GUT MICROBES
2025, VOL. 17, NO. 1, 2473518
https://doi.org/10.1080/19490976.2025.2473518

Taylor & Francis
Taylor & Francis Group

The global stress response regulator *oxyS* in an adherent-invasive *Escherichia coli* strain attenuates experimental colitis

Diana Arsene[a,b], Sandrine Y. Tchaptchet[a], and Jonathan J. Hansen[a,b,c]

[a]Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [b]Department of Medicine, Division of Gastroenterology and Hepatology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [c]Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

ABSTRACT

Crohn's disease and ulcerative colitis in humans and experimental immune-mediated colitis in mice are likely due in part to overactive immune responses to resident intestinal bacteria, including certain strains of adherent-invasive *Escherichia coli* (*E. coli*) such as *E. coli* NC101. We have previously shown that specific *E. coli* NC101 stress responses are upregulated during experimental colitis and attenuate inflammation. However, the roles of broader stress response pathways in *E. coli* NC101 during experimental colitis are unknown. We hypothesize that the global stress response regulator in *E. coli*, *oxyS*, also reduces experimental colitis. We show that intestinal *E. coli* NC101 upregulate *oxyS* expression during colitis in monocolonized interleukin-10 deficient mice. Furthermore, we demonstrate that *oxyS*-sufficient *E. coli* NC101 have decreased motility and biofilm formation *in vitro* and attenuated intestinal translocation and colitogenic potential *in vivo* compared with *oxyS*-deficient *E. coli*. These data suggest that activation of a generalized *E. coli* stress response, *oxyS*, reduces experimental colitis and may be a potential therapeutic target.

ARTICLE HISTORY
Received 12 August 2024
Revised 13 January 2025
Accepted 21 February 2025

KEYWORDS
Stress response; interleukin-10; flagellum; motility; translocation

Introduction

Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis, are chronic immune-mediated diseases that cause significant morbidity. While the pathogenesis of IBDs is not completely understood, most recent evidence suggests that they are due to a combination of genetic susceptibility and environmental exposures that lead to sustained T cell–mediated immune responses in the intestines.[1] Of the many potential environmental factors that may contribute to IBDs, resident intestinal bacteria are likely dominant.

The human intestine is home to over 1000 species of bacteria that normally peacefully coexist with the mucosal immune system. In patients with IBDs, the composition and function of the resident enteric bacteria are altered in ways that might cause chronic intestinal inflammation. For instance, multiple studies demonstrate decreased numbers of Firmicutes and increased number of Enterobaceriacea including adherent-invasive strains of *Escherichia coli* (*E. coli*) in patients with IBDs compared with healthy controls.[2–4] Whether these bacteria are the cause or consequence of human IBDs is difficult to prove. However, animal models of IBDs permit direct testing of this question.

While no animal model perfectly resembles human IBDs, almost all of them require the presence of intestinal microbes in order for disease to manifest.[5,6] This is particularly true of the interleukin-10 deficient ($Il10^{-/-}$) mouse model of colitis.[7,8] When raised in germ-free conditions, the mice remain healthy. However, colonization of the mice with a variety nonpathogenic bacteria results in rapid onset chronic, T cell–mediated colon inflammation that resembles some aspects of human IBDs. Moreover, we and others have shown that monocolonizing the $Il10^{-/-}$ mice with a single strain of adherent-invasive *E. coli*, *E. coli* NC101, also induces colitis.[9–11] While some other models such as the Vill-hCC6 mouse model are particularly useful to study interactions with human IBD-associated strains of adherent-

CONTACT Jonathan J. Hansen jjhansen@med.unc.edu Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill, CB #7032, 7341 MBRB, 111 Mason Farm Rd, Chapel Hill, NC 27599-7032, USA

invasive *E. coli*,[12] the *Il10*$^{-/-}$ colitis model has advantages because germ-free colonies are readily available and because it harbors an immunological defect that has been associated with very early onset IBD.[13]

The interactions between *E. coli* NC101 and the host immune system are bi-directional. In addition to *E. coli* NC101 stimulating innate and adaptive immune responses in the colon, colitis also affects *E. coli* NC101 function. For instance, we have previously shown that intestinal inflammation induces transcription of the specific bacterial stress response genes, *ibpAB* and *gadAB*, in luminal *E. coli* NC101.[11,14] We have also determined that the presence of these stress response pathways attenuates experimental colitis. However, the effects of colitis on global stress response regulators such as *oxyS* in *E. coli* NC101 are unknown.

