]	0.29 [0.18 – 0.42]	49.77 [32.10 – 104.10]
SD	53.37 ** [51.61 – 55.73]	3.22 [3.02 – 3.84]	7.15 [6.86 – 7.45]	13.16 [10.50 – 13.89]	0.60 * [0.30 – 0.74]	73.83 [46.06 – 135.90]

Inflammatory markers in plasma samples were quantified to assess the systemic immune response. An increase in the concentration of these markers in serum of SPF wild type mice orally treated daily with *B. wadsworthia* for seven days was reported before [21]. In the present study, the plasma concentration of none of the analyzed markers from SD-fed IL-10$^{-/-}$ mice differed from those of the control group (Table 12).

Table 12: Inflammatory markers in plasma of conventional IL-10$^{-/-}$ mice fed the Spirulina diet (SD) or control diet (CD) for three weeks. IFN-ɣ: interferon-gamma, TNF-α: tumor necrosis factor-alpha, LCN 2: lipocalin 2, SAA: serum amyloid A, n. d.: not detected. Data are shown as median [Q1 – Q3], n = 3 – 4.

Mouse group	IFN-ɣ (pg/ml)	TNF-α	LCN 2 (ng/ml)	SAA (µg/ml)
CD	22.47 [16.81 – 27.63]	n. d.	67.72 [59.71 – 77.31]	19.83 [8.94 – 28.49]
SD	19.54 [16.21 – 29.31]	n. d.	159.41 [90.90 – 359.40]	34.78 [25.13 – 73.90]

3.2.3 The intestinal microbiota was altered due to Spirulina feeding

Oral administration of Spirulina was shown to have diverse effects on intestinal microbiota composition [116, 117]. According to the hypothesis investigated in the current PhD-project, the growth of bacteria involved in the degradation of SQDG would be stimulated by Spirulina feeding. However, the cell number of *B. wadsworthia* was lower in SD-fed mice compared to CD-fed mice in cecum and colon contents and in feces from day 18 (Fig. 16A). This indicates that either SQDG is not converted to DHPS or DHPS formed in the intestine is not used as a growth substrate by *B. wadsworthia*. It is also conceivable that another ingredient of the SD suppresses the growth of *B. wadsworthia*. Similarly, the abundance of sulfite-reducing bacteria was lower in cecum and colon of SD-fed mice than in CD-fed mice (Fig. 16B). SQ-converting bacteria were rarely detected and their absolute abundance did not differ between the groups (Fig. 16C). This indicated that neither sulfite-reducing nor SQ-converting bacteria profit from the SQDG content of the SD.

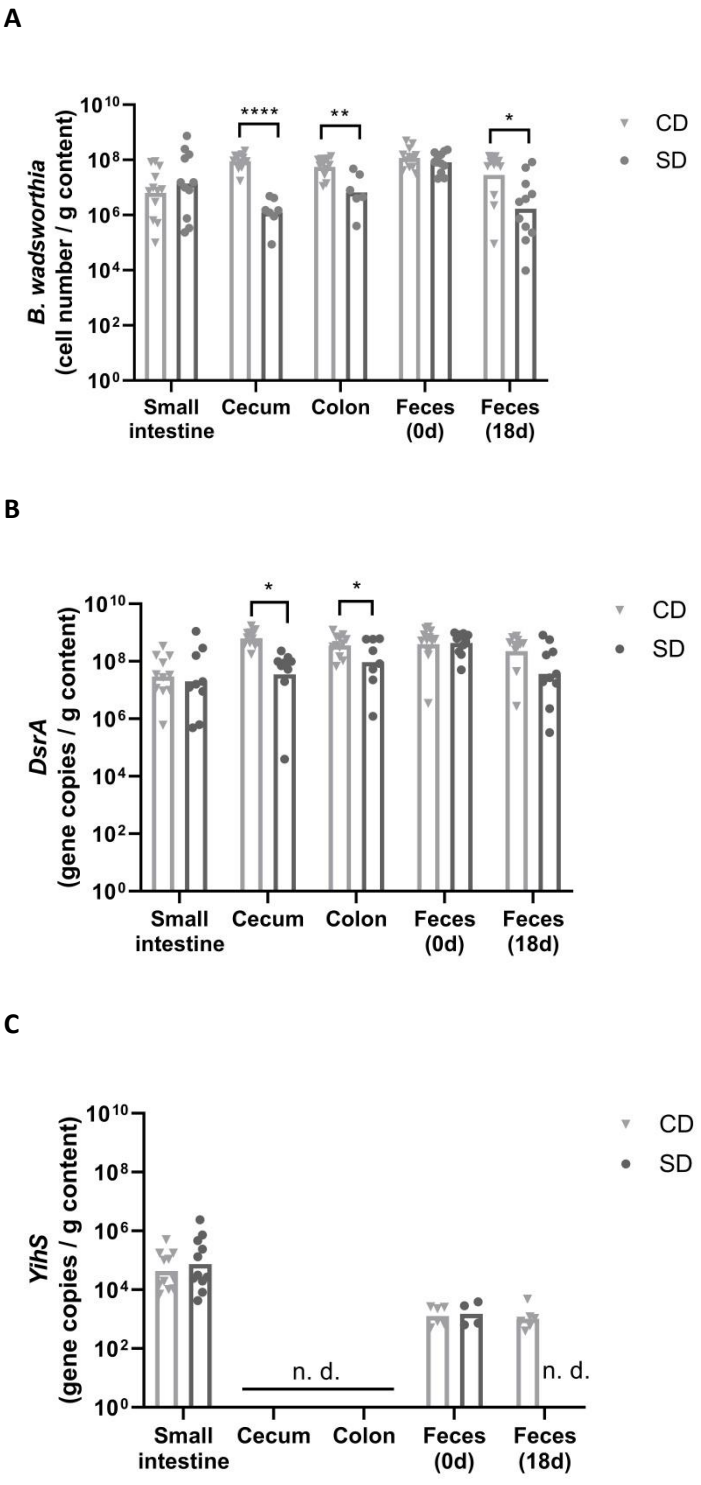


Figure 16: Quantification of *B. wadsworthia*, sulfite-reducing and sulfoquinovose-converting bacteria in intestinal contents and feces (0 and 18 d) of IL-10$^{-/-}$ mice fed the Spirulina diet (SD) or control diet (CD) for three weeks. Cell numbers and gene copies were determined by qPCR. (A) *B. wadsworthia* was detected by species-specific primers targeting the *16S rRNA* gene. (B) Bacteria equipped with the dissimilatory sulfite reductase A (*dsrA*) gene and (C) bacteria equipped with the SQ isomerase (*yihS*) gene were detected by gene-specific primers. Data shown as values of individual animals with median, **P* < 0.05, ***P* < 0.01, *****P* < 0.0001, n = 12, n. d.: not detected.

In accordance with these results the relative abundance of the *Desulfovibrionaceae*, which include *B. wadsworthia*, was lower in SD-fed mice compared with CD-fed mice (Fig. 17A). Furthermore, the

relative abundance of *Porphyromonadaceae* in feces and cecal contents was higher in SD-fed mice than in CD-fed mice (Fig. 17B). There were no further differences in the abundance of other bacterial taxa between the two groups (data not shown).

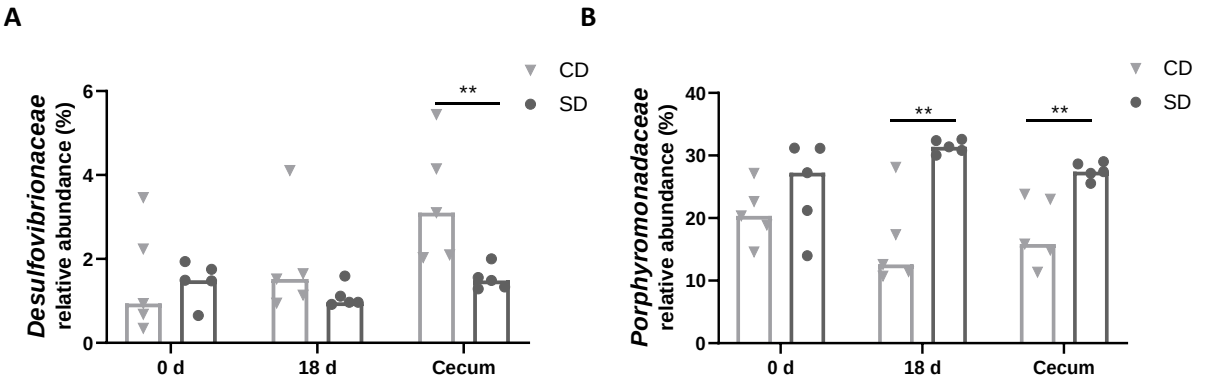


Figure 17: Relative bacterial abundance in feces from day zero (0 d) and day 18 (18 d) and cecal contents of conventional IL-10$^{-/-}$ mice fed the Spirulina diet (SD) or control diet (CD) for three weeks. The abundance of (A) *Desulfovibrionaceae* and (B) *Porphyromonaceae* were quantified by partial *16S rRNA* gene sequencing. Data shown for each animal with mean, ** *P* < 0.01, n = 5.

The microbiota composition was affected by Spirulina feeding as shown by NMDS plots based on bacterial profiles in feces (Fig. 18A, B, C, D) and cecal contents (Fig. 18E). No differences were observed between SD and CD mice at the beginning of the intervention (Fig. 18 A). While the profiles in feces from CD mice did not change from day 0 to day 18 (Fig. 18B), alterations were observed in feces from SD mice of day 0 to 18 (Fig. 18C). These differences were also detectable in feces (18 d) or cecal contents of SD and CD mice (Fig. 18D, E). However, bacterial richness and α-diversity did not differ between or within SD- and CD-fed mice (data not shown).

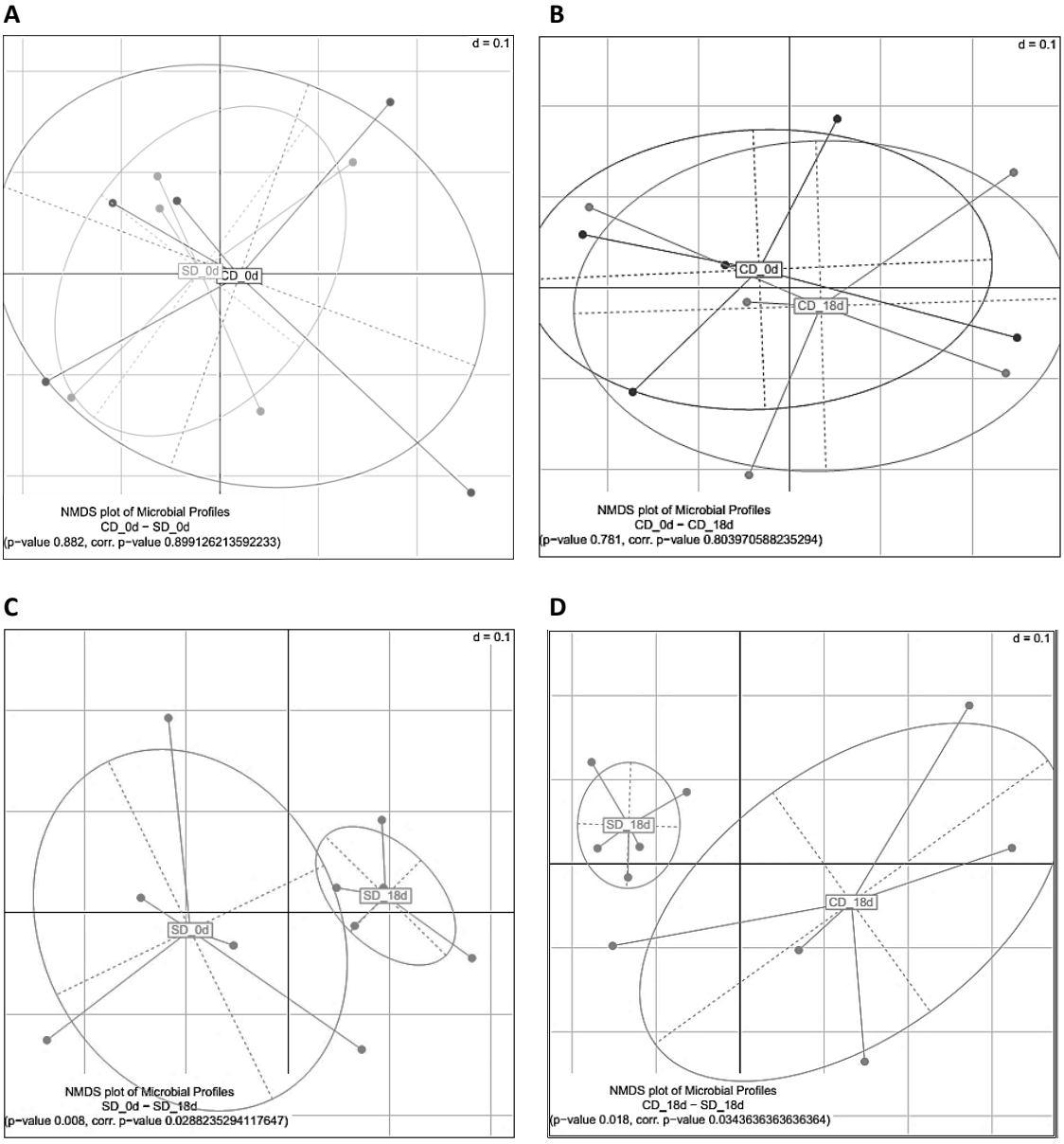
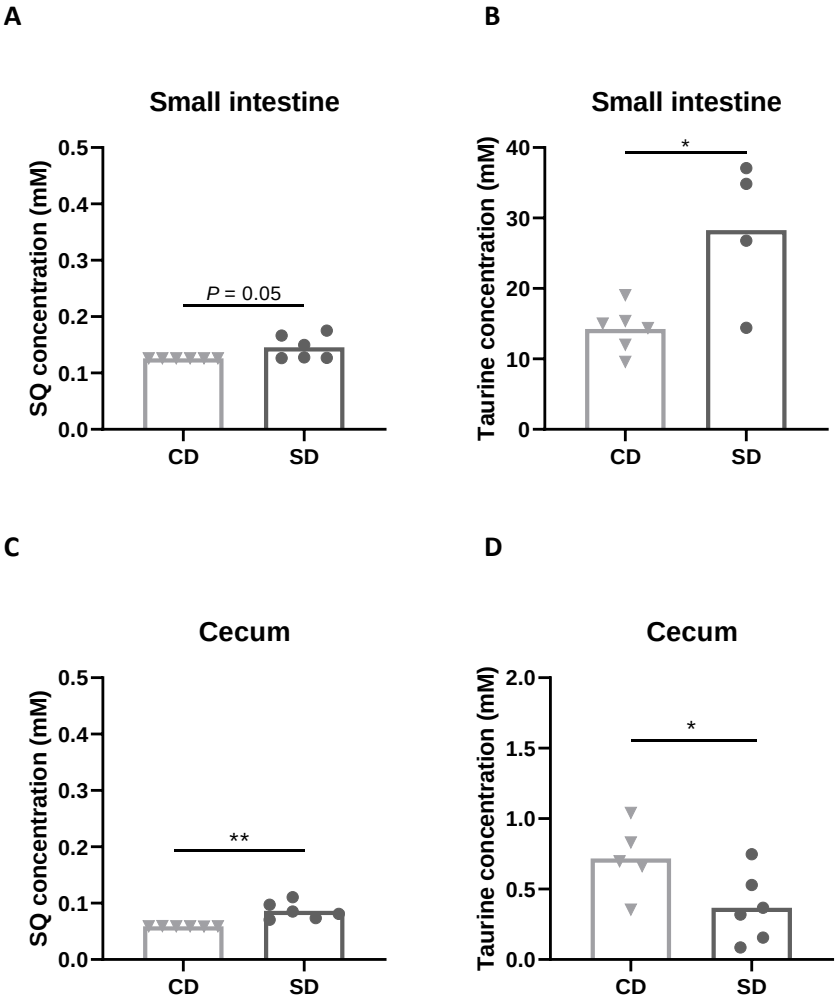


Figure 18: Microbial profiles in feces (0d and 18d) and cecal contents of conventional IL-10$^{-/-}$ mice fed Spirulina diet (SD) or control diet (CD) for three weeks. Plots were generated by *16S rRNA* gene-sequence analyses. Comparison of (A) SD and CD mice at day 0 in feces, (B) CD mice at days 0 and 18 in feces, (C) SD mice at days 0 and 18 in feces, (D) SD and CD mice at day 18 in feces and (E) SD and CD mice at day 21 in cecal contents. Data presented in non-metric multi-dimensional scaling (NMDS) plots, n = 5.

3.2.4 SD feeding increased the concentration of SQ in intestinal contents and feces

SQ, DHPS, taurine and isethionate were quantified in intestinal contents and feces to find out, whether their concentration in the gut was higher due to SD feeding. These sulfonates could then serve as sulfite-source for intestinal bacteria. The fate of SQDG has only been investigated in guinea pigs, which were orally treated with radioactively labeled SQDG [42]. However, the authors did not determine SQ and they only analyzed the mucosa of the small intestine, not the colon. Therefore, the investigations of the present study provide new insights into the delivery of substrates for bacterial metabolism through the diet. The SQDG-rich SD resulted in somewhat higher SQ concentrations in cecal and colonic contents and feces on day 18 compared to the CD, with the highest value of 0.28 mM reached in the latter (Fig. 19A, C, E, G, I). Taurine concentrations were higher in the small intestine (up to 37.08 mM) but lower in the cecum of SD mice compared to CD mice. Taurine was not detected in every sample (Fig. 19B, D, F, H, J). DHPS was detected in the small intestine of only two SD mice (1.83 and 0.39 mM) and in colonic contents of one of these mice (6.48 mM), while isethionate was not detected in any of the mice (data not shown).



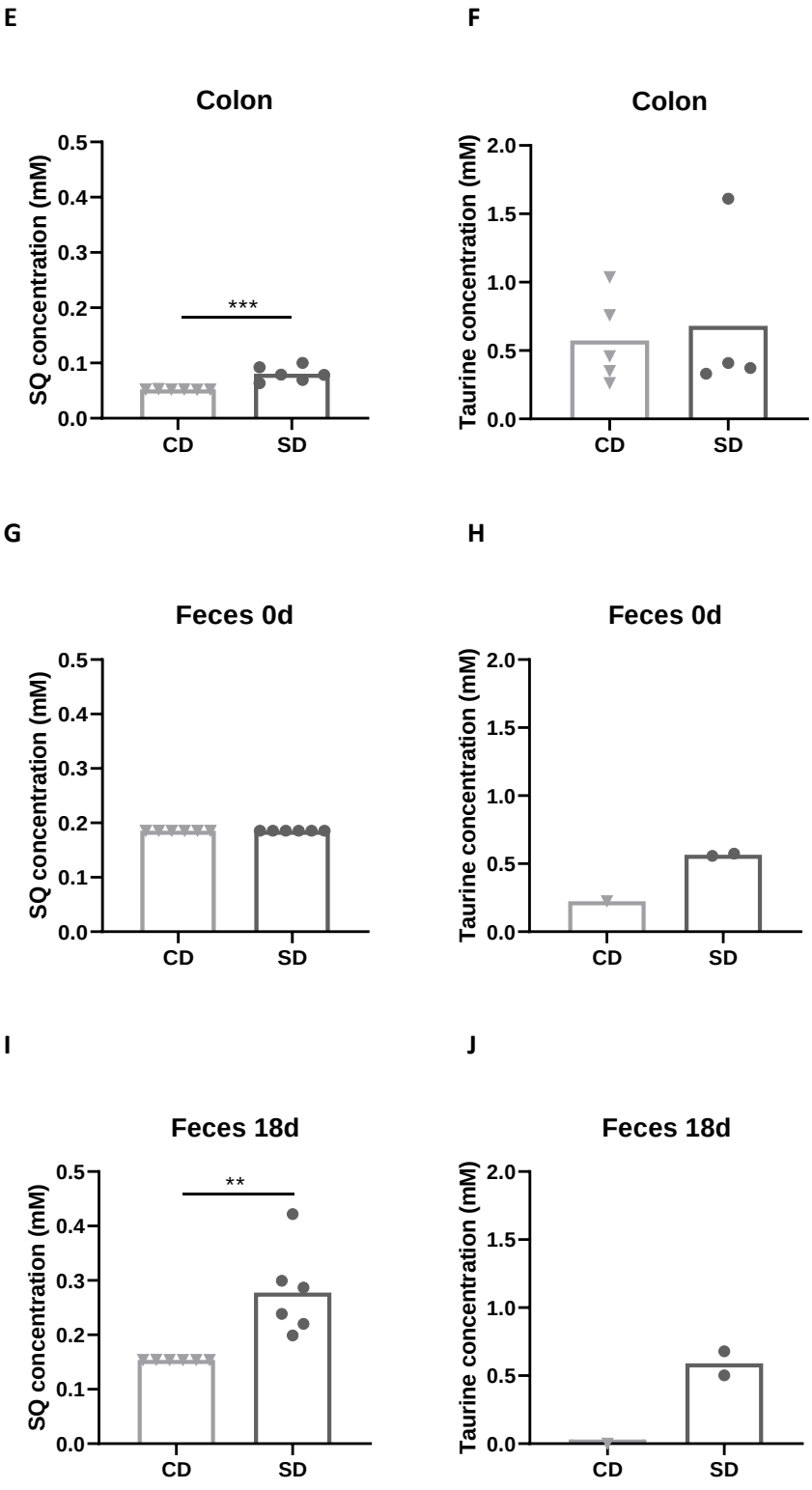


Figure 19: Sulfoquinovose (SQ) and taurine concentrations in intestinal contents and feces from conventional IL-10$^{-/-}$ mice fed the Spirulina diet (SD) or the control diet (CD) for three weeks. SQ in (A) small intestine, (C) cecum, (E) colon, (G) feces from day zero and (I) feces from day 18. Taurine in (B) small intestine, (D) cecum, (F) colon, (H) feces from day zero and (J) feces from day 18. Note that taurine was not detected in all samples. Data presented as values for individual mice and mean, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n = 6.

3.2.5 The Spirulina diet did not increase sulfide detoxification in cecum or colon

The postulated reduction of dietary sulfonates to sulfide by intestinal bacteria would increase the intestinal sulfide concentration, which would reduce the mitochondrial ATP production and trigger various detoxification processes [59]. The latter include the elevation of the *Sqr* expression and, if a certain threshold is overcome, the induction of hypoxia in epithelial mitochondria followed by transcription of the *Hif-1α* gene. Therefore, the expression of both genes was assessed in cecal and colonic mucosa to get an indirect measure for the intestinal sulfide concentration. However, analysis of the *Sqr* and *Hif-1α* expression was not indicative of sulfide concentrations exceeding the physiological nanomolar range in cecum or colon or of a hypoxic state of the mitochondria in the mucosa of these intestinal sites in SD-fed versus CD-fed mice (Table 13).

Table 13: Relative expression of *Sqr* and *Hif-1α* in cecal and colonic mucosa of conventional IL-10$^{-/-}$ mice fed a Spirulina diet (SD) or control diet (CD) for three weeks. *Sqr*: sulfide:quinone oxidoreductase, *Hif-1α*: hypoxia-inducible factor-1alpha. Data are presented as mean ± σ, n = 12.

	CD		SD	
	Cecum	**Colon**	**Cecum**	**Colon**
Sqr	1.00 ± 0.16	1.00 ± 0.25	0.88 ± 0.20	1.18 ± 0.22
Hif-1 α	1.00 ± 0.46	1.00 ± 0.38	0.66 ± 0.27	1.04 ± 0.24

3.2.6 Conversion of primary to secondary bile acids was increased in SD-fed mice

The bile acid pool is a diet-mediated factor that may affect the intestinal microbiota [1]. Accumulation of secondary bile acids has also been linked to a number of diseases such as gallstones and colon cancer [25]. Changes in bile-acid composition contribute to the initiation of inflammation in high-fat diet-fed IL-10$^{-/-}$ mice [2]. Due to the various effects associated with alterations in the bile-acid composition, investigation of the impact of a dietary intervention on bile-acid composition is necessary. In the present study, the cecal bile-acid profile of CD- and SD-fed mice revealed a higher proportion of unconjugated versus conjugated bile acids in both groups and higher concentrations of the secondary bile acids DCA (2.4-fold) and LCA (2.7-fold) in SD-fed mice than in CD-fed mice (Table 14).

