Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Quorum Quenching Lactonase SsoPox Ameliorates DSS-Induced Colitis

Matthew Dietz
University of Minnesota
Zachary Ziegert
University of Minnesota
Maria Martell
University of Minnesota
Marjais McBride
University of Minnesota
Rakesh Sikdar
University of Minnesota
Cyrus Jahansouz
University of Minnesota
Subbaya Subramanian
University of Minnesota
Mikael Elias
University of Minnesota
Christopher Staley
cmstaley@umn.edu

University of Minnesota

Research Article

Keywords: acyl-homoserine lactone, autoinducer, biofilm, immunity, inflammatory bowel disease, lactic acid bacteria, microbiome, microbiota therapeutics, quorum sensing

Posted Date: June 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9750568/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: Competing interest reported. MHE is a co-founder, a former CEO, and an equity holder of Gene&GreenTK, a company that holds the license to WO2014167140 A1, FR 3068989 A1, and FR 19/02834. MHE has filed the patents EP3941206 and WO2020185861A1. MHE has an active research collaboration with Gene&GreenTK. MHE interests with Gene&GreenTK have been reviewed and managed by the University of Minnesota in accordance with its Conflict-of-Interest policies. The remaining authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Abstract

Background

Increasing evidence suggests that dysbiosis of the gut microbiome plays a significant role in the onset and pathogenesis of inflammatory bowel disease (IBD). Many of the pathogenic functions of these gut bacteria involve community-wide coordination using various autoinducer molecules and associated receptors, collectively known as quorum sensing (QS) systems. Disruption of these QS systems, a process known as quorum quenching (QQ), has shown promise in sharply inhibiting these pathogenic functions and has become a target of interest in microbiota-focused therapies. Here, we utilize SsoPox, a QQ enzyme that degrades long-chain acyl-homoserine lactones (AHLs), an autoinducer-1 QS signal, to examine the role QS plays in dextran-sulfate sodium (DSS)-induced colitis.

Results

Mice were given water supplemented with SsoPox at either 1mg/mL or 2mg/mL for 6 weeks, with DSS supplementation during the latter 3 weeks. We found that male and female mice administered SsoPox at 2mg/ml had longer colons compared to their respective drinking water controls (ANOVA F = 10.218, 3.663, *P* = 0.005, 0.072, respectively) while also exhibiting greater fecal concentrations of IgA (ANOVA F = 10.403, 4.374, *P* = 0.005, 0.052, respectively). SsoPox treatment was also observed to significantly increase the total transcript abundances of lactic acid-producing bacteria, including members of the families Bifidobacteriaceae and Lactobacillaceae (Kruskal-Wallis K = 6.361, 5.763, *P* = 0.012, 0.016, respectively).

Conclusions

These data suggest that SsoPox may cause community-level changes that promote activity of lactic-acid-producing taxa and potentially increase lactic acid production, representing a possible mechanism for the anti-inflammatory and anti-pathogenic effects of SsoPox supplementation reported here.

BACKGROUND

The intestinal microbiome is the most densely populated microbial community in the human body, contributing to nutrition, immune system development, and protection against enteric pathogens [1–3]. Optimal functioning of this microbial community relies on maintaining balance and species diversity, involving a complex interplay between the various taxa within the intestinal microbiome and their host [4]. A disruption of this equilibrium, often referred to as *dysbiosis*, is known to play a role in many diseases, including inflammatory bowel disease (IBD) and colorectal cancer (CRC) [5]. The onset of microbial dysbiosis can be initiated by a number of external factors, including oral antibiotic use, lifestyle

choices, or other disease processes, leading to an expansion of pathogenic bacteria within the gut microbiome [6, 7].

Gut bacteria communicate with one another through a process known as quorum sensing (QS), excreting autoinducer molecules that allow for community-wide functional coordination in a density-dependent manner [8–11]. Gram-negative bacteria predominantly use acyl-homoserine lactones (AHLs) as autoinducer-1 QS signaling molecules [12, 13]. Gram-positive bacteria use different QS systems and do not produce AHLs of their own; however, they are also able to cleave AHLs via quorum quenching (QQ) enzymes to disrupt their Gram-negative competition [12, 14, 15]. This results in a natural homeostasis between competing QS and QQ systems, which is present in nearly all environments [16]. This balance has become a point of interest for microbiota-targeted therapeutics, as AHL serum concentrations were found to be higher in Crohn's disease (CD) patients compared to healthy controls, regardless of whether they were experiencing active inflammation [17]. Longer-chain AHLs, in particular, were found at higher concentrations in patients with actively inflamed CD than in both uninflamed CD and healthy controls [17]. While other QQ enzymes degrade AHLs broadly, the lactonase SsoPox shows preference for long-chain AHLs and has already been shown to sharply inhibit bacterial biofilm and virulence factor production in several taxa *in vitro* [15, 18]. Clinically, recent research has begun to demonstrate strong evidence that SsoPox may be useful in treating murine models of gut diseases: In 2025, Yang et al. demonstrated that a lactonase was capable of suppressing the pathogenicity of enterotoxigenic bacterial infections [19], and our own research has demonstrated the ability of SsoPox to impact the metastatic rate of CRC in an aggressive orthotopic murine model [20].

Our objective was to determine the effects of the AHL lactonase SsoPox on host-microbe interactions in the context of dextran sulfate sodium (DSS)-induced colitis, a common model for studying IBD and associated microbial dysbiosis [21, 22]. We hypothesized that the administration of SsoPox would alleviate inflammation from DSS-induced colitis by preventing coordination of pathogenic processes. Microbial composition was assessed using 16S rRNA amplicon sequencing of murine fecal pellets, and inflammation was characterized by molecular assays for immunoglobulin A (IgA) and lipocalin 2 (Lcn2), along with colon length, at the time of necropsy. RNAseq was performed on a subset of fecal samples prior to the initiation of DSS to capture changes in transcript abundances associated with SsoPox. Results of this study elucidate the effects of SsoPox on host-microbe interactions and provide foundational information on the potential of SsoPox, and QQ enzymes more broadly, to become microbiome-targeted precision therapeutics.

METHODS

Mice and Dextran Sulfate Sodium (DSS) Model

This study was approved by the University of Minnesota Institutional Animal Care and Use Committee, protocol 2403-41874A. C57BL/6 mice were purchased at 35–41 days of age from Jackson Laboratory. Male and female mice (n = 40) were each divided into two groups (n = 10/sex/group) and given drinking

water supplemented with 1mg/mL of SsoPox enzyme (SsoPox) [23] or unamended drinking water (DW) (Fig. 1). This experiment was conducted a second time with another order of mice (n = 40, 10/sex/group) to explore the effects of dose, with mice from the second experiment receiving 2mg/mL SsoPox. Unless otherwise stated, SsoPox-treated mice were compared to only the controls from their order to minimize spurious differences due to batch effects. Mice were housed individually under conventional housing for six weeks. Beginning at week 4, all drinking water was additionally supplemented with 1.5% wt/vol DSS (Sigma-Aldrich, Burlington, MA, USA) for three more weeks to induce colitis [24, 25]. A DSS concentration of 1.5% was chosen based on successful implementation of the DSS murine model while minimizing mortality in our prior studies [26]. Throughout DSS treatment, all mice were monitored for evidence of loose stools, weight loss, and the presence of blood in cages to ensure successful implementation of the DSS model [25]. Successful implementation of the DSS model and the severity of resulting colitis were measured using the Disease Activity Index for animal colitis as described in Kim et al., which grades mice on a 0–4 scale for each of three categories: weight loss, stool consistency, and bleeding [27]. We calculated an average DAI score across the final two weeks for each mouse, with a higher DAI indicating more severe colitis. Fecal pellets were collected weekly and stored at -80°C for DNA extraction, with pellets for RNA extraction stored in RNAlater. At the study endpoint or when moribund, mice were euthanized with carbon dioxide and necropsies were performed. Aesthesia was not necessary throughout the study due to the lack of surgical procedures.

Lactonase Preparation

SsoPox is a highly thermostable phosphotriesterase-like lactonase that has demonstrated protease resistance, which is key to enabling this enzyme to remain active while passing through the digestive tract [28, 29]. Here, we used the W263I variant SsoPox lactonase, referred to throughout this manuscript as *SsoPox*, which is improved for activity [28]. SsoPox was overexpressed in *Escherichia coli* BL21 Star™ (DE3; Invitrogen, Carlsbad, CA, USA) containing the pGro7 plasmid (TakaRa Bio, San Jose, CA, USA). The enzyme was produced using a 75 L fermentation system (New Brunswick Scientific, Edison, NJ, USA) operated by the University of Minnesota BioResource Center and purified as previously described, leveraging its high thermal stability [28, 30]. As reported earlier [31], The enzyme preparation was assayed for quality and purity using SDS-PAGE (6–12%) and tested for activity against 5-thiobutyl-γ-butyrolactone (TBBL) substrate (synthesized by Enamine LTD, Kyiv, Ukraine) in an activity buffer (50-mM HEPES pH 8.0, 150-mM NaCl, 0.2 -mM CoCl2) containing 0.5-mM TBBL and 1-mM Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB] as previously described [32], with a specific activity for TBBL of the purified lyophilized SsoPox at 28,410 µmol/min/mg [31].

