

Article

Bovine Bile and Oxygen Availability Impact *Escherichia coli* O157:H7 Metabolism and Adherence Factor Expression

Joel J. Maki ^{1,*}  and Randy Ortiz ^{1,2}¹ Food Safety and Enteric Pathogens Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA 50010, USA² Genome Informatics Facility, Iowa State University, 2200 Osborn Drive, Ames, IA 50011, USA

* Correspondence: joel.maki@usda.gov

Abstract

Escherichia coli O157:H7 represents a major food safety concern, with cattle serving as the primary reservoir host. A better understanding of the interactions between O157 and the potential physiochemical indicators to which it is exposed to in the cattle hindgut would lend insight into the O157 colonization process and aid in the development of effective preharvest interventions to control O157 in its reservoir host. Bovine bile and oxygen are two universal cues that O157 is exposed to upon entry into the cattle hindgut and that vary between different segments of the gastrointestinal tract. Here, we exposed *E. coli* O157:H7 strain EDL933 to whole bovine bile powder and oxygen, both individually and in combination, and assessed the transcriptomic and phenotypic responses. Under aerobic conditions, EDL933 increased the expression of *adiC*, and increased biofilm production in vitro, while anaerobic conditions increased the expression of fimbrial genes, including *ydeR* and *fimA*. Bovine bile induced the expression of pathways responsible for quorum sensing and the utilization of mucus-derived sugars. These findings suggest that bovine bile and oxygen impact the transcriptomic and phenotypic characteristics of EDL933 in vitro.

Keywords: *E. coli*; O157:H7; EHEC; cattle; bile

1. Introduction

Escherichia coli O157:H7 (O157) is an important foodborne pathogen capable of causing severe illness, ranging from diarrheal disease to hemorrhagic colitis and hemolytic uremic syndrome. O157 is one of the most well-known serotypes of Shiga toxin-producing *E. coli* (STEC), a pathogroup comprising over 400 serotypes of *E. coli*, all of which can produce Shiga toxin (Stx). Upon ingestion, STEC commonly infect the gastrointestinal tract (GIT) of humans, producing Stx and causing a variety of symptoms ranging from severe hemorrhagic gastroenteritis to hemolytic uremic syndrome, resulting in acute kidney failure and, in extreme cases, death [1]. O157 is responsible for >50,000 infections and dozens of deaths in the USA on a yearly basis [2]. The natural reservoir for O157 is the cattle GIT. O157 is present in multiple intestinal compartments of the cattle hindgut but prefers colonization of the rectoanal junction (RAJ), where it forms microcolonies and is readily shed in fecal material [3,4]. Contaminated fecal material allows O157 transmission between hosts and contaminates beef and dairy products and, increasingly, produce such as lettuce and spinach [5]. O157 contamination of food and water places a significant burden on the agricultural industry, requiring additional testing, control measures, and, in the worst-case scenarios, product recalls [6–8]. A better understanding of O157 colonization dynamics



Academic Editors: Alba Blesa,
Lucía García Pastor and
Mercedes Sánchez-Costa

Received: 9 June 2026

Revised: 25 July 2026

Accepted: 27 July 2026

Published: 1 August 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

in the cattle GIT could enable development of targeted preharvest interventions that help reduce industry costs and protect consumers.

Upon entering the cattle GIT, O157 and other bacteria must contend with a multitude of drastic environmental shifts. Upon ingestion, O157 enters the rumen, which is generally considered to provide an inhospitable environment for O157 persistence, necessitating the activation of numerous stress response and survival pathways [9,10]. O157 exits the rumen and enters the highly acidic abomasum, a similarly stressful environment for O157, before entering the cattle hindgut [4]. The cattle hindgut offers a more hospitable environment for O157 [9]. However, O157 still must contend with a variety of signals and stressors as it transits the different compartments of the small and large intestine, and these signals, both individually and in combination, impact the ability of O157 to survive, colonize, and persist within the cattle host.

One of these signals is the secretion of liquid bile. Bile is a complex biofluid composed of a variety of solutes dissolved in water [11]. Over half of the solutes found in bile are the bile acids, a group of cholesterol-derived molecules responsible for the absorption of fats and lipid-soluble vitamins from the diet [12]. Additional solutes present in bile include phospholipids, fatty acids, cholesterol, and bilirubin, among others [11]. After synthesis in hepatocytes, bile is stored in the gallbladder and, upon feeding, bile is transported via the bile duct and secreted into the duodenum to aid in digestion [13]. In cattle, bile is an especially interesting signal because it demarcates the transition from the rumen, a stressful environment that significantly decreases viable O157 counts, to the duodenum, the beginning of the more hospitable hindgut [4]. Bile is a major constituent of the early compartments of the hindgut, but its various components are broken down as the intestinal contents move along the GIT. Bile acid-modifying organisms begin the process of transforming the primary, host-synthesized bile acids into microbially modified secondary bile acids [14,15]. The phospholipids and proteins are quickly utilized by other bacteria, while the bilirubin is degraded by the anaerobes present in the terminal ileum and colon [16]. Modification of the different bile components by commensal microbes in the GIT make it a temporary indicator of location for O157, with bile presence mainly restricted to the duodenum, jejunum, and proximal ileum [12,17–19]. Previous studies examining the O157 response to bile have exclusively focused on bile acids, a single component of liquid bile. These studies have largely relied on commercially available blends of deconjugated bile acids, which can impact bacterial physiology differently from the host-derived, conjugated bile acids that are typically present in the liquid bile and small intestine of cattle [20–22]. Moreover, the composition of these commercial bile salt mixes does not reflect the bile acid profiles of any individual segment of the cattle hindgut, making it difficult to put these results into a physiologically relevant context [12,23]. However, challenges related to sourcing and cost make the utilization of liquid bovine bile for in vitro studies challenging. Bovine bile powders, sometimes referred to as oxgall powders, represent an inexpensive and readily available alternative to liquid bovine bile for in vitro studies. Bovine bile powder is compositionally similar across manufacturers and closely resembles the bovine bile acid pool in the gallbladder and duodenum in vivo, with conjugated bile acids representing >95% of the bile acid species present, predominantly taurocholic acid (TCA; >30%) and glycocholic acid (GCA; >20%) [12,23]. Evaluating the response of EDL933, a strain of O157, to whole bovine bile powder would provide a more accurate representation of the physiological conditions encountered by O157 upon entry into, and traversal of, the bovine small intestine.

Oxygen is another molecule that varies considerably between compartments of the cattle GIT, as well as within a given gut compartment. The rumen is a strictly anaerobic environment, supporting a host of obligately anaerobic bacteria such as Lachnospiraceae and Prevotellaceae [24,25]. As O157 moves from the rumen into the hindgut, there is an increase in oxygen concentration, either due to the diffusion of oxygen across the thinner mucus layer found in the duodenum and jejunum or to oxygen being introduced alongside liquid bile as it enters the duodenum from the bile duct, potentially a combination of the two [26–28]. Enough oxygen exists in the upper small intestine that strict anaerobes capable of bile acid modification are inhibited, limiting bile acid modification until a more anaerobic environment is achieved in the terminal ileum [29]. The terminal ileum and large intestine of cattle are predominantly devoid of oxygen, which requires a shift in metabolism and other physiological processes for a facultative anaerobe like O157 [28]. The large intestine terminates in the RAJ, a short segment where lymphoid tissue-rich columnar epithelium transitions to the keratinized squamous epithelium of the anus. This region serves as the primary colonization site for EDL933, as well as other strains of O157 [30]. The reduced thickness of the mucus layer and proximity to the anus make oxygen diffusion likely in this segment, representing a transition back to an aerobic, or at least microaerobic, environment, further exemplified by an increase in aerobic and aerotolerant bacteria at the rectal mucosa of other mammalian species [31–34].

The fluctuating oxygen levels along the cattle GIT, along with other compartment-specific signals such as bile, could serve as important indicators for EDL933, serving as a sort of “roadmap” that impacts gene expression and promotes successful colonization. Understanding these different signals and the way they impact the transcription of colonization and metabolic pathways would aid in the development of targeted preharvest interventions to reduce EDL933 colonization and dissemination, reducing product contamination and instances of foodborne illness. Here, we assessed the hypothesis that physiologically relevant concentrations of cattle bile powder and oxygen availability act as key environmental signals shaping EDL933 gene expression and physiology in ways that may influence its ability to determine locality within the cattle GIT, an important component of the colonization process. These *in vitro* experiments will also help determine how bovine bile and oxygen affect EDL933 growth and the regulation of metabolic and colonization-associated traits and pathways, including biofilm formation, allowing for a controlled evaluation of individual physiochemical signals. While the *in vitro* assays utilized here cannot fully mimic the complexity of interactions among host tissues, physiochemical gradients, and the native microbiota within the cattle GIT, this work provides foundational observations for the generation of hypotheses about EDL933 behavior in the cattle hindgut.

2. Materials and Methods

2.1. Bacterial Strains and Growth Conditions

Escherichia coli O157 strain EDL933 (ATCC 43895) was initially obtained from the American Type Culture Collection (ATCC) and maintained aerobically in Tryptic Soy Agar (TSA; BD Difco, Franklin Lakes, NJ, USA) at 39 °C or Tryptic Soy Broth (TSB; BD Difco) supplemented with 20 mM MOPS (TSB+MOPS; Sigma-Aldrich, Burlington, MA, USA) at 39 °C unless otherwise noted. For assays conducted under anaerobic conditions (5% H₂:10% CO₂: 85% N₂), a vinyl anaerobic chamber (Coy Laboratories, Grass Lake, MI, USA) was used.

2.2. Bacterial Growth Curve Analysis

EDL933 was grown in TSB overnight at 39 °C under aerobic or anaerobic conditions as described above. Cells were pelleted at 5000× *g* for 10 min, and the supernatant was removed. Cells were washed twice with 1× PBS (pH 7.0; Sigma-Aldrich) before resuspension in fresh TSB + MOPS to an OD₆₀₀ of 0.05 (±0.01) using a WPA Biowave CO8000 Cell Density Meter (Biochrom, Cambridge, UK). Ten µL of the OD₆₀₀-adjusted cell suspension was inoculated into a 96-well cell culture plate containing 190 µL of TSB + MOPS or TSB + MOPS supplemented with either 0.02% (*w/v*), 0.2% (*w/v*), or 2.0% (*w/v*) dry, unfractionated bovine bile powder (pH 7.0; Sigma-Aldrich). A SmartReader 96 MR9600-T instrument (Accuris, Edison, NJ, USA) was used to incubate the plate at 39 °C for 24 h and record serial OD₆₃₀ measurements every 15 min under aerobic or anaerobic conditions. Each set of conditions was conducted with 12 biological replicates. The resultant OD₆₃₀ measurements were imported into R (v4.5.1) to plot growth curves and calculate the time to midlog phase (*T*_{mid}), mean generation times (*T*_{Gen}), and mean area under the logistic curve (AUC) values for EDL933 grown under each set of conditions with the GrowthCurveR package (v0.3.1) [35,36]. Statistical analysis (wilcox.test) with a Bonferroni correction and figure generation were conducted with the stats (v4.3.1), ggsignif (v0.6.4), ggpubr (v0.6.1), ggprism (v1.0.6), and ggplot2 (v3.5.2) packages [37–40].