Table 14: Concentrations of cecal primary and secondary bile acids of conventional IL-10$^{-/-}$ mice fed a Spirulina diet (SD) or control diet (CD) for three weeks. Values were determined by HPLC-MS. CA: cholic acid, TCA: taurocholic acid, GCA: glycocholic acid, CDCA: chenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, GCDCA: glycochenodeoxycholic acid, UDCA: ursodeoxycholic acid, MCA: muricholic acid, T-β-MCA: tauro-beta-MCA, DCA: deoxycholic acid, TDCA: taurodeoxycholic acid, GDCA: glycodeoxycholic acid, LCA: lithocholic acid, n. d.: not detected. Data presented in nmol per g content as median [Q1 – Q3], **$P < 0.01$, n = 6.

	CD	SD
Primary bile acids (nmol / g)		
CA	196.79 [25.88 – 503.95]	69.08 [29.84 – 103.58]
TCA	0.42 [0.12 – 0.78]	0.21 [0.15 – 0.28]
GCA	0.02 [0.00 – 0.05]	n. d.
CDCA	n. d.	n. d.
TCDCA	0.04 [0.02 – 0.06]	n. d.
GCDCA	n. d.	n. d.
α-MCA	64.85 [19.90 – 103.02]	60.61 [50.76 – 82.43]
β-MCA	92.19 [37.58 – 197.85]	93.44 [69.18 – 118.65]
T-β-MCA	0.51 [0.12 – 1.15]	0.39 [0.30 – 0.57]
Secondary bile acids (nmol / g)		
DCA	208.44 [143.98 – 310.84]	491.41 ** [358.81 – 694.94]
TDCA	0.37 [0.06 – 0.45]	0.42 [0.32 – 0.47]
GDCA	0.06 [0.04 – 0.17]	0.09 [0.06 – 0.15]
UDCA	9.07 [6.36 – 20.12]	14.41 [6.22 – 26.64]
LCA	19.48 [14.37 – 26.13]	52.40 ** [35.80 – 70.97]
ω-MCA	272.81 [142.30 – 457.60]	369.74 [261.20 – 546.15]

3.3 Sulfonate application experiments with conventional IL-10$^{-/-}$ mice

The oral application of the sulfonated bile acid TC has been shown to evoke an overgrowth of *B. wadsworthia*, which was accompanied by intestinal inflammation [2]. Therefore, one of the aims of the present PhD-project was to find out whether the diet-derived sulfonate SQ could stimulate the growth of *B. wadsworthia* and elicit intestinal inflammation as well. The impact of the SQDG-metabolite SQ on the murine health status and the intestinal microbiota was investigated in conventional IL-10$^{-/-}$ mice. The mice were orally treated with SQ for three weeks. Additional mouse groups treated with water or TC served as negative and positive control, respectively.

3.3.1 SQ and TC did not lead to body weight loss but TC partly reduced feed intake

The enrichment of *B. wadsworthia* in SPF wild type mice reduces the body weight of mice within one week [21]. Under the assumption that the orally applied sulfonates SQ and TC had the same effect, body weight and feed intake were measured twice per week. The body-weight development and diet consumption did not differ between SQ and control mice (Fig. 20). Furthermore, the body-weight development was not affected by TC application (Fig. 20A), even though TC mice consumed 0.8 – 1.0 g less feed from day three to day seven and from day 14 to day 18 in comparison to control mice (Fig. 20B).

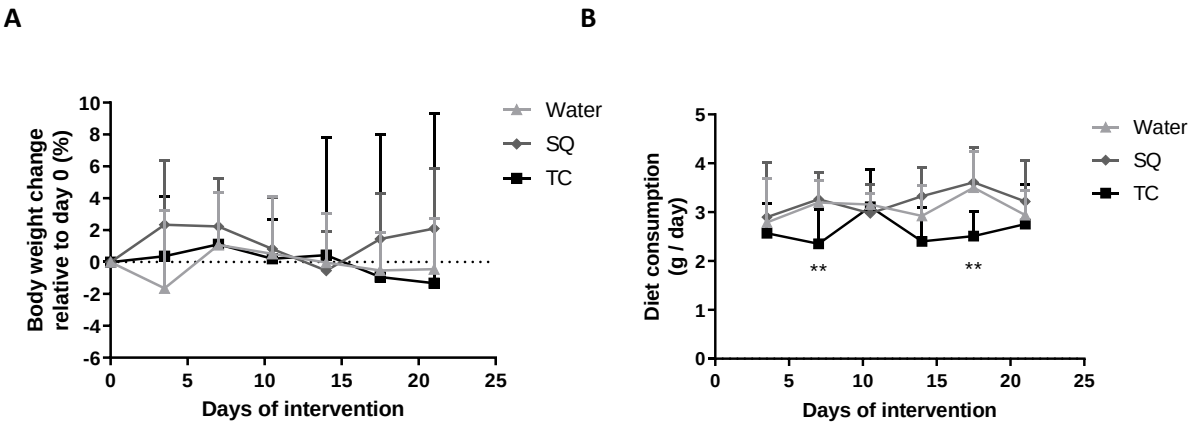


Figure 20: Body-weight development and diet consumption of conventional IL-10$^{-/-}$ mice gavaged with water (n = 12), sulfoquinovose (SQ, n = 12) or taurocholate (TC, n = 10) for three weeks. (A) Body-weight change relative to day zero. (B) Diet consumption per mouse and day. Data are presented as mean + σ, **$P < 0.01$.

3.3.2 SQ evoked no inflammation-associated changes but TC mildly stimulated an inflammatory response

Mice fed a high-fat diet and orally gavaged with *B. wadsworthia* (three times) have a dysfunctional intestinal barrier and higher cytokine concentrations in various tissues [22]. If the sulfonates applied in this study had a similar growth-stimulating effect on *B. wadsworthia*, the consequences for the murine health could be detrimental. However, the FITC-dextran test conducted after two weeks of intervention was not indicative of an increased colonic permeability caused by SQ or TC treatment (Fig. 21). In contrast to that, the expression of *Tjp 1* was lower in the cecal mucosa of TC-gavaged mice relative to water-gavaged mice (Table 15), indicating the weakening of the cellular junctions in the intestinal epithelium [113].

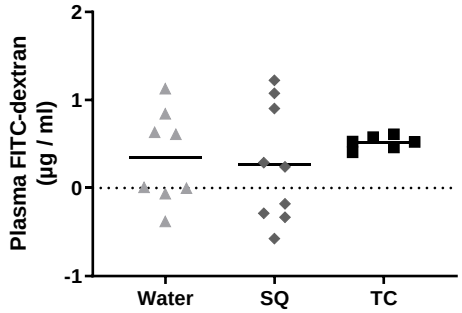


Figure 21: Colon permeability test in conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) after two weeks of intervention. Plasma fluorescein isothiocyanate (FITC)-dextran concentrations presented as values of individual mice and mean (n = 6 - 8 receiving FITC-dextran).

Table 15: Relative expression of *Tjp 1* and *Ocln* in cecal and colonic mucosa of conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Tjp 1: tight junction protein 1, Ocln: occludin. Data are shown as median [Q1 – Q3], *$P < 0.05$, n = 10 – 12.

	Water		SQ		TC	
	Cecum	Colon	Cecum	Colon	Cecum	Colon
Tjp 1	1.00 [0.86 – 1.35]	1.00 [0.58 – 1.12]	0.95 [0.72 – 1.19]	0.99 [0.84 – 1.25]	0.73 * [0.62 – 0.94]	1.07 [0.77 – 1.11]
Ocln	1.00 [0.87 – 1.11]	1.00 [0.73 – 1.35]	1.06 [0.65 – 1.20]	1.15 [0.86 – 1.37]	0.91 [0.83 – 1.05]	0.80 [0.56 – 1.08]

Colonic histopathology scores and mucosal cytokine concentrations were reported to be strongly increased in TC-gavaged IL-10$^{-/-}$ mice relative to control mice after 21 days of intervention [2]. Therefore, these parameters were assessed in cecum and colon of SQ- and TC-gavaged mice using histopathology scores (Fig. 22) and gene expression analysis of cytokines in mucosa (Table 16). The evaluation of the first parameter revealed that no inflammation-associated changes were detected in cecal tissue of SQ- or TC-gavaged mice compared to control mice (Fig. 22A). However, the histopathology score of colonic tissue was higher in TC-gavaged mice (3.5 ± 0.5) than in control mice (0.0 ± 0.0, Fig. 22B). Cytokine-gene expression was partly elevated in cecal mucosa but not in colonic mucosa of TC-gavaged mice. Higher relative expression was detected for *Ifn-γ, Il-1β* and *Il-6* with median values of 4.4, 8.1 and 1.8, respectively (Table 16).

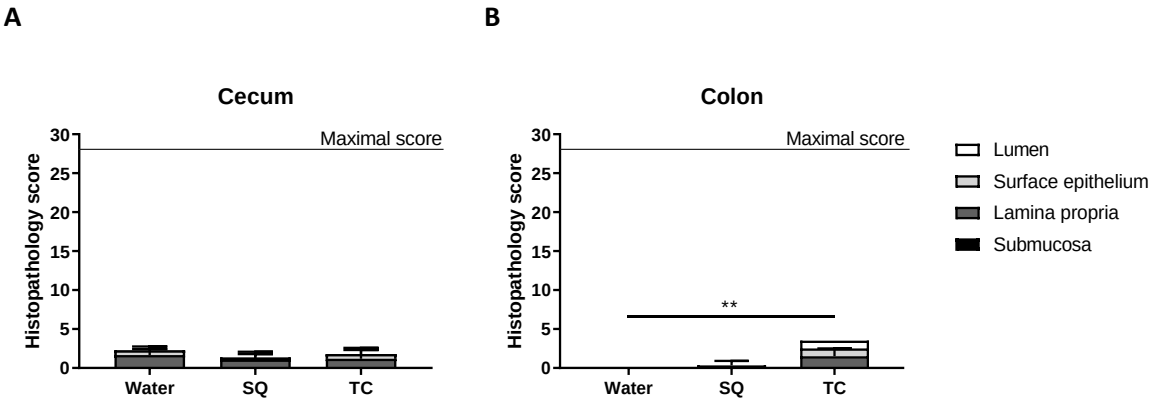


Figure 22: Intestinal histopathology scores of IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Scores of (A) cecal (n = 10 – 12) and (B) colonic tissue preparations (n = 3 – 4). Data are presented as mean + σ, **$P < 0.01$.

Table 16: Cytokine-gene expression assessed in cecal and colonic mucosa of conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. *Ifn-ɣ*: interferon-gamma, *Tnf-α*: tumor necrosis factor-alpha, *Il*: interleukin. Data presented as median [Q1 – Q3], **P* < 0.05, ***P* < 0.01, n = 10 - 12.

	Water		SQ		TC	
Cytokine	**Cecum**	**Colon**	**Cecum**	**Colon**	**Cecum**	**Colon**
Ifn-ɣ	1.00 [0.73 – 1.80]	1.00 [0.32 – 1.89]	2.49 [0.47 – 3.59]	2.19 [0.37 – 3.26]	4.43 * [2.23 – 7.19]	1.52 [1.01 – 3.70]
Tnf-α	1.00 [0.52 – 2.21]	1.00 [0.71 – 2.34]	1.10 [0.48 – 2.30]	0.75 [0.54 – 2.33]	2.07 [0.91 – 2.72]	1.04 [0.82 – 2.04]
Il-1ß	1.00 [0.81 – 2.43]	1.00 [0.56 – 4.10]	1.24 [0.85 – 1.58]	1.39 [0.74 – 2.16]	8.10 ** [5.75 – 11.31]	1.77 [0.61 – 2.08]
Il-6	1.00 [0.93 – 1.14]	1.00 [0.80 – 1.57]	1.00 [0.75 – 1.56]	1.01 [0.53 – 1.49]	1.83 ** [1.42 – 2.24]	1.15 [0.28 – 10.92]
Il-12a	1.00 [0.57 – 1.70]	1.00 [0.42 – 1.83]	1.94 [0.82 – 2.86]	0.76 [0.23 – 1.51]	1.87 [0.78 – 4.68]	0.66 [0.38 – 1.17]

No differences in small intestinal and colonic length (Fig. 23A, B) or in cecum (Fig. 23C), liver and spleen weight (Table 17) were detected among the groups. Mice receiving SQ had heavier kidneys, whereas those receiving TC displayed a reduced mLN size (Table 17). The microscopic examination of liver, spleen, kidney and small intestinal tissue slices was not indicative of any sulfonate-induced abnormalities (data not shown).

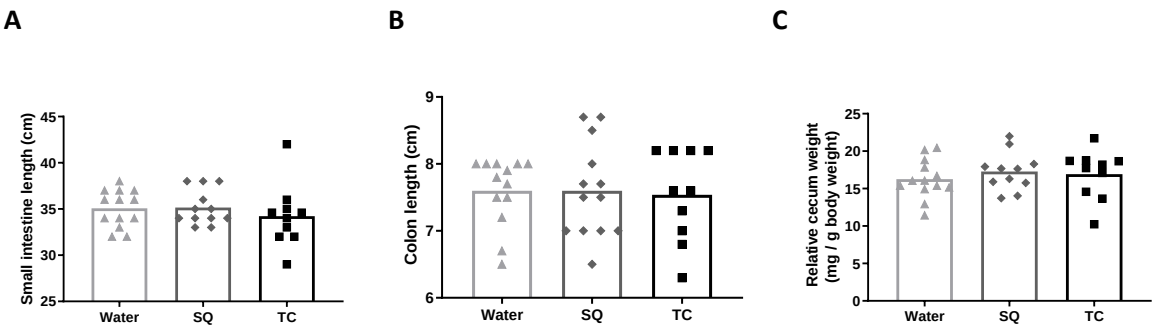


Figure 23: Measures of intestinal organs of conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. (A) Length of the small intestine, (B) colon length and (C) cecum weight relative to the murine body weight are presented as values of individual mice and mean (n = 10 – 12).

Table 17: Measures of organs and tissues from conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. N. r. = not recorded, data shown as median [Q1 – Q3], *$P < 0.05$, n = 10 – 12.

Organ	Water	SQ	TC
Liver (mg / g)	56.31 [52.36 – 57.60]	59.55 [56.25 – 63.57]	57.90 [53.37 – 61.15]
Spleen (mg / g)	3.05 [2.89 – 3.65]	3.22 [2.83 – 4.21]	3.18 [2.90 – 3.79]
Kidney (mg / g)	7.20 [6.86 – 7.55]	8.11 * [7.66 – 8.47]	7.40 [6.94 – 8.00]
mWAT (mg / g)	n. r.	n. r.	9.50 [8.38 – 10.46]
mLN (mm²)	135.10 [87.96 – 176.30]	135.10 [64.40 – 175.90]	83.25 * [43.59 – 94.25]
Gall bladder (mg / g)	n. r.	n. r.	0.91 [0.44 – 1.19]

No hints for systemic inflammation were detected, as inflammatory markers in plasma did not differ among the groups (Table 18).

Table 18: Plasma concentrations of inflammatory markers in conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. N. d.: not detected. Data are shown as median [Q1 – Q3], n = 3 – 4.

Protein	Water	SQ	TC
IFN-γ (pg / ml)	18.88 [16.34 – 20.78]	19.51 [14.38 – 41.26]	13.95 [12.34 – 33.99]
TNF-α	n. d.	n. d.	n. d.
LCN 2 (ng / ml)	109.46 [99.25 – 190.95]	73.16 [69.81 – 18.25]	159.50 [107.82 – 206.87]
SAA (µg / ml)	16.99 [7.69 – 49.47]	16.48 [8.02 – 24.51]	35.87 [15.28 – 110.44]

3.3.3 Unlike TC, SQ had little impact on the intestinal microbiota

In vitro incubation experiments with murine fecal bacteria and SQ or TC indicated a growth-stimulating effect of both SQ and TC on sulfite-reducing bacteria such as *B. wadsworthia* (chapter 3.1.1). However, *in vivo* the abundance of *B. wadsworthia* and, in general, of sulfite-reducing bacteria was not altered in SQ-gavaged mice, but was higher in cecum contents of TC-gavaged mice than in control mice (Fig. 24A, B). Bacteria carrying the SQ isomerase gene (*yihS*) were detected in few samples only and if so, in much lower abundance than observed for the sulfite-reducing bacteria (Fig. 24C). Owing to the low number of samples tested positively for *yihS*, statistically relevant differences were not detected. Nevertheless, it is important to note that bacteria equipped with the *yihS* gene were more often detected in intestinal contents of TC-gavaged mice than in any other of the mouse groups.

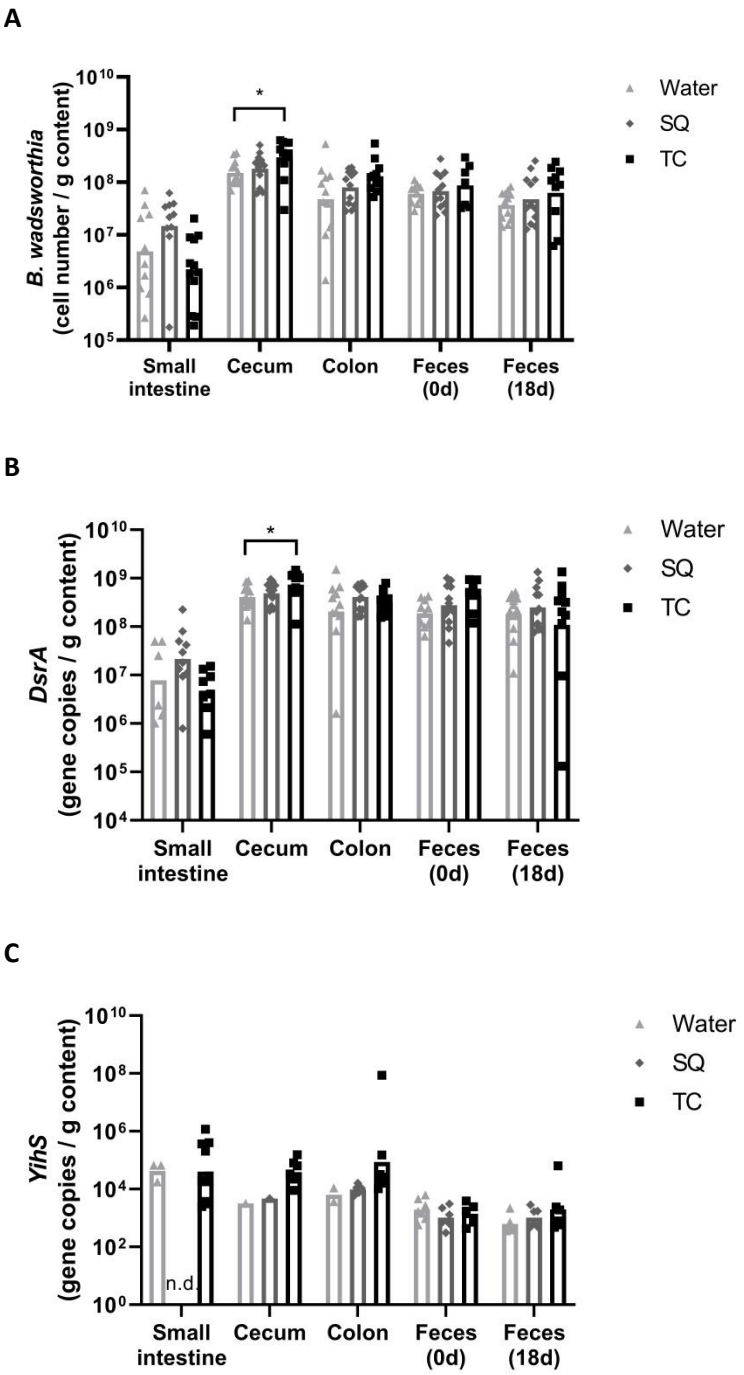


Figure 24: Bacterial abundance in small intestinal, cecal and colonic contents and in feces from day zero (0 d) and 18 (18 d) of conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Cell numbers and gene copies of (A) *B. wadsworthia*, (B) sulfite-reducing bacteria and (C) SQ-converting bacteria were determined by qPCR. *DsrA*: dissimilatory sulfite reductase A gene, *yihS*: SQ isomerase gene, n. d.: not detected. Note that *yihS* was not detected in all samples. Data are presented as values of individual mice and median, **P* < 0.05, n = 10 – 12.

Analysis of the 16S rRNA gene sequencing data confirmed the TC-induced higher cecal abundance of *B. wadsworthia*, which belongs to *Desulfovibrionaceae*. Additionally, the analysis revealed an increased relative abundance of members of this family in feces from day 18 of mice treated with SQ or TC compared to feces collected at the beginning of the experiment. Mice gavaged with TC displayed a higher relative abundance of *Bacteroidaceae* (genus *Bacteroides*) and *Lactobacillaceae* (genus *Lactobacillus*) and a lower relative abundance of *Erysipelotrichaceae* (genus *Allobaculum*) and

Porphyromonadaceae (unknown genus) than control mice treated with water (Fig. 25). An increased relative abundance in feces of TC-gavaged mice was also observed for strains of *Clostridium* cluster IV (data not shown). Differences in other genera were not observed (data not shown).

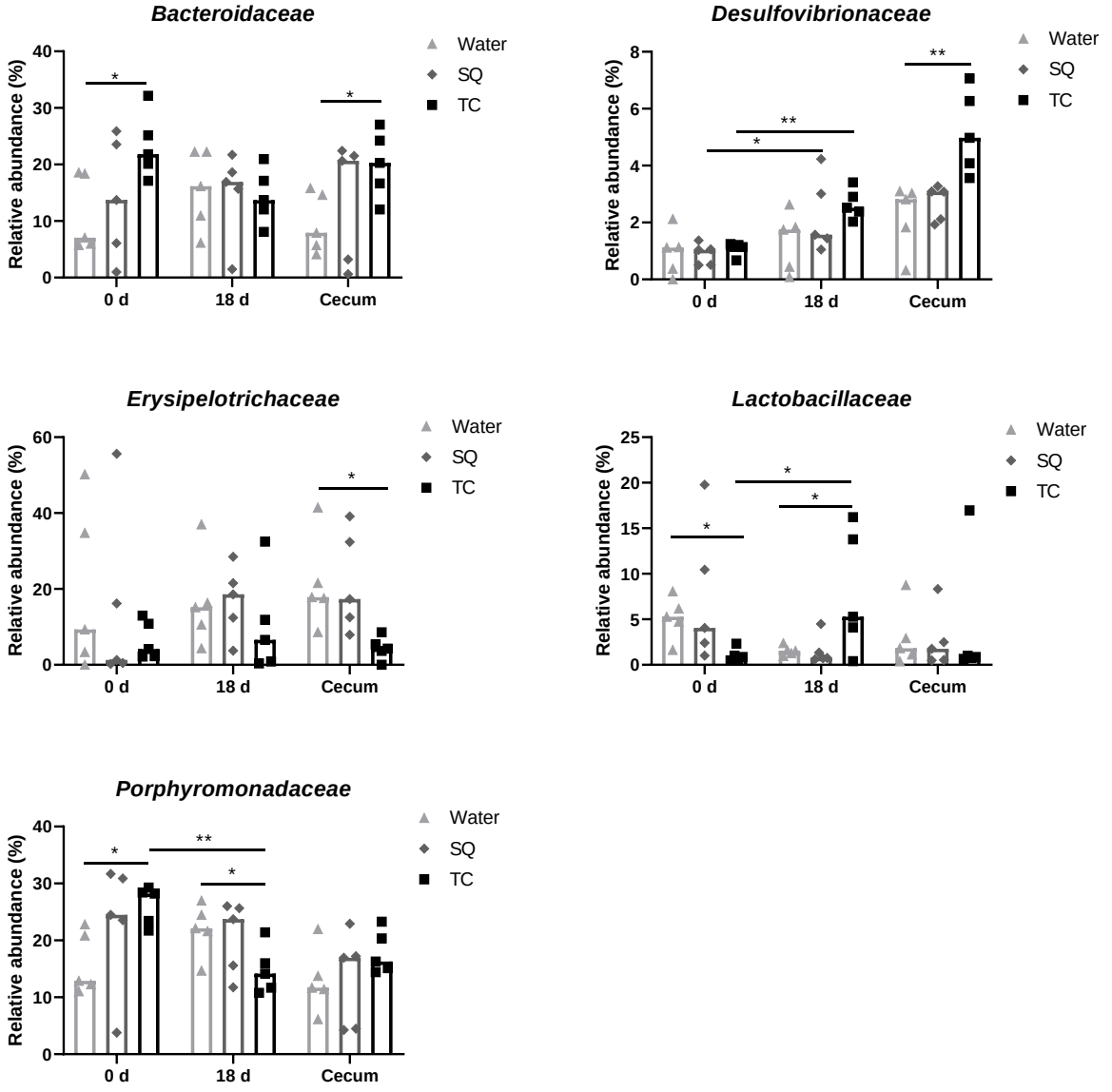


Figure 25: Relative abundance of *Bacteroidaceae, Desulfovibrionaceae, Erysipelotrichaceae, Lactobacillaceae* and *Porphyromonadaceae* in feces from day zero (0 d) and 18 (18 d) as well as cecal contents of conventional IL-10$^{-/-}$ mice orally treated with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Bacterial abundance was determined by *16S rRNA* gene sequencing. Data are presented as values of individual mice and median, * $P < 0.05$, * $P < 0.01$, n = 5.