E. coli oxyS is important to normal bacterial physiology and disease pathogenesis. The primary function of *oxyS* in *E. coli* physiology is to protect bacteria from oxidative damage such as that induced by hydrogen peroxide treatment.[15] Mechanistically, *oxyS* impairs cell division,[16,17] decreases intracellular peroxide concentrations,[18,19] decreases translation of rpoS,[20] and downregulates formate metabolism.[21] Pathologically, *oxyS* has been shown to facilitate *E. coli* colonization of the mouse urinary tract in models of urinary tract infection.[22] However, the role of *E. coli oxyS* in gastrointestinal pathology has not been previously described.

Methods

Bacterial strains and bacterial lysates

The nonpathogenic murine *E. coli* strain NC101 was originally isolated from a wild-type 129/SvEv mouse housed in specific pathogen free conditions as described previously.[9] NC101Δ*oxyS* was constructed using the λ-red recombinase method as described elsewhere.[23] Briefly, the FRT-flanked kanamycin cassette in pKD4 was amplified using primers *oxyS*KOF 5'-g cat agc aac gaa cga tta tcc cta tca agc att ctg act gtg tag gct gga gct gct tc-3' and *oxyS*KOR 5'-acc gtt cta tca ggc tct ctt gct gtg ggc ctg tag aat cat atg aat atc ctc ctt agt tcc-3' each of which contains 40 nucleotides that are homologous to the 5′ and 3′ regions of the *oxyS* gene in NC101, respectively. The PCR product was gel-purified, digested with *DpnI*, and transformed into NC101 that had previously been transformed with pKD46. A kanamycin resistant clone in which the *oxyS* gene was replaced by the kanamycin resistance cassette was transformed with pCP20 in order to remove the kanamycin resistance cassette. Loss of pCP20 and pKD46 from the resultant colonies was confirmed by replicate plating onto kanamycin and carbenicillin agar plates. The *oxyS* gene region on the NC101 chromosome was sequenced to confirm deletion of the coding region. Bacterial lysates for *ex vivo* mesenteric lymph node cell stimulation were prepared as previously described.[9]

Mice

Germfree *Il10*$^{-/-}$ and wild-type mice on the 129S6/SvEV background were originally derived under sterile conditions by hysterectomy at the Gnotobiotic Laboratory (University of Wisconsin, Madison) and have since been continuously maintained in germ-free conditions at the University of North Carolina National Gnotobiotic Rodent Resource Center. Germ-free mice were monocolonized with bacterial strains by adding 50 mL of overnight bacterial cultures to food pellets and bedding. Mice were maintained in gnotobiotic isolators at the National Gnotobiotic Rodent Resource Center at the University of North Carolina, Chapel Hill, for the indicated times. The absence of isolator contamination was confirmed by Gram stain and culture of cecal contents on brain heart infusion (BHI) agar plates under aerobic and anaerobic conditions. All animal protocols were approved by the UNC Chapel Hill Institutional Animal Care and Use Committee.

RNA isolation from cecal contents and bacterial cultures

100–300 mg of freshly harvested cecal contents were snap frozen in liquid nitrogen and stored at −80°C. Frozen samples were thawed into 1 mL of Bacterial RNAProtect (Qiagen) while vortexing, incubated at 25°C for 5 min, and bacterial RNA was isolated as described previously.[11] To measure *flhD* and *fliC* transcript abundance, overnight bacterial cultures grown in Luria

broth (LB) were diluted 1:1000 in fresh LB and incubated at 37°C with shaking for the indicated times. Two mL of culture were removed and bacteria pelleted by centrifugation for 15 sec at 12,000×g after which the pellet was resuspended in 0.5 mL of Bacterial RNAProtect and RNA isolated using RNeasy Mini columns (Qiagen) according to the manufacturer's instructions. Purified RNA was treated with either on-column DNase treatment (Qiagen) or Baseline-Zero DNase (Epicentre) according to the manufacturer's instructions.