2.3. RNA Sequencing (RNA-Seq) Sample Preparation and Analysis

EDL933 was grown overnight in TSB + MOPS as described above. Cells were pelleted at 5000× *g* for 10 min, and supernatant was removed. Cells were washed twice with PBS before resuspension in fresh TSB+MOPS to an OD₆₀₀ of 0.05 (±0.01) using the WPA Biowave CO8000 Cell Density Meter. Two hundred µL of OD₆₀₀-adjusted cell suspension was inoculated into 4 mL of TSB + MOPS (1:20 dilution) with or without supplementation with 0.2% (*w/v*) dry, unfractionated bovine bile powder (pH 7.0). Cultures were incubated at 39 °C until reaching the mid-log phase of growth as determined by previously generated growth curve data (Table S1). Upon reaching the mid-log phase, cultures were vortexed and 500 µL of broth culture was inoculated directly into 1 mL of RNeasy Protect (Qiagen, Hilden, Germany). After 5 min, the tubes were centrifuged at 5000× *g* for 10 min after which the supernatant was removed. Cell pellets were stored at −80 °C prior to RNA extraction. Samples were prepared under both aerobic and anaerobic conditions. For anaerobically prepared samples, all steps were conducted in a vinyl anaerobic chamber under anaerobic atmospheric conditions with pre-reduced media to limit exposure of EDL933 to oxygen. Three biological replicates were conducted for each set of conditions. RNA was extracted with an RNeasy kit (Qiagen) according to the manufacturers' instructions. Quality of extracted RNA (RINe) was measured using an RNA ScreenTape (Agilent, Santa Clara, CA, USA) on the 4200 TapeStation system (Agilent) following the manufacturer's instructions. RNA quantity was determined with the Broad Range RNA kit (Qubit, Boston, MA, USA) on a Qubit 4 Fluorometer instrument (Qubit) according to the manufacturer's instructions. RNA samples were submitted to the Iowa State University DNA Facility where bacterial rRNA was depleted using a NEBNext rRNA Depletion kit for bacteria (New England Biolabs, Ipswich, MA, USA) and mRNASeq libraries were prepared using an Illumina Stranded mRNA Prep kit (Illumina, San Diego, CA, USA), both according to the manufacturer's instructions. Resulting mRNA libraries were sequenced on a NovaSeq6000 instrument (Illumina) in paired-end mode with 150 cycles.

Each sample was sequenced across two lanes of an SP1 flow cell (Illumina), yielding two sets of paired-end FASTQ files per sample. The paired FASTQ files generated from the two sequencing runs were merged to produce one consolidated forward and reverse paired dataset. Merged reads were assessed for quality using FastQC (v0.12.1) [41]. Adapter

and low-quality base trimming were performed with cutadapt (v5.2) [42]. High-quality reads were aligned to the *E. coli* O157:H7 str. EDL933 (ASM73296v1) reference genome using bowtie2 (v2.5.4) with the very-sensitive alignment parameter [43]. The resulting SAM files were converted to BAM format and indexed with samtools (v1.21) [44]. Gene-level expression was quantified using featureCounts (v2.1.1) with default settings and additional flags to count only read pairs for which both reads aligned and to exclude chimeric pairs [45].

All downstream analyses were conducted in R. Count data from featureCounts were analyzed with DESeq2 (v1.50.2) for differential expression analysis [46]. Our design formula was ~group where the groups were factored as Aerobic Bile, Anaerobic Bile, Aerobic TSB, and Anaerobic TSB. Genes with counts of less than 10 across samples were filtered out. The remaining counts were normalized via the median ratio standard DESeq2 command and subjected to variance-stabilized transformation (VST). The comparisons conducted were (1) Aerobic Bile vs. Anaerobic Bile, (2) Aerobic TSB vs. Anaerobic TSB, (3) Aerobic Bile vs. Aerobic TSB, and (4) Anaerobic Bile vs. Anaerobic TSB, with an alpha value of 0.01 and a gene significance threshold of $\text{padj} < 0.01$ and absolute fold change ≥ 2 . Results were subjected to log-fold change shrinkage using the ashhr method (ashr v2.2.63) [47]. No DEGs of interest were detected in comparison (4) Anaerobic Bile vs. Anaerobic TSB.

Gene annotation data were parsed from the GCF_000732965.1_ASM73296v1_genomic.gff file for *E. coli* O157:H7 str. EDL933 using rtracklayer (v1.70.1) for gene ontology (GO) terms [48]. GO terms were binned into either Biological Process, Molecular Function, or Cellular Component using GO.db (v3.22.0) for GO full names and classification. Enrichment analysis of GO terms was performed using the clusterProfiler (v4.18.4) over-representation analysis (enricher) using the upregulated and downregulated DEGs within those groups against all GO-annotated genes [49]. Multiple testing correction was performed using the Benjamini–Hochberg false discovery rate (FDR) procedure and GO terms with an adjusted p (FDR) < 0.01 were considered significantly enriched (p -value cutoff < 0.01 , q -value cutoff < 0.01).

2.4. Biofilm Formation Assay

Biofilm formation was assessed using previously described methods with minor modifications [50,51]. Briefly, overnight cultures of EDL933 were prepared in TSB + MOPS under aerobic and anaerobic conditions as described above. The overnight culture was adjusted to an OD₆₀₀ of 0.05 (± 0.01) using sterile TSB + MOPS and the WPA Biowave CO8000 Cell Density Meter. Ten μL of the OD₆₀₀-adjusted overnight culture was inoculated into a 96-well culture plate pre-inoculated with 190 μL of TSB + MOPS or 190 μL of TSB + MOPS supplemented with 0.2% (w/v) dry, unfractionated bovine bile powder. Plates were incubated at 39 °C for 24 h, after which the supernatant was carefully removed so as to not disturb any exopolysaccharide (EPS) matrix present. The wells were gently washed with PBS, and plates were inverted and allowed to dry for 30 min. A 0.5% (w/v) crystal violet solution (Fisher Scientific, Waltham, MA, USA) was applied to the wells and incubated for 5 min at room temperature. The supernatant was carefully removed, and the wells were washed five times with $1\times$ PBS to remove any non-adherent bacteria. The plate was again inverted to dry in a dark location. Wells were inoculated with 200 μL of 95% ethanol and incubated for 30 min on an Orbit 300 orbital shaker (Labnet International, Edison, NJ, USA) at 4 °C to solubilize the crystal violet for visualization. The plate was placed on an Accuris SmartReader 96 MR9600-T instrument, and read at OD₆₃₀ to quantitate EPS. Twenty-four biological replicates were conducted for each set of conditions. Resultant OD₆₃₀ measurements were imported into R, and statistical analyses (wilcox.test) with a

Bonferroni correction, as well as plotting were conducted with the stats, ggsignif, ggpubr, ggprism, and ggplot2 packages.

2.5. Metabolic Characterization

EDL933 was grown overnight on TSA under the conditions described above. A well-isolated colony was selected with a sterile cotton swab and resuspended in IF-A Gen III inoculating fluid (Biolog, Hayward, CA, USA). The tube was vortexed, and the OD₆₀₀ was adjusted to 0.05 (+/−0.01). Ninety µL of cell suspension was added to a Gen III microbial identification test panel microplate (Biolog) which was pre-inoculated with PBS or PBS supplemented with 0.2% (*w/v*) dry, unfractionated bovine bile powder (pH 7.0). An Accuris SmartReader 96 MR9600-T instrument was used to incubate the plate at 39 °C for 24 h and record serial OD₆₃₀ measurements every 15 min. Plates were prepared under both aerobic and anaerobic conditions. For anaerobically prepared plates, all steps were conducted in a vinyl anaerobic chamber under anaerobic atmospheric conditions with pre-reduced media to limit the exposure of EDL933 to oxygen. Plates prepared and incubated under each set of conditions were repeated in triplicate, resulting in 3 biological replicates. Resultant OD₆₃₀ measurements were imported into R to plot growth curves and calculate mean area under the logistic curve (AUC) values for EDL933 grown under each set of conditions with GrowthCurveR. Statistical analysis (wilcox.test) with a Bonferroni correction and figure generation were conducted with the stats, ggsignif, ggpubr, ggprism, and ggplot2 packages in R.

3. Results

3.1. Growth Dynamics of EDL933 When Exposed to Bovine Bile and Oxygen

Growth performance of *E. coli* O157 strain EDL933 was examined in response to bovine bile powder concentrations of 0.02% (*w/v*), 0.2% (*w/v*), and 2.0% (*w/v*) under aerobic and anaerobic conditions, with 2.0% (*w/v*) and 0.2% (*w/v*) representing the upper and lower physiological range of bovine bile in the proximal small intestine, and 0.02% (*w/v*) approximating bovine bile concentrations in the distal small intestine [12,52]. EDL933 grown under anaerobic conditions produced significantly lower area under the curve (AUC) values for all media types tested than EDL933 grown under aerobic conditions ($p < 0.001$) (Figures 1A and S1). Significant differences in AUC were also observed for EDL933 exposed to different bovine bile powder concentrations within an atmospheric condition (Figure 1B). Under aerobic conditions, EDL933 grown in TSB supplemented with 0.02% (*w/v*) bovine bile powder produced a significantly higher AUC than EDL933 grown in TSB alone ($p < 0.05$) and EDL933 grown in TSB supplemented with 2.0% (*w/v*) bovine bile powder ($p < 0.01$). Under anaerobic conditions, EDL933 grown in TSB supplemented with 2.0% (*w/v*) bovine bile powder produced significantly lower AUC values than TSB-alone ($p < 0.001$), TSB supplemented with 0.02% (*w/v*) bovine bile powder ($p < 0.001$), and TSB supplemented with 0.2% (*w/v*) bovine bile powder ($p < 0.001$) (Figure 1B). The bovine bile powder concentration of 0.2% (*w/v*) was selected for additional analyses as it did not significantly impact EDL933 growth metrics and better represented the conditions in the proximal small intestine.

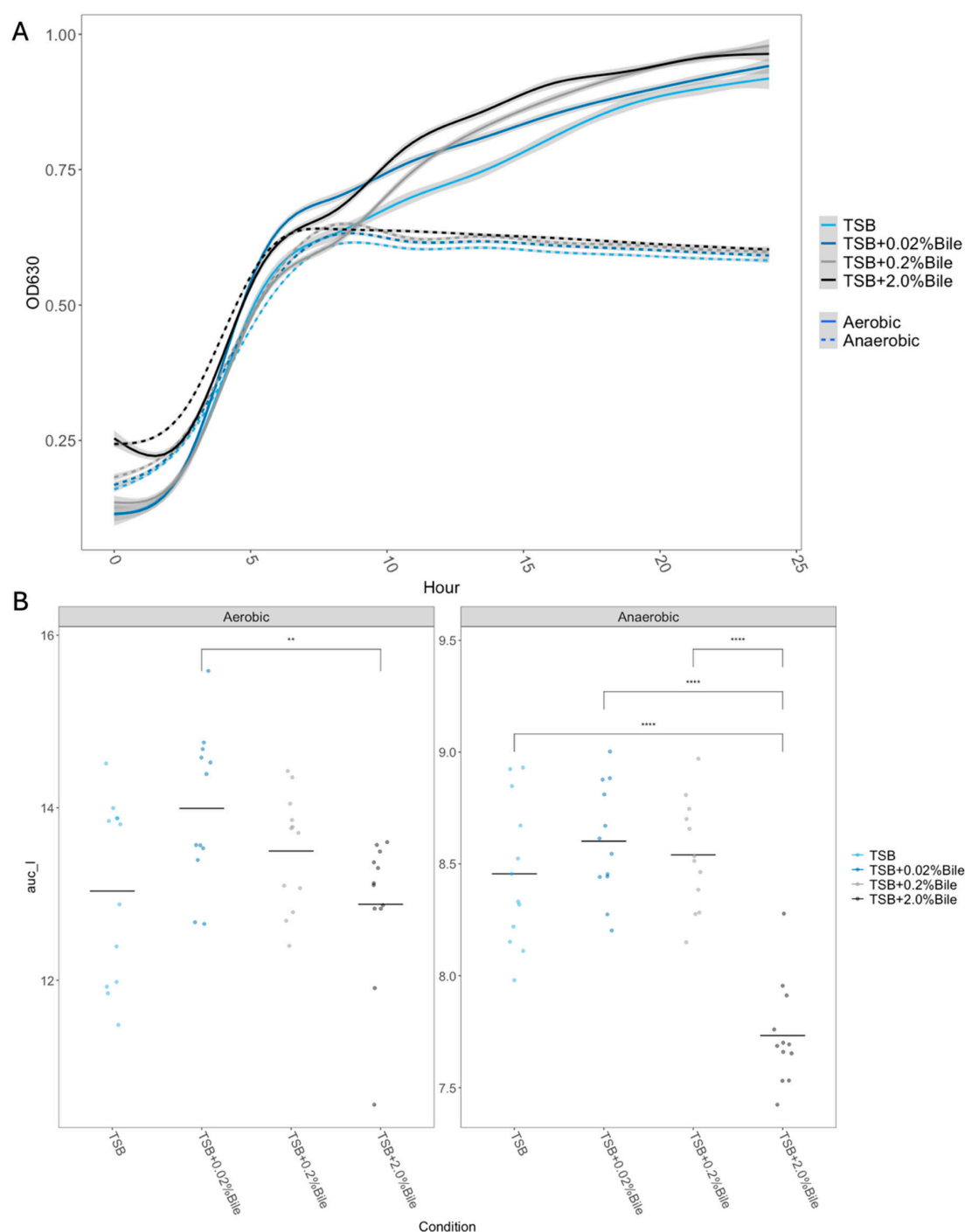


Figure 1. Growth of EDL933 exposed to different concentrations of bovine bile powder under aerobic and anaerobic conditions. **(A)** Growth curves and **(B)** Area under the curve (AUC) values of EDL933 exposed to different concentrations of bovine bile powder under aerobic and anaerobic conditions. EDL933 was grown in TSB alone or supplemented with 0.02% (*w/v*), 0.2% (*w/v*), or 2.0% (*w/v*) dry, unfractionated bovine bile under either aerobic or anaerobic conditions. Growth was measured at an optical density of 630 nm (OD_{630}). Replicate growth curves for each condition were condensed, and trend lines and confidence regions were determined using the `geom_smooth` command with default settings (method = LOESS) using `ggplot2` (v3.5.2) in R. AUC values were calculated from the growth curve data using `GrowthCurveR` (v0.3.1). Twelve biological replicates were conducted for each set of conditions. The solid black bar indicates the mean AUC for a given condition. Statistical comparisons (`wilcox.test`) with Bonferroni correction were made between conditions using the `stats` (v4.3.1), `ggsignif` (v0.6.4), and `ggpubr` (v0.6.1) packages in R. ** indicates $p < 0.01$; **** indicates $p < 0.0001$.