No data exist about the impact of SQ supplementation on the microbiota composition, while the oral application of TC affect the microbial community strongly [2]. Therefore, the microbial profiles of feces and cecal contents were assessed in the present study by *16S rRNA* gene sequencing. The microbial profiles of feces did not differ between SQ- and water-gavaged mice before the intervention (Fig. 26A), after 18 days of intervention (Fig. 26B), nor did they differ in cecal content (Fig. 26E). Furthermore, the microbial profiles of mice gavaged with water or SQ did not differ between days 0 and 18 (Fig. 26C, D). In contrast to that, the fecal microbial profiles of mice gavaged

with TC or water differed at day 0 (Fig. 26F), at day 18 (Fig. 26G) and in cecal content (Fig. 26I). The fecal microbial profiles of mice orally treated with TC changed between day 0 and 18 (Fig. 26H).

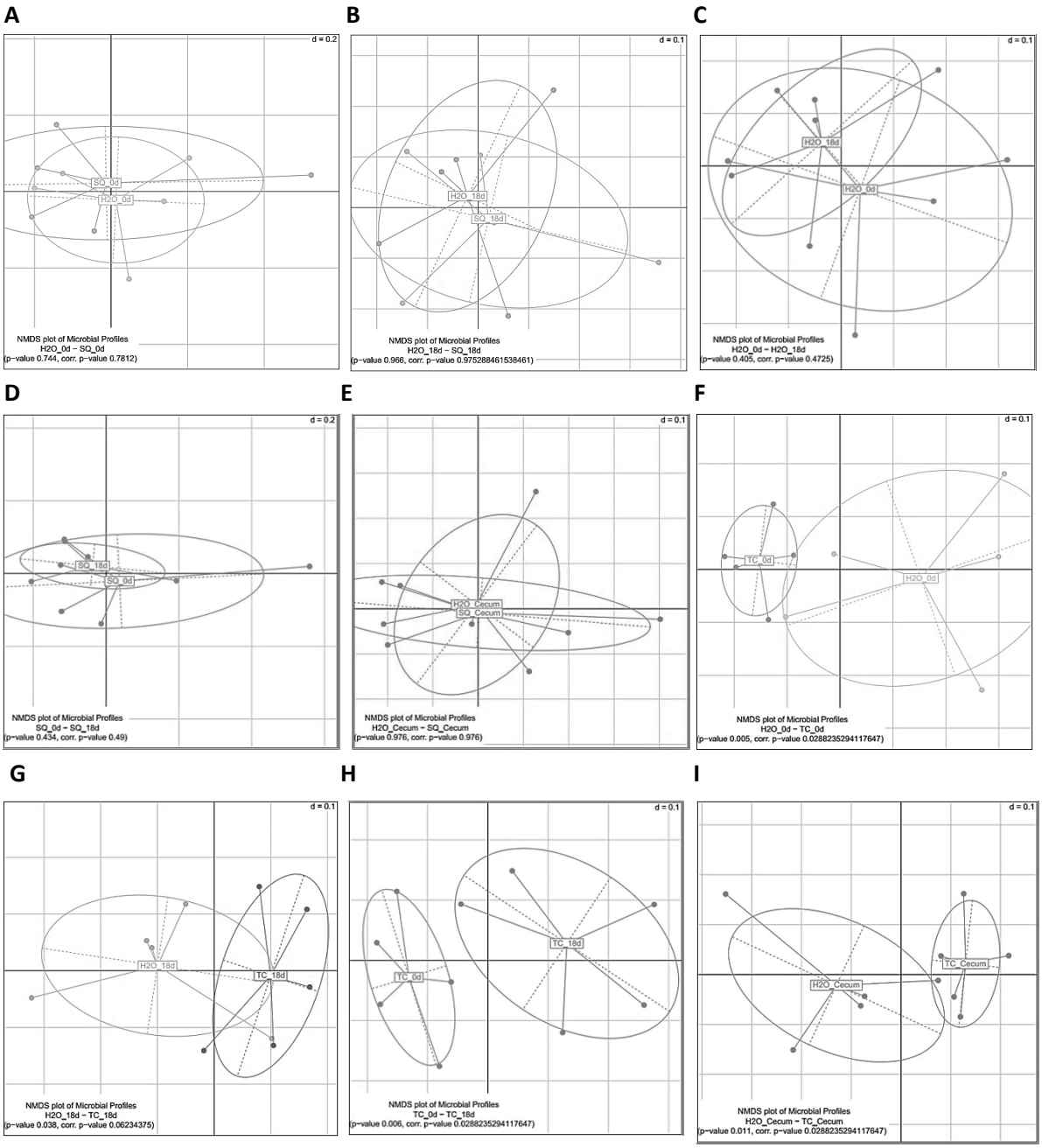
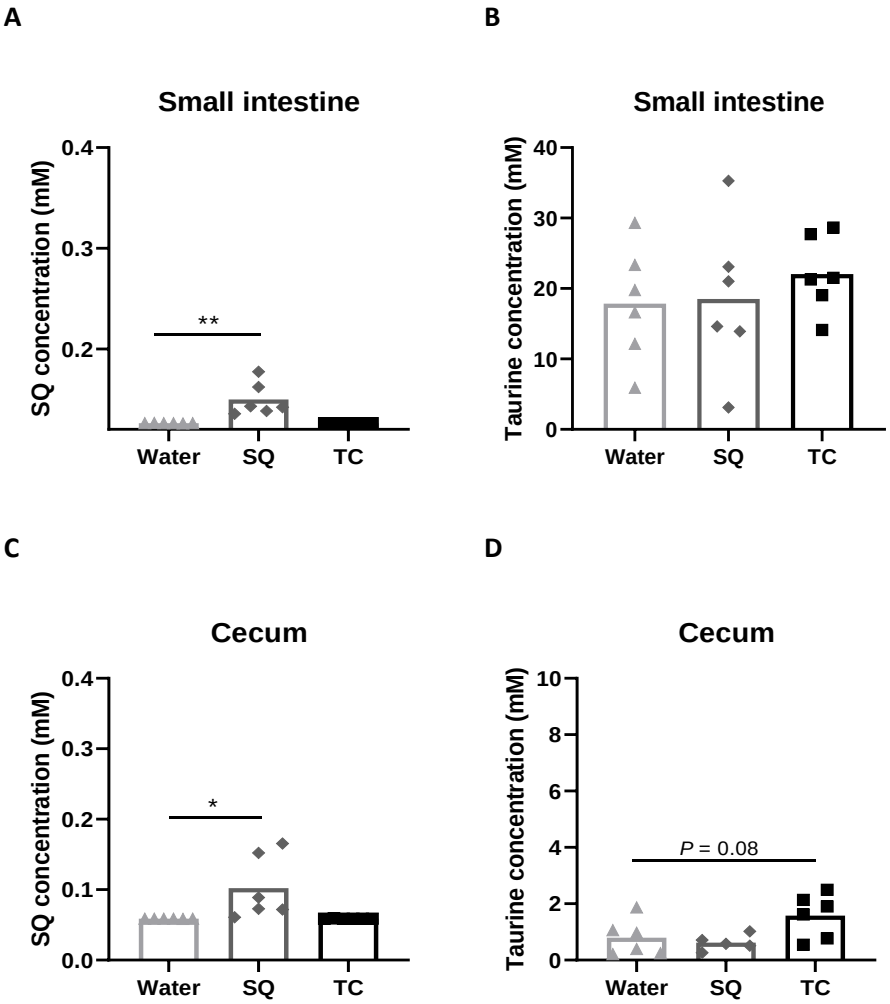


Figure 26: Microbial profiles of feces from day 0 (0d) and 18 (18d) and of cecal content from conventional IL-10$^{-/-}$ mice orally treated with water (H2O), sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Comparison of fecal microbial profiles of mice treated with (A) SQ or (F) TC versus water at day 0, (B) SQ or (G) TC versus water at day 18, (C) treated with water at days 0 versus 18, treated with (D) SQ or (H) TC at days 0 versus 18, and comparison of cecal microbial profiles of mice treated with (E) SQ or (I) TC versus water at day 21. Data presented as non-metric multi-dimensional scaling (NMDS) plots with *P*-values in the lower left corner of each plot, n = 5.

3.3.4 SQ concentrations were elevated in intestinal contents and feces of SQ-treated mice while the taurine concentrations did not differ among the groups

To answer the question, whether SQ and TC reached the large intestine and underwent bacterial degradation, the concentrations of SQ, its degradation product DHPS, taurine and isethionate were

quantified in intestinal contents from different gut sections and in feces. SQ treatment led to higher SQ concentrations in small intestinal, cecal and colonic contents and in feces from day 18 of the intervention compared mice treated with water (Fig. 27A, C, E, G, I). In contrast, taurine concentrations did not differ between mice treated with TC or water (Fig. 27B, D, F, H, J). Isethionate was solely detected in feces collected at day 18 of the intervention from one mouse treated with water (0.26 mM) and from another mouse treated with SQ (0.10 mM). DHPS was not detected in any of the mice (data not shown).



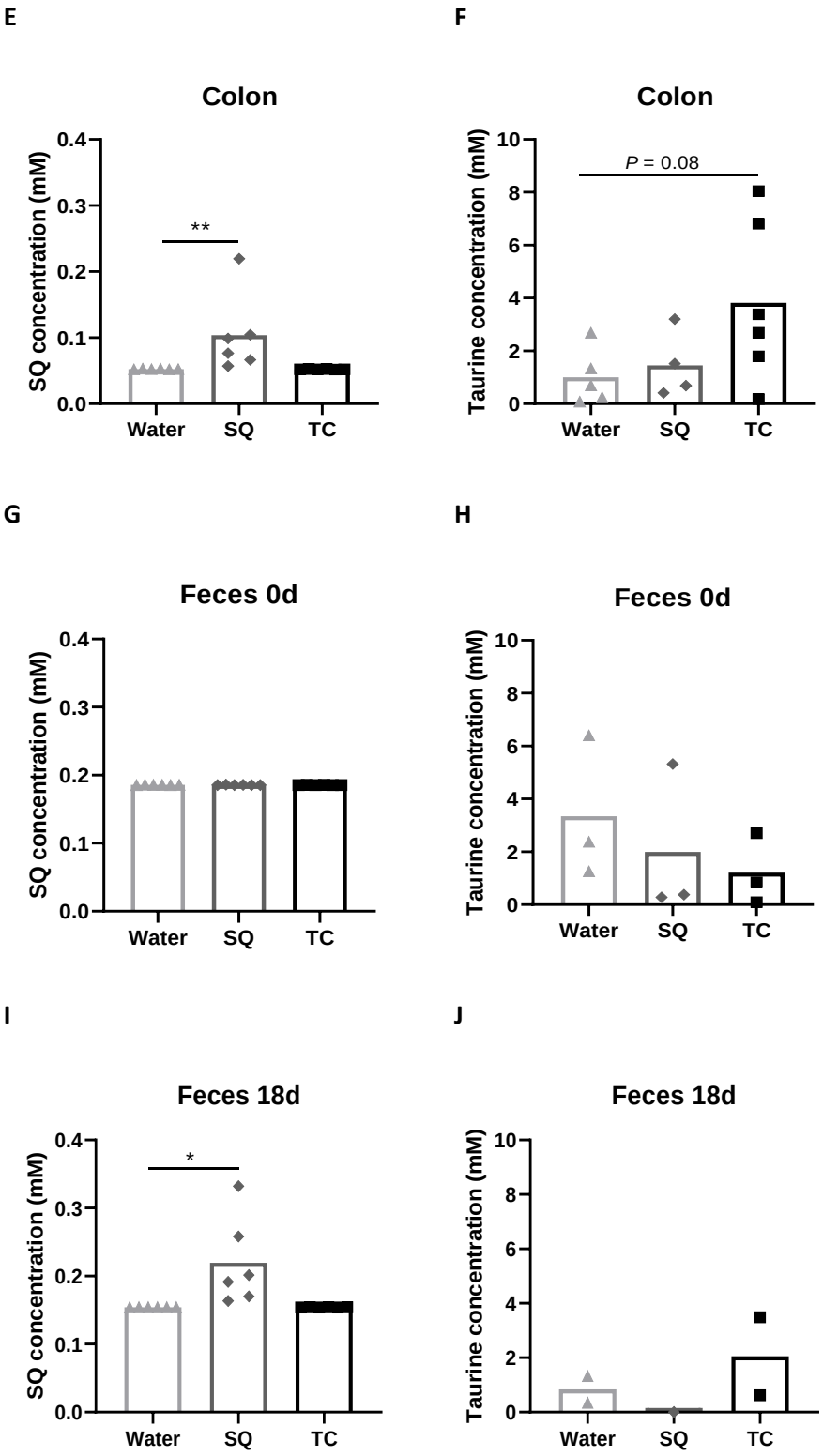


Figure 27: Sulfoquinovose (SQ) and taurine concentrations in intestinal contents and feces of conventional IL-10$^{-/-}$ mice gavaged with water, SQ or taurocholate (TC) for three weeks. Left panel: SQ in (A) small intestine, (C) cecum, (E) colon, (G) feces from day zero and (I) feces from day 18. Right panel: taurine in (B) small intestine, (D) cecum, (F) colon, (H) feces from day zero and (J) feces from day 18. Note that taurine was not detected in all samples. Data shown as values of individual mice and mean, *P < 0.05, **P < 0.01, n = 6.

3.3.5 SQ and TC did not induce the expression of *Sqr* and *Hif-1α*

Complete bacterial conversion of SQ and TC results in the formation of sulfide, which could have detrimental consequences for host health [58]. However, neither SQ nor TC increased the sulfide formation in cecum and colon sufficiently enough to enhance the expression of *Sqr* or caused hypoxia in mitochondria of epithelial cells, as assessed by *Hif-1α* expression analysis (Table 19).

Table 19: Relative expression of *Sqr* and *Hif-1α* in cecal and colonic mucosa of conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Sqr: sulfide:quinone oxidoreductase, Hif-1α: hypoxia-inducible factor-1alpha. Data presented as mean ± σ, n = 12.

	Water		SQ		TC	
	Cecum	Colon	Cecum	Colon	Cecum	Colon
Sqr	1.00 ± 0.32	1.00 ± 0.36	0.90 ± 0.18	0.88 ± 0.28	1.22 ± 0.32	1.22 ± 0.24
Hif-1α	1.00 ± 0.43	1.00 ± 0.44	0.76 ± 0.36	0.79 ± 0.19	1.07 ± 0.64	1.15 ± 0.49

3.3.6 The oral application of SQ or TC affected the bile-acid profile

The effect of sulfonate supplementation on bile-acid composition was investigated in cecal contents of the mice. Bile-acid secretion can be influenced by the diet or by specific bacteria and, by modulating the bile-acid composition, the signaling of bile acid receptors can be affected [118]. The analysis was also performed to assess the impact of TC-gavages on cecal TCA and CA concentrations. In the present study, the majority of bile acids was detected in their deconjugated forms and SQ as well as TC treatment reduced the concentration of LCA (both 2.0-fold) compared the treatment with water. Additionally, β-MCA concentrations were 3.1-fold lower in mice gavaged with TC than in mice gavaged with water (Table20).

Table 20: Concentrations of primary and secondary bile acids in cecal contents of conventional IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. CA: cholic acid, TCA: taurocholic acid, GCA: glycocholic acid, CDCA: chenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, GCDCA: glycochenodeoxycholic acid, UDCA: ursodeoxycholic acid, MCA: muricholic acid, T-β-MCA: tauro-beta-MCA, DCA: deoxycholic acid, TDCA: taurodeoxycholic acid, GDCA: glycodeoxycholic acid, LCA: lithocholic acid, n. d.: not detected. Data presented in nmol per g content as median [Q1 – Q3], *$P < 0.05$, n = 6.

	Water	SQ	TC
Primary bile acids (nmol / g)			
CA	265.55 [208.60 – 365.08]	230.65 [92.63 – 527.00]	347.67 [224.60 – 490.26]
TCA	0.58 [0.22 – 1.95]	0.29 [0.08 – 0.99]	0.60 [0.37 – 0.82]
GCA	0.11 [0.00 – 0.14]	0.11 [0.05 – 0.29]	0.00 [0.00 – 0.04]
CDCA	n. d.	n. d.	n. d.
TCDCA	0.06 [0.00 – 0.23]	0.03 [0.00 – 0.08]	0.05 [0.03 – 0.06]
GCDCA	n. d.	n. d.	n. d.
α-MCA	92.64 [62.97 – 123.37]	68.94 [41.44 – 103.67]	42.08 [13.28 – 87.07]
β-MCA	138.68 [76.18 – 208.82]	110.10 [58.98 – 141.36]	44.62 * [18.03 – 116.85]
T-β-MCA	0.57 [0.34 – 2.17]	0.36 [0.12 – 0.62]	0.26 [0.10 – 0.36]
Secondary bile acids (nmol / g)			
DCA	240.19 [157.35 – 391.06]	199.33 [75.00 – 253.72]	389.28 [211.70 – 697.08]
TDCA	0.44 [0.19 – 1.04]	0.12 [0.09 – 0.38]	1.01 [0.49 – 1.80]
GDCA	0.72 [0.38 – 1.19]	0.54 [0.39 – 1.09]	0.09 [0.04 – 0.52]
UDCA	11.41 [5.97 – 18.84]	9.75 [4.25 – 13.69]	7.26 [2.85 – 13.24]
LCA	31.60 [18.29 – 44.99]	16.19 * [8.77 – 20.78]	15.60 * [13.49 – 20.71]
ω-MCA	437.84 [313.26 – 554.37]	354.99 [247.50 – 587.16]	248.83 [102.39 – 400.81]

3.4 Sulfonate application experiments in gnotobiotic IL-10$^{-/-}$ mice

By reducing the complexity of the intestinal microbiota, the characteristics of specific bacteria can be investigated in a standardized setting. Various gnotobiotic animal models have been established for dissecting microbiota-associated research questions [84]. The SIHUMI consortium was established with the aim to meet as many microbiota-associated characteristics as possible and to combine those bacteria that are prominent members of the human intestine [83]. This microbial community can be expanded by adding one bacterium, which enables the investigation of this new community member in a defined environment. In the present study, the metabolic and colitogenic capacity of *B. wadsworthia* was evaluated by applying SQ and TC orally to IL-10$^{-/-}$ mice harboring the SIHUMI consortium in the absence or presence of *B. wadsworthia* (SIHUMI + Bw). Effects of these sulfonates on the composition of the simplified microbial consortium and on the murine inflammatory response were assessed.

3.4.1 Neither the oral application of sulfonates nor the presence of *B. wadsworthia* affected weight gain or feed intake of gnotobiotic IL-10$^{-/-}$ mice

As Feng et al. [21] reported that *B. wadsworthia* induced body-weight reduction in SPF wild type mice, body weight and feed intake of mice were also monitored in the present study. However, the relative body-weight development (Fig. 28A, B) and diet consumption (Fig. 28C, D) were not different among the groups and were, furthermore, not affected by the presence of *B. wadsworthia*.

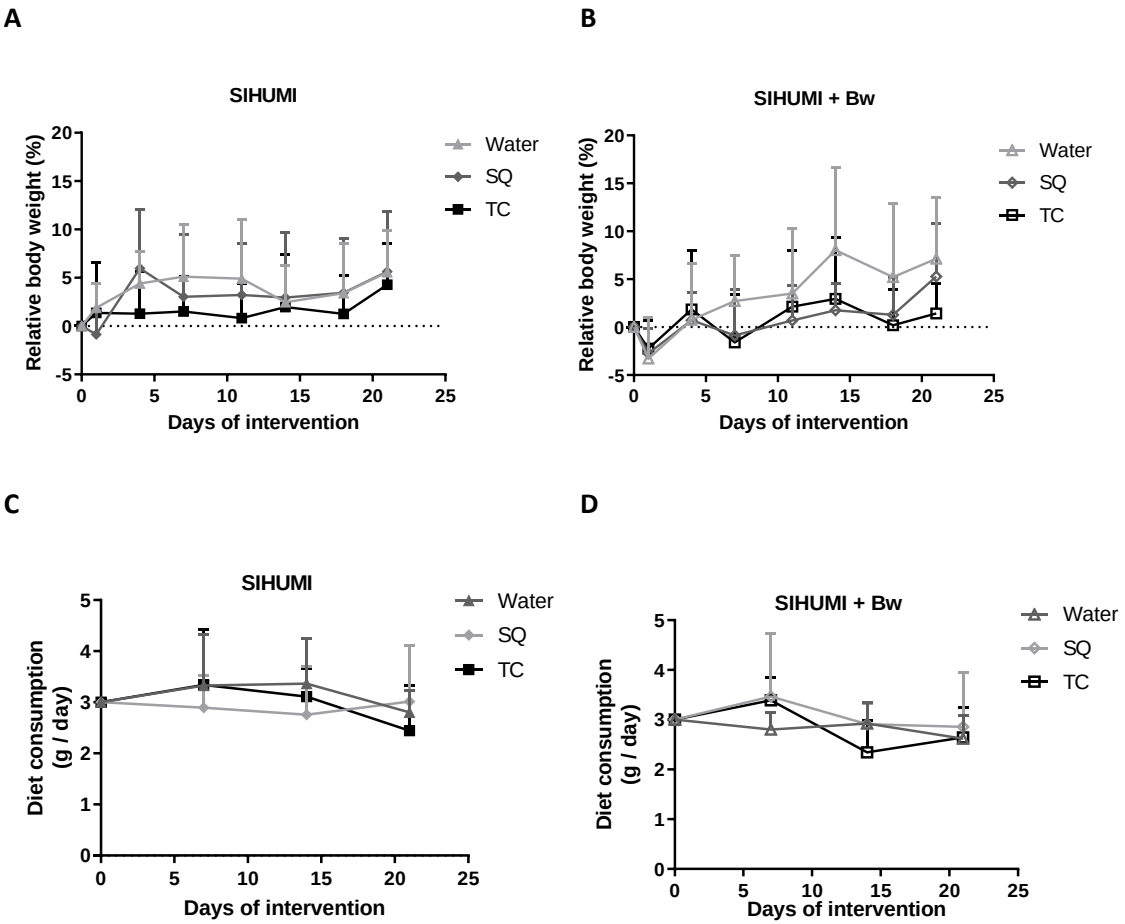


Figure 28: Body-weight development and diet consumption of gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) with or without *B. wadsworthia* (Bw) and gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Body weight relative to day zero of (A) SIHUMI and (B) SIHUMI + Bw mice. Diet consumed per mouse and day of (C) SIHUMI and (D) SIHUMI + Bw mice. Data presented as mean + σ, n = 8.

3.4.2 TC increased the intestinal permeability and mildly stimulated the cytokine gene expression of SIHUMI + Bw mice

Changes in intestinal permeability were assessed by the FITC-dextran test and expression analysis of *Tjp 1* and *Ocln* in cecal and colonic mucosa. *TJP 1* and *OCLN* are tight-junction proteins, which are necessary for cell-to-cell adhesion [113]. Both methods revealed a higher permeability in the group of SIHUMI + Bw mice gavaged with TC in comparison to water-gavaged mice (Fig. 29 and Table 21). The expression of *Tjp 1* in cecal mucosa of TC-gavaged SIHUMI + Bw mice was also lower than of TC-gavaged SIHUMI mice (Table 21).

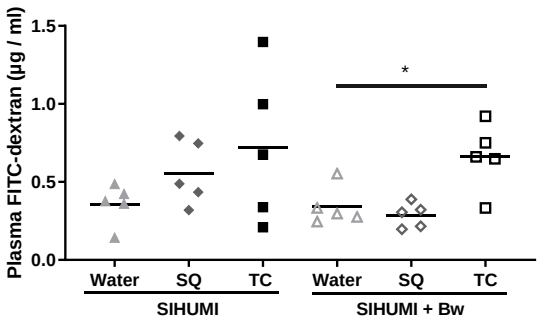


Figure 29: Plasma concentration of FITC-dextran in gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw) and gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. FITC: fluorescein isothiocyanate. Data shown as values of individual mice and mean, *$P < 0.05$, n = 5 treated with FITC-dextran.

Table 21: Gene expression of tight junction protein 1 (*Tjp 1*) and occludin (*Ocln*) in cecal and colonic mucosa of gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw). The mice were gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Data are shown as median [Q1 – Q3], *$P < 0.05$ versus SIHUMI + Bw water, $^{\#}P < 0.05$ versus SIHUMI TC, n = 8.