DNA Extraction and Sequencing

DNA was extracted from thawed mouse pellets (~ 100mg) using the DNeasy PowerSoil Pro kit (QIAGEN, Hilden, Germany) on the QIAcube platform. The V4 region of the 16S rRNA gene was amplified using the 515F/806R primer set by the University of Minnesota Genomics Center (UMGC) [33]. Paired-end

sequencing was done on the Illumina MiSeq platform (Illumina, Inc., San Diego, CA, USA) at a read length of 301 nucleotides by UMGC [34]. Sequences were processed using mothur (ver. 1.41.1) and our previously published pipeline [35]. Briefly, sequences were aligned against the SILVA database (ver. 138.1) [36] and clustered at 99% sequence similarity using the OptiClust algorithm [37]. Samples were rarified to 5,000 sequence reads by random subsampling [38], and taxonomic classification was performed against the Ribosomal Database Project (ver. 18) [39].

RNA Extraction and Sequencing

RNA was extracted from thawed pellets (~ 100mg) from mice treated with SsoPox at 2mg/mL at baseline and week 3. The RNeasy Mini kit (QIAGEN, Hilden, Germany) was used for extraction in accordance with the manufacturer's recommendations on the QIAcube platform. RNA sequencing (RNAseq) was done by UMGC on the Element AVITI (Element Biosciences, San Diego, USA) sequencer using the Illumina Stranded Total RNA with RiboZero Plus kit (Illumina, Inc.). The resulting linear libraries were sequenced directly on the AVITI using the Cloudbreak Freestyle sequencing kit, which enables circularization of third-party libraries on the flow cell [40]. Sequence data were processed first through kneaddata (ver. 0.7.4) to remove host contamination, with trimmomatic (ver. 0.39) to trim out low-quality transcripts, trf (ver. 4.10.Orc2) to identify and locate tandem repeats, and bowtie2 (ver. 2.5.4) for read alignment [41–44]. The remaining sequences were then processed with HUMAnN3 (ver. 3.9) for functional profiling and Kraken 2 and Bracken (ver. 2.1.3) for taxonomic classification and diversity [45, 46]. Functional annotation was performed using the UniRef90 database (ver. 2019.1), remapped to level-4 Enzyme Commission categories and KEGG orthogroups; transcript abundances were normalized to copies per million (cpm) for statistical analysis [47].

Sequencing data were deposited in the National Center for Biotechnology's Sequence Read Archive under BioProject accession number SRP529236.

Assays

Assays for the fecal concentrations of IgA and Lcn2 at endpoint were performed using ELISA assays from Abcam (ab157717 and ab199083; Cambridge, UK). For both assays, 10mg of frozen stool was suspended in 250µL sterile phosphate-buffered saline. Samples were homogenized by vortex at max speed (3200 rpm) on a Vortex-Genie 2 (Scientific Industries, Bohemia, New York, USA) followed by sonication at 40kHz with a 2.8L ultrasonic bath (Fisher Scientific, Hampton, New Hampshire, USA) for ten minutes each. Samples were then centrifuged at 13,000 × *g*, and the supernatant for each sample was transferred to a new sterile tube. For IgA, supernatants were diluted to 1:500 (vol/vol) in the kit-provided diluent before the ELISA assay. For Lcn2, a 1:5 dilution (vol/vol) with the kit-provided diluent was used. Both assays were performed following the manufacturer's protocols and read on a Synergy 2 Multi-Mode Microplate Reader (BioTek Instruments, Vermont, USA) and read at an absorbance of 450nm.

Statistics

Shannon and Chao1 indices for 16S rRNA amplicon data were calculated using mothur [48]. Beta diversity was also evaluated in mothur using Bray-Curtis distances for analysis of similarity (ANOSIM) [49]. Ordination was performed by principal coordinate analysis (PCoA). Differences in alpha diversity and analyte concentrations were determined by ANOVA using Tukey's *post-hoc* test. Relative abundances of taxa were compared with Kruskal-Wallis test, performed using XLSTAT ver. 2022.1.1.1243, Excel ver. 16.0 (Lumivero, Denver, CO, USA). Spearman correlations and redundancy analyses were both also run in XLSTAT. Wald tests were performed with DESeq2 (ver. 1.46.0) in R (ver. 4.4.2) [50]. All statistics were evaluated at $\alpha = 0.05$ with a Bonferroni correction made for multiple comparisons.

RESULTS

16S rRNA Sequencing Reveals Distinct Changes in Diversity and Composition

1mg/mL Diversity and Composition

Minimal changes in diversity and taxonomic composition were observed among male and female mice that received 1mg/ml of the lactonase SsoPox over the first three weeks. Alpha diversity between DW controls and SsoPox-treated mice did not differ among baseline, week 3, or endpoint time points in either sex (ANOVA $F < 3.611$, $P > 0.075$, Tables 1 & 2). Similarly, the beta diversity of both male and female SsoPox-treated mice did not differ from their respective DW controls at baseline (ANOSIM R = 0.093, -0.005, $P = 0.090$, 0.429, Fig. 2A). After three weeks of SsoPox treatment, the overall microbiome composition of SsoPox-treated male mice showed significant separation from their controls (ANOSIM R = 0.257, $P < 0.001$). This difference was not present among female mice (ANOSIM R = 0.107, $P = 0.799$).

Table 1
Shannon index (mean ± standard deviation).

Experiment	Sex	Timepoint	DW	SsoPox	F	P-value
	Female	**Baseline**	**2.20 ± 0.36**	**2.37 ± 0.29**	**1.367**	**0.258**
1mg/mL		Week 3	2.48 ± 0.41	2.62 ± 0.36	0.650	0.431
		Endpoint	2.70 ± 0.15	2.84 ± 0.17	0.319	0.579
	Male	Baseline	2.84 ± 0.45	2.57 ± 0.62	1.267	0.275
		Week 3	2.77 ± 0.28	2.92 ± 0.38	1.056	0.318
		Endpoint	2.70 ± 0.15	2.84 ± 0.17	3.610	0.075
	Female	Baseline	3.17 ± 0.39	2.37 ± 0.25	29.694	< 0.001
2mg/mL		Week 3	2.84 ± 0.44	2.60 ± 0.33	2.040	0.170
		Endpoint	2.94 ± 0.19	2.85 ± 0.26	0.908	0.353
	Male	Baseline	2.54 ± 0.20	2.72 ± 0.39	1.700	0.210
		Week 3	2.58 ± 0.22	2.44 ± 0.24	1.900	0.185
		Endpoint	2.72 ± 0.12	2.76 ± 0.24	0.141	0.712

Table 2
Chao index (mean ± standard deviation)

Experiment	Sex	Timepoint	DW	SsoPox	F	P-value
	Female	**Baseline**	**208 ± 22**	**202 ± 36**	**0.188**	**0.670**
1mg/mL		Week 3	226 ± 28	202 ± 32	0.848	0.369
		Endpoint	180 ± 47	186 ± 27	2.757	0.114
	Male	Baseline	204 ± 20	219 ± 30	1.667	0.213
		Week 3	230 ± 24	223 ± 27	1.134	0.301
		Endpoint	194 ± 28	204 ± 26	0.502	0.488
	Female	Baseline	239 ± 67	187 ± 43	4.039	0.061
2mg/mL		Week 3	237 ± 36	228 ± 50	0.848	0.369
		Endpoint	270 ± 46	224 ± 74	2.757	0.114
	Male	Baseline	197 ± 17	196 ± 52	0.005	0.943
		Week 3	194 ± 56	186 ± 31	1.134	0.301
		Endpoint	233 ± 163	196 ± 36	0.502	0.488

Similar to diversity, the baseline relative abundances of predominant taxa in the gut microbiomes of male and female mice did not differ between mice assigned to the SsoPox and control groups (Kruskal-Wallis K ≤ 3.227, *P* ≥ 0.072; Figs. 2B & 2C). However, after three weeks of SsoPox treatment, the relative abundances of *Paramuribaculum* and *Lactobacillus* were lower, and the relative abundance of *Bifidobacterium* was greater in SsoPox-treated male mice compared to DW controls (K = 7.000, 4.806, 8.170, *P* = 0.008, 0.028, 0.004; respectively, **Supplemental Figures S1A-S1C**). Similarly, among SsoPox-treated male mice, the relative abundance of *Lactobacillus* dropped significantly between baseline and week 3 (K = 5.143, *P* = 0.023; **Supplemental Figure S2A**). The relative abundances of *Bifidobacterium* and Muribaculaceae, which had not been detected at baseline, had also increased by week 3 (K = 9.638, 4.677, *P* = 0.002, 0.031, respectively; **Supplemental Figures S2B-S2C**). Among female mice at week 3, the relative abundances of *Turicibacter* and *Clostridium sensu stricto* were lower in SsoPox-treated mice compared to DW controls (K = 3.866, 4.123, *P* = 0.049, 0.042, respectively; **Supplemental Figures S1E-S1F**). Similar to SsoPox-treated males, the relative abundance of *Lactobacillus* dropped from baseline to week 3 in SsoPox-treated females (K = 6.223, *P* = 0.013; **Supplemental Figure S2H**).