Real-time PCR

The following real-time polymerase chain reaction oligonucleotide primers were designed using Primer 3 software based on *E. coli* NC101 genomic DNA sequences published in GenBank: *E. coli* 16S, 16SF 5'-gaatgccacggtgaatacgtt-3', 16SR 5'-acccactcccatggtgtga-3'; *E. coli oxyS*, *oxyS*F 5'-gaatttgcccgctaagcgtcgg-3', *oxyS*R 5'-ggcagtt ttgtttttgcttcctt-3'; *E. coli flhD*, *flhD*F 5'-cagcgtctgattgttcagga-3', *flhD*R 5'- gcttaaccatttgcg-gaaga-3'; *E. coli fliC*, *fliC*F 5'-gccaccgacggtatttctgt -3', *fliC*R 5'-tagtcccggtagaagcctga-3'. First-strand cDNA was synthesized were performed as described previously,[11] and quantitative real-time PCR was performed using Bio-Rad iTaq SYBR green PCR Supermix in the QuantStudio 6 Flex real-time PCR machine (Applied Biosystems) per manufacturer instructions. Relative gene expression compared to *E. coli* 16S rRNA was calculated using the ΔΔCt method.

In vitro *bacterial survival assays in H_2O_2*

Overnight bacterial cultures grown in LB were diluted 1:100 in fresh LB and incubated aerobically at 37°C until cultures reached mid-log growth phase (approximately 2 hours). Phosphate buffered saline or a sufficient volume of 8.82 M H_2O_2 stock was added to 6 mL aliquots of mid-log bacterial culture to achieve the indicated final concentrations of H_2O_2. Cultures were then incubated at 37°C for 30 min with shaking and then serial dilutions were plated onto LB agar to determine bacterial concentrations.

In vitro *bacterial motility assays*

Overnight bacterial cultures grown in LB were diluted 1:10 in LB and then 20 µL of the diluted bacteria were injected with a pipet tip into the center of 15 cm diameter plates of Tryptic Broth containing 0.3% agar. The plates were incubated at 37°C for 12 hrs, then 25°C for 9 hrs at which point the diameter of the bacterial growth ring was measured.

Quantification of luminal bacteria

Concentrations of luminal bacteria were measured as previously described.[11]

Histological scoring

Histologic inflammation scores of colon segments were determined as previously described.[11] Briefly, individual sections of cecum, proximal colon, mid colon, and distal colon were scored in a blinded fashion based on the following four parameters: crypt epithelial hyperplasia, goblet cell dropout, lamina propria mononuclear cell infiltration, and transmural inflammation/crypt abscesses. A score of 1–4 (4 most severe) for each of the four parameters was assigned to each section and then the 4 scores were averaged for each colon section. The average score for each of the 4 sections from a given mouse were then summed and reported as composite histological inflammation score with a range of 0–16 (16 most severe).

Mesenteric lymph node preparation and culture

Mesenteric lymph node cells were isolated and stimulated *ex vivo* with *E. coli* NC101 bacterial lysate for 72 hours as previously described.[11]

Cytokine measurements

IFN-γ concentrations in supernatants of stimulated mesenteric lymph node cells were measured by enzyme-linked immunosorbent assays as previously described.[11]

In vitro *biofilm assays on polystyrene*

Overnight bacterial cultures were diluted 1:100 in LB and 130 µL/well was added to 96-well non-tissue culture treated polystyrene plates (Costar #3370) and incubated at 37°C for 24 hrs. Supernatant was removed by gentle aspiration and each well was gently washed twice with 200 µL of PBS after which 150 µL of 1% crystal violet solution was added to each well and the plate incubated at 25°C for 20 min. The supernatant was aspirated and each well washed twice with 300 µL PBS after which 200 µL of 95% ethanol was added to each well and the plate incubated at 25°C for 20 min with occasional rocking. The absorbance of each well at 570 nm was then measured using a spectrophotometer and background absorbance from media-only wells was subtracted.