	SIHUMI			SIHUMI + Bw		
	Water	**SQ**	**TC**	**Water**	**SQ**	**TC**
Cecum						
Tjp 1	1.00 [0.87 – 1.22]	1.17 [0.60 – 1.37]	1.24 [1.06 – 1.56]	1.33 [0.98 – 1.60]	0.98 [0.67 – 1.20]	0.74 *$^{\#}$ [0.55 – 0.99]
Ocln	1.00 [0.78 – 1.24]	1.15 [0.97 – 1.42]	1.09 [0.81 – 1.48]	1.08 [0.67 – 1.39]	0.95 [0.59 – 1.30]	1.06 [0.65 – 1.25]
Colon						
Tjp 1	1.00 [0.68 – 2.36]	0.62 [0.33 – 1.37]	0.87 [0.56 – 1.23]	1.09 [0.88 – 3.00]	1.21 [0.52 – 2.29]	1.07 [0.68 – 1.46]
Ocln	1.00 [0.83 – 1.14]	1.03 [0.84 – 1.31]	0.80 [0.61 – 1.00]	1.02 [0.54 – 1.31]	0.97 [0.87 – 1.17]	0.96 [0.63 – 1.49]

Mono-association of germ-free mice with *B. wadsworthia* was reported to elicit intestinal inflammation, characterized by a strong increase of the histological colitis score, in TC-gavaged mice [2]. However, in the current PhD-project, tissue-specific inflammatory changes were not observed in cecum and colon of gnotobiotic IL-10$^{-/-}$ mice orally treated with SQ or TC, as assessed by histopathological scoring (Fig. 30).

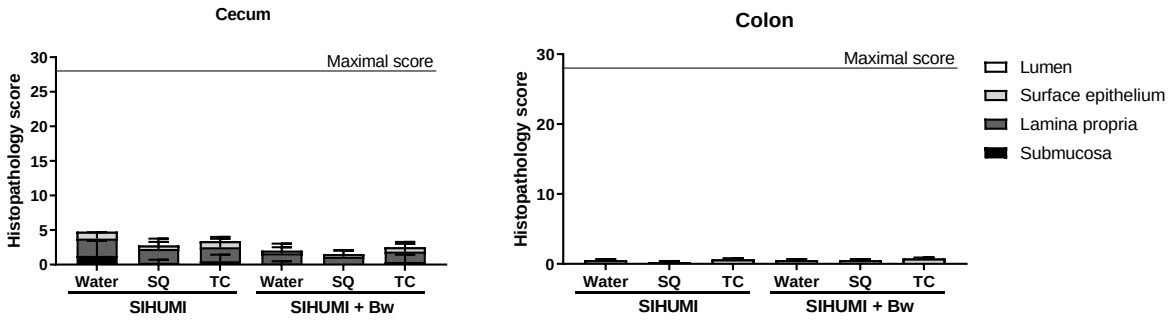


Figure 30: Cecal and colonic histopathology scores of gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw). The mice were gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Data shown as mean + σ, n = 8.

Mice associated with a microbial community known as Altered Schaedler Flora and fed a high-fat diet were reported to display increased levels of several cytokines in the cecum, mLN and spleen in response to the colonization with *B. wadsworthia* [22]. In the present study, the expression of pro-inflammatory cytokines in cecal and colonic mucosa did not differ among the groups, except *Ifn-ɣ* and *Il-6*, which were more highly expressed in TC-gavaged SIHUMI + Bw mice than in SIHUMI mice. In addition, *Ifn-ɣ* expression was higher in SIHUMI + Bw mice gavaged with TC than with water. Gnotobiotic mice treated with SQ did not have a higher gene expression of cytokines in cecal or colonic mucosa; at the contrary, the colonic expression of *Tnf-α* in SQ-gavaged SIHUMI + Bw mice was lower than in water-gavaged SIHUMI + Bw mice (Table 22).

Table 22: Gene expression of cytokines in cecum and colon mucosa of IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw). The mice were gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. *Ifn-γ*: interferon-gamma, *Tnf-α*: tumor necrosis factor-alpha, *Il*: interleukin, n. d.: not detected. Data presented as median [Q1 – Q3], **P* < 0.05 water vs. TC mice with same gnotobiotic status, $^{\#}$*P* < 0.05, $^{\#\#}$*P* < 0.01 TC-gavaged SIHUMI vs. TC-gavaged SIHUMI + Bw mice, n = 8.

	SIHUMI			SIHUMI + Bw		
Cytokine	**Water**	**SQ**	**TC**	**Water**	**SQ**	**TC**
Cecum						
Ifn-γ	1.00 [0.11–1.79]	0.32 [0.12 – 1.96]	0.08 * [0.04 – 0.16]	0.61 [0.21 – 1.00]	0.21 [0.19 – 0.95]	1.77 * $^{\#\#}$ [1.12 – 18.58]
Tnf-α	1.00 [0.53 – 6.01]	0.74 [0.37 – 0.90]	0.75 [0.43 – 1.06]	0.65 [0.44 – 1.04]	0.67 [0.58 – 0.90]	1.70 * $^{\#}$ [1.15 – 4.28]
Il-1β	1.00 [0.51 – 2.84]	0.55 [0.28 – 1.01]	0.89 [0.63 – 7.34]	2.06 [0.61 – 2.82]	1.17 [0.40 – 1.42]	3.50 [1.33 – 11.78]
Il-6	1.00 [0.48 – 1.54]	0.66 [0.51 – 1.07]	0.44 [0.39 – 0.69]	0.89 [0.69 – 1.01]	0.71 [0.51 – 1.17]	1.18 $^{\#}$ [1.11 – 1.37]
Il-12a	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Colon						
Ifn-γ	1.00 [0.23 – 2.06]	0.36 [0.21 – 1.03]	0.30 [0.15 – 0.69]	0.95 [0.27 – 1.60]	0.21 [0.15 – 0.97]	0.58 [0.30 – 1.51]
Tnf-α	1.00 [0.51 – 1.58]	1.10 [0.61 – 2.20]	0.80 [0.41 – 1.05]	1.65 [0.88 – 2.36]	0.51 * [0.38 – 0.70]	1.29 [0.71 – 2.02]
Il-1β	1.00 [0.60 – 2.11]	0.74 [0.35 – 1.33]	0.46 [0.14 – 0.91]	0.61 [0.50 – 0.93]	0.19 [0.06 – 0.80]	0.69 [0.33 – 2.05]
Il-6	1.00 [0.47 – 3.13]	1.03 [0.09 – 2.12]	1.55 [0.33 – 3.37]	0.86 [0.45 – 2.23]	0.53 [0.19 – 0.85]	2.55 [0.51 – 5.22]
Il-12a	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.

A higher abundance of *B. wadsworthia* induced by a milk-fat diet was linked to a reduced cecum size in mice mono-associated with this bacterium [2]. In agreement with this, the cecum weight was 1.4-fold lower in TC-gavaged SIHUMI + Bw mice than in those gavaged with water. In contrast to that, the relative cecum weight of SIHUMI mice treated with SQ was 1.2-fold higher than of SIHUMI mice gavaged with water (Fig. 31). The size or weight of visceral organs did not differ among the groups, except for the relative gall bladder weight, which was 2.4-fold higher in SIHUMI + Bw mice gavaged with TC compared to SIHUMI + Bw mice gavaged with water (Table 23). No indications for tissue-level alterations were observed in tissue slices of liver, spleen, kidney and small intestine (data not shown).

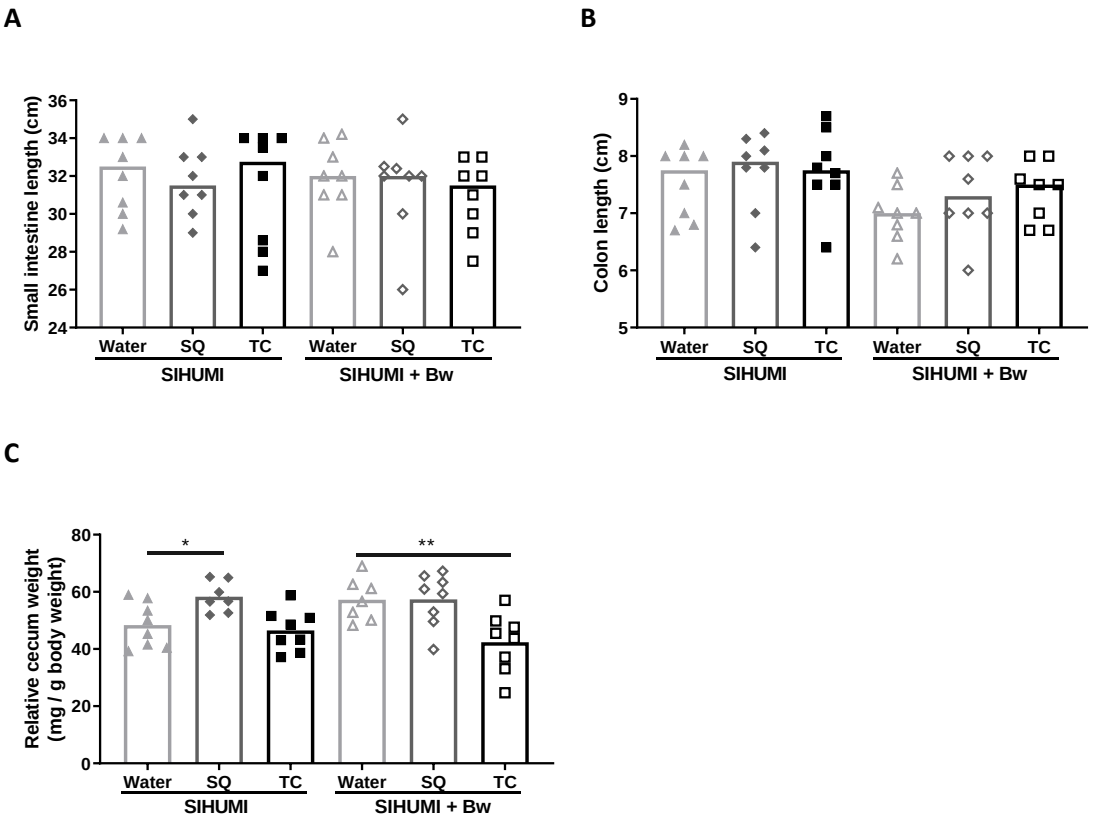


Figure 31: Measures of intestinal organs from gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw). The mice were gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. (A) Small intestinal length, (B) colonic length and (C) cecum weight relative to the murine body weight. Date presented as values of individual mice and mean, **P* < 0.05, ***P* < 0.01, n = 8.

Table 23: Measures of visceral organs from gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw). The mice were gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Data shown as mean ± σ, ***P* < 0.01 compared to water-gavaged SIHUMI + Bw mice, n = 8.

	SIHUMI			SIHUMI + Bw		
Organ	**Water**	**SQ**	**TC**	**Water**	**SQ**	**TC**
Liver	59.63	56.77	61.06	62.98	59.03	60.87
(mg / g)	± 4.55	± 8.26	± 3.65	± 5.04	± 3.03	± 5.48
Spleen	3.72	3.54	3.37	3.34	3.09	3.36
(mg / g)	± 0.43	± 0.71	± 0.64	± 0.81	± 0.66	± 0.45
Kidney	6.12	6.34	6.50	6.36	5.67	5.79
(mg / g)	± 0.51	± 0.78	± 0.65	± 0.30	± 1.84	± 2.03
mWAT	11.84	10.83	8.97	11.32	9.81	11.19
(mg / g)	± 4.42	± 2.11	± 3.31	± 4.18	± 1.56	± 4.06
mLN (mm²)	34.78	30.51	32.99	39.60	31.05	38.85
	± 10.20	± 10.06	± 13.44	± 17.04	± 8.10	± 7.93
Gall bladder	1.06	0.97	1.80	0.94	1.08	2.25 **
(mg / g)	± 0.60	± 0.60	± 0.74	± 0.12	± 0.91	± 0.87

The reported ability of *B. wadsworthia* to elicit systemic inflammation even in SPF wild-type mice [21] was also investigated in the current PhD-project. However, no systemic inflammation was

detected in SIHUMI and SIHUMI + Bw mice, as inflammatory markers in plasma samples were low and did not differ among the groups (Table 24).

Table 24: Inflammatory markers in plasma samples of gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw). The mice were gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. IFN-ɣ: interferon-gamma, TNF-α: tumor necrosis factor-alpha, LCN: lipocalin, SAA: serum amyloid A, n. d.: not detected. Data shown as median [Q1 – Q3], n = 3 – 4.

Plasma	SIHUMI			SIHUMI + Bw		
	Water	SQ	TC	Water	SQ	TC
IFN-ɣ (pg / ml)	21.12 [20.92 – 23.15]	32.95 [20.58 – 43.13]	19.90 [11.81 – 22.47]	24.75 [21.59 – 27.73]	22.61 [16.57 – 25.45]	27.85 [20.45 – 35.86]
TNF-α	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
LCN 2 (ng / ml)	149.60 [42.07 – 197.09]	81.80 [58.19 – 86.26]	119.95 [107.44 – 132.17]	74.71 [63.14 – 76.79]	44.94 [34.61 – 96.87]	89.34 [53.07 – 95.69]
SAA (µg / ml)	11.27 [8.43 – 106.85]	10.12 [6.63 – 13.20]	31.16 [19.37 – 53.60]	7.71 [6.15 – 47.99]	4.88 [3.49 – 14.77]	8.33 [2.71 – 63.09]

3.4.3 Sulfonate treatments had a minor impact on bacterial cell numbers in gnotobiotic mice

Cell numbers of SIHUMI bacteria and *B. wadsworthia* determined in small intestinal, cecal and colonic contents and feces from day zero and day 18 showed that the impact of SQ and TC gavage was minor and region-dependent (Fig. 32). In the small intestine, the abundance of *B. thetaiotaomicron* was 1.2 $\log_{10}$ lower in TC-gavaged SIHUMI mice, while *C. ramosum* (0.8 $\log_{10}$) and *E. coli* (0.7 $\log_{10}$) numbers were lower in TC-gavaged SIHUMI + Bw mice compared to the respective control mice (Fig. 32 A). The cecal abundance of *E. coli* was lower by 0.3 $\log_{10}$ in SQ-treated than in water-treated SIHUMI mice. In mice harboring SIHUMI + Bw the abundance of *B. wadsworthia* was 0.4 $\log_{10}$ higher in SQ-treated than in water-treated mice. In colonic contents, the abundance of *C. ramosum* was 0.3 $\log_{10}$ lower in TC versus water-gavaged SIHUMI + Bw mice. In fecal samples from day zero, no differences in bacterial abundance were detected. In fecal samples from day 18, the cell numbers of *A. caccae* were higher in mice gavaged with SQ or TC (0.4 $\log_{10}$ each), while the cell numbers of *B. producta* (SQ: 0.3 $\log_{10}$; water: 0.3 $\log_{10}$) and *C. butyricum* (SQ: 0.3 $\log_{10}$) were reduced in response to the presence of *B. wadsworthia*. This indicates that the growth of *A. caccae* is enhanced by *B. wadsworthia*, potentially through cross-feeding, and the proliferation of *B. product* and *C. butyricum* is reduced by *B. wadsworthia*, potentially because of substrate competition.

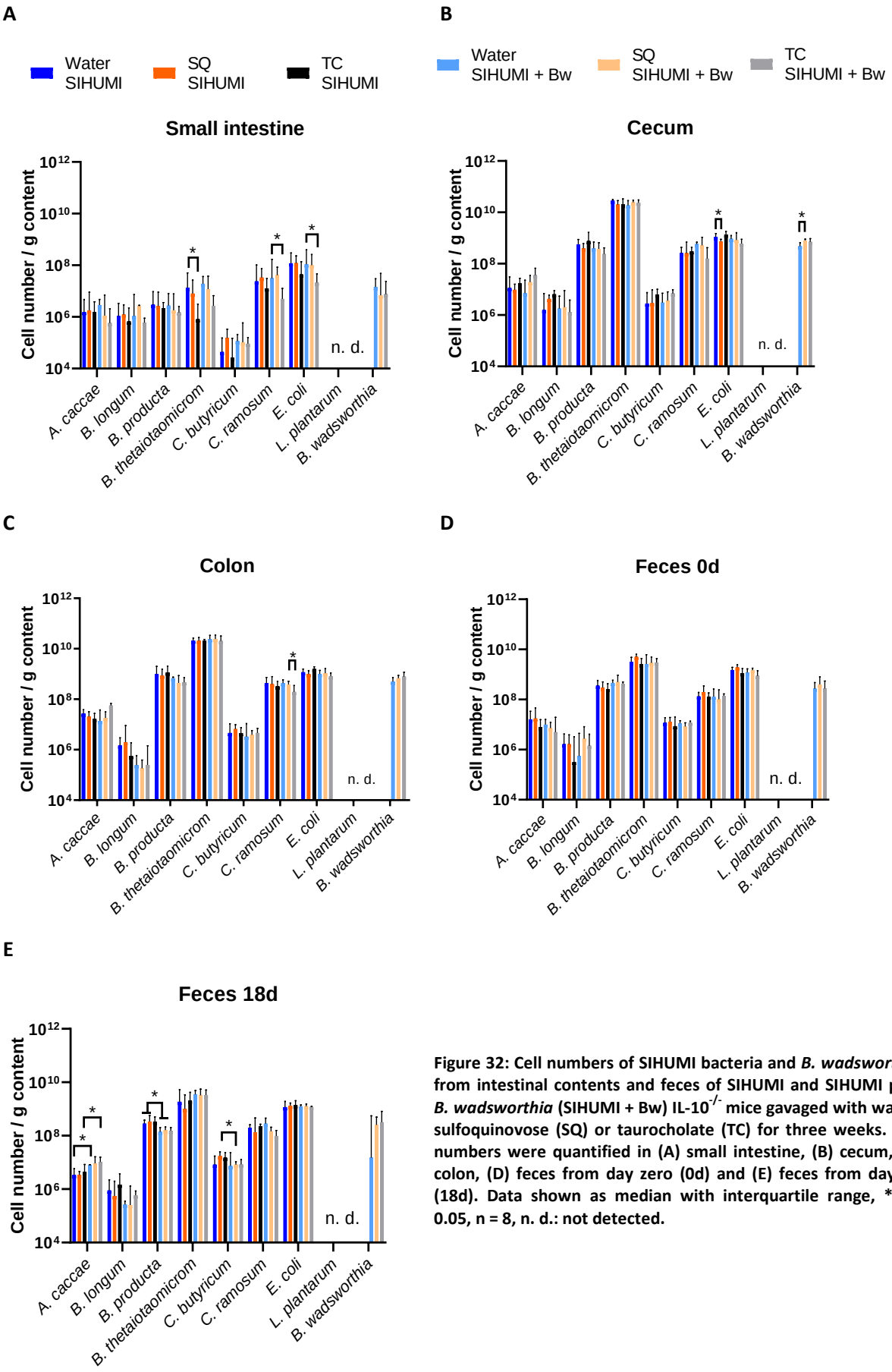
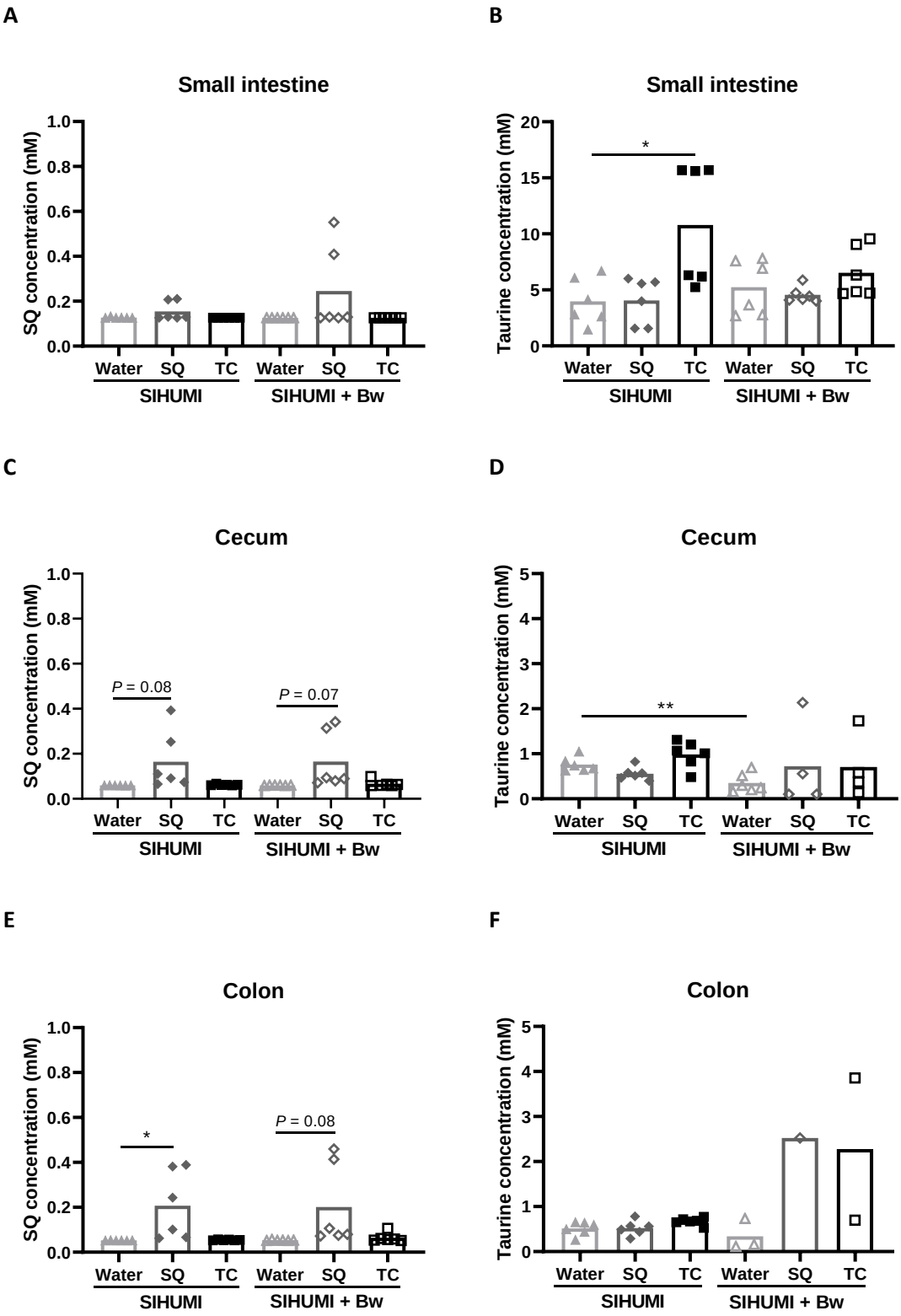


Figure 32: Cell numbers of SIHUMI bacteria and *B. wadsworthia* from intestinal contents and feces of SIHUMI and SIHUMI plus *B. wadsworthia* (SIHUMI + Bw) IL-10$^{-/-}$ mice gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Cell numbers were quantified in (A) small intestine, (B) cecum, (C) colon, (D) feces from day zero (0d) and (E) feces from day 18 (18d). Data shown as median with interquartile range, *P < 0.05, n = 8, n. d.: not detected.

3.4.4 SQ, DHPS and taurine concentrations were partly elevated in mice treated with SQ or TC

Sulfonates reaching the large intestine could serve as sulfite source for sulfite-reducing bacteria (chapter 3.1.1). However, the availability of dietary sulfonates for bacteria in the large intestine has not been investigated. Also, nothing is known about the *in vivo* formation of degradation products by intestinal bacteria, such as DHPS formed from SQ and taurine or isethionate formed from TC. In the present study, intestinal contents and feces of SQ- and TC-gavaged gnotobiotic mice were analyzed for the presence of such sulfonates. SIHUMI mice orally treated with SQ only had higher SQ concentrations in the colon when compared to SIHUMI mice treated with water (Fig. 33E). SIHUMI mice gavaged with TC had higher taurine concentrations in the small intestine than SIHUMI mice gavaged with water (Fig. 33B). In the presence of *B. wadsworthia*, SQ concentrations in feces from day 18 of mice gavaged with SQ were higher than those of mice gavaged with water (Fig. 33I). Lower concentrations of taurine were detected in SIHUMI + Bw mice compared to SIHUMI mice, both gavaged with water (Fig. 33D). DHPS was detected exclusively in cecal and colonic contents was well as feces collected on day 18 of all SIHUMI mice gavaged with SQ with mean (± σ) concentrations of 0.45 mM (± 0.12 mM), 0.39 mM (± 0.13 mM) and 1.16 mM (± 0.45 mM), respectively. Isethionate was not detected in any mouse (data not shown).