Following completion of DSS administration at week 6, beta diversity of both male and female SsoPox-treated mice differed from the beta diversity of DW controls, with female mice reaching statistical significance (ANOSIM R = 0.101, 0.147, *P* = 0.083, 0.042, respectively; Fig. 2A). Endpoint SsoPox-treated male mice had a greater relative abundance of Muribaculaceae compared to controls (K = 3.93, *P* = 0.048; **Supplemental Figure S1D**). At endpoint, SsoPox-treated male mice had a lower relative abundance of Lachnospiraceae and greater relative abundances of *Akkermansia*, Clostridiales, and *Clostridium sensu stricto* when compared to their pre-DSS microbiomes at week 3 (K = 9.606, 13.166, 3.863, 5.491, *P* = 0.002, < 0.001, 0.049, 0.019, respectively; **Supplemental Figure S2D-S2G**). SsoPox-treated female mice, at endpoint, had lower relative abundances of *Lactobacillus* and *Clostridium sensu stricto* and a greater relative abundance of *Akkermansia* compared to their DW controls (K = 5.23, 4.17, 4.86, *P* = 0.022, 0.041, 0.027, respectively; **Supplemental Figure S1G-S1I**). Similar to male mice, SsoPox-treated female mice had a lower relative abundance of Lachnospiraceae and greater relative abundances of *Akkermansia* and *Clostridium sensu stricto* at endpoint when compared to their pre-DSS microbiomes at week 3 (K = 9.143, 11.659, 7.189, *P* = 0.002, 0.001, 0.007, respectively; **Supplemental Figure S2I-2K**). Clostridiales trended towards a lower relative abundance in endpoint SsoPox-treated female mice compared to their week 3 microbiomes, but this did not reach statistical significance (K = 3.571, *P* = 0.059; Fig. 2C).

2mg/mL Diversity and Composition

No significant differences in alpha (Tables 1 & 2) or beta diversity were detected between DW controls and 2mg/ml SsoPox-treated male mice at baseline, similar to observations at 1mg/mL (ANOSIM R = -0.012, *P* = 0.533; Fig. 3A). However, in female mice, Shannon indices differed between SsoPox-treated mice and DW controls at baseline (ANOVA F = 29.69, *P* < 0.001; Table 1), though Chao1 did not differ (Table 2). In contrast to results observed at 1mg/ml and among males at 2mg/ml, the baseline beta diversity of female mice assigned to the SsoPox group differed from that of the control group (ANOSIM

R = 0.475, *P* < 0.001; Figs. 3A & 3C). At week 3 and endpoint, similar to the 1mg/mL experiment, neither Shannon nor Chao1 indices showed significant differences between 2mg/mL SsoPox-treated mice and their respective DW controls in either sex (ANOVA F ≤ 2.758, *P* ≥ 0.114; Tables 1 & 2). In contrast to 1mg/ml results, however, the gut microbiome compositions of both male and female 2mg/mL SsoPox-treated mice did not differ from the compositions of their respective DW controls at week 3(ANOSIM R = 0.036, -0.010, *P* = 0.250, 0.518, respectively; Fig. 3A).

At baseline, predominant taxa did not significantly differ in relative abundance between SsoPox-treated males and controls (K ≤ 3.527, *P* ≥ 0.060; Figs. 3B & 3C). In contrast, among females, the baseline relative abundances of Lachnospiraceae, *Turicibacter*, and Clostridiales were all lower, and the relative abundance of *Lactobacillus* was greater in SsoPox-treated female mice (K = 8.691, 5.356, 6.606, 9.606, *P* = 0.003, 0.021, 0.010, 0.002, respectively; **Supplemental Figures S3A-3D**). At week 3, unlike their 1mg/mL counterparts but similar to the 2mg/ml baseline, no predominant taxa differed in relative abundance between SsoPox-treated male mice and DW controls (K ≤ 1.286, all *P* ≥ 0.257; Fig. 3B). However, among females, Ruminococcaceae was less abundant in the SsoPox group (K = 4.806, *P* = 0.028; **Supplemental Figure S3E**). Among SsoPox-treated animals, the relative abundance of Lachnospiraceae dropped from baseline to week 3 among males (K = 4.507, *P* = 0.034; **Supplemental Figure S4A**), while the relative abundance of Muribaculaceae increased from baseline to week 3 (K = 6.611, *P* = 0.010; **Supplemental Figure S4E**).

Following completion of DSS administration, the gut microbiome compositions of both 2mg/mL SsoPox-treated male and female mice did not differ from those of DW controls (ANOSIM R = 0.082, 0.077, *P* = 0.103, 0.082, respectively, Fig. 3A), in contrast to observations at 1mg/ml. Moreover, no differences in the relative abundance of predominant taxa were observed between SsoPox-treated males or females and their respective DW controls (Figs. 3B & 3C). At endpoint, SsoPox-treated male mice had a lower relative abundance of *Lactobacillus* and, like 1mg/mL SsoPox-treated males, greater relative abundances of *Akkermansia* and Clostridiales when compared to their week 3 microbiomes (K = 14.286, 11.063, 9.143, *P* = < 0.001, 0.001, 0.002, respectively; **Supplemental Figures S4B-S4D**). Similarly, SsoPox-treated female mice had a lower relative abundance of *Lactobacillus* and greater relative abundances of *Akkermansia* and Clostridiales, as well as a greater relative abundance of *Clostridium sensu stricto*, when compared to their pre-DSS microbiomes at week 3 (K = 13.720, 9.606, 5.491, 8.757, *P* = < 0.001, 0.002, 0.019, 0.003, respectively; **Supplemental Figures S4F-S4I**), a pattern similar to what was seen in 1mg/mL SsoPox-treated females.

Symptoms of Colitis were Exacerbated by the Lactonase SsoPox at Lower Dose (1mg/mL) and Alleviated at Higher Dose (2mg/mL)

1mg/mL Endpoints and Correlations

SsoPox at 1mg/mL appeared to exacerbate symptoms of colitis in both sexes. At endpoint, both SsoPox-treated male and female mice had similar fecal concentrations of IgA when compared to DW controls (ANOVA: F = 0.981, 0.849, *P* = 0.335, 0.370, respectively; Table 3). Across all mice in the 1mg/mL

experiment, no predominant taxa were correlated with fecal concentration of IgA. In contrast to IgA, both SsoPox-treated males and females had greater fecal concentrations of Lcn2 compared to controls (ANOVA F = 5.093, 5.140, *P* = 0.043, 0.043, respectively; Table 3). Across all mice (SsoPox-treated + DW controls) in the 1mg/mL experiment, *Lactobacillus* was the only taxon whose relative abundance was negatively correlated with fecal Lcn2 concentration at endpoint (Spearman ρ = -0.417, *P* = 0.023; Fig. 4A); no positive correlations were observed.

Table 3
Endpoint concentrations (mean ± standard deviation) of immunoglobulin-A (IgA) and lipocalin-2 (Lcn2), as well as colon length and Disease Activity Index (DAI) for animal colitis.

Assay	Experiment	Sex	DW	SsoPox	F	P-value	
IgA	1mg/mL	Female	7.06 ± 1.68	7.73 ± 1.46	0.849	0.37	
		Male	7.17 ± 1.13	7.75 ± 1.46	0.981	0.335	
	2mg/mL	Female	7.90 ± 3.77	21.79 ± 19.55	4.374	0.052	
		Male	10.88 ± 6.26	31.01 ± 18.72	10.403	0.005	
Lcn2	1mg/mL	Female	227 ± 195	13730 ± 11627	5.14	0.043	
		Male	3600 ± 5107	28229 ± 30424	5.093	0.043	
	2mg/mL	Female	479 ± 504	175 ± 136	3.049	0.099	
		Male	866 ± 935	598 ± 640	0.542	0.472	
Colon Length		1mg/mL	Female	71.0 ± 9.2	59.6 ± 14.8	3.856	0.067
			Male	75.7 ± 10.1	61.5 ± 15.7	5.824	0.027
		2mg/mL	Female	69.3 ± 5.5	74.3 ± 6.2	3.663	0.072
			Male	65.1 ± 5.9	76.1 ± 9.1	10.218	0.005
DAI Score		1mg/mL	Female	1.20 ± 1.75	3.70 ± 2.95	5.320	0.033
			Male	4.60 ± 2.50	6.10 ± 2.28	1.960	0.178
		2mg/mL	Female	1.70 ± 2.45	1.30 ± 1.89	0.167	0.688
			Male	4.10 ± 2.88	3.00 ± 2.00	0.982	0.335

SsoPox-treated male mice had shorter colons, and SsoPox-treated female mice trended towards shorter colons, relative to controls, although this did not reach significance (ANOVA F = 5.824, 3.856, *P* = 0.027, 0.067, respectively; Table 3). Conversely, SsoPox-treated female mice had a higher DAI score relative to DW controls, and SsoPox-treated male mice trended towards a higher DAI score but did not reach significance (ANOVA F = 5.320, 1.960, *P* = 0.033, 0.178, respectively; Table 3). Across all SsoPox-treated mice, colon length was positively correlated with the endpoint relative abundance of Lachnospiraceae (Spearman ρ = 0.500, *P* = 0.031; Fig. 4A). Colon length negatively correlated with fecal Lcn2 concentration (Spearman ρ = -0.557, *P* = 0.002).

2mg/mL Endpoints and Correlations

In contrast to SsoPox at 1mg/mL, 2mg/mL alleviated colitis symptoms. SsoPox-treated male mice had greater endpoint fecal IgA concentrations than controls, and female mice trended towards the same (ANOVA F = 10.403, 4.374, *P* = 0.005, 0.052, respectively; Table 3). Across all SsoPox-treated mice, the fecal concentration of IgA was positively correlated with the endpoint relative abundances of *Akkermansia* and Clostridiales (Spearman ρ = 0.650, 0.660, *P* = 0.002, 0.002, respectively; Fig. 4B), and negatively correlated with the endpoint relative abundances of *Turicibacter* and *Bifidobacterium* (Spearman ρ = -0.573, -0.475, *P* = 0.009, 0.036, respectively; Fig. 4B). Both SsoPox-treated male and female mice had similar endpoint fecal concentrations of Lcn2 compared to DW controls, with Lcn2 concentration even trending lower in SsoPox-treated females compared to female controls (ANOVA F = 0.542, 3.049, *P* = 0.472, 0.099, respectively; Table 3). Among all mice in the 2mg/mL experiment, no predominant taxa correlated with fecal Lcn2 concentration (Fig. 4B).