Quantification of mucus-associated bacteria

Whole intestinal pieces without luminal contents were filleted open longitudinally, cut into 1 cm-long pieces, and vigorously washed in PBS followed by three 5-minute washes with 1 mL/cm tissue of 0.016% dithiothreitol (DTT) in PBS while vortexing the tissue at 150rpm. Combined DTT washes were diluted and plated onto LB agar for enumeration. The intestinal tissue was then briefly blotted on a sterile paper towel to remove excess liquid and weighed.

Quantification of intra-epithelial bacteria

Whole intestinal pieces without luminal contents were filleted open, cut into 1 cm-long pieces, and vigorously washed in PBS. To remove epithelial cells, and kill extracellular bacteria, the pieces were transferred to 1.5 µM EDTA containing 50 µg/mL gentamicin. Pieces were rocked gently at 45 rpm at 4°C for 30 minutes, transferred to 5 mL of PBS, and shaken vigorously at 3 shakes/second x 5 seconds to remove epithelial cells. 10 µl aliquots of epithelial cell suspensions were removed and crypts containing live epithelial cells were quantified using the trypan blue method. The epithelial cell suspensions were centrifuged at 300×g for 5 minutes at 4°C, and pellets were resuspended in 250 µl of 0.05% Triton X-100 in water by vigorously pipetting up and down to disrupt epithelial cells. Intraepithelial bacteria were diluted in PBS and plated onto LB agar.

Quantification of sub-epithelial tissue-associated bacteria

Whole intestinal pieces without luminal contents were filleted open, cut into 1 cm-long pieces, and vigorously washed in PBS, followed by 1.5 µM EDTA in PBS with 50 µg/mL gentamicin to remove epithelial cells and kill extracellular bacteria. Epithelial crypts were enumerated as described above. Following wash steps, the remaining tissue was homogenized in 1 mL of molecular grade water using a disperser (IKA WorksT25 basic). Subepithelial tissue-associated bacteria was diluted in PBS and plated onto LB agar.

Statistical analysis

Data are presented as the mean ± SEM. p values were calculated using the unpaired, 2-tailed Student's T-test.

Results

E. coli *upregulate* oxyS *expression during experimental colitis*

We have previously shown that *E. coli* upregulate specific oxidative stress (*ibpAB*) and acid stress (*gadAB*) response genes during experimental colitis. Therefore, we hypothesized that *E. coli* may also increase expression of the global stress response regulator, *oxyS*, that is upregulated during oxidative stress and controls expression of the stress response genes *gadB*, *rpoS*, and *yhiV*. To test this, we monocolonized germ-free interleukin 10 knockout ($Il10^{-/-}$) and wild-type (wt) mice with *E. coli* NC101 and quantified severity of colitis as well as *E. coli* NC101 *oxyS* expression. We previously reported that histological colon inflammation scores and spontaneous IL-12/23p40 secretion by colon explant cultures increases over time in the $Il10^{-/-}$, but not wt, mice.[11] Additionally, we have previously reported similar *E. coli* concentrations