3.2. Impact of Bovine Bile Powder and Oxygen Presence on EDL933 Transcriptome

To assess the impact of bovine bile powder and atmospheric conditions on the EDL933 transcriptome, a global transcriptional analysis was conducted on EDL933 grown to mid-log phase in TSB or TSB supplemented with 0.2% (*w/v*) bovine bile powder under aerobic and anaerobic conditions (Table S1). Samples yielded an average of 15 million reads per sample, with >98.7% of reads aligning to the EDL933 genome. Two samples, S114 (1.4 M reads) and S90 (1.4 M reads), displayed lower sequencing depth than the other samples but did not substantially diminish the overall performance of the dataset. Gene ontology (GO) terms were assigned to differentially expressed genes (DEGs), identifying multiple genes and pathways that were upregulated and downregulated between the different conditions (Figures 2 and S2; Tables S2–S5).

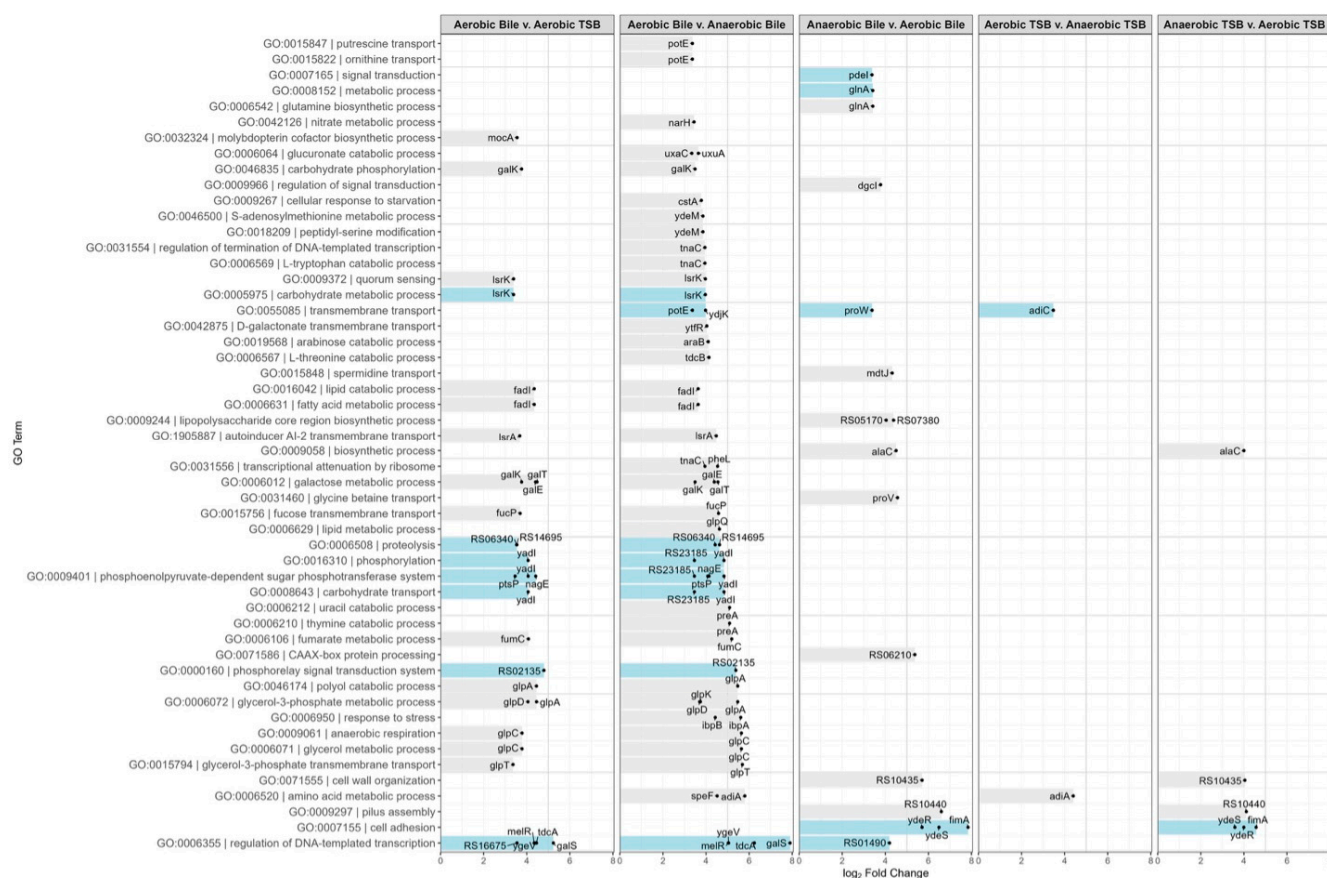


Figure 2. Transcriptomic response (absolute fold change ≥ 10) of EDL933 incubated aerobically or anaerobically with and without exposure to 0.2% (*w/v*) bovine bile powder. Bars represent the max absolute fold change ≥ 2 per Biological Process GO term, limited to DEGs with an absolute fold change ≥ 10 . Individual DEGs are represented as dots labeled by gene symbol. Enriched GO terms are denoted by light-blue filled bars.

Under aerobic conditions, EDL933 grown in TSB supplemented with 0.2% (*w/v*) bovine bile displayed 24 DEGs (24 up, 0 down; absolute fold change ≥ 10) across multiple physiological pathways compared to EDL933 grown in TSB alone (Figures 2 and S2; Table S3). Bile powder exposure increased the expression of numerous pathways, including those for PEP-dependent sugar phosphotransferase systems (*yadI*, *ptsP*, *nagE*) and regulation of DNA-templated transcription (*RS16675*, *ygeV*, *meIR*, *tcdA*, and *galS*), among others. Other DEGs highly expressed (absolute fold change ≥ 10) when exposed to both bile and oxygen included several genes involved in metabolic processes for galactose (*galk*, *gelE*, and *galT*) and fucose (*RS14695* and *fucP*), glycerol (*glpA*, *glpC*, *glpD*, and *glpT*), and fatty

acids (*fadL*) (Figure 2; Table S5). Additional trends were observed when analyzing all DEGs displaying an absolute fold change ≥ 2 , with EDL933 grown in TSB supplemented with bovine bile either increasing or decreasing the expression of genes involved in cell adhesion, DNA repair, and peptidoglycan biosynthetic processes, among numerous other pathways, compared to EDL933 grown in TSB alone (Figure S2; Table S5).

To assess the impact of oxygen presence on the EDL933 transcriptome, growth in TSB under ambient air and anaerobic conditions was compared. Under aerobic conditions, EDL933 displayed multiple highly expressed DEGs (2 up, 6 down; absolute fold change ≥ 10) compared to EDL933 grown under anaerobic conditions (Figure 2; Table S3). The two DEGs (absolute fold change ≥ 10) that were increased under aerobic conditions were involved in transmembrane transport (*adiC*) and amino acid metabolic processes (*adiA*) (Figure 2; Table S5). Other DEGs displaying an absolute fold change ≥ 2 under aerobic conditions compared to anaerobic conditions included genes for various stages of the TCA cycle, the electron transport chain, and DNA repair (Figure S2; Table S5). EDL933 grown under anaerobic conditions displayed several DEGs (absolute fold change ≥ 10) with higher expression levels than under aerobic conditions, including those involved in cell adhesion (*ydeR*, *ydeS*, and *fimA*), cell wall organization (RS10435), and pilus assembly (RS10440), with additional DEGs (absolute fold change ≥ 2) belonging to several other pathways, including carbohydrate metabolic processes and transmembrane transport (Figures 2 and S2; Table S5).

To assess the impact of oxygen presence on the EDL933 transcriptome when bovine bile powder was also present, EDL933 grown under aerobic conditions with bile exposure saw differential expression of numerous genes (42 up, 16 down; absolute fold change ≥ 10) across multiple physiological pathways compared to EDL933 grown in TSB+bile under anaerobic conditions (Figure 2; Table S3). Numerous DEGs (absolute fold change ≥ 10) were increased in EDL933 exposed to bile under aerobic conditions compared to bile exposure under anaerobic conditions. These DEGs were involved in transmembrane transport (*ydjK* and *potE*), quorum sensing (*lsrA* and *lsrK*), and numerous metabolic pathways, including those for nitrate (*narH*), glucuronate (*uxaA* and *uxaC*), galactose (*galE*, *galK*, and *galT*), galactonate (*ytfR*), arabinose (*araB*), fucose (*fucP*), amino acids (*adiA*, *speF*, and *tcdB*), and lipids and fatty acids (*fadI* and *glpQ*), among others. Other DEGs (absolute fold change ≥ 2) that were enriched in aerobically grown, bile-exposed EDL933 included several involved in type VI secretion system protein secretion, cell adhesion, and DNA recombination, among others (Figure S2; Table S5). When exposed to bile under anaerobic conditions, EDL933 increased the expression of genes (absolute fold change ≥ 10) involved in signal transduction (*pdeI* and *dcgL*), cell adhesion (*ydeR*, *ydeS*, and *fimA*), spermidine transport (*mdtJ*), and transmembrane transport (*proV* and *proW*), among others, as compared to bile-exposed EDL933 under aerobic conditions (Figures 2 and S2; Table S5).

There were no highly expressed DEGs (absolute fold change ≥ 10) present when comparing the transcriptome of EDL933 grown under anaerobic conditions in TSB and EDL933 grown under anaerobic conditions in TSB supplemented with bovine bile powder. There were 11 DEGs present at the absolute fold change ≥ 2 level, all of which were increased in anaerobically grown EDL933 exposed to bovine bile powder compared to TSB alone. These DEGs included multiple genes involved in translation (*ahpC*, *rpsR*, *rplR*, and *rpsQ*), and *ahpF*, a gene involved in the response to reactive oxygen species (Figure S2; Table S5). A full list of DEGs with an absolute fold change ≥ 2 and associated GO terms can be found in Supplementary Table S5.

3.3. EDL933 Biofilm Formation Under Different Conditions

A biofilm assay was used to assess whether the increased expression of genes involved in adherence and biofilm formation was associated with increased biofilm formation *in vitro* (Figure 3). There were no significant differences in biofilm formation ($p > 0.05$) when comparing EDL933 grown in TSB with and without bovine bile powder supplementation under either aerobic or anaerobic conditions. Biofilm formation between EDL933 grown in the same media type, either TSB alone or TSB supplemented with 0.2% (*w/v*) bovine bile powder, under different atmospheric conditions displayed significant differences, with the aerobic cultures forming significantly more biofilm than the anaerobic cultures for EDL933 grown in TSB ($p < 0.001$) and TSB supplemented with bile powder ($p < 0.001$) (Figure 3).