At endpoint, SsoPox-treated male mice had longer colons than DW controls, and SsoPox-treated female mice trended towards longer colons, though this did not reach significance (ANOVA F = 10.218, 3.663, *P* = 0.005, 0.072, respectively; Table 3). The DAI scores of both male and female SsoPox-treated mice did not differ from their DW controls (ANOVA F = 0.982, 0.167, *P* = 0.335, 0.688, respectively; Table 3). No predominant taxa were associated with colon length in SsoPox-treated mice, though colon length was positively correlated with fecal IgA concentration (Spearman ρ = 0.337, *P* = 0.034; Fig. 4B).

Endpoint Correlations Across Experiments

Across all endpoint samples in both experiments, the fecal concentration of IgA was positively correlated with the relative abundance of Muribaculaceae and negatively correlated with the relative abundances of *Turicibacter*, *Lactobacillus*, and *Clostridium sensu stricto* (Spearman ρ = 0.365, -0.238, -0.240, -0.269, *P* < 0.001, 0.037, 0.036, 0.018, respectively; Fig. 4C). Fecal Lcn2 concentration was positively correlated with the relative abundance of Muribaculaceae and negatively correlated with the relative abundance of *Lactobacillus* (Spearman ρ = 0.276, -0.249, *P* = 0.021, 0.038; Fig. 4C). Colon length was positively correlated with the relative abundance of *Bifidobacterium* and negatively correlated with fecal Lcn2 concentration (Spearman ρ = 0.231, -0.312, *P* = 0.045, 0.009, respectively; Fig. 4C).

Baseline Gut Microbiome Compositions Did Not Impact Colitis in Controls

Despite several differences in relative abundances among predominant taxa between the baseline of both experiments (**Supplemental Figure S5**), the endpoint fecal concentrations of IgA and Lcn2 did not differ between the 1mg/mL and 2mg/mL DW controls (ANOVA F = 2.812, 0.186, *P* = 0.103, 0.700, respectively; Table 3). Similarly, while DW controls in the 1mg/mL experiment trended towards slightly longer colons than their 2mg/mL counterparts, this difference did not reach significance (ANOVA F = 4.009, *P* = 0.053; Table 3). For DSS severity, the DAI scores of both male and female DW controls did not differ between experiments (ANOVA F = 0.171, 0.275, *P* = 0.684, 0.606, respectively; Table 3). These results suggest that, despite batch differences in the microbiome, DSS colitis was successfully and comparably induced in both 1mg/ml and 2mg/ml experiments.

SsoPox Triggers Increased Transcript Abundance of Lactate-Utilizing Bacterial Families and Enzymes

RNAseq was performed at baseline and week 3 for fecal samples from SsoPox-treated mice in the 2mg/mL experiment to determine the effects of SsoPox on bacterial transcript abundances. In male mice, the only predominant genus with greater total transcript abundances following three weeks of SsoPox treatment was *Bifidobacterium* (Wald z = 4.383, *P* < 0.001; Fig. 5A). In female mice, no predominant genera differed by transcript abundance before and after three weeks of SsoPox treatment. When male and female samples were combined, both *Bifidobacterium* and *Lactobacillus* were found to have increased in transcript abundance following SsoPox treatment (Wald z = 3.442, 2.190, *P* = 0.0004, 0.026, respectively; Figs. 5B-5C).

Analysis was then additionally performed at family level, with sexes combined to outline broader trends within SsoPox-treated mice. Bifidobacteriaceae, Lactobacillaceae, Enterobacteriaceae, and Enterococcaceae were found to have greater transcript abundances after SsoPox treatment (Wald z = 4.090, 2.423, 7.585, 6.390, *P* ≤ 0.001, respectively; Figs. 6A-6B). Butyricicoccaceae was the only predominant family that was found to have lower transcript abundances after SsoPox treatment (Wald z = -2.589, *P* = 0.015; Figs. 6C-6D).

Among functional genes, RNA transcripts involved in lactate formation and utilization were more abundant following three weeks of SsoPox treatment, including phosphoenolpyruvate (PEP) protein phosphotransferase, alpha glucan branching enzyme, lactate dehydrogenase, pyruvate kinase, and formyl CoA transferase (Wald z = 1.426, 1.742, 0.012, 0.807, 5.126, *P* = 0.036, 0.036, 0.037, 0.035, < 0.001, respectively; Fig. 6E). Transcripts that encoded the cyclic lactone autoinducer peptide, the precursor to the autoinducing peptide (AIP) used exclusively by Gram-positive bacteria, were also less abundant after SsoPox treatment (Wald z = -0.002, *P* = 0.003; Fig. 6E). Multiple transcripts for bacterial replication or repair had greater transcript abundances after SsoPox treatment, including AbrB family transcriptional factor, cell wall-associated hydrolase fragment, and twenty ribosomal subunits (Wald z > 0.02, *P* < 0.029).

RNA transcript abundance for methylglyoxal synthase, repressor LexA, and methionine adenosyltransferase, as well as transcript abundances of MraZ protein and S-adenosyl methyltransferase, were lower after SsoPox treatment (Wald z = -2.571, -2.074, -0.979, -3.261, -3.440, *P* = 0.006, 0.015, 0.035, < 0.001, < 0.001, respectively; Figs. 6E-6F).

DISCUSSION

While the baseline gut microbial compositions of mice differed between the 1mg/mL and 2mg/mL experiments, this was not surprising. The experiments were performed sequentially, and temporal shift of gut microbiome composition within mice from a given vendor has been previously reported [51]. Nonetheless, the effects of DSS administration on fecal IgA concentration, Lcn2 concentration, colon length, and DAI scores were consistent across the DW controls in both experiments. At endpoint, both male and female mice treated with 1mg/ml SsoPox had increased severity of intestinal colitis [52, 53]. However, mice treated with 2mg/mL SsoPox had less severe colitis, as evidenced by longer colon lengths and a smaller change in overall community composition compared to their respective controls. This follows a similar trend seen in antimicrobials, where a subinhibitory concentration can, in some situations, cause a pro-inflammatory or pro-pathogenic effect [54]. While the lactonase SsoPox (and QQ generally) is non-bactericidal, this effect could be part of a general stress response [54], and this experiment may provide some of the first evidence that sub-therapeutic doses of QQ enzymes may also exhibit this pro-inflammatory effect. These findings are similar to our previously reported results, where we noted dose-specific effects of SsoPox in a diet-induced obesity model [23]. Additionally, we have previously shown that administration of 1mg/ml SsoPox reduced the metastatic rate of aggressive CRC in male mice, indicating that 1mg/mL may be a sufficiently therapeutic dose for males in some situations [20].

SsoPox administration among female mice had less impact on their microbiomes than that of males, and this was also observed among markers of inflammation. SsoPox-treated female mice trended towards shorter colons at 1mg/ml and greater fecal IgA concentrations, and towards longer colons at 2mg/ml, but did not reach significance, in contrast to males. This observed difference was also observed in previous work, with SsoPox having a weaker effect in females in the diet-induced obesity model mentioned earlier [23]. Interestingly, in the aggressive CRC model, female mice treated with SsoPox had an increased metastatic rate of CRC, opposite to its effect in male mice [20]. While this was initially reported as a sex-specific effect, the findings of the current study raise the intriguing possibility that females receive a subtherapeutic dose of SsoPox at 1mg/ml, which may be related to the previously reported pro-metastatic effect. At present, our data suggest that SsoPox at 2mg/ml may be a more effective dose for delivering reproducible results across both sexes and further highlight the need to better interrogate sex differences in quorum dynamics.

Our RNA-seq analysis revealed that three weeks of SsoPox treatment reduced transcript abundance of the AIP precursor, a QS molecule used exclusively by Gram-positive bacteria, possibly signaling a community-wide shift in cellular processes. At the same time, we also detected greater transcript

abundances of PEP phosphotransferase and lactate dehydrogenase, the latter utilized by lactic-acid-producing bacteria (LAB) and signaling an increase in lactic acid production [55]. Formyl CoA transferase, which had greater transcript abundances in SsoPox-treated mice after three weeks and is known to be primarily utilized by LAB for oxalate metabolism in the lower pH ranges within the gut [56], provided further evidence of increased LAB activity. Common LAB include members of Lactobacillaceae and Enterococcaceae, and many researchers now consider members of Bifidobacteriaceae to be functionally similar to LAB (the larger group sometimes differentiated as LAB&B) [57–61]. All three lactic acid-associated families showed increased transcript abundance after SsoPox treatment in the present study. Additionally, *Lactobacillus* and *Bifidobacterium*, predominant members of LAB&B families, were associated with markers of alleviated colitis following DSS administration. Enterobacteriaceae, the only other predominant family observed to have higher transcript abundances after SsoPox treatment, has also recently been shown to utilize lactic acid during DSS-induced colitis [62]. Notably, while these LAB&B families showed significantly increased transcript abundances after three weeks of treatment with 2mg/mL SsoPox, an increase in the relative abundance was not observed in the 16S rRNA data. This may signal an important functional change in LAB&B, specifically, or a more general metabolic shift throughout the colon, and further investigation is required to validate this hypothesis.

An increase in LAB activity could be a potential mechanism for the observed anti-inflammatory and anti-pathogenic effects of SsoPox administration, as supplementation with LAB, lactic acid, or their common fermentation product, butyrate, are all known to reduce gut inflammation and boost intestinal wall integrity [63, 64]. This upregulation of LAB&B activity and lactic acid production can have downstream effects that shift the entire microbiome towards an anti-colitic state, with several LAB metabolites known to have anti-inflammatory properties [65, 66]. SsoPox, which causes this upregulation of lactic acid production, is also supported by a recent study in the oral microbiome, which demonstrated for the first time that SsoPox supplementation increased sucrose fermentation to lactate [67].