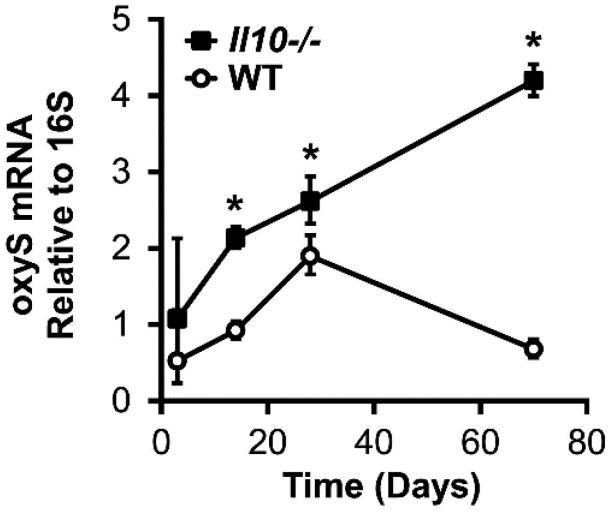

Figure 1. *E. coli oxyS* expression levels in luminal bacteria during experimental colitis. *E. coli oxyS* transcripts relative to bacterial 16S transcripts were quantified in cecal bacterial RNA from wild-type (WT) and interleukin-10 deficient ($Il10^{-/-}$) mice monocolonized with *E. coli* NC101 for the indicated times. Data are expressed as means ± SEM (n=4–5 mice/group, *p<0.05 $Il10^{-/-}$ v. WT).

in colon luminal contents from *E. coli* NC101 monocolonized $Il10^{-/-}$ and wt mice and absent histological colon inflammation in germ-free $Il10^{-/-}$ and wt mice.[14] In the current studies, we find that *oxyS* transcript abundance as measured by quantitative PCR (qPCR) continuously increases with time in cecal bacteria from $Il10^{-/-}$ mice with colitis, but only transiently increases in cecal bacteria from healthy wt mice (Figure 1). These data show that colitis is associated with increased *oxyS* expression in luminal *E. coli* and suggest that resident intestinal bacteria respond to chronic immune-mediated colitis by activating global stress response pathways.

E. coli oxyS *impairs bacterial motility*

The role of *E. coli oxyS* in protecting *E. coli* from oxidative stress is well studied.[15] Since NC101 also upregulates acid tolerance genes during experimental colitis,[14] we tested whether *oxyS* confers a growth advantage to NC101 in acidic environments and found no significant effect (data not shown). In addition to regulating stress response pathways, *E. coli oxyS* also impacts expression of genes important for bacterial motility. Others have previously shown that overexpression of *oxyS* impairs expression of *flhD*, a master transcriptional regulator of flagellar genes that are important for bacterial motility.[24] However, whether physiologically normal levels of *oxyS* decrease expression of *flhD* is unknown. Moreover, the effects of physiologic levels of *oxyS* on the expression of *fliC*, the primary monomeric subunit of the flagellar filament, as well as bacterial motility are unknown. To examine the effects of endogenous levels of *oxyS* on flagellar gene expression and bacterial motility, we first generated a deletion mutant of *oxyS* in *E. coli* NC101 (NC101Δ*oxyS*) using the λ-red recombinase method. We showed that the absence of *oxyS* had minimal effect on the growth of NC101 in Luria-Bertani (LB) liquid media (Figure 2(a)). We confirmed the genetic absence of *oxyS* using PCR and the functional absence of *oxyS* by challenging the bacteria with hydrogen peroxide. As expected, hydrogen peroxide is more toxic to NC101Δ*oxyS* compared with the parental strain (Figure 2(b)). We then measured *flhD* and *fliC* transcript abundance in the mutant and parental strains grown in LB media and detected increased levels of both *flhD* and *fliC* mRNA in NC101Δ*oxyS* compared with the parental strain (Figure 2(d-e)). Motility assays in soft agar also revealed that NC101Δ*oxyS* is more motile than the parental strain (Figure 2(c)). These data indicate that the presence of physiologic levels of *oxyS* decreases flagellar gene expression and motility in *E. coli*.

E. coli oxyS *attenuates experimental colitis*

Others have previously shown that the flagellum, particularly FliC, promotes adherence and invasion of *E. coli* in intestinal epithelial cell monolayers and exacerbates chemically induced colitis.[25–30] Since the presence of *oxyS* is associated with decreased levels of FliC and bacterial motility, we hypothesized that *oxyS* would also attenuate chronic, immune-mediated colitis in mice. To test this, we mono-colonized germ-free $Il10^{-/-}$ mice with NC101Δ*oxyS* or the parental strain for up to 10 weeks and quantified intestinal inflammation. As predicted, we detected less histological inflammation in colon tissue and decreased IFNγ secretion by stimulated mesenteric lymph node cells from $Il10^{-/-}$ mice colonized with NC101 compared with NC101Δ*oxyS* (Figure 3(b-d)). We have