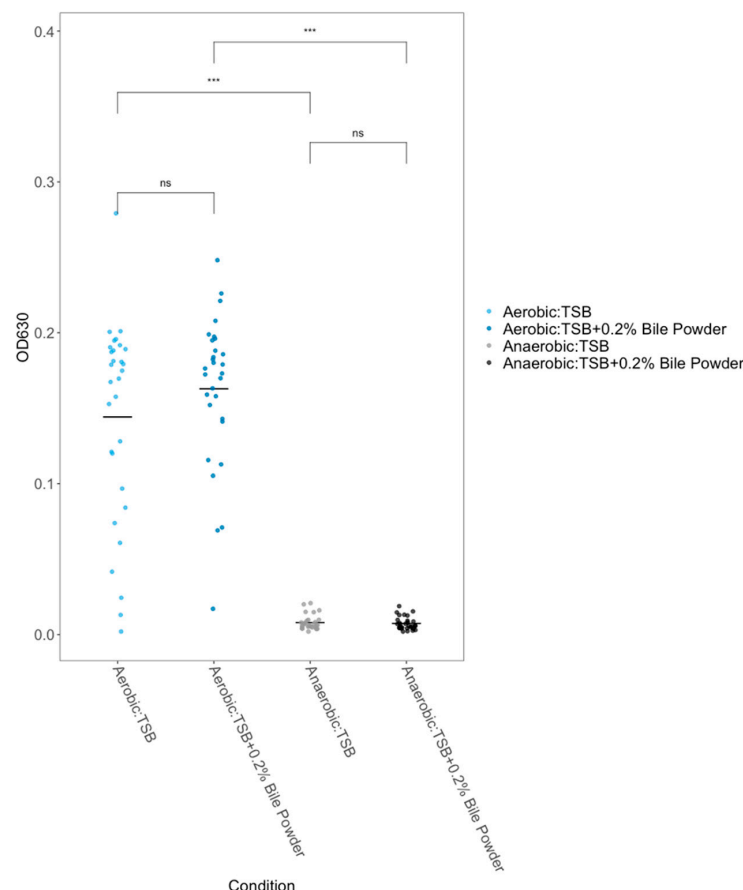


Figure 3. Biofilm formation of EDL933 grown aerobically or anaerobically with and without exposure to 0.2% (*w/v*) bovine bile powder. Biofilm biomass was measured at an optical density of 630 nm (OD_{630}). Twenty-four biological replicates were conducted for each set of conditions. The solid black bar indicates the mean OD_{630} for a given condition. Statistical comparisons (wilcox.test) with Bonferroni correction were made between conditions using the stats (v4.3.1), ggsignif (v0.6.4), and ggpubr (v0.6.1) packages in R. *** indicates $p < 0.001$; “ns” indicates $p > 0.05$.

3.4. EDL933 Metabolic Characterization Under Different Conditions

The transcriptomic data showed that genes from numerous metabolism-related DEGs as either upregulated or downregulated in EDL933 when comparing bovine bile exposure and atmospheric conditions. To assess whether these differences in gene expression were reflected in the metabolic phenotype, Biolog GenIII microplates were used to profile nutrient utilization patterns of EDL933 under the conditions tested above (Figure 4; Tables S6 and S7). Comparisons of the positive and negative control wells on the GenIII microplates confirmed that the incorporation of bovine bile powder did not significantly impact absorbance readings or growth profiles of EDL933 (Figures S3 and S4). Nutrient utilization profiles

varied across media types and atmospheric conditions (Figure 4). The area under the curve (AUC) values from the growth curves generated for each well were used as a proxy to assess growth performance. There were significant differences in AUC values observed for 14 different nutrients between EDL933 grown under different conditions (Figure 4). Most of the significant differences were observed between EDL933 grown under aerobic and anaerobic conditions without the addition of 0.2% (*w/v*) bovine bile powder to the media. D-fucose ($p < 0.05$), gentiobiose ($p < 0.05$), and mucic acid ($p < 0.001$), among several other nutrients, produced significantly greater AUC values for EDL933 incubated under anaerobic conditions than under aerobic conditions (Table S7). EDL933 grown under aerobic conditions produced significantly lower AUC values in the presence of 3-methyl glucose than both EDL933 grown under aerobic conditions supplemented with 0.2% (*w/v*) bovine bile powder ($p < 0.05$) and EDL933 grown under anaerobic conditions ($p < 0.01$). In the presence of bromo-succinic acid, EDL933 supplemented with 0.2% (*w/v*) bovine bile powder produced significantly greater AUC values ($p < 0.05$) under anaerobic conditions than under aerobic conditions (Tables S6 and S7).

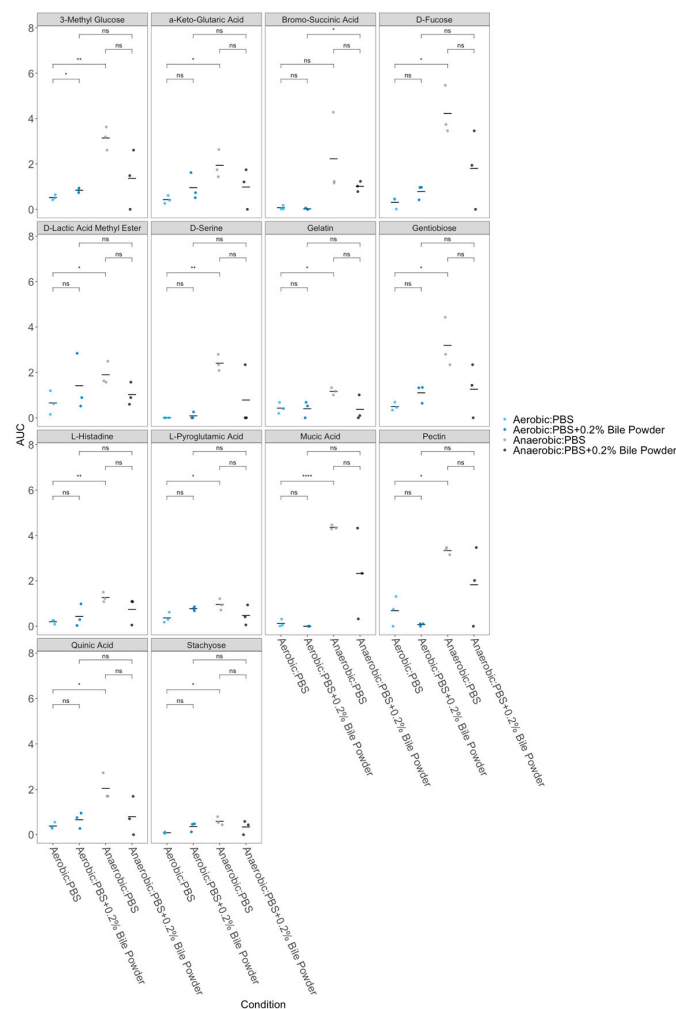


Figure 4. Nutrients on the Biolog GenIII microplates producing significantly different AUC values for EDL933 grown under aerobic and anaerobic atmosphere with and without exposure to 0.2% (*w/v*) bovine bile powder. Mean AUC values were determined from OD₆₃₀ growth curve data through analysis with GrowthCurveR (v0.3.1). Three biological replicates were conducted for each set of conditions. The solid black bar indicates the mean AUC for a given condition. Statistical comparisons (wilcox.test) with Bonferroni correction were made between conditions using the stats (v4.3.1), ggsignif (v0.6.4), and ggpubr (v0.6.1) packages in R. * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.0001$; “ns” indicates $p > 0.05$.

4. Discussion

Escherichia coli O157 represents an important foodborne pathogen. Understanding the interplay of the different physiochemical signals to which O157 is exposed to within the bovine gastrointestinal tract (GIT) and how these signals, both individually and in combination, impact the growth and transcriptomic responses of EDL933, a well-studied strain of *E. coli* O157, provides important information on the factors driving colonization of the bovine hindgut. Delineating the roles of these different GIT signals will aid in identifying intervention points for the development of preharvest controls to reduce O157 colonization, shedding, or downstream product contamination. Here, we investigated the impact of bovine bile and oxygen presence, two physiochemical cues in the bovine hindgut, on the growth, transcriptome, and various phenotypic traits of the O157 strain EDL933.

Oxygen and bile concentrations vary between intestinal compartments as EDL933 transits the cattle hindgut. Oxygen levels vary both radially and longitudinally within the GIT, with higher concentrations present at the beginning of the small intestine (duodenum) and nearer the intestinal epithelial cell surface, and lower concentrations found in the large intestine and in the intestinal lumen, though the rectoanal junction (RAJ), a primary site of O157 colonization in cattle, may present elevated oxygen levels due to mucus layer thinning, allowing increased oxygen diffusion at this site [28,29,34,53–55]. Bile is abundant in the small intestine, where it is secreted into the duodenum, but is modified by the gut microbiota as the intestinal contents move from the small intestine to the large intestine, with the majority of the biliary components being actively reabsorbed prior to entering the colon [12,13]. Together, bile and oxygen represent two conserved physiochemical stimuli in the bovine hindgut. Both stimuli vary within and between segments of the GIT, and the combination of the two could be utilized by EDL933, and potentially other strains of O157, to determine location within the bovine hindgut, potentially controlling virulence, colonization, and metabolic responses.

Significant differences in overall EDL933 growth were observed under aerobic and anaerobic conditions, with EDL933 growing significantly better under aerobic conditions regardless of bile concentration. Reduced growth of O157 under anaerobic conditions has been observed previously, likely stemming from limitations in energy generation between anaerobic fermentation and aerobic respiration [55–58]. When grown aerobically, lower concentrations of bovine bile powder either minimally impacted, or significantly improved, EDL933 growth. This is largely in line with previous reports of O157 growth characteristics in the presence of commercial bile acid blends [20,22,59]. Bile concentrations at the upper physiological limit, 2.0% (*w/v*), were detrimental to EDL933 growth. High bile and bile acid concentrations are associated with membrane-disruptive and bactericidal effects, resulting in growth inhibition in *E. coli* [60–62]. These results confirm that bile concentrations at the high end of the physiological range impede overall EDL933 growth, while lower concentrations have minimal or even positive impacts on growth, depending on the surrounding atmosphere.

Prior studies suggest that oxygen is required for *E. coli* to successfully colonize the GIT [63]. Here, EDL933 displayed significant shifts in several transcriptional pathways in response to an aerobic atmosphere, with the largest increases in expression associated with *adiA* and *adiC*, two genes involved in arginine metabolism and transport, a finding in line with previous transcriptomic studies involving O157 and non-O157 strains of *E. coli* [64,65]. Increased expression of the agmatine transporter encoded by *adiC* improves adherence to bovine rectoanal junction (RAJ) cells, the primary colonization site of EDL933 and other O157 strains [66]. The agmatine transporter encoded by *adiC* has also been implicated in *E. coli* biofilm formation, which may partially explain the significantly increased biofilm formation observed under aerobic conditions compared to anaerobic conditions [67]. However,

the role of *adiC* in EDL933 biofilm formation remains speculative, and additional studies must be conducted to determine what role, if any, *adiC* plays in this process. While the mechanisms of biofilm formation in EDL933 remain somewhat unclear, increased biofilm formation may enhance EDL933 colonization both in the GIT and the external environment, aiding its survival and dissemination to new hosts [68–70].

In unsupplemented TSB under anaerobic conditions, EDL933 significantly increased the expression of several genes involved in fimbriae production, namely the *ydeR*, *ydeS*, and *fimA* genes. The *ydeR* and *ydeS* genes are two putative fimbrial proteins involved in colonization of the GIT by other *E. coli* [71,72]. The *fimA* gene is also involved in the early phases of intestinal colonization by O157 and other enteric pathogens [73–75]. Anaerobiosis has been shown to increase fimbrial expression in O157, as well as in other *E. coli* and even *Salmonella*, potentially in response to stressors encountered as these organisms transit the GIT [76–79]. Smaller increases in transcription were observed under anaerobic conditions for genes involved in glycine betaine transport. Glycine betaine functions as an osmoprotectant for *E. coli*, an important defense mechanism against the osmotic stresses encountered in the intestinal lumen during transit [69,70]. Together, these transcriptional changes suggest that EDL933 could use a lack of oxygen presence as a physiochemical indicator to increase fimbrial production in the intestinal lumen, where it begins to cope with osmotic stress and other physiological hardships.