The current study has important limitations. Chief among them is the fact that this study did not measure lactic acid concentration; future studies will preserve blood and tissue for this purpose. Additionally, this study represents the latest in a growing body of work indicating that the QQ lactonase SsoPox may have a weaker effect in females, and future research should examine the interaction between sex and quorum dynamics. There is emerging research on the interactions between the gut microbiome and sex hormones, termed the “microgenderome”, but the complex interplay remains poorly understood, and there is a paucity of research to explain the sex-based differences in quorum dynamics observed in this experiment [68]. Finally, this study’s design allowed us to explore the effects of SsoPox throughout the first three weeks and its ability to prevent colitis in the final three weeks. We did not, however, examine the ability of SsoPox to reverse active colitis. A future study wherein colitis is induced prior to SsoPox treatment will be needed to explore the preclinical potential of QQ treatment.

CONCLUSIONS

This experiment explored the effects of SsoPox treatment on DSS-induced colitis at two different doses. Mice treated with SsoPox at 1mg/mL demonstrated an increased severity in several markers of colitis, while mice treated with SsoPox at 2mg/mL demonstrated an alleviation of colitis markers. Mice treated with SsoPox at 2mg/mL also showed changes in the abundance of transcripts related to LAB&B metabolic function, as measured by RNA sequencing, without an associated increase in LAB&B abundance as determined by 16S rRNA sequencing, suggesting a likely functional metabolic change within the microbiome. This demonstrates a potential mechanism by which SsoPox exerts its anti-inflammatory and anti-pathogenic effects, and future studies should closely examine the role of SsoPox supplementation in lactic acid and butyrate production.

Abbreviations

AHL	*N*-acyl homoserine lactone
AIP	*Autoinducing peptide*
ANOSIM	Analysis of similarity
ANOVA	Analysis of variation
CD	Crohn's disease
CRC	Colorectal cancer
DAI	Disease activity index
DSS	Dextran-sulfate sodium
DW	Drinking water
IACUC	Institutional Animal Care and Use Committee
IBD	Inflammatory bowel disease
IgA	Immunoglobulin A
LAB	Lactic acid bacteria
LAB&B	LAB & Bifidobacteriaceae
Lcn2	Lipocalin-2
PCoA	Principal coordinate analysis
PEP	phosphoenolpyruvate
QQ	Quorum quenching
QS	Quorum sensing
RNAseq	RNA sequencing
TBBL	5-thiobutyl-γ-butyrolactone
UMGC	University of Minnesota Genomics Center

Declarations

Ethics approval and consent to participate

The experiment was approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC), protocol 2403-41874A.

Consent for publication

Not applicable.

Competing Interests

MHE is a co-founder, a former CEO, and an equity holder of Gene&GreenTK, a company that holds the license to WO2014167140 A1, FR 3068989 A1, and FR 19/02834. MHE has filed the patents EP3941206 and WO2020185861A1. MHE has an active research collaboration with Gene&GreenTK. MHE interests with Gene&GreenTK have been reviewed and managed by the University of Minnesota in accordance with its Conflict-of-Interest policies. The remaining authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This research was funded through a 2023 Norm and Pat Wells grant and a 2024 research grant, both from the Minnesota Colorectal Cancer Research Foundation. Other funds include the MnDrive Initiative.

Author Contribution

This experiment was conceptualized by MD, MHE, CJ, SS, and CS. MHE and RS were responsible for lactonase preparation. MD, ZZ, MM, and MB were responsible for investigation, data curation, and formal analysis, with MD responsible for final visualization and authoring the final draft. CJ and SS provided review and editing. CS was responsible for project administration, supervision, and funding acquisition. All authors read and approved the final manuscript.

Acknowledgement

We would like to acknowledge the Minnesota Supercomputing Institute, whose resources were used for data storage, processing, and analysis. We would also like to sincerely thank Kelli Tourand for administrative support. We are grateful to Dr. Frederick Schendel, Plant Manager, University of Minnesota Biotechnology Resource Center (BRC), St. Paul, MN, Tom Douville, and the BRC staff for assistance with protein production.

Data Availability

The dataset(s) supporting the conclusions of this article are available in the Sequence Read Archive of the National Center for Biotechnology Information, BioProject accession number SRP529236.

References

1. Forgie AJ, Fouhse JM, Willing BP. Diet-Microbe-Host Interactions That Affect Gut Mucosal Integrity and Infection Resistance. Front Immunol. 2019;10. https://doi.org/10.3389/fimmu.2019.01802.
2. Sbihi H, Boutin RCT, Cutler C, Suen M, Finlay BB, Turvey SE. Thinking bigger: How early-life environmental exposures shape the gut microbiome and influence the development of asthma and allergic disease. Allergy. 2019;74:2103–15. https://doi.org/10.1111/all.13812.
3. Sanz Y, Cryan JF, Deschasaux-Tanguy M, Elinav E, Lambrecht R, Veiga P. The gut microbiome connects nutrition and human health. Nat Rev Gastroenterol Hepatol. 2025;22:534–55. https://doi.org/10.1038/s41575-025-01077-5.
4. Staley C, Kaiser T, Khoruts A. Clinician guide to microbiome testing. Dig Dis Sci. 2018;63:3167–77. https://doi.org/10.1007/s10620-018-5299-6.
5. Quaglio AEV, Grillo TG, De Oliveira ECS, Di Stasi LC, Sassaki LY. Gut microbiota, inflammatory bowel disease and colorectal cancer. World J Gastroenterol. 2022;28:4053–60. https://doi.org/10.3748/wjg.v28.i30.4053.
6. Wu H, Lv B, Zhi L, Shao Y, Liu X, Mitteregger M, et al. Microbiome–metabolome dynamics associated with impaired glucose control and responses to lifestyle changes. Nat Med. 2025;31:2222–31. https://doi.org/10.1038/s41591-025-03642-6.
7. Effect of diet and lifestyle on microbiome composition. In: International Review of Cell and Molecular Biology. Academic Press; 2025. p. 157–74. https://doi.org/10.1016/bs.ircmb.2024.12.008.
8. Mukherjee S, Bassler BL. Bacterial Quorum Sensing in Complex and Dynamically Changing Environments. Nat Rev Microbiol. 2019;17:371–82. https://doi.org/10.1038/s41579-019-0186-5.
9. Maldonado-Barragán A, West SA. The Cost and Benefit of Quorum Sensing-Controlled Bacteriocin Production in *Lactobacillus plantarum*. J Evol Biol. 2020;33:101–11. https://doi.org/10.1111/jeb.13551.
10. Packiavathy IASV, Kannappan A, Thiyagarajan S, Srinivasan R, Jeyapragash D, Paul JBJ, et al. AHL-Lactonase Producing *Psychrobacter* Sp. from Palk Bay Sediment Mitigates Quorum Sensing-Mediated Virulence Production in Gram Negative Bacterial Pathogens. Front Microbiol. 2021;12:634593. https://doi.org/10.3389/fmicb.2021.634593.
11. Ziegert Z, Dietz M, Hill M, McBride M, Painter E, Elias MH, et al. Targeting quorum sensing for manipulation of commensal microbiota. BMC Biotechnol. 2024;24:106. https://doi.org/10.1186/s12896-024-00937-3.
12. Utari PD, Vogel J, Quax WJ. Deciphering Physiological Functions of AHL Quorum Quenching Acylases. Front Microbiol. 2017;8. https://doi.org/10.3389/fmicb.2017.01123.
13. Billot R, Plener L, Jacquet P, Elias M, Chabrière E, Daudé D. Engineering Acyl-Homoserine Lactone-Interfering Enzymes Toward Bacterial Control. The Journal of Biological Chemistry. 2020;295:12993. https://doi.org/10.1074/jbc.REV120.013531.
14. Schwab M, Bergonzi C, Sakkos J, Staley C, Zhang Q, Sadowsky MJ, et al. Signal Disruption Leads to Changes in Bacterial Community Population. Front Microbiol. 2019;10.

https://doi.org/10.3389/fmicb.2019.00611.