A
Small intestine
SQ concentration (mM)
1.0
0.8
0.6
0.4
0.2
0.0
Water SQ TC Water SQ TC
SIHUMI
SIHUMI + Bw
B
Small intestine
Taurine concentration (mM)
20
15
10
5
0
*
Water SQ TC Water SQ TC
SIHUMI
SIHUMI + Bw
C
Cecum
SQ concentration (mM)
P = 0.08
P = 0.07
Water SQ TC Water SQ TC
SIHUMI
SIHUMI + Bw
D
Cecum
Taurine concentration (mM)
5
4
3
2
1
0
**
Water SQ TC Water SQ TC
SIHUMI
SIHUMI + Bw
E
Colon
SQ concentration (mM)
*
P = 0.08
Water SQ TC Water SQ TC
SIHUMI
SIHUMI + Bw
F
Colon
Taurine concentration (mM)
Water SQ TC Water SQ TC
SIHUMI
SIHUMI + Bw

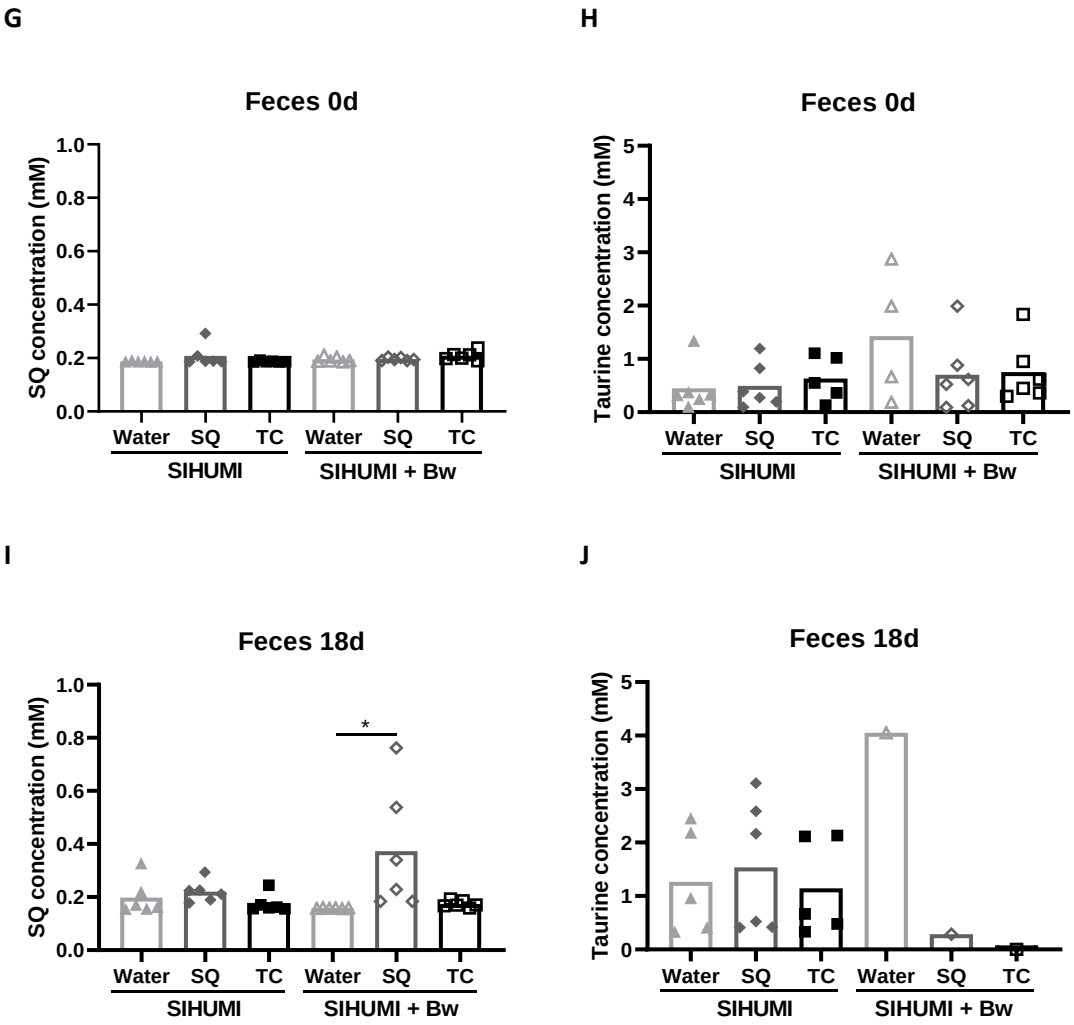


Figure 33: Sulfoquinovose (SQ) and taurine concentrations in intestinal contents and feces of SIHUMI and SIHUMI plus *B. wadsworthia* (SIHUMI + Bw) IL-10$^{-/-}$ mice. The mice were gavaged with water, SQ or taurocholate (TC) for three weeks. Left panel: SQ in (A) small intestine, (C) cecum, (E) colon, (G) feces from day zero and (I) feces from day 18. Right panel: taurine in (B) small intestine, (D) cecum, (F) colon, (H) feces from day zero and (J) feces from day 18. Note that taurine was not detected in all samples. Data shown as values of individual mice and mean, *$P < 0.05$, **$P < 0.01$, n = 6.

3.4.5 Sulfonate treatment of gnotobiotic mice did not increase sulfide concentrations above physiological levels

As an indirect measure of sulfide formation, the relative expression of *Sqr* and *Hif-1α* was determined. Neither the oral application of SQ or TC, nor additional colonization of SIHUMI mice with *B. wadsworthia* evoked an increase in the expression of these genes in cecal or colonic mucosa relative to the respective controls (Table 25).

Table 25: Expression of *Sqr* and *Hif-1α* in cecal and colonic mucosa of gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw). The mice were gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. Sqr: sulfide:quinone oxidoreductase, Hif-1α: hypoxia-inducible factor-1 alpha. Data shown as mean ± σ, n = 8.

	SIHUMI			SIHUMI + Bw		
	Water	**SQ**	**TC**	**Water**	**SQ**	**TC**
Cecum						
Sqr	1.00 ± 0.58	0.96 ± 0.64	1.39 ± 1.13	0.76 ± 0.55	1.20 ± 0.97	0.96 ± 0.76
Hif-1α	1.00 ± 0.42	0.64 ± 0.37	1.17 ± 0.50	0.94 ± 0.35	0.76 ± 0.33	1.28 ± 0.35
Colon						
Sqr	1.00 ± 0.37	0.93 ± 0.47	1.00 ± 0.55	0.83 ± 0.30	0.95 ± 0.59	0.71 ± 0.51
Hif-1α	1.00 ± 0.83	0.54 ± 0.43	0.77 ± 0.57	0.71 ± 0.46	1.44 ± 1.11	0.57 ± 0.40

3.4.6 Sulfonate treatment and presence of *B. wadsworthia* affected the bile-acid profile of gnotobiotic mice tremendously

Bacterial conversion of bile acids can have a remarkable impact on host health and disease [119]. Therefore, it is crucial to know how specific bacteria, such as *B. wadsworthia*, influence the bile-acid metabolism. The cecal bile-acid profile of SIHUMI mice differed considerably from that of SIHUMI + Bw (Table 26) and conventional mice (Tables 14, 20). Bile acids in SIHUMI mice were marginally deconjugated and converted into secondary bile acids. In SIHUMI + Bw mice, TCA was deconjugated to CA and T-β-MCA was deconjugated to β-MCA. In SIHUMI mice gavaged with TC, the β-MCA concentrations were 2.1-fold lower than in SIHUMI mice gavaged with water. Furthermore, the concentrations of α-MCA and CDCA were much higher in SIHUMI + Bw than in SIHUMI mice. The presence of *B. wadsworthia* did not lead to an increase in the conversion of primary to secondary bile acids. Only ω-MCA was detected in considerable amounts in SIHUMI + Bw mice treated with TC, which was not observed in any other mouse group (Table 26). Independent of the microbial status, mice gavaged with TC had much higher CA concentrations compared to mice gavaged with water.

Table 26: Concentrations of primary and secondary bile acids in cecal contents of gnotobiotic IL-10$^{-/-}$ mice harboring a simplified human intestinal microbiota (SIHUMI) or SIHUMI with *B. wadsworthia* (SIHUMI + Bw). The mice were gavaged with water, sulfoquinovose (SQ) or taurocholate (TC) for three weeks. CA: cholic acid, TCA: taurocholic acid, GCA: glycocholic acid, CDCA: chenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid, GCDCA: glycochenodeoxycholic acid, UDCA: ursodeoxycholic acid, MCA: muricholic acid, T-β-MCA: tauro-beta-MCA, DCA: deoxycholic acid, TDCA: taurodeoxycholic acid, GDCA: glycodeoxycholic acid, LCA: lithocholic acid, n. d.: not detected. Data presented in nmol per g content as median [Q1 – Q3], *$P < 0.05$, **$P < 0.01$ versus water group with same colonization status, $^{\#\#}P < 0.01$ SIHUMI versus SIHUMI + Bw, n = 6.

	SIHUMI			SIHUMI + Bw		
	Water	**SQ**	**TC**	**Water**	**SQ**	**TC**
Primary bile acids (nmol / g)						
CA	21.52 [16.89 – 68.61]	28.13 [21.34 – 37.98]	113.02 * [69.89 – 204.20]	708.74 ## [337.66 – 1498.09]	505.44 ## [401.56 – 1405.17]	3889.05 ** ## [2808.43 – 5530.45]
TCA	135.64 [63.93 – 421.79]	194.74 [118.72 – 242.84]	451.01 [326.69 – 1099.55]	0.69 ## [0.23 – 1.47]	0.31 ## [0.10 – 0.93]	1.57 ## [0.78 – 3.17]
GCA	0.06 [0.00 – 0.16]	0.03 [0.00 – 0.12]	0.20 [0.10 – 0.31]	0.03 [0.00 – 0.14]	0.00 [0.00 – 0.11]	0.29 [0.11 – 0.49]
CDCA	2.57 [1.83 – 3.43]	2.43 [2.03 – 3.35]	2.92 [1.73 – 3.41]	14.16 ## [7.32 – 18.04]	8.11 ## [7.30 – 19.95]	15.73 ## [11.43 – 24.09]
TCDCA	5.05 [3.54 – 25.22]	6.49 [3.95 – 12.12]	11.80 [7.98 – 22.53]	0.06 ## [0.03 – 0.09]	0.00 ## [0.00 – 0.07]	0.00 ## [0.00 – 0.92]
GCDCA	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
α-MCA	0.00 [0.00 – 14.56]	n. d.	n. d.	76.50 ## [36.05 – 111.44]	61.63 ## [46.15 – 125.93]	63.08 ## [56.47 – 83.11]
β-MCA	12.66 [9.34 – 20.78]	11.49 [8.81 – 13.69]	6.08 ** [3.83 – 9.39]	384.67 ## [284.47 – 520.49]	399.12 ## [346.45 – 671.84]	191.18 ## [132.64 – 313.66]
T-β-MCA	240.46 [188.88 – 641.63]	349.30 [284.17 – 358.34]	120.49 [99.87 – 284.21]	1.16 ## [0.84 – 2.08]	1.42 ## [0.37 – 2.30]	0.14 ## [0.09 – 0.50]
Secondary bile acids (nmol / g)						
DCA	0.31 [0.13 – 0.37]	0.21 [0.03 – 0.37]	0.15 [0.00 – 0.43]	n. d. #	n. d. #	n. d.
TDCA	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
GDCA	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
UDCA	0.00 [0.00 – 0.82]	0.00 [0.00 – 0.10]	n. d.	7.16 ## [5.10 – 21.32]	7.40 ## [5.84 – 23.42]	4.35 ## [2.76 – 9.81]
LCA	5.69 [3.58 – 8.05]	4.22 [2.91 – 5.96]	5.16 [3.28 – 6.94]	5.53 [4.16 – 7.12]	5.16 [4.38 – 7.40]	3.22 [2.89 – 4.05]
ω-MCA	n. d.	n. d.	n. d.	0.00 [0.00 – 10.59]	0.00 [0.00 – 9.55]	40.44 ** ## [29.41 – 47.63]

4 Discussion

The degradation of dietary sulfonates by intestinal bacteria and the resulting consequences for host health have not been studied to date. However, possible detrimental effects of associated degradation products and of bacteria involved in the degradation have been suggested [2, 57, 58]. Therefore, the effects of dietary sulfonates on gut microbiota composition and host-health were investigated in conventional and gnotobiotic IL-$10^{-/-}$ mice. The gnotobiotic mice harbored the SIHUMI consortium with or without *B. wadsworthia*. The dietary sulfonates under investigation were the sulfolipids SQDG, which are highly abundant in the cyanobacterium Spirulina (*A. platensis*), and their degradation product SQ. The sulfonated bile acid TC was included as a positive control.

4.1 A complex microbiota and a minimal microbial consortium degraded SQ and TC to sulfide

To test fecal bacteria from conventional mice for their ability to convert SQ to sulfide, incubation experiments were conducted. The decline in the concentration of the sulfonates was accompanied by the formation of sulfide. Furthermore, SQ stimulated the growth of *B. wadsworthia*, as cell numbers of this bacterium increased to a larger extent than in the absence of SQ. Experiments that tested the ability of *B. wadsworthia* to grow on various sulfonates in pure culture revealed that the bacterium is unable to grow on SQ. However, addition of DHPS to the medium led to sulfide formation and enabled B*. wadsworthia* to grow, even though to a lesser extent than with taurine [156]. The pathway for the conversion of DHPS to sulfide has been characterized in sulfate- and sulfite-reducing bacteria including *B. wadsworthia* [41]. Although *E. coli* was not detected by qPCR, it is conceivable that this or another bacterium converted SQ to DHPS [35, 39] and *B. wadsworthia* utilized the latter as an electron acceptor for its growth. This would explain the observed sulfide formation in fecal SQ incubations and the stronger growth of *B. wadsworthia*. The gene cluster for the degradation of SQ is widely distributed among *Enterobacteriaceae* and strictly anaerobic clostridia and a co-culture of *E. coli* K12 and *Desulfovibrio* sp. strain DF1 has been demonstrated to convert SQ to sulfide [35, 39, 40].

This co-operation of bacterial strains was also observed in experiments with SIHUMI or SIHUMI + Bw incubated with SQ. Here, sulfide formation in quantitative amounts occurred in the presence of *B. wadsworthia* only, whereas in its absence only DHPS was formed. In this minimal consortium, *E. coli* was detected by qPCR but its growth was not enhanced by SQ supplementation. This might be explained by a previously reported 50% slower growth rate of *E. coli* K12 with SQ than with glucose. The reason for that is the fact that *E. coli* is not able to gain energy from the degradation of DHPS, which was found to be secreted into the medium [40]. Sulfide formation from SQ and increased cell numbers of *B. wadsworthia* observed in incubation experiments with complex and minimal bacterial consortia makes it conceivable that the *in vivo* conversion of SQ could have detrimental effects on the host health. Both, the accumulation of sulfide and growth stimulation of *B. wadsworthia* have been associated with intestinal inflammation [2, 58].

The addition of *B. wadsworthia* to the minimal bacterial community was a prerequisite for the formation of sulfide from TC and for the stimulation of its growth. The ability of *B. wadsworthia* to

utilize bile as a growth substrate was documented in the first description of *B. wadsworthia* [14]. Interestingly, this bacterium is devoid of any of the known bile salt hydrolase genes, which are widely distributed among bacteria [28], and no other enzyme catalyzing the deconjugation of bile acids has been described so far. Nevertheless, *B. wadsworthia* does cleave the taurine moiety from TC and, in the presence of an electron donor such as formate, converts taurine to ammonia, acetate and sulfide [111].

The deconjugation of the bile acid was not dependent on the presence of *B. wadsworthia* because TC hydrolysis was catalyzed by SIHUMI bacteria as well. In fact, the ability to deconjugate TC has already been described for *C. ramosum*, *B. longum* and *E. coli* [120]. However, the conversion of taurine to sulfide by the SIHUMI bacteria was not observed in the present study, even though *C. butyricum* was reported to possess a gene cluster for taurine degradation similar to that found in *B. wadsworthia* [49]. In general, it has been reported that the human microbiota degrades taurine under anoxic conditions to a small extent and that the microbial composition is not impacted by this sulfonated amino acid [48]. In conclusion, a complex microbiota and a minimal consortium including *B. wadsworthia* have been demonstrated to be capable of converting SQ and TC to sulfide and the growth of *B. wadsworthia* was thereby promoted.

4.2 Spirulina feeding mildly stimulated an immune response and modulated the intestinal microbiota composition

Conventional IL-$10^{-/-}$ mice were fed an SQDG-rich SD for three weeks to investigate the effects of this dietary sulfonate on microbiota composition and host-health status. Spirulina is the commercial name of the cyanobacterium *A. platensis*, which is widely used as a food supplement [121]. SD-fed mice consumed approximately 23 g of Spirulina per kg body weight, which corresponds to an estimated intake of 200 mg SQDG per kg body weight and day, based on the lipid content of the cyanobacterium and literature data on the percentage of SQDG in the lipid fraction [122]. The relatively high SQDG content of Spirulina was supposed to increase the SQ concentration in the murine intestinal tract and, thereby, serve as a substrate for the degradation to sulfide (see chapter 4.1). Indeed, the concentration of SQ was higher in cecal and colonic contents and feces collected on day 18 of SD-fed than of CD-fed mice. This observation suggests that SQDG was liberated from the Spirulina cells and converted to SQ, possibly by *E. coli* [34]. The liberation of SQ from Spirulina in the intestinal tract of mice has not been described in the literature so far.

Mice fed SD displayed a higher cecal histopathology score, higher relative expression of *Tnf-α* in cecal and colonic mucosa and lower cecal expression of *Ocln* compared to mice fed CD. The differences between the mouse groups were small, but in sum they indicate a mild activation of the immune system in the mice fed SD. An immunomodulatory effect of Spirulina has been described previously [31] and a slightly increased TNF-α secretion has been reported for wild type mice fed a low dose of Spirulina [123]. Furthermore, 24-month-old mice fed a diet supplemented with 5% Spirulina exhibited higher gene expression of inflammatory markers including *Ifn-γ* and *Il-6* in the cecum compared to mice fed a control diet [124]. However, the majority of studies investigating the effect of Spirulina report anti-inflammatory, anti-oxidant and other health-promoting effects of this cyanobacterium [31]. Moreover, increased histopathology scores in TNBS-treated rats were lowered

by a single gavage of a Spirulina solution (2 g/kg body weight) and markers for oxidative stress were reduced [125]. However, it has to be pointed out that histopathological scores reached in the present study were low compared to those of severely inflamed mice [94, 126] and, thus, are rather signs of immune cell activation than intestinal inflammation.

Beneficial characteristics described for Spirulina were mainly attributed to its richness in bioactive ingredients, including vitamins, anti-oxidants, trace elements, minerals, essential amino acids, unsaturated fatty acids, β-carotene, calcium spirulan (anti-viral), immulina (immune-stimulating) and C-phycocyanin (anti-oxidative, anti-microbial) [31, 32]. Owing to these health-promoting substances, Spirulina is considered as a functional food [127] and rated as safe in the United States Pharmacopeia [128]. In addition, SQDG has been shown to inhibit the activity of human DNA polymerases, which could be used to stop the proliferation of cancer cells. Therefore, SQDG was speculated to be a useful anti-cancer therapeutic. However, incubating human cancer cell lines with SQDG did not reduce their growth. Furthermore, reports on the ability of SQDG to inhibit angiogenesis, which is an essential mechanism for cancer-cell growth, were inconsistent between different test assays [129]. This indicates that the effects of Spirulina supplementation still need to be studied in more detail.

Assessment of body-weight gain and feed intake revealed an initial increase of both parameters in mice fed SD compared to mice fed CD. However, body-weight gain and feed intake did not differ between the mouse groups at the end of the intervention and no fat accumulation was observed in mWAT or intestinal organs. Spirulina supplementation has previously been reported to result in an increase in body weight in broiler chicken, even though the Spirulina content was lower (up to 1%) than in the present study (20%). In contrast to the observations made in the present PhD-project, the increased body weight was solely observed in the fourth and last week of the intervention [130]. Apart from this study, there are no further publications that report an increase in body weight or feed intake in response to Spirulina feeding in animals. In contrast, supplementation of the drinking water with Spirulina reduced the diet-induced weight gain of mice fed a Western diet [131].

Another intriguing observation was the effect of Spirulina feeding on bile-acid composition and on bile-associated organs. A lower liver weight, higher gall bladder weight and two-fold higher cecal concentrations of secondary bile acids, namely DCA and LCA, were observed in SD-fed compared to CD-fed mice. This suggests that Spirulina impacts the enterohepatic circulation and bile-acid composition. The increase in secondary bile acids could possibly be mediated by an increased abundance of bacteria catalyzing 7α/β-dehydroxylation of primary bile acids [25] (see below). Alternatively, the high DCA concentration might be linked to the low abundance of *B. wadsworthia*. A negative correlation of these two has been described recently [132]. Secondary bile acids are more hydrophobic and excess concentrations thereof are associated with gallstone formation, liver cancer and colorectal cancer [23]. DCA was further found to elicit anti-microbial and intestinal barrier-disrupting effects [133, 134]. The down-regulation of *Ocln* expression observed in the present study may be considered as an indication for a disrupted intestinal barrier. However, intestinal permeability was not higher in mice fed SD versus mice fed CD, as assessed by the FITC-dextran assay. This might indicate that down- regulation of the *Ocln* gene does not affect the physiological function of the gut barrier and/or that the lower expression of *Ocln* is merely the first step in a cascade ultimately leading to increased intestinal permeability in the long run. Furthermore, the higher gall-bladder weight could indicate an increased bile acid production or a reduced secretion of bile acids from the gall bladder into the small intestine. Higher production rates of bile acids in the liver are generally attributed to a higher fat content in the diet [135]. However, the fat content of the

CD and SD were the same and, thus, are unlikely to be responsible for the increased gall-bladder weight. Another possible mechanism that increases gall-bladder size is the agonistic stimulation of the intestinal TGR5 receptor by DCA and LCA, which elicits smooth muscle relaxation of the gall bladder and, thereby, facilitates its filling [136]. To date, there are no publications hinting at an impact of Spirulina feeding on bile-acid composition and no direct link between Spirulina intake and liver injury [137]. On the contrary, feeding rats a diet supplemented with 20% Spirulina for four weeks was considered safe and did not cause an increased body-weight gain or alterations in visceral organs [138].

The Spirulina-induced higher concentration of SQ in cecum and colon of conventional mice was postulated to promote the growth of sulfite-reducing bacteria, such as *B. wadsworthia* and, thus, lead to an altered composition of beneficial and harmful bacteria in the gut. However, feeding Spirulina to conventional mice for three weeks strongly reduced the abundance of *B. wadsworthia* and of sulfite-reducing bacteria. Furthermore, Spirulina feeding affected the β-diversity but not the bacterial richness and α-diversity. The observation of distinct clustering of microbial profiles in response to the diet is shared by other observational studies, and the re-establishment of a beneficial microbial composition by Spirulina supplementation was suggested [139]. As an example, mice fed a control diet or a diet supplemented with 5% Spirulina for four weeks displayed diet-induced alterations in their gut microbiota profiles [114]. In the present study, an unknown genus of the family *Porphyromonadaceae* was enriched in feces and cecal contents. *Porphyromonadaceae* are common members of the human and animal gut, but some species thereof were associated with infections [140]. The abundance of *B. wadsworthia* was reduced by approximately two orders of magnitude, which is consistent with a lower relative abundance of the family *Desulfovibrionaceae* detected in cecal content of SD mice. This is partly in accordance with literature data, in which the cell numbers of *Bacteroides*, *Clostridium* XIVa and *Barnesiella* species (the latter belonging to the family *Porphyromonadaceae*) were increased in cecal content and feces after 21 days of intervention of mice gavaged daily with Spirulina (1.5 or 3.0 g/kg body weight). Lower cell numbers of *Flavonifractor* and *Desulfovibrio* species (closely related to *B. wadsworthia*) were reported in cecal content of mice gavaged with Spirulina compared to control mice. Spirulina applications affected β-diversity but not bacterial richness [117]. However, an increase in the relative abundance of *Clostridium* cluster XIVa, which includes bacteria able to convert primary into secondary bile acids [134], was not observed in the present PhD-project, even though higher concentrations of secondary bile acids were detected.

Feeding a diet supplemented with 5% Spirulina to 24-month-old mice increased bacterial diversity but lowered the total bacterial cell number. This was accompanied by an increase in the abundance of *Roseburia* and *Lactobacillus*, whereas the tendency towards a lower abundance of *Desulfovibrionaceae* was not significant [124]. That these observations do not match those of the present study might be explained by the age difference.