The presence or absence of oxygen impacted EDL933 metabolism, both transcriptomically and phenotypically. Under aerobic conditions, EDL933 increased expression of genes and pathways involved in aerobic respiration, including those for various components of the TCA cycle and electron transport chain, consistent with known *E. coli* physiology [80,81]. In anaerobic TSB, EDL933 increased expression of genes involved in fucose metabolism and peptide transport, among a variety of other metabolic pathways. In vitro nutrient utilization profiles broadly reflected transcriptional trends, with EDL933 increasing overall growth in the presence of fucose and various amino acids under anaerobic conditions. EDL933 also significantly increased growth in media containing mucic acid and fucose, two byproducts of mucus layer degradation, under anaerobic conditions. This is consistent with previous reports of O157 nutritional requirements in the GIT [82]. Interestingly, several uncommon nutrient sources enhanced EDL933 growth under anaerobic conditions in vitro, including stachyose and alpha-keto-glutaric acid. This may represent a metabolic shift to focus on alternative, more readily available carbon and nitrogen sources, highlighting the need for flexibility in EDL933 metabolism to compete in the anaerobic and microbially dense intestinal lumen [81,82]. Together, the transcriptomic and nutrient utilization data suggest that EDL933 shifts to alternative nutrient sources within the anoxic lumen of the bovine hindgut, focusing on growth through the acquisition and utilization of the readily available nutrients not commonly utilized by the native gut microbiota.

Bile and bile acids impact the colonization success of other foodborne pathogens, suggesting that these physiochemical signals would impact EDL933, as well as other O157 strains, during the colonization process [52,83,84]. Under aerobic conditions, the presence of bile increased EDL933 expression of genes involved in numerous genetic pathways. Several genes involved in quorum sensing, including *lsrA*, *lsrK*, and *ygeV*, were upregulated in aerobically grown EDL933 exposed to bile compared to EDL933 grown in TSB alone. Quorum sensing in the cattle gastrointestinal tract is vital for O157, playing a critical role in its stable colonization of the hindgut [85–88]. Upregulation of EDL933 quorum sensing pathways upon bovine bile exposure suggests that bile may serve as a signal to EDL933 that it has entered the microbially rich hindgut where it must begin interacting with, and competing against, the resident microbiota. EDL933 increased expression of *yadL* in response to bovine bile under aerobic atmosphere as well. *yadL*

encodes a fimbrial adhesin that is important for colonization of the cattle GIT, indicating bovine bile may act as a signal for EDL933 to begin the hindgut colonization process in cattle [89,90]. Numerous DEGs belonging to a variety of metabolic pathways saw increased expression when EDL933 was exposed to bovine bile under aerobic conditions, including multiple genes involved in metabolic pathways for galactose, fucose, N-acetylglucosamine, galactonate, and glucuronate, among others, when compared to EDL933 grown aerobically in TSB alone and EDL933 exposed to bile under anaerobic conditions. Increased utilization of the above mucin sugars, such as fucose and N-acetylglucosamine, which are released during the degradation of intestinal mucus layer by the native microbiota, is critical for O157 colonization and survival in the bovine GIT [82,91–94]. These results indicate EDL933 upregulates metabolic pathways for mucus-derived sugars when oxygen and bovine bile are both present, likely reflecting the conditions present in the duodenum, jejunum, and proximal ileum.

Interestingly, there were few significantly differentially expressed genes when comparing EDL933 grown under anaerobic conditions with and without bovine bile, none of which were highly expressed (absolute fold change ≥ 10). This may suggest a substantial metabolic shift is required for anaerobic growth, which might supersede large-scale, bile-specific transcriptional responses. However, this interpretation may also be impacted by our sample size, growth-phase effects, or other experimental variables. Even with these caveats in mind, this observation is consistent with previous studies of *E. coli* metabolism [95–97]. These results may indicate anaerobic atmospheres represent an overwhelming signal for EDL933, largely masking responses to other stimuli, such as bovine bile. However, smaller changes in gene expression and metabolic phenotype under anaerobic conditions with and without bovine bile present suggest that metabolic and transcriptional nuances exist that warrant further study.

Previous reports on O157 and other pathogenic *E. coli* suggest a general decrease in the expression and activity of virulence and colonization factors in response to bile salts [20,22,98,99]. We did not identify significant shifts in the expression of the LEE or other colonization factors when exposing EDL933 to bovine bile powder under either atmosphere. This likely reflects differences in the composition of the bile salts within the dried bovine bile supplement used in our study and the bile salts mixes utilized in prior studies, which are primarily composed of the bile salts deoxycholic acid (DCA) and cholic acid (CA) [20,22,98,99]. CA and DCA are microbially modified bile acids which are more prevalent in the distal hindgut, where bile acid concentrations are generally lower [12,15]. Whole bovine bile preparations such as the one used in this work have bile acid compositions more reflective of the duodenum, jejunum, and proximal ileum, with conjugated bile acids, such as TCA and GCA, making up the vast majority of bile acids while unconjugated bile acids, such as CA and DCA, represent <1% of the bile acids present [23]. The conjugation status of bile acids significantly impacts the effects on *E. coli* and other enteric pathogens, making it important to select bile acids reflective of the specific GIT compartments being queried [60,100,101]. Additionally, whole bile preparations contain phospholipids, cholesterol, bile pigments, and other biliary components that are present in vivo, making bile powder a more physiologically relevant proxy for the small intestinal environment in cattle [11].

Taken together, these results suggest a speculative model of predicted EDL933 behavior in the cattle hindgut. Upon entering the duodenum and along the jejunal and ileal mucosa, oxygen levels are relatively high and bile is abundant. Under these in vitro conditions, EDL933 upregulates pathways involved in the metabolism of mucin-derived monomers. This may be consistent with early colonization along the small intestine mucosa, an area prior studies have identified as a key colonization and carriage site of O157

in cattle [65,82,102,103]. Outside of the highly oxygenated duodenum, the lumen of the jejunum and ileum is mainly anaerobic, with bile concentrations remaining high. When exposed to bovine bile powder under anaerobic atmosphere in vitro, EDL933 seems to shift its metabolic profile and upregulates the expression of fimbriae, potentially in response to osmotic or other stressors present in the intestinal lumen [104]. Upon reaching the large intestine, most bile acids and biliary components have been reabsorbed or microbially transformed, which may diminish the direct impacts of bile on EDL933 [14,105,106]. As EDL933 transits the large intestine, the lumen is anoxic and largely devoid of biliary components. When simulating these conditions in vitro, EDL933 increased transcription of fimbriae genes and nutrient utilization of metabolic byproducts or other nutrients that may be readily available as they are not commonly utilized by the hindgut microbiota [12]. However, the distal hindgut is not completely devoid of oxygen, with both the crypts of the large intestine and the RAJ exhibiting localized oxygen gradients [107]. When exposed to oxygen in vitro, EDL933 increased *adiC* expression and biofilm formation, which might help facilitate colonization of the bovine intestinal epithelia in the terminal hindgut [66]. The in vitro observations here may partially explain the sporadic O157 colonization within the large intestine and the tropism that EDL933 displays for the RAJ [17,28,98,103,107–109]. When shed from the cattle host, the oxygen-driven increases in biofilm formation may also enhance environmental survival, facilitating transmission between hosts as well as contamination of meat, dairy, and produce [17,98,108,110,111]. However, the model above remains a working hypothesis, and additional testing, especially in vitro tissue adherence assays, intestinal organ culture, and in vivo studies, is required to more fully characterize the role that bovine bile and oxygen presence have on EDL933 colonization dynamics in cattle. Additional strains of O157 will also need to be tested, both in vitro and in vivo, to confirm the generalizability of the results obtained in this work and the robustness of the speculative model proposed here.

This work is not without limitations. While bovine bile powder, such as the preparation used in this work, is compositionally similar to the bile acid pool observed in the bovine small intestine in vivo, additional work should be conducted with liquid bovine bile to confirm the transcriptional and phenotypic trends observed here. Additionally, the batch-specific bile acid composition and osmolarity were not determined for the bovine bile powder utilized in this work. The relatively small sample size likely reduced the statistical power and limited the complexity of models available for transcriptomic analysis. Differences in growth phase and exposure duration may have confounded the transcriptomic responses observed here. Only a single strain of O157, EDL933, was used, which limits the generalizability of the results. While numerous DEGs were identified as being impacted by bovine bile powder and/or oxygen presence, additional RT-qPCR and functional assays, including direct adherence assays, are required to more fully discern the role of these DEGs in the EDL933 response to bovine bile and oxygen, and how these DEGs may impact colonization dynamics. The results described here were solely based on in vitro work, and further in vivo colonization studies are required to more fully capture the physiological complexity of the O157 colonization process in cattle. Future work incorporating larger numbers of biological replicates, additional O157 strains, and functional assays under a greater variety of physiologically relevant conditions would improve statistical power and help capture both inter- and intrastrain variations in the physiological response of O157 to bovine bile and oxygen.

Ultimately, EDL933 colonization of the cattle GIT is a highly complex process involving multiple physiochemical signals. The work presented here suggests that both oxygen availability and bovine bile presence are two important signals that might impact the EDL933 transcriptional response and may affect adherence and metabolism, both facilitat-

ing successful colonization and persistence in the cattle gut. Further studies into additional chemical signals found within the cattle gastrointestinal tract, both individually and in combination, would lend additional insight into the factors that enhance or inhibit EDL933 colonization and persistence in cattle.

5. Conclusions

Here, we assessed the transcriptomic and phenotypic responses of *E. coli* O157 strain EDL933 to the presence of oxygen and whole bovine bile, two major signals present in the gastrointestinal tract of cattle. Under aerobic conditions, EDL933 increased the expression of the *adiC* gene, an adherence and biofilm formation factor, and increased biofilm formation in vitro. Anaerobic conditions induced significant shifts in numerous metabolic pathways, enhancing the ability of EDL933 to utilize alternative and more readily available nutrient sources that can be found in the cattle gut. Bovine bile impacted EDL933 expression of pathways responsible for quorum sensing and the utilization of mucus-derived sugars. Together, both bovine bile and oxygen presence impacted the transcriptome and phenotypic characteristics of EDL933, suggesting that both may serve as signals for EDL933 during colonization of the cattle gastrointestinal tract.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/bacteria5030044/s1>, Figure S1: AUC values for EDL933 grown aerobically and anaerobically under similar media conditions; Figure S2: Transcriptomic Response (absolute fold change ≥ 2) of EDL933 Grown in TSB with and without 0.2% (*w/v*) dry, unfractionated bovine bile under aerobic conditions; Figure S3: Difference in optical density (OD₆₃₀) in the negative control wells of Biolog GenIII microplates under aerobic and anaerobic conditions with and without 0.2% (*w/v*) dry, unfractionated bovine bile; Figure S4: AUC values for the positive control wells of the Biolog GenIII microplates under aerobic and anaerobic conditions with and without 0.2% (*w/v*) dry, unfractionated bovine bile; Figure S5: Mean area under the curve (AUC) values for EDL933 grown under aerobic and anaerobic atmosphere with and without exposure to 0.2% (*w/v*) bovine bile powder for nutrients on the Biolog GenIII microplate; Table S1: Time to mid-log (T_{mid}) for EDL933 grown in TSB under aerobic and anaerobic conditions with and without 0.2% (*w/v*) dry, unfractionated bovine bile; Table S2: Transcriptomic Sequencing Statistics: FastQC, bowtie2, and featureCounts Summary; Table S3: Total number of upregulated and downregulated DEGs (absolute fold change ≥ 10); Table S4: Total number of upregulated and downregulated DEGs (absolute fold change ≥ 2); Table S5: Differentially expressed genes (absolute fold change ≥ 2), Gene Ontology, and Enrichment Results for EDL933 grown in TSB under aerobic and anaerobic conditions with and without 0.2% (*w/v*) dry, unfractionated bovine bile; Table S6: Nutrient utilization profiles of EDL933 from Biolog GenIII microplates grown under different conditions; Table S7: Statistical comparisons of AUC values of EDL933 growth in Biolog GenIII microplates under different conditions.