15. Guendouze A, Plener L, Bzdrenga J, Jacquet P, Rémy B, Elias M, et al. Effect of Quorum Quenching Lactonase in Clinical Isolates of *Pseudomonas aeruginosa* and Comparison with Quorum Sensing Inhibitors. Front Microbiol. 2017;8. https://doi.org/10.3389/fmicb.2017.00227.
16. Tu Y, Li H, Huo J, Gou L, Wen X, Yu X, et al. Disrupting the bacterial language: quorum quenching and its applications. Critical Reviews in Microbiology. 2025;51:1066–80. https://doi.org/10.1080/1040841X.2025.2466472.
17. O'Connor G, Quintero MA, Deo SK, Abreu MT, Daunert S. Bacterial Quorum-Sensing Molecules in Serum: A Potential Tool for Crohn's Disease Management. Clinical and Translational Gastroenterology. 2022;13:e00547. https://doi.org/10.14309/ctg.0000000000000547.
18. Bergonzi C, Schwab M, Naik T, Elias M. The Structural Determinants Accounting for the Broad Substrate Specificity of the Quorum Quenching Lactonase GcL. ChemBioChem. 2019;20:1848–55. https://doi.org/10.1002/cbic.201900024.
19. Yang Y, Shao J, Han Z, Li J, Fang Q, Zhu G. AiiA Lactonase Suppresses ETEC Pathogenicity Through 3OC12-HSL Quenching in a Murine Model. Microbiology Research. 2025;16. https://doi.org/10.3390/microbiolres16080166.
20. Dietz M, Gates TJ, Sikdar R, Subramanian S, Elias MH, Staley C. Impeding Quorum Sensing Among the Intestinal Microbiota Impacts the Metastatic Rate of Colorectal Cancer. Cancer Med. 2025;14:e71009. https://doi.org/10.1002/cam4.71009.
21. Yang C, Merlin D. Unveiling Colitis: A Journey through the Dextran Sodium Sulfate-induced Model. Inflammatory Bowel Diseases. 2024;30:844–53. https://doi.org/10.1093/ibd/izad312.
22. Mukhopadhyay S, Ray P, Aich P. A comparative analysis of gut microbial dysbiosis by select antibiotics and DSS to understand the effects of perturbation on the host immunity and metabolism. Life Sciences. 2023;312:121212. https://doi.org/10.1016/j.lfs.2022.121212.
23. Syal A, Martell M, Sikdar R, Dietz M, Ziegert Z, Jahansouz C, et al. Quorum quenching enzymes disrupt bacterial communication in a sex- and dose-dependent manner. Animal Model Exp Med. 2025;8:473–82. https://doi.org/10.1002/ame2.12520.
24. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran Sulfate Sodium (DSS)-Induced Colitis in Mice. Current Protocols in Immunology. 2014;104:15.25.1-15.25.14. https://doi.org/10.1002/0471142735.im1525s104.
25. Eichele DD, Kharbanda KK. Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. World J Gastroenterol. 2017;23:6016–29. https://doi.org/10.3748/wjg.v23.i33.6016.
26. Martell M, Quarnstrom CF, Khoruts A, Vezys V, Staley C, Shmidt E. Chronic Intestinal Inflammation and Microbial Dysbiosis Are Associated With Female Reproductive Outcomes in a Mouse Model of Inflammatory Bowel Disease. Gastro Hep Adv. 2025;4:100670. https://doi.org/10.1016/j.gastha.2025.100670.

27. Kim JJ, Shajib MdS, Manocha MM, Khan WI. Investigating Intestinal Inflammation in DSS-induced Model of IBD. J Vis Exp. 2012;:3678. https://doi.org/10.3791/3678.
28. Hiblot J, Gotthard G, Elias M, Chabriere E. Differential Active Site Loop Conformations Mediate Promiscuous Activities in the Lactonase SsoPox. PLOS ONE. 2013;8:e75272. https://doi.org/10.1371/journal.pone.0075272.
29. Rémy B, Plener L, Poirier L, Elias M, Daudé D, Chabrière E. Harnessing hyperthermostable lactonase from Sulfolobus solfataricus for biotechnological applications. Sci Rep. 2016;6:37780. https://doi.org/10.1038/srep37780.
30. Bergonzi C, Schwab M, Elias M. The quorum-quenching lactonase from Geobacillus caldoxylosilyticus: purification, characterization, crystallization and crystallographic analysis. Acta Crystallogr F Struct Biol Commun. 2016;72 Pt 9:681–6. https://doi.org/10.1107/S2053230X16011821.
31. Impeding Quorum Sensing Among the Intestinal Microbiota Impacts the Metastatic Rate of Colorectal Cancer - Dietz – 2025 - Cancer Medicine - Wiley Online Library. https://onlinelibrary.wiley.com/doi/10.1002/cam4.71009. Accessed 11 May 2026.
32. Khersonsky O, Tawfik DS. The histidine 115-histidine 134 dyad mediates the lactonase activity of mammalian serum paraoxonases. J Biol Chem. 2006;281:7649–56. https://doi.org/10.1074/jbc.M512594200.
33. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012;6:1621–4. https://doi.org/10.1038/ismej.2012.8.
34. Gohl DM, Vangay P, Garbe J, MacLean A, Hauge A, Becker A, et al. Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. Nat Biotechnol. 2016;34:942–9. https://doi.org/10.1038/nbt.3601.
35. Staley C, Kaiser T, Vaughn BP, Graiziger CT, Hamilton MJ, Rehman T ur, et al. Predicting recurrence of Clostridium difficile infection following encapsulated fecal microbiota transplantation. Microbiome. 2018;6:166. https://doi.org/10.1186/s40168-018-0549-6.
36. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, et al. SILVA: A Comprehensive Online Resource for Quality Checked and Aligned Ribosomal Rna Sequence Data Compatible with ARB. Nucleic Acids Research. 2007;35:7188–96. https://doi.org/10.1093/nar/gkm864.
37. Westcott SL, Schloss PD. OptiClust, an Improved Method for Assigning Amplicon-Based Sequence Data to Operational Taxonomic Units. mSphere. 2017;2:10.1128/mspheredirect.00073 – 17. https://doi.org/10.1128/mspheredirect.00073-17.
38. Schloss PD. Rarefaction is currently the best approach to control for uneven sequencing effort in amplicon sequence analyses. mSphere. 2024;9:e0035423. https://doi.org/10.1128/msphere.00354-23.
39. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, et al. The Ribosomal Database Project: Improved Alignments and New Tools for rRNA Analysis. Nucleic Acids Research. 2009;37

suppl_1:D141–5. https://doi.org/10.1093/nar/gkn879.

40. Arslan S, Garcia FJ, Guo M, Kellinger MW, Kruglyak S, LeVieux JA, et al. Sequencing by avidity enables high accuracy with low reagent consumption. Nat Biotechnol. 2024;42:132–8. https://doi.org/10.1038/s41587-023-01750-7.
41. KneadData – The Huttenhower Lab. https://huttenhower.sph.harvard.edu/kneaddata/. Accessed 8 Mar 2026.
42. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30:2114–20. https://doi.org/10.1093/bioinformatics/btu170.
43. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9. https://doi.org/10.1038/nmeth.1923.
44. Benson G. Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Res. 1999;27:573–80. https://doi.org/10.1093/nar/27.2.573.
45. Beghini F, McIver LJ, Blanco-Míguez A, Dubois L, Asnicar F, Maharjan S, et al. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. eLife. 2021;10:e65088. https://doi.org/10.7554/eLife.65088.
46. Wood DE, Lu J, Langmead B. Improved metagenomic analysis with Kraken 2. Genome Biol. 2019;20:257. https://doi.org/10.1186/s13059-019-1891-0.
47. Suzek BE, Wang Y, Huang H, McGarvey PB, Wu CH, the UniProt Consortium. UniRef clusters: a comprehensive and scalable alternative for improving sequence similarity searches. Bioinformatics. 2015;31:926–32. https://doi.org/10.1093/bioinformatics/btu739.
48. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. Applied and Environmental Microbiology. 2009;75:7537–41. https://doi.org/10.1128/AEM.01541-09.
49. Clarke KR. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology. 1993;18:117–43. https://doi.org/10.1111/j.1442-9993.1993.tb00438.x.
50. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550. https://doi.org/10.1186/s13059-014-0550-8.
51. Mandal RK, Denny JE, Waide ML, Li Q, Bhutiani N, Anderson CD, et al. Temporospatial shifts within commercial laboratory mouse gut microbiota impact experimental reproducibility. BMC Biol. 2020;18:83. https://doi.org/10.1186/s12915-020-00810-7.
52. Kim JJ, Shajib MdS, Manocha MM, Khan WI. Investigating Intestinal Inflammation in DSS-induced Model of IBD. J Vis Exp. 2012;:3678. https://doi.org/10.3791/3678.
53. Stallhofer J, Friedrich M, Konrad-Zerna A, Wetzke M, Lohse P, Glas J, et al. Lipocalin-2 Is a Disease Activity Marker in Inflammatory Bowel Disease Regulated by IL-17A, IL-22, and TNF-α and Modulated by IL23R Genotype Status. Inflammatory Bowel Diseases. 2015;21:2327–40. https://doi.org/10.1097/MIB.0000000000000515.