In accordance with the decreased abundance of sulfite-reducing bacteria in the present PhD-project no increased expression of host genes responsible for sulfide detoxification were observed. The expression of genes transcribed when intestinal epithelial cells enter a hypoxic state was also not elevated.

In conclusion, Spirulina feeding to conventional mice mildly stimulated the murine immune system and strongly affected the intestinal microbiota composition. However, due to the richness of Spirulina in bioactive compounds it is not possible to tell whether SQDG or another compound caused the described effects.

4.3 Mice orally treated with SQ did not develop bacteria-induced intestinal inflammation

To elucidate the effects of SQ on the intestinal bacterial community and host-health status, conventional mice were gavaged daily with SQ, while SIHUMI and SIHUMI + Bw mice were gavaged with SQ twice per week for three weeks. A single dose of SQ was equimolar to that of TC but the amount of SQ per gavage (1.37 mmol/kg body weight) was higher than the estimated amount of SQ taken up with the SD (0.38 mmol/kg body weight). The administered SQ increased the SQ concentration in small intestinal, cecal and colonic contents and in feces collected on day 18 of conventional mice and those in colonic contents of SIHUMI mice and feces from day 18 of SIHUMI + Bw mice relative to the respective controls. DHPS was solely detected in cecal and colonic contents and in feces from day 18 of SIHUMI mice. The higher amounts of SQ detected in conventional mice compared to gnotobiotic mice might be due to the higher frequency of SQ gavages to the former group of mice. The detection of DHPS solely in SIHUMI mice and not in SIHUMI + Bw mice indicates that SQ was converted to DHPS by *E. coli* but in the absence of *B. wadsworthia*, DHPS was not degraded any further to sulfide. The conversion of DHPS to sulfide was mediated by the DHPS-sulfolyase (HpsG) present in *B. wadsworthia* [41]. This is in line with results obtained in the *in vitro* incubation experiments with SQ and the SIHUMI consortium (see chapter 4.1). Apart from *E.coli*, no other SIHUMI member is known so far to possess the gene cluster for SQ degradation [35, 39].

The oral application of SQ to conventional and gnotobiotic IL-10$^{-/-}$ mice did not induce an immune response, increase the colonic permeability or the histopathology score. On the contrary, SIHUMI + Bw mice gavaged with SQ had a lower relative expression of *Tnf-α* than SIHUMI + Bw mice gavaged with water. Furthermore, body weight and feed intake were not affected by SQ. Only the kidney of conventional mice and the cecum of SIHUMI mice were heavier compared to their respective controls. However, no explanations for these observed effects can be found in the literature. To date, no information is available about the fate of SQ in the mammalian intestinal tract or its effect on mammalian physiology.

The oral application of SQ led to a two-fold lower LCA concentration in the cecal content of conventional mice, which is in contrast to observations made in Spirulina-fed mice. Overall, alterations resulting from Spirulina feeding such as immune-cell activation and increased formation of secondary bile acids were not equally elicited by gavaging pure SQ to conventional mice, which argues against SQDG as key component responsible for the effects described in chapter 4.2.

The observed changes in gut bacterial community composition in SQ-gavaged conventional and gnotobiotic mice are inconsistent. The relative abundance of *Desulfovibrionaceae* in fecal samples of conventional mice gavaged with SQ was slightly increased, but the cell number of *B. wadsworthia* was not elevated in this group. SIHUMI mice gavaged with SQ harbored lower cell numbers of *E. coli* in their cecal content and higher cell numbers of *B. producta* in their feces than SIHUMI control mice

gavaged with water. SIHUMI + Bw mice treated with SQ had a higher cecal abundance of *B. wadsworthia* compared to SIHUMI + Bw mice gavaged with water and *C. butyricum* was less abundant in these mice than in SIHUMI mice treated with SQ. Interestingly, although the cell number of *E. coli* decreased, DHPS formation from SQ was catalyzed by this bacterium, as DHPS accumulated in intestinal contents of SIHUMI mice treated with SQ. The higher abundance of *B. wadsworthia* is in accordance with the SIHUMI + Bw incubation experiments, which showed a growth-stimulating effect of SQ on *B. wadsworthia*. Nevertheless, even though the relative abundance of *Desulfovibrionaceae* and *B. wadsworthia* was partly increased in conventional and gnotobiotic mice, this did not lead to intestinal inflammation. Overall, the alterations in the abundance of bacterial population groups due to SQ treatment were small and no differences in α- and β-diversity were observed.

In the present PhD-project, cecal and colonic mucosa of SQ-gavaged mice displayed no sulfide-induced higher expression of *Sqr* or *Hif-1α* relative to water-gavaged mice. This indicates that even though the microbial community is capable of converting SQ to sulfide in the presence of *B. wadsworthia*, the intestinal sulfide concentrations did not increase beyond physiological levels and induce hypoxia in mitochondria of mucosal epithelial cells. The detoxification of sulfide as described in the literature seems to be effective in preventing the accumulation of sulfide. In conclusion, even though sulfide might be formed from dietary SQ, no intestinal inflammation or bacterial alteration was observed in the present study.

4.4 TC orally applied to mice induced a mild immune response and alterations of the intestinal microbiota composition

Based on the reported capacity of TC to elicit bacteria-induced colitis in genetically susceptible mice [2], TC was applied as a positive control to IL-10$^{-/-}$ mice harboring a complex microbiota, the SIHUMI consortium or SIHUMI + Bw. The TC concentration per gavage was the same as in the study by Devkota et al. [2] and the dose (1.86 mmol/kg body weight) is comparable the amount of TC excreted with bile into the small intestine of age-matched C57BL/6 mice fed a low fat diet per day (1.73 mmol/kg body weight) [141].

However, in intestinal contents of conventional mice neither TCA nor taurine concentrations were higher than those of control mice. In SIHUMI mice treated with TC, taurine concentrations were 2.7-fold higher in the small intestine compared to SIHUMI mice treated with water. However, this increase was only observed in three of the six samples tested. No other differences in taurine or TCA concentrations were detected in the gnotobiotic mice. This might be explained by the low frequency of TC-applications (twice weekly), the high efficiency of taurine absorption in mice [142] or by the efficient degradation of taurine to sulfide by intestinal bacteria [49].

In conventional mice, oral applications of TC to mice led to a reduced feed intake, lower expression of *Tjp 1* in cecal mucosa, a higher colonic histopathology score and higher relative expression of *Ifn-ɣ*, *Il-1ß* and *Il-6* in cecal mucosa relative to control mice. SIHUMI mice gavaged with TC showed no inflammation-associated alterations, but in the presence of *B. wadsworthia,* the intestinal permeability was increased (higher plasma FITC-dextran concentrations and lower cecal *Tjp 1*

expression) and the cecal expression of *Ifn-ɣ* and *Tnf-α* was higher than in mice gavaged with water, while the expression of *Il-6* and *Tnf-α* was higher in TC-gavaged SIHUMI + Bw mice than in those without *B. wadsworthia*. These alterations indicate a mild inflammatory response following TC application in the presence of *B. wadsworthia* together with a decreased integrity of the intestinal barrier. Indeed, a link between secretion of cytokines, especially IFN-ɣ, and damaged tight junctions ultimately leading to a dysfunctional intestinal barrier has been described [143]. However, the immune response observed in the present study was much lower than that reported in the literature. Especially the strongly increased histopathology scores and the highly elevated levels of Th1-induced cytokines such as IFN-ɣ in IL-$10^{-/-}$ mice gavaged with TC reported previously [2] are in contrast to the observations made in the current PhD-project. This discrepancy is surprising, because the experimental set-up, the animal model and substance applied were adapted from the study performed by Devkota and colleagues.

Another conflicting observation was made in gnotobiotic mice. In the study by Devkota et al. mono-association of germ-free mice with *B. wadsworthia* could only be established by the simultaneous administration of TC [2]. In contrast to that, SIHUMI + Bw mice gavaged with water or SQ were stably colonized by *B. wadsworthia* in the present PhD-project. Furthermore, the immune response of SIHUMI + Bw mice gavaged with TC was much milder than that described for *B. wadsworthia*-mono-associated mice [2], which was assessed by histopathology scores and quantification of cytokines. This might be explained by the presence of other bacteria limiting the expansion of the pathobiont by competing for substances required for the growth of *B. wadsworthia*. At the same time, the SIHUMI bacteria could produce substances that are used by *B. wadsworthia* for its growth, a mechanism referred to as cross-feeding [144]. These factors need to be considered when interpreting data from mono-association studies and comparing them to experiments with a minimal consortium.

The outcome of animal experiments is further subject to environmental conditions, which vary considerably between animal facilities. It has been shown that IL-$10^{-/-}$ mice kept in Bar Harbor (Maine, USA) had significantly higher cecal and colonic histopathology scores than IL-$10^{-/-}$ mice kept in Hannover (Germany), even though treatment and the pathologist scoring the tissue samples were the same [79]. This discrepancy may result from divergent animal handling, feed composition and water treatment (acidified, autoclaved or untreated).

In the literature, TC-application studies were mostly conducted with lower doses of TC and aimed to increase the solubility and absorption of hydrophobic drugs, which would otherwise not be able to elicit the intended effect [145]. Only few studies have so far investigated the effect of TC on mucosal barrier integrity or modulation of the immune system. Indeed, some studies indicate that TC elicits mucosal injury [146] and modulates the immune response towards different stimuli *in vitro* and *in vivo* [147].

Bile acids were quantified in cecal contents of conventional and gnotobiotic mice after three weeks of TC-treatment to quantify the amount of TC reaching the cecum and to clarify the impact of this bile acid on the overall bile acid composition. The deconjugation and dehydroxylation of TC could alter the bile-acid composition considerably. In conventional mice, TC-applications led to lower concentrations of the primary bile acid β-MCA (3-fold reduction) and of the secondary bile acid LCA (2-fold reduction). In SIHUMI and SIHUMI + Bw mice gavaged with TC, CA concentrations were five times higher than in mice gavaged with water, while the ω-MCA concentration was exclusively

increased in SIHUMI + Bw mice gavaged with TC. This indicates that TC is deconjugated to taurine and cholate by SIHUMI bacteria, but secondary bile acids are not formed. This is in accordance with results from *in vitro* incubation experiments described in chapter 3.1.3. In general, the additional colonization of SIHUMI mice with *B. wadsworthia* strongly increased the deconjugation of bile acids, especially of primary bile acids. This observation is in accordance with reports stating that *B. wadsworthia* is highly effective in converting conjugated to unconjugated bile acids [148]. However, the dehydroxylation of primary to secondary bile acids was strongly reduced in SIHUMI and SIHUMI + Bw mice compared to conventional mice. It appears that *B. wadsworthia* is able to convert α- or β-MCA to ω-MCA but the reason, why this conversion only takes place in SIHUMI + Bw mice treated with TC cannot be found.

It is conceivable that TC is deconjugated in both gnotobiotic mouse groups leading to increased concentrations of CA and taurine in mice gavaged with TC. Not only *B. wadsworthia*, but also *Lactobacillus*, *Bifidobacterium*, *Bacteroides* and *Clostridium* possess a TC-deconjugating BSH [28, 149]. While the taurine concentration in the small intestine of SIHUMI mice increased in response to TC, in SIHUMI + Bw mice taurine was degraded to sulfide by *B. wadsworthia*. Another interesting, but yet unexpected observation was the increased size of the gall bladder in TC-gavaged mice. The above observations indicate that TC-supplementation has a strong impact on the enterohepatic circulation of bile acids. However, this needs to be further investigated.

The postulated TC-induced growth stimulation of sulfite-reducing bacteria, such as *B. wadsworthia,* was investigated by quantifying gut bacteria by *16S rRNA* gene sequencing and by qPCR analysis. The relative abundance of *Desulfovibrionaceae* and cell numbers of sulfite-reducing bacteria, especially of *B. wadsworthia*, increased two-fold in response to TC, but only in the cecum of conventional mice. The stimulating effect of bile acids on the growth of *B. wadsworthia* is in accordance with the literature [2, 14], but an increase by 2000-fold as reported by Devokta et al. was not observed in the present study. Differences in β-diversity were detected in feces of mice gavaged with TC between day zero and day 18 of the intervention and between mice treated with TC or water. The microbiota-modulating potential of bile acids is well known and includes cell membrane-damaging, other bacteriocidal effects and growth stimulation of certain bacteria [150]. The relative abundance of *Lactobacillus* and strains of the *Clostridium* cluster IV increased, while the relative abundance of *Porphyromonadaceae* strains decreased in feces of mice gavaged with TC. *Lactobacillus* strains have been reported to be capable of deconjugating but not dehydroxylating bile acids and, contrary to observations in the present PhD-project, their *in vitro* growth was reported to be inhibited by bile acids [151]. Contrarily, feeding mice a milk-fat diet led to an increased secretion of TC and thereby stimulated the growth of *Lactobacillus* [2]. *Clostridium* cluster IV belongs to the small group of bacteria able to convert primary into secondary bile acids [25], which explains their expansion in mice gavaged with TC. The observed decrease of *Porphyromonadaceae* in fecal samples of TC-gavaged conventional mice is in accordance with a previous study which reported a higher abundance of this family in patients with liver cirrhosis and explained this to result from lower intestinal bile acid concentrations [134]. Sequencing results of the present PhD-project further indicate that in cecal contents of mice gavaged with TC another member of the *Bacteroidetes*, namely *Bacteroides*, increased and *Allobaculum* (*Firmicutes*) decreased in its relative abundance compared to mice gavaged with water. The higher abundance of *Bacteroides* strains might be explained by the ability of some *Bacteroides* spp. to dehydroxylate CA to DCA [152]. The growth of *Allobaculum* species was reported to be stimulated by dietary CA supplementation of mice [153], which was not the case in the present PhD-project. The latter is in agreement with a lower relative

abundance of *Allobaculum* species in milk-fat-fed mice reported by Devkota et al. [2]. In conclusion, it appears that some observations regarding bile acid-induced alterations in the abundance of bacterial taxa are shared among some studies whereas others are not. It can only be speculated that this is due to differences in experimental set-up or divergence in microbiota composition at the beginning of the experiment.

In summary, the inflammatory response and growth stimulation of *B. wadsworthia* observed in mice treated with TC was much milder than described in the literature. Furthermore, transferability of these data from mice to humans is difficult because the bile-acid composition of humans is different from that of mice [154].

4.5 *B. wadsworthia* elicits a mild colitogenic response in gnotobiotic mice orally treated with TC

The comparison of mice harboring the SIHUMI consortium with *B. wadsworthia* additionally present or not allows investigating the role of this pathobiont in a defined setting. Overall, neither feed intake nor body-weight gain was affected negatively by *B. wadsworthia*. Differences in microbiota composition between SIHUMI and SIHUMI + *B. wadsworthia* mice were small and only detected in feces collected on day 18 of the intervention. TC-treated gnotobiotic mice displayed a higher relative gene expression of a number of cytokines in cecal mucosa and a higher intestinal permeability when *B. wadsworthia* was present. However, these alterations were not as strong as those described in other studies [2, 21, 22]. Gnotobiotic Altered Schaedler Flora wild-type mice additionally colonized with *B. wadsworthia* responded with elevated cytokine concentrations in cecum, mLN and spleen to this bacterium, which was partly reversed by adding the probiotic bacterium *Lactobacillus rhamnosus* [22]. This indicates that the composition of the bacterial community affects the inflammatory potential of *B. wadsworthia*. Furthermore, the *B. wadsworthia* strain used by Devkota et al. and Natividad et al. differed from that used in the present PhD-project. They used the type strain *B. wadsworthia* ATCC 49260 [2, 22], which was isolated from a patient's perforated appendiceal abscess; *B. wadsworthia* DSM 11045, which was used in the present study, was isolated from anoxic sewage sludge, indicating a possible human fecal source. The expression of virulence genes and resulting cytotoxicity of both strains could vary considerably even though both of them belong to the same species. The genetic diversity of these two sub-strains was demonstrated by PCR fingerprinting [155], which could possibly explain the divergent experimental outcomes. Variations of this kind can be observed for example in *E. coli*, which includes the probiotic strain *E. coli* Nissle 1917 and the pathogenic adherent-invasive *E. coli* LF 82 [84]. However, this needs to be clarified in future studies, and it does not explain the discrepancies observed in conventional mice. The sub-strains present in conventional mice were not identified any further by Devkota et al. and also not in the present study. However, both *B. wadsworthia* strains have in common that they were unable to cause inflammation in wild-type mice [2, 23]. Interestingly, a *B. wadsworthia* isolate from a LADA patient caused systemic inflammation in SPF wild-type mice [21]. Symptoms included body-weight and fat-mass reduction, hepatosplenomegaly and higher concentrations of inflammatory markers in serum compared to control mice. However, these effects were observed only when the mice were gavaged daily with *B. wadsworthia* for one week, indicating that a high abundance of this bacterium is required to cause inflammation. This is in accordance with other experiments showing that the slightly increased

abundance of *B. wadsworthia* in mice fed a high-fat diet was not sufficient to elicit inflammatory and metabolic effects. Only when the cell number of *B. wadsworthia* was increased by oral applications of this bacterium on three consecutive days, inflammation and metabolic dysfunctions occurred [22].

Strain *B. wadsworthia* DSM11045 was also used in conjunction with a minimal consortium called B4PC2 [23], which is composed of bacteria known to deconjugate and dehydroxylate bile acids, as verified by an *in vivo* study in wild-type mice. However, no adverse effects or inflammation were reported even though the relative abundance of *B. wadsworthia* was as high as 14.7%. In addition to the high abundance, transcriptomic analysis revealed that the genes involved in taurine respiration were up-regulated, indicating a high activity of this bacterium concomitant with formation of sulfide [23].

In summary, the colitogenic effect of *B. wadsworthia* seems to be dependent on multiple factors, including environmental conditions, microbiota composition, abundance and strain of *B. wadsworthia*. Furthermore, even though *B. wadsworthia* is capable of converting sulfonates such as taurine and DHPS to sulfide, this does not necessarily lead to intestinal inflammation.

5 Conclusion and perspectives

Interactions between gut microbiota and host play a pivotal role in health and disease. The diet consumed by the host can modify the intestinal microbiota composition and stimulated the growth of beneficial or harmful bacteria [1]. Therefore it is important to identify dietary components that stimulate or suppress the growth of pathogenic bacteria. Devkota et al. [2] reported a causal link between a high-fat diet, the sulfonated bile acid TC and bacteria-induced intestinal inflammation in susceptible mice. This led to the hypothesis that dietary sulfonates could stimulate the growth of colitogenic bacteria as well and thereby elicit colitis in the host. Based on this assumption, the effects of the dietary sulfolipids SQDG on the intestinal microbiota composition and host health were investigated in the present PhD-project.

Supplementation of a low-fat diet with Spirulina led to a mild immune response, affected the intestinal microbiota composition and impacted the bile acid metabolism. However, the hypothesized sulfonate-mediated stimulation of *B. wadsworthia* accompanied by intestinal inflammation was not observed. Nevertheless, it cannot be excluded that the SD-mediated immune response get stronger over time and lead to severe inflammation in a prolonged intervention period. Further studies are necessary to clarify this assumption. Furthermore, as the effects elicited by SD feeding were different from those elicited by gavaging SQ, it is conceivable that the effects were not mediated by SQDG.

Oral treatment of conventional and gnotobiotic mice with SQ did not cause any immune response, even though the growth of *Desulfovibrionaceae* in conventional mice and of *B. wadsworthia* in SIHUMI + Bw mice was slightly stimulated. *In vitro* experiments revealed that microbial communities converted both SQ and TC to sulfide provided *B. wadsworthia*-was present. However, *in vivo* experiments did not provide any evidence for sulfide-induced adverse effects in the host. Solely the integrity of the intestinal barrier was slightly disturbed in mice fed SD or treated with TC. This might be due to the sulfide-mediated cleavage of sulfur bonds in the mucosa of these animals [61].

Mice orally treated with TC showed a number of colitis-associated effects, which, however, were only observed in the presence of *B. wadsworthia* and not in SIHUMI mice, indicating that growth stimulation of this bacterium is detrimental for the host. This finding corresponds to previous observations made by Devkota et al. [2] but the effect observed in the present study was much less pronounced. Higher abundance of *B. wadsworthia* was solely observed in conventional mice, which was accompanied by a distinct clustering of the microbial profiles compared to control mice.

To date, the mechanism by which *B. wadsworthia* evokes an inflammatory response is unknown. It has been suggested that the production of sulfide by this bacterium causes inflammation [16] but data obtained in this study argue against this explanation. Another proposed mechanism is the increased production of secondary bile acids [2], which are more hydrophobic than primary bile acids and could cause leakage of the intestinal barrier [25]. However, in the present study the cecal concentration of LCA was lower in conventional mice treated with TC than in such mice treated with water, while the concentrations of other secondary bile acids did not differ. Nevertheless, the concentration of some bile acids differed considerably between SIHUMI and SIHUMI + Bw mice, indicating that *B. wadsworthia* plays an important role in the bile acid metabolism, even though its relative abundance is low. Finally, the pathogenic characteristic of *B. wadsworthia* itself could cause

inflammation [20]. The authors underlined that a high abundance of *B. wadsworthia* is necessary for its immune cell-activating properties, which might explain the minor effects observed in the present study. Discrepancies in the colitogenic potential of *B. wadsworthia* between this and other studies might also result from the use of different strains isolated from different sources.

In summary, the sulfonates tested in this study were converted to sulfide by intestinal bacteria and SIHUMI + Bw, which slightly stimulated the growth of *B. wadsworthia*. However, intestinal inflammation was not observed in response to SQ, whereas Spirulina-feeding and TC-applications mildly activated the immune system of IL-10$^{-/-}$ mice. No detrimental effects caused by sulfide released from dietary sulfonates were observed. This indicates that SQDG-containing food such as spinach or Spirulina do not increase the risk of colitis for humans.

Appendix I

If not indicated otherwise, all solutions were prepared with ultra-pure distilled water from a water purification system (Ultra Clear, Siemens Water Technologies, Günsburg, Germany) and chemicals and solvents were purchased from Fluka, Oxoid, Roth, Sigma-Aldrich or Merck.

Table A1: Media and solutions used for bacterial cultivations and quantification methods.