Author Contributions: Conceptualization, J.J.M.; Methodology, J.J.M. and R.O.; Software, J.J.M. and R.O.; Formal Analysis, J.J.M. and R.O.; Investigation, J.J.M. and R.O.; Resources, J.J.M.; Data Curation, J.J.M. and R.O.; Writing—Original Draft Preparation, J.J.M.; Writing—Review and Editing, J.J.M. and R.O.; Visualization, J.J.M. and R.O.; Supervision, J.J.M.; Project Administration, J.J.M.; Funding Acquisition, J.J.M. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the USDA under USDA-ARS CRIS project 5030-32000-225-00D. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw transcriptomic sequencing data generated in this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive

(SRA) database under accession number PRJNA1437027. Additional materials associated with this paper, including the code for the transcriptomic analysis, the sample metadata table, count matrix, and DeSeq2 comparison tables, have been made publicly available in the AgData Commons repository (via FigShare) under the following link: <https://figshare.com/s/0c56910f0ad59b8f64df> (Accessed on 26 July 2026).

Acknowledgments: The authors would like to thank Morgan Smith for excellent technical assistance. The authors also thank Nathan Peroutka-Bigus and Crystal Loving for assistance with coordinating the transcriptomic sequencing.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

GIT	Gastrointestinal tract
O157	<i>Escherichia coli</i> O157:H7
EDL933	<i>Escherichia coli</i> O157:H7 strain EDL933
RAJ	Rectoanal junction
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
DEGs	Differentially expressed genes
GO	Gene ontology
EPS	Exopolysaccharide
AUC	Area under the logistic curve

References

1. Alhadlaq, M.A.; Aljurayyad, O.I.; Almansour, A.; Al-Akeel, S.I.; Alzahrani, K.O.; Alsaman, S.A.; Yahya, R.; Al-Hindi, R.R.; Hakami, M.A.; Alshahrani, S.D.; et al. Overview of pathogenic *Escherichia coli*, with a focus on Shiga toxin-producing serotypes, global outbreaks (1982–2024) and food safety criteria. *Gut Pathog.* **2024**, *16*, 57. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Scallan, E.; Hoekstra, R.M.; Angulo, F.J.; Tauxe, R.V.; Widdowson, M.-A.; Roy, S.L.; Jones, J.L.; Griffin, P.M. Foodborne Illness Acquired in the United States—Major Pathogens. *Emerg. Infect. Dis. J.* **2011**, *17*, 7. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Kolodziejek, A.M.; Minnich, S.A.; Hovde, C.J. *Escherichia coli* O157:H7 virulence factors and the ruminant reservoir. *Curr. Opin. Infect. Dis.* **2022**, *35*, 205–214. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Sapountzis, P.; Segura, A.; Desvaux, M.; Forano, E. An Overview of the Elusive Passenger in the Gastrointestinal Tract of Cattle: The Shiga Toxin Producing *Escherichia coli*. *Microorganisms* **2020**, *8*, 877. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Tack, D.M.; Kisselburgh, H.M.; Richardson, L.C.; Geissler, A.; Griffin, P.M.; Payne, D.C.; Gleason, B.L. Shiga Toxin-Producing *Escherichia coli* Outbreaks in the United States, 2010–2017. *Microorganisms* **2021**, *9*, 1529. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Beauvais, W.; Gart, E.V.; Bean, M.; Blanco, A.; Wilsey, J.; McWhinney, K.; Bryan, L.; Krath, M.; Yang, C.-Y.; Manriquez Alvarez, D.; et al. The prevalence of *Escherichia coli* O157:H7 fecal shedding in feedlot pens is affected by the water-to-cattle ratio: A randomized controlled trial. *PLoS ONE* **2018**, *13*, e0192149. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Schroeder, T.; Mark, D. How can the beef industry recapture lost consumer demand. *J. Anim. Sci.* **2000**, *77*, 1–13. [\[CrossRef\]](#)
8. Koohmaraie, M.; Arthur, T.M.; Bosilevac, J.M.; Brichta-Harhay, D.M.; Kalchayanand, N.; Shackelford, S.D.; Wheeler, T.L. Interventions to reduce/eliminate *Escherichia coli* O157:H7 in ground beef. *Meat Sci.* **2007**, *77*, 90–96. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Lange, M.E.; Uwiera, R.R.E.; Inglis, G.D. Enteric *Escherichia coli* O157:H7 in Cattle, and the Use of Mice as a Model to Elucidate Key Aspects of the Host-Pathogen-Microbiota Interaction: A Review. *Front. Vet. Sci.* **2022**, *9*, 937866. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Kudva, I.T.; Stanton, T.B.; Lippolis, J.D. The *Escherichia coli* O157:H7 bovine rumen fluid proteome reflects adaptive bacterial responses. *BMC Microbiol.* **2014**, *14*, 48. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Russell, D.W. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev. Biochem.* **2003**, *72*, 137–174. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Lin, L.; Lai, Z.; Yang, H.; Zhang, J.; Qi, W.; Xie, F.; Mao, S. Genome-centric investigation of bile acid metabolizing microbiota of dairy cows and associated diet-induced functional implications. *ISME J.* **2023**, *17*, 172–184. [\[PubMed\]](#)
13. Hundt, M.; Basit, H.; John, S. *Physiology, Bile Secretion*; StatPearls Publishing: Treasure Island, FL, USA, 2025.

14. Ridlon, J.M.; Harris, S.C.; Bhowmik, S.; Kang, D.-J.; Hylemon, P.B. Consequences of bile salt biotransformations by intestinal bacteria. *Gut Microbes* **2016**, *7*, 22–39. [[CrossRef](#)] [[PubMed](#)]
15. Ridlon, J.M.; Kang, D.J.; Hylemon, P.B. Bile salt biotransformations by human intestinal bacteria. *J. Lipid Res.* **2006**, *47*, 241–259. [[CrossRef](#)] [[PubMed](#)]
16. Edwinston, A.L.; Yang, L.; Peters, S.; Hanning, N.; Jeraldo, P.; Jagtap, P.; Simpson, J.B.; Yang, T.-Y.; Kumar, P.; Mehta, S.; et al. Gut microbial β -glucuronidases regulate host luminal proteases and are depleted in irritable bowel syndrome. *Nat. Microbiol.* **2022**, *7*, 680–694. [[CrossRef](#)] [[PubMed](#)]
17. Doranga, S.; Conway, T. OmpC-Dependent Bile Tolerance Contributes to *E. coli* Colonization of the Mammalian Intestine. *Microbiol. Spectr.* **2023**, *11*, e0524122. [[CrossRef](#)] [[PubMed](#)]
18. Jia, H.; Dong, N. Effects of bile acid metabolism on intestinal health of livestock and poultry. *J. Anim. Physiol. Anim. Nutr.* **2024**, *108*, 1258–1269. [[CrossRef](#)] [[PubMed](#)]
19. Kipp, Z.A.; Pauss, S.N.; Martinez, G.J.; Hinds, T.D.; Lee, W.-H. Bilirubin Hepatic and Intestinal Transport and Catabolism: Physiology, Pathophysiology, and Benefits. *Antioxidants* **2025**, *14*, 1326. [[CrossRef](#)] [[PubMed](#)]
20. Hamner, S.; McInerney, K.; Williamson, K.; Franklin, M.J.; Ford, T.E. Bile salts affect expression of *Escherichia coli* O157: H7 genes for virulence and iron acquisition, and promote growth under iron limiting conditions. *PLoS ONE* **2013**, *8*, e74647. [[CrossRef](#)] [[PubMed](#)]
21. Gadishaw-Lue, C.; Banaag, A.; Birstonas, S.; Francis, A.-S.; Barnett Foster, D. Bile salts differentially enhance resistance of enterohemorrhagic *Escherichia coli* O157: H7 to host defense peptides. *Infect. Immun.* **2021**, *89*, e00719-20. [[CrossRef](#)] [[PubMed](#)]
22. Kus, J.V.; Gebremedhin, A.; Dang, V.; Tran, S.-L.; Serbanescu, A.; Foster, D.B. Bile salts induce resistance to polymyxin in enterohemorrhagic *Escherichia coli* O157: H7. *J. Bacteriol.* **2011**, *193*, 4509–4515. [[CrossRef](#)] [[PubMed](#)]
23. Hu, P.-L.; Yuan, Y.-H.; Yue, T.-L.; Guo, C.-F. Bile acid patterns in commercially available oxgall powders used for the evaluation of the bile tolerance ability of potential probiotics. *PLoS ONE* **2018**, *13*, e0192964. [[CrossRef](#)] [[PubMed](#)]
24. Seshadri, R.; Leahy, S.C.; Attwood, G.T.; Teh, K.H.; Lambie, S.C.; Cookson, A.L.; Elie-Fadrosch, E.A.; Pavlopoulos, G.A.; Hadjithomas, M.; Varghese, N.J.; et al. Cultivation and sequencing of rumen microbiome members from the Hungate1000 Collection. *Nat. Biotechnol.* **2018**, *36*, 359–367. [[CrossRef](#)] [[PubMed](#)]
25. Mizrahi, I.; Wallace, R.J.; Morais, S. The rumen microbiome: Balancing food security and environmental impacts. *Nat. Rev. Microbiol.* **2021**, *19*, 553–566. [[CrossRef](#)] [[PubMed](#)]
26. Sanz-Fernandez, M.V.; Daniel, J.-B.; Seymour, D.J.; Kvidera, S.K.; Bester, Z.; Doelman, J.; Martín-Tereso, J. Targeting the Hindgut to Improve Health and Performance in Cattle. *Animals* **2020**, *10*, 1817. [[CrossRef](#)] [[PubMed](#)]
27. Plaizier, J.C.; Danesh Mesgaran, M.; Derakhshani, H.; Golder, H.; Khafipour, E.; Kleen, J.L.; Lean, I.; Loores, J.; Penner, G.; Zebeli, Q. Review: Enhancing gastrointestinal health in dairy cows. *Animal* **2018**, *12*, s399–s418. [[CrossRef](#)] [[PubMed](#)]
28. Singhal, R.; Shah, Y.M. Oxygen battle in the gut: Hypoxia and hypoxia-inducible factors in metabolic and inflammatory responses in the intestine. *J. Biol. Chem.* **2020**, *295*, 10493–10505. [[CrossRef](#)] [[PubMed](#)]
29. Firman, J.; Friedman Elliot, S.; Hecht, A.; Strange, W.C.; Narowe Adrienne, B.; Mahalak, K.; Wu Gary, D.; Liu, L. Preservation of conjugated primary bile acids by oxygenation of the small intestinal microbiota in vitro. *mBio* **2024**, *15*, e00943-24. [[CrossRef](#)] [[PubMed](#)]
30. Kudva, I.T.; Stasko, J.A. Bison and bovine rectoanal junctions exhibit similar cellular architecture and *Escherichia coli* O157 adherence patterns. *BMC Vet. Res.* **2013**, *9*, 266. [[CrossRef](#)] [[PubMed](#)]
31. Kudva, I.T.; Biernbaum, E.N.; Cassmann, E.D.; Palmer, M.V. Bovine Rectoanal Junction In Vitro Organ Culture Model System to Study Shiga Toxin-Producing *Escherichia coli* Adherence. *Microorganisms* **2023**, *11*, 1289. [[CrossRef](#)] [[PubMed](#)]
32. Yin, X.-F.; Ye, T.; Chen, H.-L.; Liu, J.; Mu, X.-F.; Li, H.; Wang, J.; Hu, Y.-J.; Cao, H.; Kang, W.-Q. The microbiome compositional and functional differences between rectal mucosa and feces. *Microbiol. Spectr.* **2024**, *12*, e03549-23. [[CrossRef](#)] [[PubMed](#)]
33. Jones, R.B.; Zhu, X.; Moan, E.; Murff, H.J.; Ness, R.M.; Seidner, D.L.; Sun, S.; Yu, C.; Dai, Q.; Fodor, A.A.; et al. Inter-niche and inter-individual variation in gut microbial community assessment using stool, rectal swab, and mucosal samples. *Sci. Rep.* **2018**, *8*, 4139. [[CrossRef](#)] [[PubMed](#)]
34. Albenberg, L.; Esipova, T.V.; Judge, C.P.; Bittinger, K.; Chen, J.; Laughlin, A.; Grunberg, S.; Baldassano, R.N.; Lewis, J.D.; Li, H.; et al. Correlation Between Intraluminal Oxygen Gradient and Radial Partitioning of Intestinal Microbiota. *Gastroenterology* **2014**, *147*, 1055–1063.e8. [[CrossRef](#)] [[PubMed](#)]
35. Ihaka, R.; Gentleman, R. R: A Language for Data Analysis and Graphics. *J. Comput. Graph. Stat.* **1996**, *5*, 299–314. [[CrossRef](#)]
36. Sprouffske, K.; Wagner, A. Growthcurver: An R package for obtaining interpretable metrics from microbial growth curves. *BMC Bioinform.* **2016**, *17*, 172. [[CrossRef](#)] [[PubMed](#)]
37. Ahlmann-Eltze, C.; Patil, I. Ggsignif: R Package for Displaying Significance Brackets for 'ggplot2'. *PsyArxiv* **2021**. [[CrossRef](#)]
38. Kassambara, A. Ggpubr: 'ggplot2' Based Publication Ready Plots, R package version 0.6.1. CRAN: Contributed Packages. 2025. Available online: <https://CRAN.R-project.org/package=ggpubr> (accessed on 26 July 2026).