54. Ranieri MR, Whitchurch CB, Burrows LL. Mechanisms of biofilm stimulation by subinhibitory concentrations of antimicrobials. Current Opinion in Microbiology. 2018;45:164–9. https://doi.org/10.1016/j.mib.2018.07.006.
55. Sheridan PO, Louis P, Tsompanidou E, Shaw S, Harmsen HJ, Duncan SH, et al. Distribution, organization and expression of genes concerned with anaerobic lactate utilization in human intestinal bacteria. Microbial Genomics. 2022;8:000739. https://doi.org/10.1099/mgen.0.000739.
56. Youssef HIA. Detection of oxalyl-CoA decarboxylase (oxc) and formyl-CoA transferase (frc) genes in novel probiotic isolates capable of oxalate degradation in vitro. Folia Microbiol. 2024;69:423–32. https://doi.org/10.1007/s12223-024-01128-5.
57. Rossi F. Special Issue “Functional Characterization of Lactic Acid Bacteria”: Editorial. Microorganisms. 2023;11. https://doi.org/10.3390/microorganisms11051190.
58. Valero-Cases E, Nuncio-Jáuregui N, Frutos MJ. Influence of Fermentation with Different Lactic Acid Bacteria and in Vitro Digestion on the Biotransformation of Phenolic Compounds in Fermented Pomegranate Juices. J Agric Food Chem. 2017;65:6488–96. https://doi.org/10.1021/acs.jafc.6b04854.
59. Mora-Villalobos JA, Montero-Zamora J, Barboza N, Rojas-Garbanzo C, Usaga J, Redondo-Solano M, et al. Multi-Product Lactic Acid Bacteria Fermentations: A Review. Fermentation. 2020;6. https://doi.org/10.3390/fermentation6010023.
60. Liu W, Chen M, Duo L, Wang J, Guo S, Sun H, et al. Characterization of potentially probiotic lactic acid bacteria and bifidobacteria isolated from human colostrum. Journal of Dairy Science. 2020;103:4013–25. https://doi.org/10.3168/jds.2019-17602.
61. Vaughan EE, Heilig HGHJ, Ben-Amor K, de Vos WM. Diversity, vitality and activities of intestinal lactic acid bacteria and bifidobacteria assessed by molecular approaches. FEMS Microbiol Rev. 2005;29:477–90. https://doi.org/10.1016/j.fmrre.2005.04.009.
62. Taylor SJ, Winter MG, Gillis CC, Silva LA da, Dobbins AL, Muramatsu MK, et al. Colonocyte-derived lactate promotes E. coli fitness in the context of inflammation-associated gut microbiota dysbiosis. Microbiome. 2022;10:200. https://doi.org/10.1186/s40168-022-01389-7.
63. Yang Y, Hong J, Zhang Z, Zheng M, Zhao J, Fang X, et al. Oral supplementation with lactic acid bacteria improve the intestinal epithelial barrier and gut microbiota of broiler chicks to alleviate *Salmonella* Enteritidis infection. Poultry Science. 2024;103:104385. https://doi.org/10.1016/j.psj.2024.104385.
64. Chen W, Ma Q, Li Y, Wei L, Zhang Z, Khan A, et al. Butyrate Supplementation Improves Intestinal Health and Growth Performance in Livestock: A Review. Biomolecules. 2025;15. https://doi.org/10.3390/biom15010085.
65. Guo W, Mao B, Tang X, Zhang Q, Zhao J, Zhang H, et al. Improvement of inflammatory bowel disease by lactic acid bacteria-derived metabolites: a review. Critical Reviews in Food Science and Nutrition. 2025;65:1261–78. https://doi.org/10.1080/10408398.2023.2291188.

66. Li D, Liu Z, Fan X, Zhao T, Wen D, Huang X, et al. Lactic Acid Bacteria–Gut-Microbiota-Mediated Intervention towards Inflammatory Bowel Disease. Microorganisms. 2024;12. https://doi.org/10.3390/microorganisms12091864.
67. Sikdar R, Beauclaire MV, Herzberg MC, Lima BP, Elias MH. N-acyl homoserine lactone signaling modulates bacterial community associated with human dental plaque. npj Biofilms Microbiomes. 2025;11:204. https://doi.org/10.1038/s41522-025-00846-z.
68. Yoon K, Kim N. Roles of Sex Hormones and Gender in the Gut Microbiota. Journal of Neurogastroenterology and Motility. 2021;27:314. https://doi.org/10.5056/jnm20208.

Figures

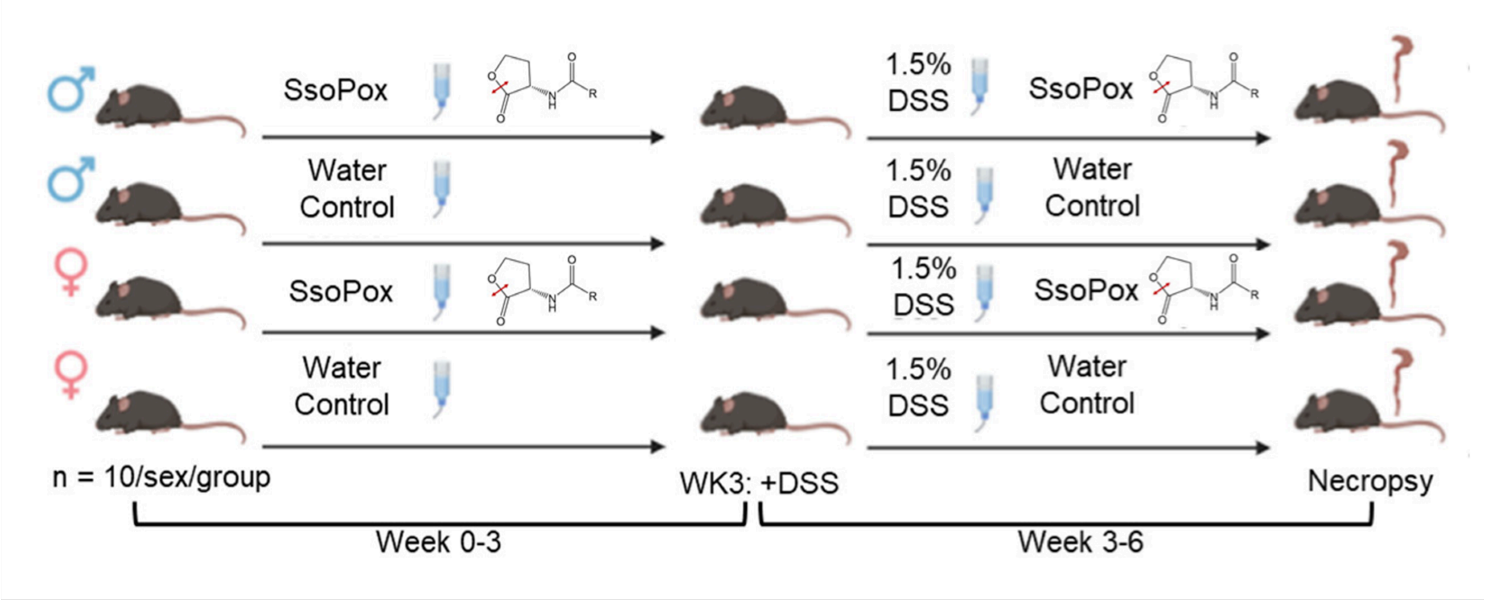


Figure 1

The experimental design consisted of male and female mice divided into two groups and given either drinking water supplemented with SsoPox at a concentration of 1mg/mL or an un-supplemented drinking water control (DW) for a total of 40 mice (n = 10/group/sex). This experiment was then repeated to explore the effects of dose, with SsoPox at 2mg/mL, resulting in a total of eight treatment groups (n = 10/group).

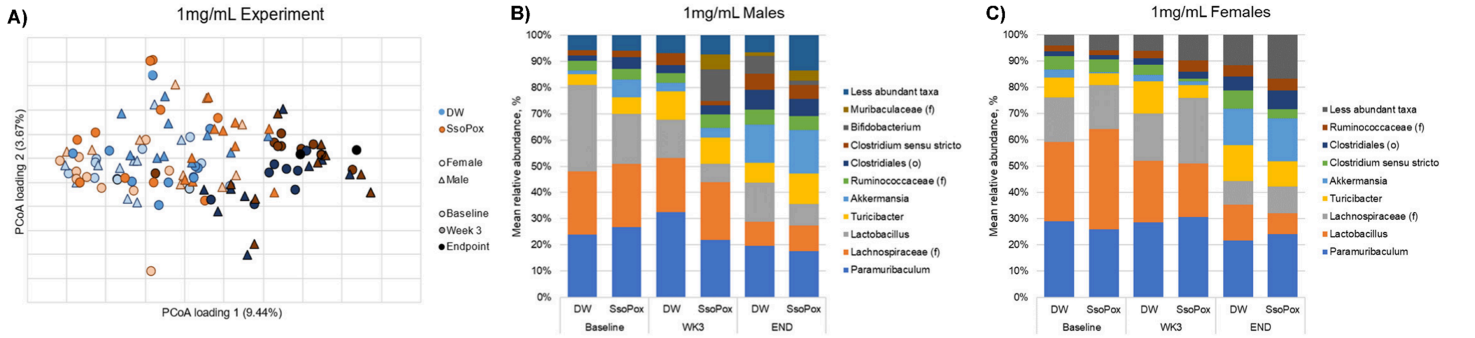


Figure 2

(A) Principal coordinate analysis (PCoA) of the beta diversity from fecal pellets of the initial experiment where SsoPox was administered at 1mg/mL. (B)(C) The gut microbiome compositions of male (B) and female (C) fecal samples in the 1mg/mL experiment at baseline, week 3 (WK3), and endpoint (END).

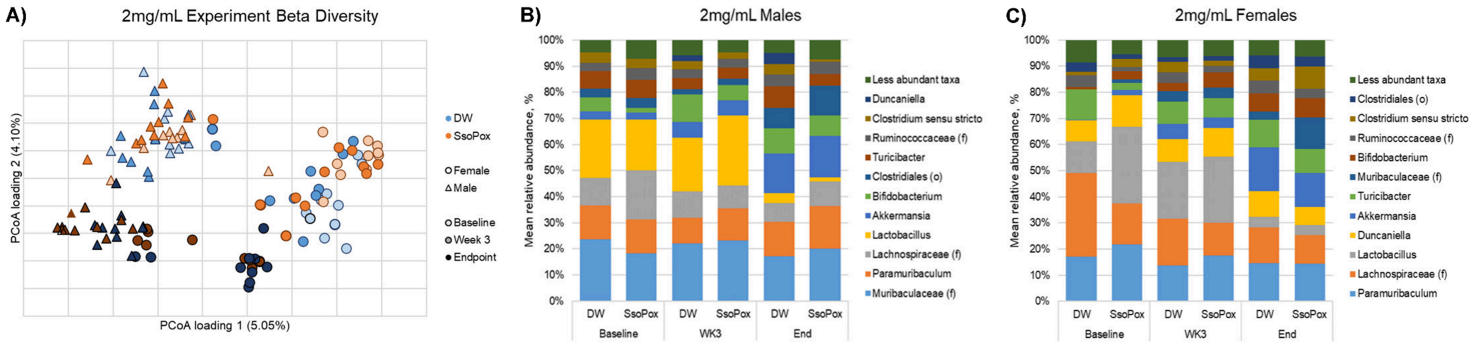


Figure 3

(A) Principal coordinate analysis (PCoA) of the beta diversity from fecal pellets of the second experiment where SsoPox was administered at 2mg/mL. (B) (C) The gut microbiome compositions of male (B) and female (C) fecal samples in the 2mg/mL experiment at baseline, week 3 (WK3), and endpoint (END).