Medium or associated solution	Concentration	Component
DS medium	19 mM	NH_4Cl
	17 mM	NaCl
	2 mM	$MgCl_2$ x 6 H_2O
	7 mM	KCl
	0.3 mM	$CaCl_2$ x 2 H_2O
	1 mM	K_2HPO_4
	40 mM	Sodium-DL-lactate
	40 mM	Sodium-format
	3.5 mg/l	Yeast extract
	1 ml/l	Selenite-tungstate solution
	1 ml/l	Trace element solution
	200 µg/l	1,4-naphtochinon
	2 µM	Resazurin
Trace element solution	10 ml/l	HCl
	1.5 g/l	$FeCl_2$ x 4 H_2O
	70 mg/l	$ZnCl_2$
	100 mg/l	$MnCl_2$ x 4 H_2O
	6 mg/l	H_3BO_3
	190 mg/l	$CoCl_2$ x 6 H_2O
	2 mg/l	$CuCl_2$ x 2 H_2O
	24 mg/l	$NiCl_2$ x 6 H_2O
	36 mg/l	Na_2MoO_4 x 2 H_2O
Selenite-tungstate solution	500 mg/l	NaOH
	3 mg/l	Na_2SeO_3 x 5 H_2O
	4 mg/l	Na_2WO_4 x 2 H_2O
7-vitamin solution	100 mg/l	Vitamin B_{12}
	80 mg/l	P-amino benzoic acid
	20 mg/l	D (+)-biotin
	200 mg/l	Nicotinic acid
	100 mg/l	Calcium pantothenate
	300 mg/l	Pyridoxine hydrochloride
	200 mg/l	Thiamine-hydrochloride x 2 H_2O
Ti (III) NTA	19.2 g/l	Nitrilotriacetic acid diluted in anoxic distilled water and adjusted to pH 9 with
	400 g/l	NaOH
	19.2 ml 20%	$TiCl_3$ (Acros)
	80 g/l	Na_2CO_3 solution to adjust to pH 7

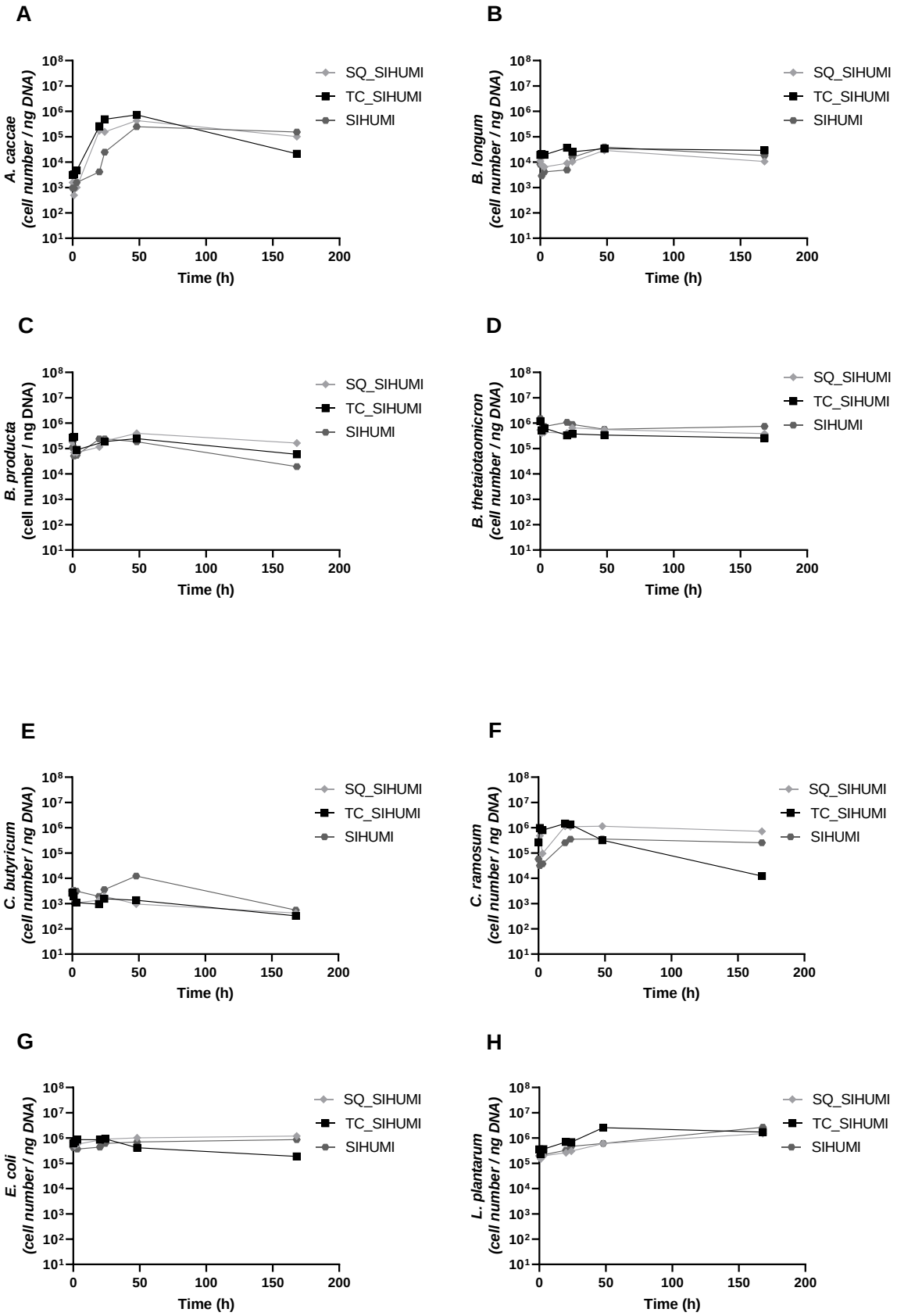
Table A1 (continued): Media and solutions used for bacterial cultivations and quantification methods.

Medium or associated solution	Concentration	Component
YH-BHI		BHI Broth
	5 g/l	Yeast extract
	10 ml/l	Hemin solution (Serva)
	1 mg/l	Resazurin
	0.5 g/l	L-cysteine HCl x H_2O
		N_2/CO_2 (80:20, v/v) as a gas phase autoclaved at 121 °C for 15 min
YCFA medium	10 g/l	Trypton/pepton from casein
	2.5 g/l	Yeast extract
	4 g/l	$NaHCO_3$
	2 g/l	Glucose
	2 g/l	Cellobiose
	2 g/l	Maltose
	150 ml/l	Mineral solution I
	150 ml/l	Mineral solution II
	3.1 ml/l	Volatile fatty acid mixture
	20 ml/l	Hemin solution
	1 ml/l	Vitamin solution I
	1 mg/l	Resazurin
		Medium was cooked and cooled down while gassed with N_2/CO_2 (80:20, v/v)
	1.44 g/l	L-cysteine hydrochloride monohydrate
		pH 7.3
		N_2/CO_2 (80:20, v/v) as gas phase autoclaved at 121 °C for 15 min
Mineral solution I	3 g/l	K_2HPO_4
Mineral solution II	3 g/l	KH_2PO_4
	6 g/l	$(NH_4)_2SO_4$
	6 g/l	NaCl
	1.22 g/l	$MgSO_4$ x 7 H_2O
	0.92 g/l	$CaCl_2$ x 2 H_2O
Hemin solution	500 mg/l	Hemin solution
	10 ml/l	NaOH (1M)
Vitamin solution I	10 mg/l	Biotin
	10 mg/l	Cobalamin
	30 mg/l	*P*-amino benzoic acid
	50 mg/l	Folic acid
	150 mg/l	Pyridoxamine
Volatile fatty acid mixture	17 ml	Acetic acid
	6 ml	Propionic acid
	1 ml	N-valeric acid
	1 ml	Isovaleric acid
	1 ml	Isobutyric acid (VEB Berlin-Adlershof, GDR)
Methylene blue detection reagent	3.45 g/l	*N,N*-dimethyl-1,4-phenylenediamine 2 HCl
	6 g/l	Fe (III)-chloride x 6 H_2O (Riedel-de Haen) dissolved in 18.5% HCl

Table A1 (continued): Media and solutions used for bacterial cultivations and quantification methods.

Medium or associated solution	Concentration	Component
DNS reagent	10 g/l	3,5-dinitrosalicylic acid
	2 g/l	Phenol
	0.5 g/l	Sodium sulfite
	200 g/l	Sodium potassium tartrate
PBS	137 mM	NaCl
	10 mM	Na_2HPO_4
	2 mM	NaH_2PO_4
		pH 7.4
		autoclaved at 121 °C for 15 min
Gitschier buffer (10 ×)	670 mM	TRIS, pH 8.8
	166 mM	NH_4SO_4
	65 mM	$MgCl_2$
		autoclaved at 121 °C for 15 min
		stored at 4 °C
	0.5%	Triton X100 (Boehringer Mannheim, Germany), added directly before use
TAE buffer	40 mM	TRIS
	20 mM	glacial acetic acid
	1 mM	EDTA
		pH 8.3
MOPS buffer (10 ×)	200 mM	MOPS
	50 mM	sodium acetate
	10 mM	EDTA
		pH 6.0
		autoclaved at 121 °C for 15 min
		stored at 4 °C

Appendix II


A
A. caccae
(cell number / ng DNA)
B
B. longum
(cell number / ng DNA)
C
B. producta
(cell number / ng DNA)
D
B. thetaiotaomicron
(cell number / ng DNA)
E
C. butyricum
(cell number / ng DNA)
F
C. ramosum
(cell number / ng DNA)
G
E. coli
(cell number / ng DNA)
H
L. plantarum
(cell number / ng DNA)
SQ_SIHUMI
TC_SIHUMI
SIHUMI
Time (h)

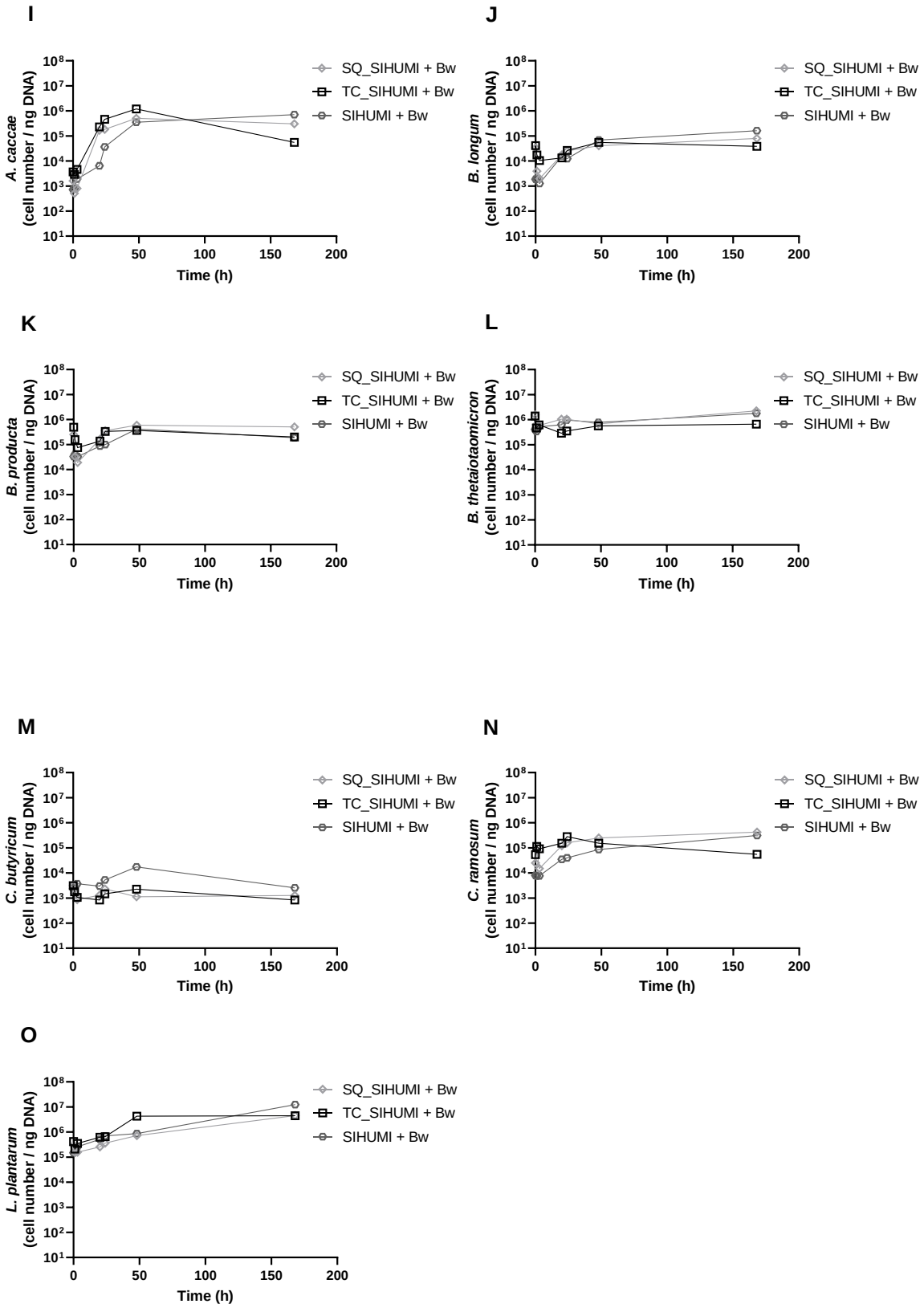


Figure A1: Cell numbers of SIHUMI bacteria incubated under anoxic conditions with sulfoquinovose (SQ), taurocholate (TC) or without sulfonates for 168 h. Cell numbers were assessed by qPCR. Incubations contained SIHUMI bacteria (A – H) or SIHUMI bacteria and *B. wadsworthia* (SIHUMI + Bw, I – O). Data presented as mean, n = 2.

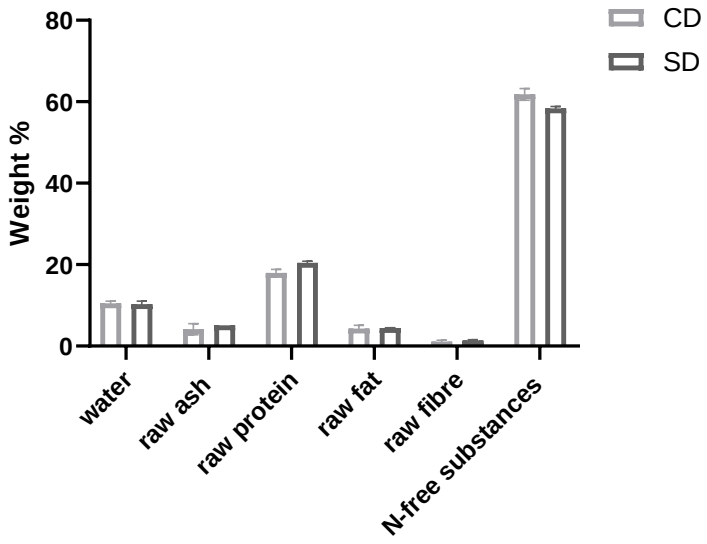


Figure A2: Analysis of dietary components of the semisynthetic control diet (CD) and the Spirulina diet (SD) performed by Weender analysis. Data presented as mean + σ, n = 2 – 4.

References

1. David LA, Maurice CF, Carmody RN et al. (2014) Diet rapidly and reproducibly alters the human gut microbiome. Nature 505:559–563. https://doi.org/10.1038/nature12820
2. Devkota S, Wang Y, Musch MW et al. (2012) Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in $Il10^{-/-}$ mice. Nature 487:104–108. https://doi.org/10.1038/nature11225
3. Turroni F, Milani C, van Sinderen D et al. (2014) Genetic strategies for mucin metabolism in *Bifidobacterium bifidum* PRL2010: An example of possible human-microbe co-evolution. Gut Microbes 2:183–189. https://doi.org/10.4161/gmic.2.3.16105
4. Backhed F (2005) Host-bacterial mutualism in the human intestine. Science 307:1915–1920. https://doi.org/10.1126/science.1104816
5. Dupont A, Heinbockel L, Brandenburg K et al. (2014) Antimicrobial peptides and the enteric mucus layer act in concert to protect the intestinal mucosa. Gut Microbes 5:761–765. https://doi.org/10.4161/19490976.2014.972238
6. Blaut M (2015) Nutrition-mediated effects of the intestinal microbiota. Ernahrungs Umschau:216–229. https://doi.org/10.4455/eu.2015.040
7. Kelly D, Conway S (2005) Bacterial modulation of mucosal innate immunity. Molecular Immunology 42:895–901. https://doi.org/10.1016/j.molimm.2004.12.003
8. DiBaise JK, Zhang H, Crowell MD et al. (2008) Gut microbiota and its possible relationship with obesity. Mayo Clin Proc 83:460–469. https://doi.org/10.4065/83.4.460
9. Frank DN, St Amand AL, Feldman RA et al. (2007) Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci U S A 104:13780–13785. https://doi.org/10.1073/pnas.0706625104
10. Sender R, Fuchs S, Milo R (2016) Revised estimates for the number of human and bacteria cells in the body. PLOS Biology 14:e1002533. https://doi.org/10.1371/journal.pbio.1002533
11. Scott KP, Duncan SH, Louis P et al. (2011) Microorganisms in the human gut: Diversity and function. The Biochemist 33:4–9. https://doi.org/10.1042/BIO03304004
12. Biasoli MS, Tosello ME, Magaró HM (2002) Adherence of *Candida* strains isolated from the human gastrointestinal tract. Mycoses 45:465–469. https://doi.org/10.1046/j.1439-0507.2002.00793.x
13. Palmer C, Bik EM, DiGiulio DB et al. (2007) Development of the human infant intestinal microbiota. PLoS Biol 5:e177. https://doi.org/10.1371/journal.pbio.0050177
14. Baron EJ, Summanen P, Downes J et al. (1989) *Bilophila wadsworthia*, gen. nov. and sp. nov., a unique gram-negative anaerobic rod recovered from appendicitis specimens and human faeces. Microbiology 135:3405–3411. https://doi.org/10.1099/00221287-135-12-3405
15. Jochum L, Stecher B (2020) Label or concept - What is a pathobiont? Trends Microbiol 28:789–792. https://doi.org/10.1016/j.tim.2020.04.011
16. Peck SC, Denger K, Burrichter A et al. (2019) A glycyl radical enzyme enables hydrogen sulfide production by the human intestinal bacterium *Bilophila wadsworthia*. Proc Natl Acad Sci U S A 116:3171–3176. https://doi.org/10.1073/pnas.1815661116
17. Finegold S, Summanen P, Hunt Gerardo S et al. (1992) Clinical importance of *Bilophila wadsworthia*. Eur J Clin Microbiol Infect Dis 11:1058–1063. https://doi.org/10.1007/BF01967799

18. Kasten MJ, Rosenblatt JE, Gustafson DR (1992) *Bilophila wadsworthia* bacteremia in two patients with hepatic abscesses. J Clin Microbiol 30:2502–2503. https://doi.org/10.1128/JCM.30.9.2502-2503.1992
19. Schumacher UK, Eiring P, Häcker F-M (1997) Incidence of *Bilophila wadsworthia* in appendiceal, peritoneal and fecal samples from children. Clin Microbiol Infect 3:134–136. https://doi.org/10.1111/j.1469-0691.1997.tb00264.x
20. Mosca A, D'Alagni M, del Prete R et al. (1995) Preliminary evidence of endotoxic activity of *Bilophila wadsworthia*. Anaerobe 1:21–24. https://doi.org/10.1016/S1075-9964(95)80379-3
21. Feng Z, Long W, Hao B et al. (2017) A human stool-derived *Bilophila wadsworthia* strain caused systemic inflammation in specific-pathogen-free mice. Gut Pathog 9:59. https://doi.org/10.1186/s13099-017-0208-7
22. Natividad JM, Lamas B, Pham HP et al. (2018) *Bilophila wadsworthia* aggravates high fat diet induced metabolic dysfunctions in mice. Nat Commun 9:2802. https://doi.org/10.1038/s41467-018-05249-7
23. Ridlon JM, Devendran S, Alves JM et al. (2020) The '*in vivo* lifestyle' of bile acid 7α-dehydroxylating bacteria: comparative genomics, metatranscriptomic, and bile acid metabolomics analysis of a defined microbial community in gnotobiotic mice. Gut Microbes 11:381–404. https://doi.org/10.1080/19490976.2019.1618173
24. García-Cañaveras JC, Donato MT, Castell JV et al. (2012) Targeted profiling of circulating and hepatic bile acids in human, mouse, and rat using a UPLC-MRM-MS-validated method. J Lipid Res 53:2231–2241. https://doi.org/10.1194/jlr.D028803
25. Ridlon JM, Kang D-J, Hylemon PB (2006) Bile salt biotransformations by human intestinal bacteria. J Lipid Res 47:241–259. https://doi.org/10.1194/jlr.R500013-JLR200
26. Martinez-Augustin O, Sanchez de Medina F (2008) Intestinal bile acid physiology and pathophysiology. World J Gastroenterol 14:5630–5640. https://doi.org/10.3748/wjg.14.5630
27. Hofmann AF (1999) The continuing importance of bile acids in liver and intestinal disease. Arch Intern Med 159:2647–2658. https://doi.org/10.1001/archinte.159.22.2647
28. Urdaneta V, Casadesús J (2017) Interactions between bacteria and bile salts in the gastrointestinal and hepatobiliary tracts. Front Med (Lausanne) 4:163. https://doi.org/10.3389/fmed.2017.00163
29. Goddard-Borger ED, Williams SJ (2017) Sulfoquinovose in the biosphere: Occurrence, metabolism and functions. Biochem J 474:827–849. https://doi.org/10.1042/BCJ20160508
30. Shimojima M (2011) Biosynthesis and functions of the plant sulfolipid. Prog Lipid Res 50:234–239. https://doi.org/10.1016/j.plipres.2011.02.003
31. Wu Q, Liu L, Miron A et al. (2016) The antioxidant, immunomodulatory, and anti-inflammatory activities of Spirulina: An overview. Arch Toxicol 90:1817–1840. https://doi.org/10.1007/s00204-016-1744-5
32. Furmaniak MA, Misztak AE, Franczuk MD et al. (2017) Edible cyanobacterial genus *Arthrospira*: Actual state of the art in cultivation methods, genetics, and application in medicine. Front Microbiol 8:2541. https://doi.org/10.3389/fmicb.2017.02541
33. Harwood JL, Nicholls RG (1979) The plant sulpholipid—a major component of the sulphur cycle. Biochem Soc Trans 7:440–447. https://doi.org/10.1042/bst0070440
34. Speciale G, Jin Y, Davies GJ et al. (2016) YihQ is a sulfoquinovosidase that cleaves sulfoquinovosyl diacylglyceride sulfolipids. Nat Chem Biol 12:215–217. https://doi.org/10.1038/nCHeMBIO.2023

35. Frommeyer B, Fiedler AW, Oehler SR et al. (2020) Environmental and intestinal phylum firmicutes bacteria metabolize the plant sugar sulfoquinovose via a 6-deoxy-6-sulfofructose transaldolase pathway. iScience 23:101510. https://doi.org/10.1016/j.isci.2020.101510
36. Liu Y, Wei Y, Zhou Y et al. (2020) A transaldolase-dependent sulfoglycolysis pathway in *Bacillus megaterium* DSM 1804. Biochem Biophys Res Commun 533:1109–1114. https://doi.org/10.1016/j.bbrc.2020.09.124
37. Roy AB, Hewlins MJE, Ellis AJ et al. (2003) Glycolytic breakdown of sulfoquinovose in bacteria: A missing link in the sulfur cycle. Appl Environ Microbiol 69:6434–6441. https://doi.org/10.1128/AEM.69.11.6434–6441.2003
38. Felux A-K, Spiteller D, Klebensberger J et al. (2015) Entner-Doudoroff pathway for sulfoquinovose degradation in *Pseudomonas putida* SQ1. Proc Natl Acad Sci U S A 112:E4298-305. https://doi.org/10.1073/pnas.1507049112
39. Denger K, Weiss M, Felux A-K et al. (2014) Sulphoglycolysis in *Escherichia coli* K-12 closes a gap in the biogeochemical sulphur cycle. Nature 507:114–117. https://doi.org/10.1038/nature12947
40. Burrichter A, Denger K, Franchini P et al. (2018) Anaerobic degradation of the plant sugar sulfoquinovose concomitant with H_2S production: *Escherichia coli* K-12 and *Desulfovibrio* sp. strain DF1 as co-culture model. Front Microbiol 9:2792. https://doi.org/10.3389/fmicb.2018.02792
41. Liu J, Wei Y, Lin L et al. (2020) Two radical-dependent mechanisms for anaerobic degradation of the globally abundant organosulfur compound dihydroxypropanesulfonate. Proc Natl Acad Sci U S A 117:15599–15608. https://doi.org/10.1073/pnas.2003434117
42. Gupta SD, Sastry PS (1987) Metabolism of the plant sulfolipid—sulfoquinovosyldiacylglycerol: Degradation in animal tissues. Archives of Biochemistry and Biophysics 259:510–519. https://doi.org/10.1016/0003-9861(87)90517-0
43. Carbonero F, Benefiel AC, Alizadeh-Ghamsari AH et al. (2012) Microbial pathways in colonic sulfur metabolism and links with health and disease. Front Physio 3. https://doi.org/10.3389/fphys.2012.00448
44. Dominy JE, Stipanuk MH (2004) New roles for cysteine and transsulfuration enzymes: Production of H_2S, a neuromodulator and smooth muscle relaxant. Nutrition Reviews 62:348–353. https://doi.org/10.1111/j.1753-4887.2004.tb00060.x
45. Loubinoux J, Bronowicki J-P, Pereira IAC et al. (2002) Sulfate-reducing bacteria in human feces and their association with inflammatory bowel diseases. FEMS Microbiology Ecology 40:107–112. https://doi.org/10.1111/j.1574-6941.2002.tb00942.x
46. Yu H, Guo Z, Shen S et al. (2016) Effects of taurine on gut microbiota and metabolism in mice. Amino Acids 48:1601–1617. https://doi.org/10.1007/s00726-016-2219-y
47. Schaffer S, Kim HW (2018) Effects and mechanisms of taurine as a therapeutic agent. Biomol Ther (Seoul) 26:225–241. https://doi.org/10.4062/biomolther.2017.251
48. Sasaki K, Sasaki D, Okai N et al. (2017) Taurine does not affect the composition, diversity, or metabolism of human colonic microbiota simulated in a single-batch fermentation system. PLoS ONE 12:e0180991. https://doi.org/10.1371/journal.pone.0180991
49. Xing M, Wei Y, Hua G et al. (2019) A gene cluster for taurine sulfur assimilation in an anaerobic human gut bacterium. Biochem J 476:2271–2279. https://doi.org/10.1042/BCJ20190486
50. Singh SB, Lin HC (2015) Hydrogen sulfide in physiology and diseases of the digestive tract. Microorganisms 3:866–889. https://doi.org/10.3390/microorganisms3040866