39. Dawson, C. *Ggprism: A 'ggplot2' Extension Inspired by 'GraphPad Prism'*, R package version 1.0.7. CRAN: Contributed Packages. 2025. Available online: <https://CRAN.R-project.org/package=ggprism> (accessed on 26 July 2026).
40. Wickham, H. Ggplot2. *WIREs Comput. Stat.* **2011**, *3*, 180–185. [CrossRef]
41. Andrews, S. *FastQC: A quality Control Tool for High Throughput Sequence Data*; Babraham Bioinformatics: Cambridge, UK, 2010. Available online: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/> (accessed on 26 July 2026).
42. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* **2011**, *17*, 10–12. [CrossRef]
43. Langmead, B.; Salzberg, S.L. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* **2012**, *9*, 357–359. [CrossRef] [PubMed]
44. Danecek, P.; Bonfield, J.K.; Liddle, J.; Marshall, J.; Ohan, V.; Pollard, M.O.; Whitwham, A.; Keane, T.; McCarthy, S.A.; Davies, R.M.; et al. Twelve years of SAMtools and BCFtools. *GigaScience* **2021**, *10*, giab008. [CrossRef] [PubMed]
45. Liao, Y.; Smyth, G.K.; Shi, W. FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **2014**, *30*, 923–930. [PubMed]
46. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550. [CrossRef] [PubMed]
47. Stephens, M. False discovery rates: A new deal. *Biostatistics* **2017**, *18*, 275–294. [CrossRef] [PubMed]
48. Lawrence, M.; Gentleman, R.; Carey, V. Rtracklayer: An R package for interfacing with genome browsers. *Bioinformatics* **2009**, *25*, 1841–1842. [CrossRef] [PubMed]
49. Yu, G.; Wang, L.-G.; Han, Y.; He, Q.-Y. ClusterProfiler: An R Package for Comparing Biological Themes Among Gene Clusters. *OMICS J. Integr. Biol.* **2012**, *16*, 284–287. [CrossRef] [PubMed]
50. Montero, D.A.; Velasco, J.; Del Canto, F.; Puente, J.L.; Padola, N.L.; Rasko, D.A.; Farfán, M.; Salazar, J.C.; Vidal, R. Locus of Adhesion and Autoaggregation (LAA), a pathogenicity island present in emerging Shiga Toxin-producing *Escherichia coli* strains. *Sci. Rep.* **2017**, *7*, 7011. [CrossRef] [PubMed]
51. Nickerson, K.P.; Faherty, C.S. Bile Salt-induced Biofilm Formation in Enteric Pathogens: Techniques for Identification and Quantification. *J. Vis. Exp.* **2018**, *135*, e57322.
52. Sistrunk, J.R.; Nickerson, K.P.; Chanin, R.B.; Rasko, D.A.; Faherty, C.S. Survival of the Fittest: How Bacterial Pathogens Utilize Bile To Enhance Infection. *Clin. Microbiol. Rev.* **2016**, *29*, 819–836. [CrossRef] [PubMed]
53. Ward, J.B.J.; Keely, S.J.; Keely, S.J. Oxygen in the regulation of intestinal epithelial transport. *J. Physiol.* **2014**, *592*, 2473–2489. [CrossRef] [PubMed]
54. Steele, M.A.; Penner, G.B.; Chaucheyras-Durand, F.; Guan, L.L. Development and physiology of the rumen and the lower gut: Targets for improving gut health1. *J. Dairy Sci.* **2016**, *99*, 4955–4966. [CrossRef] [PubMed]
55. Chaucheyras-Durand, F.; Madic, J.; Doudin, F.; Martin, C. Biotic and Abiotic Factors Influencing In Vitro Growth of *Escherichia coli* O157:H7 in Ruminant Digestive Contents. *Appl. Environ. Microbiol.* **2006**, *72*, 4136–4142. [CrossRef] [PubMed]
56. Momin, H.; Appukuttan, D.; Venkatesh, K.V. The catabolic nature of fermentative substrates influences proteomic rewiring in *Escherichia coli* under anoxic growth. *Microb. Cell Fact.* **2025**, *24*, 71. [CrossRef] [PubMed]
57. James, B.W.; Keevil, C.W. Influence of oxygen availability on physiology, verocytotoxin expression and adherence of *Escherichia coli* O157. *J. Appl. Microbiol.* **1999**, *86*, 117–124. [CrossRef] [PubMed]
58. Durso, L.M.; Smith, D.; Hutkins, R.W. Measurements of Fitness and Competition in Commensal *Escherichia coli* and *E. coli* O157:H7 Strains. *Appl. Environ. Microbiol.* **2004**, *70*, 6466–6472. [CrossRef] [PubMed]
59. Free, A.L.; Duoss, H.A.; Bergeron, L.V.; Shields-Menard, S.A.; Ward, E.; Callaway, T.R.; Carroll, J.A.; Schmidt, T.B.; Donaldson, J.R. Survival of O157:H7 and Non-O157 Serogroups of *Escherichia coli* in Bovine Rumen Fluid and Bile Salts. *Foodborne Pathog. Dis.* **2012**, *9*, 1010–1014. [CrossRef] [PubMed]
60. Begley, M.; Gahan, C.G.M.; Hill, C. The interaction between bacteria and bile. *FEMS Microbiol. Rev.* **2005**, *29*, 625–651. [CrossRef] [PubMed]
61. Gunn, J.S. Mechanisms of bacterial resistance and response to bile. *Microbes Infect.* **2000**, *2*, 907–913. [CrossRef] [PubMed]
62. Li, Y.; Hu, M.; McClements, D.J. Factors affecting lipase digestibility of emulsified lipids using an in vitro digestion model: Proposal for a standardised pH-stat method. *Food Chem.* **2011**, *126*, 498–505. [CrossRef]
63. Jones Shari, A.; Chowdhury Fatema, Z.; Fabich Andrew, J.; Anderson, A.; Schreiner Darrel, M.; House Anetra, L.; Autieri Steven, M.; Leatham Mary, P.; Lins Jeremy, J.; Jorgensen, M.; et al. Respiration of *Escherichia coli* in the Mouse Intestine. *Infect. Immun.* **2007**, *75*, 4891–4899. [CrossRef] [PubMed]
64. Kitada, Y.; Muramatsu, K.; Toju, H.; Kibe, R.; Benno, Y.; Kurihara, S.; Matsumoto, M. Bioactive polyamine production by a novel hybrid system comprising multiple indigenous gut bacterial strategies. *Sci. Adv.* **2018**, *4*, eaat0062. [CrossRef] [PubMed]
65. Bertin, Y.; Chaucheyras-Durand, F.; Robbe-Masselot, C.; Durand, A.; de la Foye, A.; Harel, J.; Cohen, P.S.; Conway, T.; Forano, E.; Martin, C. Carbohydrate utilization by enterohaemorrhagic *Escherichia coli* O157:H7 in bovine intestinal content. *Environ. Microbiol.* **2013**, *15*, 610–622. [CrossRef] [PubMed]

66. Edison, L.K.; Kudva, I.T.; Kariyawasam, S. Comparative Transcriptome Analysis of Shiga Toxin-Producing *Escherichia coli* O157:H7 on Bovine Rectoanal Junction Cells and Human Colonic Epithelial Cells during Initial Adherence. *Microorganisms* **2023**, *11*, 2562. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Xue, M.; Fu, D.; Hu, J.; Shao, Y.; Tu, J.; Song, X.; Qi, K. The Transcription Regulator YgeK Affects Biofilm Formation and Environmental Stress Resistance in Avian Pathogenic *Escherichia coli*. *Animals* **2022**, *12*, 1160. [\[CrossRef\]](#) [\[PubMed\]](#)
68. Thongbhubate, K.; Nakafuji, Y.; Matsuoka, R.; Kakegawa, S.; Suzuki, H. Effect of Spermidine on Biofilm Formation in *Escherichia coli* K-12. *J. Bacteriol.* **2021**, *203*, e00652-20. [\[CrossRef\]](#) [\[PubMed\]](#)
69. Sadeghzadeh, R.; Esfandiari, Z.; Khaneghah, A.M.; Rostami, M. A Review of Challenges and Solutions of Biofilm Formation of *Escherichia coli*: Conventional and Novel Methods of Prevention and Control. *Food Bioprocess Technol.* **2024**, *17*, 2583–2618. [\[CrossRef\]](#)
70. Yang, W.; Sun, H.; Yan, J.; Kang, C.; Wu, J.; Yang, B. Enterohemorrhagic *Escherichia coli* senses microbiota-derived nicotinamide to increase its virulence and colonization in the large intestine. *Cell Rep.* **2023**, *42*, 112638. [\[CrossRef\]](#) [\[PubMed\]](#)
71. He, Y.; Hao, F.; Fu, H.; Tian, G.; Zhang, Y.; Fu, K.; Qi, B. N-glycosylated intestinal protein BCF-1 shapes microbial colonization by binding bacteria via its fimbrial protein. *Cell Rep.* **2023**, *42*, 111993. [\[CrossRef\]](#) [\[PubMed\]](#)
72. van Diemen, P.M.; Dziva, F.; Stevens, M.P.; Wallis, T.S. Identification of Enterohemorrhagic *Escherichia coli* O26:H[−] Genes Required for Intestinal Colonization in Calves. *Infect. Immun.* **2005**, *73*, 1735–1743. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Sevrin, G.; Massier, S.; Chassaing, B.; Agus, A.; Delmas, J.; Denizot, J.; Billard, E.; Barnich, N. Adaptation of adherent-invasive *E. coli* to gut environment: Impact on flagellum expression and bacterial colonization ability. *Gut Microbes* **2020**, *11*, 364–380. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Xu, Z.; Qin, C.; Zhang, R.; Wu, M.; Cui, A.; Chen, W.; Chen, L.; Gao, D.; Shi, R. EruA, a Regulator of Adherent-Invasive *E. coli*, Enhances Bacterial Pathogenicity by Promoting Adhesion to Epithelial Cells and Survival Within Macrophages. *Biomolecules* **2026**, *16*, 152. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Miki, T.; Ito, M.; Okada, N.; Haneda, T. The CpxRA two-component system of adherent and invasive *Escherichia coli* contributes to epithelial cell invasion and early-stage intestinal fitness in a dysbiotic mouse model mediated by type 1 fimbriae expression. *Infect. Immun.* **2024**, *92*, e00132-24. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Iida, K.-I.; Mizunoe, Y.; Wai Sun, N.; Yoshida, S.-I. Type 1 Fimbriation and Its Phase Switching in Diarrheagenic *Escherichia coli* Strains. *Clin. Diagn. Lab. Immunol.* **2001**, *8*, 489–495. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Müsken, A.; Bielaszewska, M.; Greune, L.; Schweppe Christian, H.; Müthing, J.; Schmidt, H.; Schmidt, M.A.; Karch, H.; Zhang, W. Anaerobic Conditions Promote Expression of Sfp Fimbriae and Adherence of Sorbitol-Fermenting Enterohemorrhagic *Escherichia coli* O157:NM to Human Intestinal Epithelial Cells. *Appl. Environ. Microbiol.* **2008**, *74*, 1087–1093. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Rouquet, G.; Porcheron, G.; Barra, C.; Répérant, M.; Chanteloup Nathalie, K.; Schouler, C.; Gilot, P. A Metabolic Operon in Extraintestinal Pathogenic *Escherichia coli* Promotes Fitness under Stressful Conditions and Invasion of Eukaryotic Cells. *J. Bacteriol.* **2009**, *191*, 4427–4440. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Hakalehto, E.; Pesola, J.; Heitto, L.; Närvänen, A.; Heitto, A. Aerobic and anaerobic growth modes and expression of type 1 fimbriae in *Salmonella*. *Pathophysiology* **2007**, *14*, 61–69. [\[CrossRef\]](#) [\[PubMed\]](#)
80. Unden, G.; Steinmetz Philipp, A.; Degreif-Dünnwald, P. The Aerobic and Anaerobic Respiratory Chain of *Escherichia coli* and *Salmonella enterica*: Enzymes and Energetics. *EcoSal Plus* **2014**, *6*, 1–37. [\[CrossRef\]](#) [\[PubMed\]](#)
81. Sharma, V.K.; Akavaram, S.; Bayles, D.O. Genomewide transcriptional response of *Escherichia coli* O157:H7 to norepinephrine. *BMC Genom.* **2022**, *23*, 107. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Conway, T.; Cohen, P.S. Commensal and Pathogenic *Escherichia coli* Metabolism in the Gut. *Microbiol. Spectr.* **2015**, *3*, 343–362. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Hu, D.; Yang, X.; Qin, M.; La, P.; Fang, H.; Chen, P.; Ni, Y. Dietary bile acids supplementation protects against *Salmonella* Typhimurium infection via improving intestinal mucosal barrier and gut microbiota composition in broilers. *J. Anim. Sci. Biotechnol.* **2024**, *15*, 155. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Ducarmon, Q.R.; Zwiittink, R.D.; Hornung, B.V.H.; van Schaik, W.; Young, V.B.; Kuijper, E.J. Gut Microbiota and Colonization Resistance against Bacterial Enteric Infection. *Microbiol. Mol. Biol. Rev.* **2019**, *83*, e00007-1. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Sperandio, V.; Mellies, J.L.; Nguyen, W.; Shin, S.; Kaper, J.B. Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 15196–15201. [\[CrossRef\]](#) [\[PubMed\]](#)
86. Sperandio, V.; Torres Alfredo, G.; Girón Jorge, A.; Kaper, J.B. Quorum Sensing Is a Global Regulatory Mechanism in Enterohemorrhagic *Escherichia coli* O157:H7. *J. Bacteriol.* **2001**, *183*, 5187–5197. [\[CrossRef\]](#) [\[PubMed\]](#)
87. Laganenka, L.; Lee, J.-W.; Malfertheiner, L.; Dieterich, C.L.; Fuchs, L.; Piel, J.; von Mering, C.; Sourjik, V.; Hardt, W.-D. Chemotaxis and autoinducer-2 signalling mediate colonization and contribute to co-existence of *Escherichia coli* strains in the murine gut. *Nat. Microbiol.* **2023**, *8*, 204–217. [\[CrossRef\]](#) [\[PubMed\]](#)