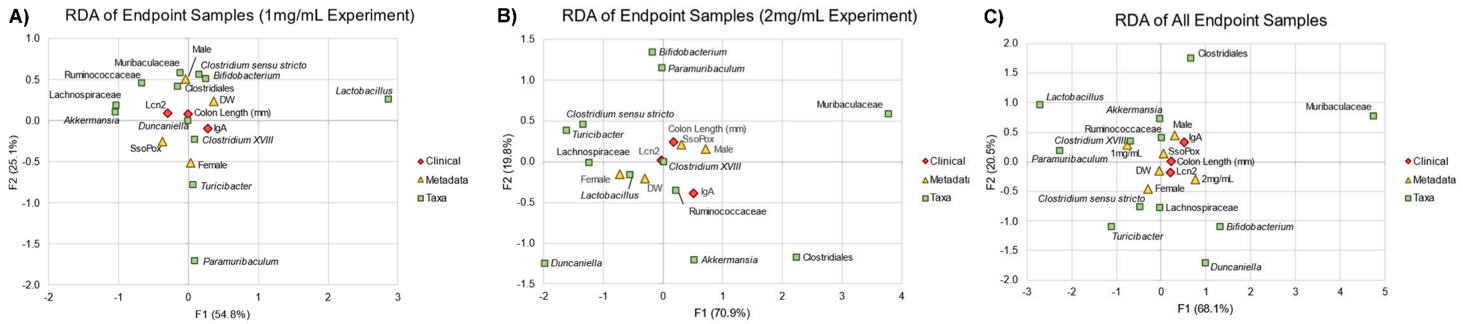


Figure 4

Redundancy analysis (RDA) was performed on endpoint samples. (A) RDA of endpoint samples from the 1mg/mL experiment. The relative abundance of *Clostridium XVIII* was positively correlated with fecal Lcn2 concentration and negatively correlated with colon length. (B) RDA of endpoint samples from the 2mg/mL experiment. The fecal concentration of IgA positively correlated with the relative abundances of *Akkermansia* and Clostridiales and colon length. (C) RDA of all endpoints from both experiments. Colon length was positively correlated with the relative abundance of *Bifidobacterium*and negatively correlated with fecal Lcn2 concentration. The relative abundance of *Lactobacillus* was negatively correlated with fecal concentrations of both IgA and Lcn2.

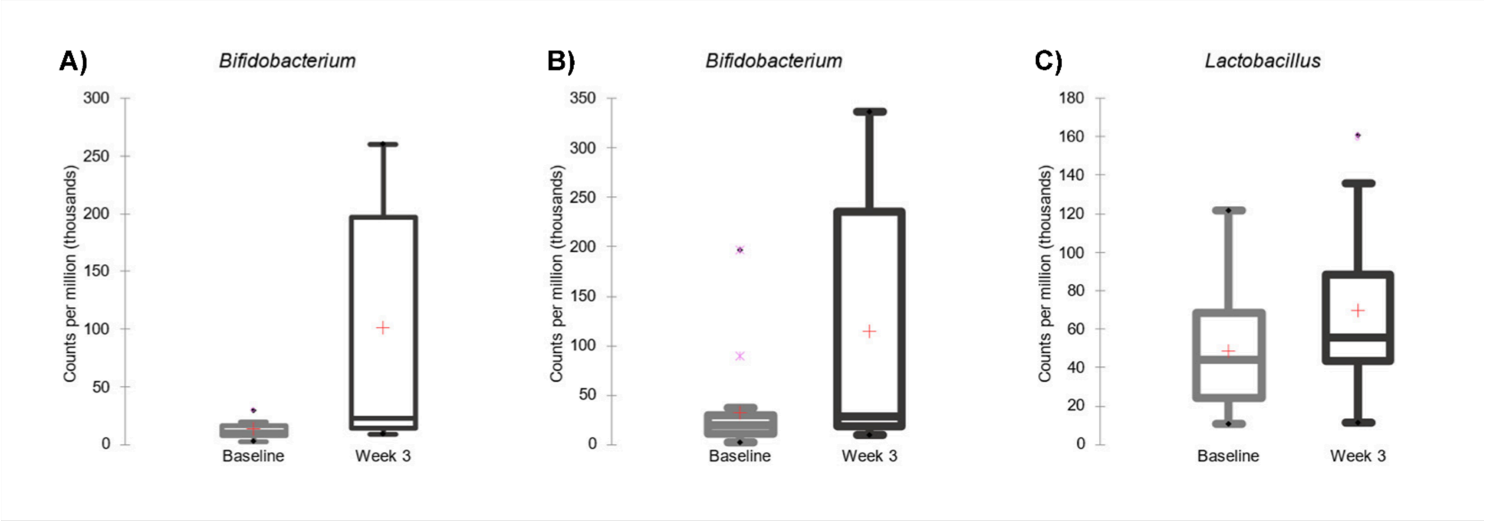


Figure 5

Box and whisker plots of predominant bacterial genera which had significantly higher transcript abundances after three weeks of treatment with SsoPox at 2mg/mL. (A) The transcript abundance of *Bifidobacterium* increased in SsoPox-treated male mice. (B & C) The transcript abundances of *Bifidobacterium* and *Lactobacillus*increased in all SsoPox-treated mice. No bacterial genera had a significant increase in transcript abundance in SsoPox-treated female mice.

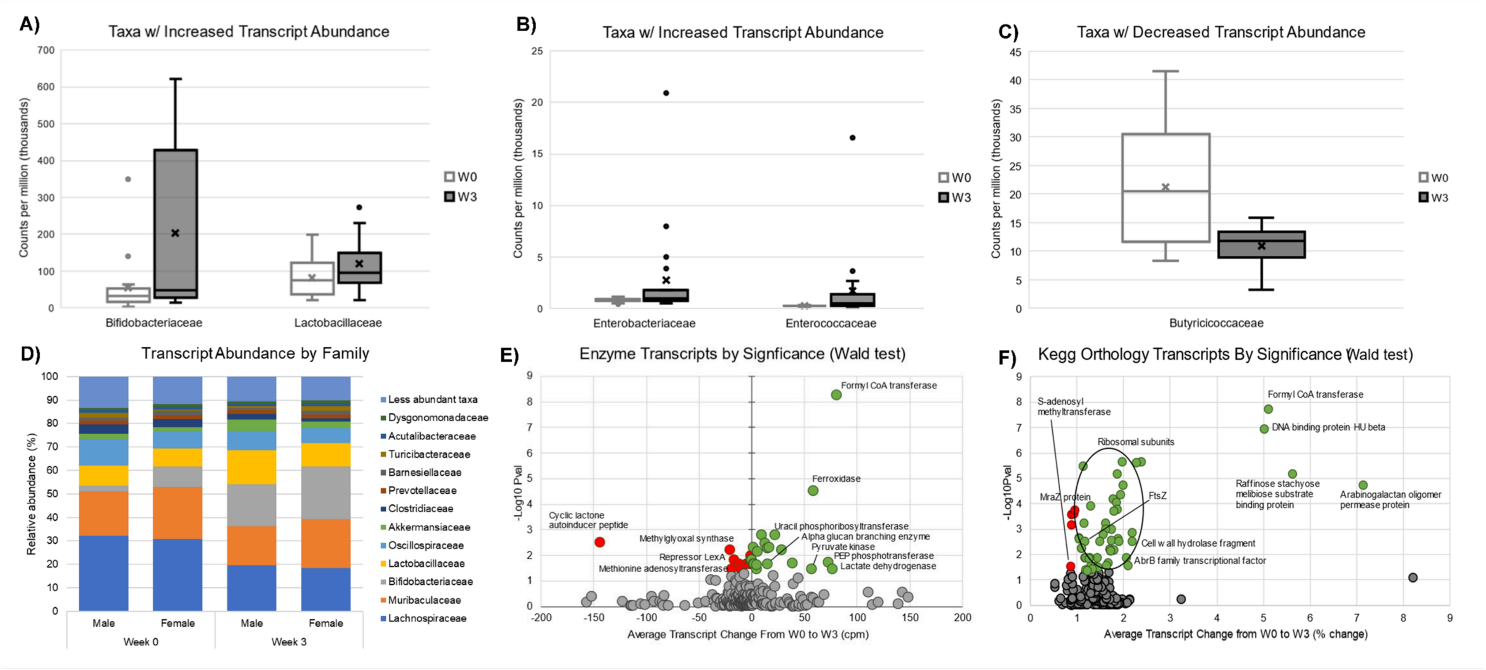


Figure 6

(A & B) Box and whisker plots of Bifidobacteriaceae, Lactobacillaceae, Enterobacteriaceae, and Enterococcaceae, predominant bacterial families which had significantly greater transcript abundance after three weeks of treatment with SsoPox at 2mg/mL. (C) A box and whisker plot of the only predominant bacterial family which had significantly lower transcript abundance after three weeks of treatment with SsoPox at 2mg/mL. (D & E) Volcano plot of enzyme and KEGG ortholog transcripts in all 2mg/mL samples, respectively. Samples with significantly higher transcript abundance after three weeks are highlighted in green, those with significantly lower transcript abundance are highlighted in red.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- QQDSSSuppFigs.docx