51. Deplancke B, Hristova KR, Oakley HA et al. (2000) Molecular ecological analysis of the succession and diversity of sulfate-reducing bacteria in the mouse gastrointestinal tract. Appl Environ Microbiol 66:2166–2174. https://doi.org/10.1128/AEM.66.5.2166-2174.2000
52. Schramm A, Santegoeds CM, Nielsen HK et al. (1999) On the occurrence of anoxic microniches, denitrification, and sulfate reduction in aerated activated sludge. Appl Environ Microbiol 65:4189–4196. https://doi.org/10.1128/AEM.65.9.4189-4196.1999
53. Santos AA, Venceslau SS, Grein F et al. (2015) A protein trisulfide couples dissimilatory sulfate reduction to energy conservation. Science 6267:1541-1545. https://doi.org/10.1126/science.aad355
54. Levine J, Ellis CJ, Furne JK et al. (1998) Fecal Hydrogen sulfide production in ulcerative colitis. American Journal of Gastroenterology 93:83–87. https://doi.org/10.1111/j.1572-0241.1998.083_c.x
55. Teigen LM, Geng Z, Sadowsky MJ et al. (2019) Dietary factors in sulfur metabolism and pathogenesis of ulcerative colitis. Nutrients 11:931. https://doi.org/10.3390/nu11040931
56. Ramasamy S, Singh S, Taniere P et al. (2006) Sulfide-detoxifying enzymes in the human colon are decreased in cancer and upregulated in differentiation. American Journal of Physiology-Gastrointestinal and Liver Physiology 291:G288-G296. https://doi.org/10.1152/ajpgi.00324.2005
57. Roediger WEW, Duncan A, Kapaniris O et al. (1993) Reducing sulfur compounds of the colon impair colonocyte nutrition: Implications for ulcerative colitis. Gastroenterology 104:802–809. https://doi.org/10.1016/0016-5085(93)91016-b
58. Babidge W, Millard S, Roediger W (1998) Sulfides impair short chain fatty acid β-oxidation at acyl-CoA dehydrogenase level in colonocytes: Implications for ulcerative colitis. Molecular and Cellular Biochemistry 181:117–124. https://doi.org/10.1023/A:1006838231432
59. Beaumont M, Andriamihaja M, Lan A et al. (2016) Detrimental effects for colonocytes of an increased exposure to luminal hydrogen sulfide: The adaptive response. Free Radical Biology and Medicine 93:155–164. https://doi.org/10.1016/j.freeradbiomed.2016.01.028
60. Blachier F, Beaumont M, Kim E (2019) Cysteine-derived hydrogen sulfide and gut health: A matter of endogenous or bacterial origin. Curr Opin Clin Nutr Metab Care 22:68–75. https://doi.org/10.1097/MCO.0000000000000526
61. Ijssennagger N, van der Meer R, van Mil SWC (2016) Sulfide as a mucus barrier-breaker in inflammatory bowel disease? Trends in molecular medicine 22:190–199. https://doi.org/10.1016/j.molmed.2016.01.002
62. Loftus EV (2004) Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. Gastroenterology 126:1504–1517. https://doi.org/10.1053/j.gastro.2004.01.063
63. Cohen RD (2017) Inflammatory bowel disease: Diagnosis and therapeutics. Clinical Gastroenterology. Springer International Publishing, Cham
64. Ng SC, Bernstein CN, Vatn MH et al. (2013) Geographical variability and environmental risk factors in inflammatory bowel disease. Gut 62:630–649. https://doi.org/10.1136/gutjnl-2012-303661
65. Xia B, Crusius JBA, Meuwissen SGM et al. (1998) Inflammatory bowel disease: Definition, epidemiology, etiologic aspects, and immunogenetic studies. World J Gastroenterol 4:446–458. https://doi.org/10.3748/wjg.v4.i5.446

66. Clavel T, Haller D (2007) Bacteria- and host-derived mechanisms to control intestinal epithelial cell homeostasis: Implications for chronic inflammation. Inflammatory Bowel Diseases 13:1153–1164. https://doi.org/10.1002/ibd.20174
67. Sartor RB (2008) Microbial influences in inflammatory bowel diseases. Gastroenterology 134:577–594. https://doi.org/10.1053/j.gastro.2007.11.059
68. Imielinski M, Hakonarson H (2010) Breaking new ground in inflammatory bowel disease genetics: Genome-wide association studies and beyond. Pharmacogenomics 11:663–665. https://doi.org/10.2217/pgs.10.56
69. Jostins L, Ripke S, Weersma RK et al. (2012) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 491:119–124. https://doi.org/10.1038/nature11582
70. Cho JH (2008) The genetics and immunopathogenesis of inflammatory bowel disease. Nat Rev Immunol 8:458–466. https://doi.org/10.1038/nri2340
71. Anderson CA, Boucher G, Lees CW et al. (2011) Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat Genet 43:246–252. https://doi.org/10.1038/ng.764
72. Kiesler P, Fuss IJ, Strober W (2015) Experimental models of inflammatory bowel diseases. Cell Mol Gastroenterol Hepatol 1:154–170. https://doi.org/10.1016/j.jcmgh.2015.01.006
73. Neurath M, Fuss I, Strober W (2009) TNBS-colitis. International Reviews of Immunology 19:51–62. https://doi.org/10.3109/08830180009048389
74. Kullberg MC, Jankovic D, Feng CG et al. (2006) IL-23 plays a key role in *Helicobacter hepaticus*–induced T cell–dependent colitis. Journal of Experimental Medicine 203:2485–2494. https://doi.org/10.1084/jem.20061082
75. Barthel M, Hapfelmeier S, Quintanilla-Martínez L et al. (2003) Pretreatment of mice with streptomycin provides a *Salmonella enterica* serovar Typhimurium colitis model that allows analysis of both pathogen and host. IAI 71:2839–2858. https://doi.org/10.1128/IAI.71.5.2839-2858.2003
76. Kühn R, Löhler J, Rennick D et al. (1993) Interleukin-10-deficient mice develop chronic enterocolitis. Cell 75:263–274. https://doi.org/10.1016/0092-8674(93)80068-P
77. Keubler LM, Buettner M, Häger C et al. (2015) A multihit model. Inflammatory Bowel Diseases 21:1967–1975. https://doi.org/10.1097/MIB.0000000000000468
78. Sellon RK, Tonkonogy S, Schultz M et al. (1998) Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. IAI 66:5224–5231. https://doi.org/10.1128/IAI.66.11.5224-5231.1998
79. Mähler M, Leiter EH (2002) Genetic and environmental context determines the course of colitis developing in IL–10-deficient mice. Inflammatory Bowel Diseases 8:347–355. https://doi.org/10.1097/00054725-200209000-00006
80. Midtvedt T, Bjørneklett A, Carlstedt-Duke B et al. (1985) The influence of antibiotics upon microflora-associated characteristics in man and mammals. Prog Clin Biol Res 181:241–244
81. Fraune S, Anton-Erxleben F, Augustin R et al. (2015) Bacteria–bacteria interactions within the microbiota of the ancestral metazoan *Hydra* contribute to fungal resistance. ISME J 9:1543–1556. https://doi.org/10.1038/ismej.2014.239
82. Dewhirst FE, Chien C-C, Paster BJ et al. (1999) Phylogeny of the defined murine microbiota: Altered Schaedler Flora. Appl Environ Microbiol 65:3287–3292. https://doi.org/10.1128/AEM.65.8.3287-3292.1999

83. Becker N, Kunath J, Loh G et al. (2011) Human intestinal microbiota: Characterization of a simplified and stable gnotobiotic rat model. Gut Microbes 2:25–33. https://doi.org/10.4161/gmic.2.1.14651
84. Clavel T, Gomes-Neto JC, Lagkouvardos I et al. (2017) Deciphering interactions between the gut microbiota and the immune system via microbial cultivation and minimal microbiomes. Immunol Rev 279:8–22. https://doi.org/10.1111/imr.12578
85. Woting A, Pfeiffer N, Loh G et al. (2014) *Clostridium ramosum* promotes high-fat diet-induced obesity in gnotobiotic mouse models. mBio 5:e01530-14. https://doi.org/10.1128/mBio.01530-14
86. Filippo C de, Cavalieri D, Di Paola M et al. (2010) Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences 107:14691–14696. https://doi.org/10.1073/pnas.1005963107
87. Eun CS, Mishima Y, Wohlgemuth S et al. (2014) Induction of bacterial antigen-specific colitis by a simplified human microbiota consortium in gnotobiotic interleukin-$10^{-/-}$ mice. IAI 82:2239–2246. https://doi.org/10.1128/IAI.01513-13
88. Brugiroux S, Beutler M, Pfann C et al. (2017) Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium. Nat Microbiol 2:793. https://doi.org/10.1038/nmicrobiol.2016.215
89. Miniats OP, Mitchell L, Barnum DA (1970) Response of gnotobiotic pigs to *Escherichia coli*. Can J Comp Med 34:269–276
90. da Silva SM, Venceslau SS, Fernandes CLV et al. (2008) Hydrogen as an energy source for the human pathogen *Bilophila wadsworthia*. Antonie Van Leeuwenhoek 93:381–390. https://doi.org/10.1007/s10482-007-9215-x
91. Hungate RE (1950) The anaerobic mesophilic cellulolytic bacteria. Bacteriol Rev 14:1–49
92. Moench TT, Zeikus JG (1983) An improved preparation method for a titanium (III) media reductant. Journal of Microbiological Methods 1:199–202. https://doi.org/10.1016/0167-7012(83)90024-6
93. Volynets V, Reichold A, Bárdos G et al. (2016) Assessment of the intestinal barrier with five different permeability tests in healthy C57BL/6J and BALB/cJ Mice. Dig Dis Sci 61:737–746. https://doi.org/10.1007/s10620-015-3935-y
94. Ring C, Klopfleisch R, Dahlke K et al. (2019) *Akkermansia muciniphila* strain ATCC BAA-835 does not promote short-term intestinal inflammation in gnotobiotic interleukin-10-deficient mice. Gut Microbes 10:188–203. https://doi.org/10.1080/19490976.2018.1511663
95. Slezak K, Hanske L, Loh G et al. (2013) Increased bacterial putrescine has no impact on gut morphology and physiology in gnotobiotic adolescent mice. Benef Microbes 4:253–266. https://doi.org/10.3920/BM2012.0047
96. Junick J, Blaut M (2012) Quantification of human fecal bifidobacterium species by use of quantitative real-time PCR analysis targeting the *groEL* gene. Appl Environ Microbiol 78:2613–2622. https://doi.org/10.1128/AEM.07749-11
97. Baldwin J, Collins B, Wolf PG et al. (2016) Table grape consumption reduces adiposity and markers of hepatic lipogenesis and alters gut microbiota in butter fat-fed mice. J Nutr Biochem 27:123–135. https://doi.org/10.1016/j.jnutbio.2015.08.027
98. Kageyama A, Benno Y, Nakase T (1999) Phylogenetic evidence for the transfer of *Eubacterium lentum* to the genus *Eggerthella* as *Eggerthella lenta* gen. nov., comb. nov. Int J Syst Bacteriol 49 Pt 4:1725–1732. https://doi.org/10.1099/00207713-49-4-1725

99. Altschul SF, Gish W, Miller W et al. (1990) Basic local alignment search tool. Journal of molecular biology 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
100. Lagkouvardos I, Kläring K, Heinzmann SS et al. (2015) Gut metabolites and bacterial community networks during a pilot intervention study with flaxseeds in healthy adult men. Mol Nutr Food Res 59:1614–1628. https://doi.org/10.1002/mnfr.201500125
101. Lagkouvardos I, Joseph D, Kapfhammer M et al. (2016) IMNGS: A comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies. Sci Rep 6:33721. https://doi.org/10.1038/srep33721
102. Lagkouvardos I, Fischer S, Kumar N et al. (2017) Rhea: a transparent and modular R pipeline for microbial profiling based on 16S rRNA gene amplicons. PeerJ 5:e2836. https://doi.org/10.7717/peerj.2836
103. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry 162:156–159. https://doi.org/10.1016/0003-2697(87)90021-2
104. Jansen GJ, Mooibroek M, Idema J et al. (2000) Rapid identification of bacteria in blood cultures by using fluorescently labeled oligonucleotide probes. J Clin Microbiol 38:814–817. https://doi.org/10.1128/JCM.38.2.814-817.2000
105. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45. https://doi.org/10.1093/nar/29.9.e45
106. Strocchi A, Furne JK, Levitt MD (1992) A modification of the methylene blue method to measure bacterial sulfide production in feces. Journal of Microbiological Methods 15:75–82. https://doi.org/10.1016/0167-7012(92)90071-B
107. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428. https://doi.org/10.1021/ac60147a030
108. Eneroth P (1963) Thin-layer chromatography of bile acids. J Lipid Res 4:11–16
109. Hofmann AF (1962) Thin-layer adsorption chromatography on microscope slides. Analytical Biochemistry 3:145–149. https://doi.org/10.1016/0003-2697(62)90104-5
110. Haange S-B, Groeger N, Froment J et al. (2020) Multiplexed quantitative assessment of the fate of taurine and sulfoquinovose in the intestinal microbiome. Metabolites 10. https://doi.org/10.3390/metabo10110430
111. Laue H, Denger K, Cook AM (1997) Taurine reduction in anaerobic respiration of *Bilophila wadsworthia* RZATAU. Appl Environ Microbiol 63:2016–2021. https://doi.org/10.1128/AEM.63.5.2016-2021.1997
112. Sivakumar PV, Westrich GM, Kanaly S et al. (2002) Interleukin 18 is a primary mediator of the inflammation associated with dextran sulphate sodium induced colitis: Blocking interleukin 18 attenuates intestinal damage. Gut 50:812–820. https://doi.org/10.1136/gut.50.6.812
113. Ulluwishewa D, Anderson RC, McNabb WC et al. (2011) Regulation of tight junction permeability by intestinal bacteria and dietary components. J Nutr 141:769–776. https://doi.org/10.3945/jn.110.135657
114. Madsen KL, Malfair D, Gray D et al. (1999) Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. Inflammatory Bowel Diseases 5:262–270. https://doi.org/10.1097/00054725-199911000-00004
115. Oh SY, Cho K-A, Kang JL et al. (2014) Comparison of experimental mouse models of inflammatory bowel disease. Int J Mol Med 33:333–340. https://doi.org/10.3892/ijmm.2013.1569

116. Rasmussen HE, Martínez I, Lee JY et al. (2009) Alteration of the gastrointestinal microbiota of mice by edible blue-green algae. J Appl Microbiol 107:1108–1118. https://doi.org/10.1111/j.1365-2672.2009.04288.x
117. Hu J, Li Y, Pakpour S et al. (2019) Dose effects of orally administered Spirulina auspension on colonic microbiota in healthy mice. Front Cell Infect Microbiol 9:243. https://doi.org/10.3389/fcimb.2019.00243
118. Ridlon JM, Kang DJ, Hylemon PB et al. (2014) Bile acids and the gut microbiome. Curr Opin Gastroenterol 30:332–338. https://doi.org/10.1097/MOG.0000000000000057
119. Jiao N, Baker SS, Chapa-Rodriguez A et al. (2018) Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. Gut 67:1881–1891. https://doi.org/10.1136/gutjnl-2017-314307
120. Chikai T, Nakao H, Uchida K (1987) Deconjugation of bile acids by human intestinal bacteria implanted in germ-free rats. Lipids 22:669–671. https://doi.org/10.1007/BF02533948
121. Vo T-S, Ngo D-H, Kim S-K (2015) Nutritional and pharmaceutical properties of microalgal Spirulina. In: Handbook of Marine Microalgae. Elsevier, pp 299–308
122. Xue C, Hu Y, Saito H et al. (2002) Molecular species composition of glycolipids from *Sprirulina platensis*. Food Chemistry 77:9–13. https://doi.org/10.1016/S0308-8146(01)00315-6
123. Shokri H, Khosravi AR, Taghavi M (2014) Efficacy of *Spirulina platensis* on immune functions in cancer mice with systemic candidiasis. JMR 1:7–13
124. Neyrinck AM, Taminiau B, Walgrave H et al. (2017) Spirulina protects against hepatic inflammation in aging: An effect related to the modulation of the gut microbiota? Nutrients 9. https://doi.org/10.3390/nu9060633
125. Coskun ZK, Kerem M, Gurbuz N et al. (2011) The study of biochemical and histopathological effects of Spirulina in rats with TNBS-induced colitis. Bratisl Lek Listy 112:235–243
126. Ganesh BP, Klopfleisch R, Loh G et al. (2013) Commensal *Akkermansia muciniphila* exacerbates gut inflammation in Salmonella Typhimurium-infected gnotobiotic mice. PLoS ONE 8:e74963. https://doi.org/10.1371/journal.pone.0074963
127. Buono S, Langellotti AL, Martello A et al. (2014) Functional ingredients from microalgae. Food Funct 5:1669–1685. https://doi.org/10.1039/c4fo00125g
128. Marles RJ, Barrett ML, Barnes J et al. (2011) United States pharmacopeia safety evaluation of spirulina. Critical Reviews in Food Science and Nutrition 51:593–604. https://doi.org/10.1080/10408391003721719
129. Maeda N, Matsubara K, Yoshida H et al. (2011) Anti-cancer effect of spinach glycoglycerolipids as angiogenesis inhibitors based on the selective inhibition of DNA polymerase activity. Mini Rev Med Chem 11:32–38. https://doi.org/10.2174/138955711793564042
130. Park JH, Lee SI, Kim IH (2018) Effect of dietary Spirulina (*Arthrospira*) *platensis* on the growth performance, antioxidant enzyme activity, nutrient digestibility, cecal microflora, excreta noxious gas emission, and breast meat quality of broiler chickens. Poult Sci 97:2451–2459. https://doi.org/10.3382/ps/pey093
131. Coué M, Tesse A, Falewée J et al. (2019) Spirulina liquid extract protects against fibrosis related to non-alcoholic steatohepatitis and increases ursodeoxycholic acid. Nutrients 11:194. https://doi.org/10.3390/nu11010194
132. Wolf PG, Devendran S, Doden HL et al. (2021) Berberine alters gut microbial function through modulation of bile acids. BMC Microbiol 21:24. https://doi.org/10.1186/s12866-020-02020-1

133. Stenman LK, Holma R, Eggert A et al. (2013) A novel mechanism for gut barrier dysfunction by dietary fat: Epithelial disruption by hydrophobic bile acids. Am J Physiol Gastrointest Liver Physiol 304:G227-34. https://doi.org/10.1152/ajpgi.00267.2012
134. Ridlon JM, Alves JM, Hylemon PB et al. (2013) Cirrhosis, bile acids and gut microbiota: Unraveling a complex relationship. Gut Microbes 4:382–387. https://doi.org/10.4161/gmic.25723
135. Cummings JH, Wiggins HS, Jenkins DJ et al. (1978) Influence of diets high and low in animal fat on bowel habit, gastrointestinal transit time, fecal microflora, bile acid, and fat excretion. J Clin Invest 61:953–963. https://doi.org/10.1172/JCI109020
136. Li T, Holmstrom SR, Kir S et al. (2011) The G protein-coupled bile acid receptor, TGR5, stimulates gallbladder filling. Mol Endocrinol 25:1066–1071. https://doi.org/10.1210/me.2010-0460
137. National Institute of Diabetes and Digestive and Kidney Diseases (2012) LiverTox: Clinical and research information on drug-induced liver injury: Spirulina, Bethesda (MD)
138. Bigagli E, Cinci L, Niccolai A et al. (2017) Safety evaluations and lipid-lowering activity of an *Arthrospira platensis* enriched diet: A 1-month study in rats. Food Res Int 102:380–386. https://doi.org/10.1016/j.foodres.2017.09.011
139. Finamore A, Palmery M, Bensehaila S et al. (2017) Antioxidant, immunomodulating, and microbial-modulating activities of the sustainable and ecofriendly Spirulina. Oxid Med Cell Longev 2017:3247528. https://doi.org/10.1155/2017/3247528
140. Sakamoto M (2013-2014) The Family *Porphyromonadaceae*. In: Rosenberg E, DeLong EF, Lory S et al. (eds) The prokaryotes, Fourth edition. Springer, Berlin, pp 811–824
141. Lickteig AJ, Zhang Y, Klaassen CD et al. (2019) Effects of absence of constitutive androstane receptor (CAR) on bile acid homeostasis in male and female mice. Toxicol Sci. https://doi.org/10.1093/toxsci/kfz143
142. Baliou S, Kyriakopoulos AM, Goulielmaki M et al. (2020) Significance of taurine transporter (TauT) in homeostasis and its layers of regulation (Review). Mol Med Rep 22:2163–2173. https://doi.org/10.3892/mmr.2020.11321
143. Lee SH (2015) Intestinal permeability regulation by tight junction: Implication on inflammatory bowel diseases. Intest Res 13:11–18. https://doi.org/10.5217/ir.2015.13.1.11
144. Pfeiffer T, Bonhoeffer S (2004) Evolution of cross-feeding in microbial populations. Am Nat 163:E126-35. https://doi.org/10.1086/383593
145. Di L, Kerns EH (eds) (druk 2019) Drug-like properties: Concepts, structure, design and methods : from ADME to toxicity optimization, Second edition. Elsevier - Academic Press, Amsterdam [i pozostałe]
146. Eastwood GL (1975) Effect of pH on bile salt injury to mouse gastric mucosa. Gastroenterology 68:1456–1465. https://doi.org/10.1016/S0016-5085(75)80132-6
147. Wang C, Li L, Guan H et al. (2013) Effects of taurocholic acid on immunoregulation in mice. International Immunopharmacology 15:217–222. https://doi.org/10.1016/j.intimp.2012.12.006
148. Narushima S, Itoha K, Miyamoto Y et al. (2006) Deoxycholic acid formation in gnotobiotic mice associated with human intestinal bacteria. Lipids 41:835–843. https://doi.org/10.1007/s11745-006-5038-1
149. Dong Z, Lee BH (2018) Bile salt hydrolases: Structure and function, substrate preference, and inhibitor development. Protein Science 27:1742–1754. https://doi.org/10.1002/pro.3484
150. Begley M, Gahan CGM, Hill C (2005) The interaction between bacteria and bile. FEMS Microbiol Rev 29:625–651. https://doi.org/10.1016/j.femsre.2004.09.003

151. Bron PA, Marco M, Hoffer SM et al. (2004) Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters *in vitro* and *in situ* in the gastrointestinal tract. J Bacteriol 186:7829–7835. https://doi.org/10.1128/JB.186.23.7829-7835.2004
152. Edenharder R (1984) Dehydroxylation of cholic acid at C12 and epimerization at C5 and C7 by Bacteroides species. Journal of Steroid Biochemistry 21:413–420. https://doi.org/10.1016/0022-4731(84)90304-2
153. Islam KBMS, Fukiya S, Hagio M et al. (2011) Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology 141:1773–1781. https://doi.org/10.1053/j.gastro.2011.07.046
154. Ridlon JM, Wolf PG, Gaskins HR (2016) Taurocholic acid metabolism by gut microbes and colon cancer. Gut Microbes 7:201–215. https://doi.org/10.1080/19490976.2016.1150414
155. Claros MC, Schumacher U, Jacob M et al. (1999) Characterization of *Bilophila wadsworthia* isolates using PCR fingerprinting. Anaerobe 5:589–593. https://doi.org/10.1006/anae.1999.0307
156. Mindermann, Nora (2019) Bachelor thesis: Die Umsetzung von Sulfonaten durch das kolitogene Bakterium *Bilophila wadsworthia in vivo* und *in vitro.* Hochschule Osnabrück

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List of original communications

Full length articles

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Burkhardt, Wiebke; Rausch, Theresa; Klopfleisch, Robert; Blaut, Michael; Braune, Annett: Impact of dietary sulfolipid-derived sulfoquinovose on gut microbiota composition and inflammatory status of colitis-prone interleukin-10-deficient mice. (Revised manuscript resubmitted to the International Journal of Medical Microbiology).

Scientific events

Burkhardt, Wiebke; Rausch, Theresa; Klopfleisch, Robert; Blaut, Michael; Braune, Annett (2019). Dietary sulfonates do not promote bacteria-induced gut inflammation. 12th Seeon Conference "Microbiota, Probiotic and Host", 27th – 30th June 2019, Monastry Seeon, Germany (oral presentation).

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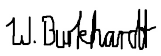
Burkhardt, Wiebke; Rausch, Theresa; Blaut, Michael; Braune, Annett (2018). Role of dietary sulfonates in the stimulation of intestinal bacteria promoting gut inflammation. 11th Seeon Conference "Microbiota, Probiotic and Host", 04th – 08th July 2018, Monastry Seeon, Germany (poster presentation).

Declaration of academic honesty

I hereby declare that my thesis entitled “Role of dietary sulfonates in the stimulation of gut bacteria promoting intestinal inflammation” is my own original work and has not previously, in part or in its entirety, been submitted at any university for a degree. Information derived from published work of others has been stated in the text and a list of references is given in the bibliography.

Potsdam,



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