88. Kumar, A.; Russell Regan, M.; Hoskan Mehmet, A.; Sperandio, V. Indole Sensing Regulator (IsrR) Promotes Virulence Gene Expression in Enteric Pathogens. *mBio* **2022**, *13*, e01939–22. [\[CrossRef\]](#) [\[PubMed\]](#)
89. Martínez, V.; Cartajena, J.T.; Méndez, E.; Dörner, J.; Méndez, D.; Arriagada, G.; Toledo, J.; Arancibia, R.; Pizarro, N.; Castro, D.; et al. Comparative genomics of Shiga toxin-producing *Escherichia coli* reveals host-specific adhesiome adaptations in humans and cattle. *Front. Vet. Sci.* **2025**, *12*, 1639243. [\[CrossRef\]](#) [\[PubMed\]](#)
90. Chinguanco, F.; Yu, Y.; Kus, J.V.; Que, L.; Lackraj, T.; Lévesque, C.M.; Barnett Foster, D. Identification of a novel adhesin involved in acid-induced adhesion of enterohaemorrhagic *Escherichia coli* O157: H7. *Microbiology* **2012**, *158*, 2399–2407. [\[CrossRef\]](#) [\[PubMed\]](#)
91. Fabich Andrew, J.; Jones Shari, A.; Chowdhury Fatema, Z.; Cernosek, A.; Anderson, A.; Smalley, D.; McHargue, J.W.; Hightower, G.A.; Smith Joel, T.; Autieri Steven, M.; et al. Comparison of Carbon Nutrition for Pathogenic and Commensal *Escherichia coli* Strains in the Mouse Intestine. *Infect. Immun.* **2008**, *76*, 1143–1152. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Leatham, M.P.; Banerjee, S.; Autieri, S.M.; Mercado-Lubo, R.; Conway, T.; Cohen, P.S. Precolonized Human Commensal *Escherichia coli* Strains Serve as a Barrier to *E. coli* O157:H7 Growth in the Streptomycin-Treated Mouse Intestine. *Infect. Immun.* **2009**, *77*, 2876–2886. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Maltby, R.; Leatham-Jensen, M.P.; Gibson, T.; Cohen, P.S.; Conway, T. Nutritional Basis for Colonization Resistance by Human Commensal *Escherichia coli* Strains HS and Nissle 1917 against *E. coli* O157:H7 in the Mouse Intestine. *PLoS ONE* **2013**, *8*, e53957. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Le Bihan, G.; Sicard, J.-F.; Garneau, P.; Bernalier-Donadille, A.; Gobert, A.P.; Garrivier, A.; Martin, C.; Hay, A.G.; Beaudry, F.; Harel, J.; et al. The NAG Sensor NagC Regulates LEE Gene Expression and Contributes to Gut Colonization by *Escherichia coli* O157:H7. *Front. Cell. Infect. Microbiol.* **2017**, *7*, 134. [\[CrossRef\]](#) [\[PubMed\]](#)
95. Partridge, J.D.; Scott, C.; Tang, Y.; Poole, R.K.; Green, J. *Escherichia coli* Transcriptome Dynamics during the Transition from Anaerobic to Aerobic Conditions. *J. Biol. Chem.* **2006**, *281*, 27806–27815. [\[CrossRef\]](#) [\[PubMed\]](#)
96. Bushell, F.; Herbert, J.M.J.; Sannasiddappa, T.H.; Warren, D.; Turner, A.K.; Falciani, F.; Lund, P.A. Mapping the Transcriptional and Fitness Landscapes of a Pathogenic *E. coli* Strain: The Effects of Organic Acid Stress under Aerobic and Anaerobic Conditions. *Genes* **2021**, *12*, 53. [\[CrossRef\]](#) [\[PubMed\]](#)
97. Liou, G.-G.; Chao Kaberdina, A.; Wang, W.-S.; Kaberdin, V.R.; Lin-Chao, S. Combined Transcriptomic and Proteomic Profiling of *E. coli* under Microaerobic versus Aerobic Conditions: The Multifaceted Roles of Noncoding Small RNAs and Oxygen-Dependent Sensing in Global Gene Expression Control. *Int. J. Mol. Sci.* **2022**, *23*, 2570. [\[CrossRef\]](#) [\[PubMed\]](#)
98. Delmas, J.; Gibold, L.; Faïs, T.; Batista, S.; Leremboure, M.; Sinel, C.; Vazeille, E.; Cattoir, V.; Buisson, A.; Barnich, N.; et al. Metabolic adaptation of adherent-invasive *Escherichia coli* to exposure to bile salts. *Sci. Rep.* **2019**, *9*, 2175. [\[CrossRef\]](#) [\[PubMed\]](#)
99. Olson, M.A.; Grimsrud, A.; Richards, A.C.; Mulvey, M.A.; Wilson, E.; Erickson, D.L. Bile Salts Regulate Zinc Uptake and Capsule Synthesis in a Mastitis-Associated Extraintestinal Pathogenic *Escherichia coli* Strain. *Infect. Immun.* **2021**, *89*, e0035721. [\[CrossRef\]](#) [\[PubMed\]](#)
100. Sannasiddappa, T.H.; Lund, P.A.; Clarke, S.R. In vitro antibacterial activity of unconjugated and conjugated bile salts on *Staphylococcus aureus*. *Front. Microbiol.* **2017**, *8*, 1581. [\[CrossRef\]](#) [\[PubMed\]](#)
101. Collins, S.L.; Stine, J.G.; Bisanz, J.E.; Okafor, C.D.; Patterson, A.D. Bile acids and the gut microbiota: Metabolic interactions and impacts on disease. *Nat. Rev. Microbiol.* **2023**, *21*, 236–247. [\[CrossRef\]](#) [\[PubMed\]](#)
102. Keen, J.E.; Laegreid, W.W.; Chitko-McKown, C.G.; Durso, L.M.; Bono, J.L. Distribution of Shiga-Toxigenic *Escherichia coli* O157 in the Gastrointestinal Tract of Naturally O157-Shedding Cattle at Necropsy. *Appl. Environ. Microbiol.* **2010**, *76*, 5278–5281. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Baines, D.; Lee, B.; McAllister, T. Heterogeneity in enterohemorrhagic *Escherichia coli* O157:H7 fecal shedding in cattle is related to *Escherichia coli* O157:H7 colonization of the small and large intestine. *Can. J. Microbiol.* **2008**, *54*, 984–995. [\[CrossRef\]](#) [\[PubMed\]](#)
104. Bessaiah, H.; Anamalé, C.; Sung, J.; Dozois, C.M. What Flips the Switch? Signals and Stress Regulating Extraintestinal Pathogenic *Escherichia coli* Type 1 Fimbriae (Pili). *Microorganisms* **2022**, *10*, 5. [\[CrossRef\]](#) [\[PubMed\]](#)
105. Hall, B.; Levy, S.; Dufault-Thompson, K.; Arp, G.; Zhong, A.; Ndjite, G.M.; Weiss, A.; Braccia, D.; Jenkins, C.; Grant, M.R.; et al. BilR is a gut microbial enzyme that reduces bilirubin to urobilinogen. *Nat. Microbiol.* **2024**, *9*, 173–184. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Stremmel, W.; Weiskirchen, R. Phosphatidylcholine in Intestinal Mucus Protects against Mucosal Invasion of Microbiota and Consequent Inflammation. *Livers* **2024**, *4*, 479–494. [\[CrossRef\]](#)
107. Nart, P.; Naylor Stuart, W.; Huntley John, F.; McKendrick Iain, J.; Gally David, L.; Low, J.C. Responses of Cattle to Gastrointestinal Colonization by *Escherichia coli* O157:H7. *Infect. Immun.* **2008**, *76*, 5366–5372. [\[CrossRef\]](#) [\[PubMed\]](#)
108. Joffre, E.; Nicklasson, M.; Álvarez-Carretero, S.; Xiao, X.; Sun, L.; Nookaew, I.; Zhu, B.; Sjöling, Å. The bile salt glycocholate induces global changes in gene and protein expression and activates virulence in enterotoxigenic *Escherichia coli*. *Sci. Rep.* **2019**, *9*, 108. [\[CrossRef\]](#) [\[PubMed\]](#)
109. Shang, T.; Zhang, R.; Liu, Y.; Shi, S. Intestinal oxygen and microbiota crosstalk: Implications for pathogenesis of gastrointestinal diseases and emerging therapeutic strategies. *Gut Pathog.* **2025**, *17*, 100. [\[CrossRef\]](#) [\[PubMed\]](#)

110. Yang, Y.; Falco, I.; Nou, X. Enhanced environmental stress tolerance in reoccurring, emerging, and persistent (REP) *Escherichia coli* O157:H7 strains from leafy green associated outbreaks. *Food Microbiol.* **2026**, *137*, 105041. [[CrossRef](#)] [[PubMed](#)]
111. Chitlapilly Dass, S.; Bosilevac, J.M.; Weinroth, M.; Elowsky, C.G.; Zhou, Y.; Anandappa, A.; Wang, R. Impact of mixed biofilm formation with environmental microorganisms on *E. coli* O157:H7 survival against sanitization. *npj Sci. Food* **2020**, *4*, 16. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.