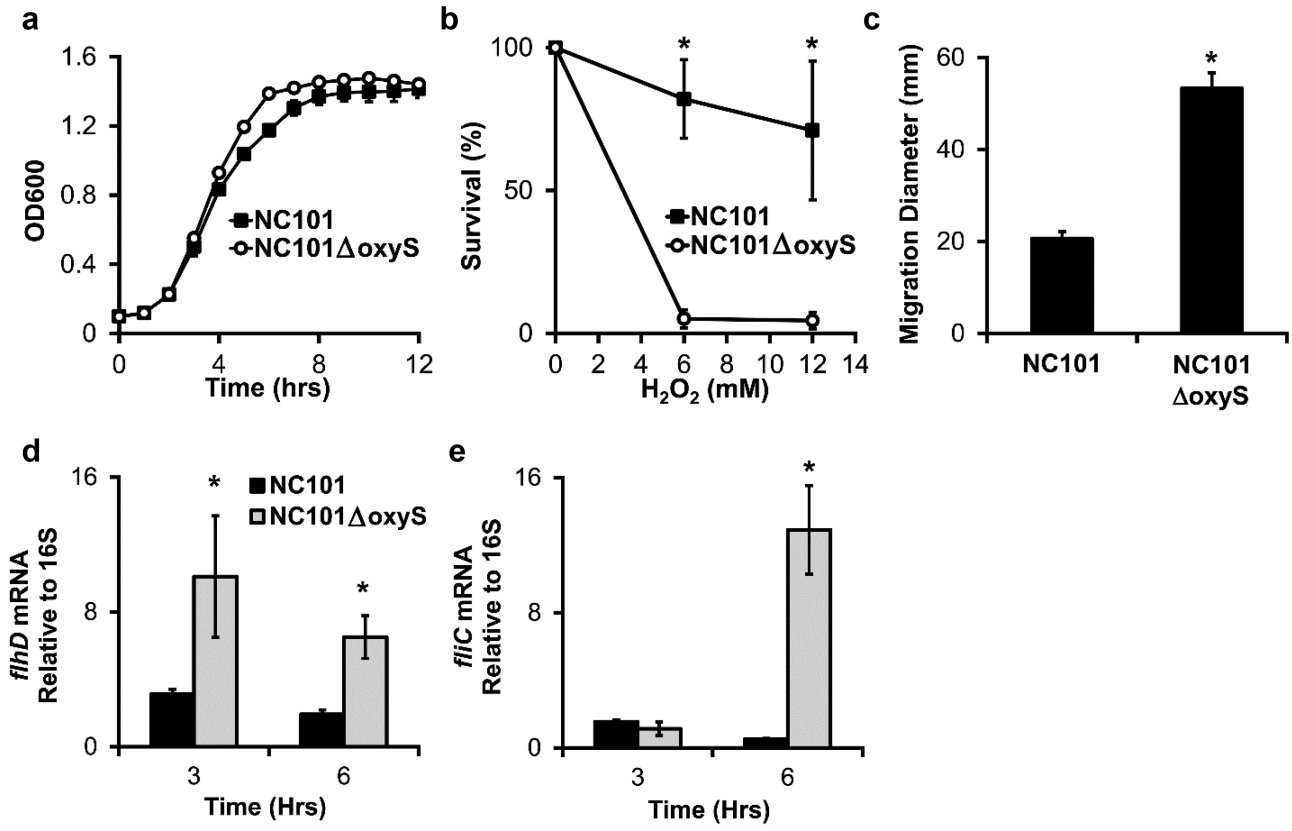

Figure 2. *In vitro* characterization of *E. coli* NC101Δ*oxyS*. a) Aerobic growth in LB liquid media measured by optical density at 600nm. Data presented as the means ± SEM (n=3 cultures/strain). b) Survival in the presence of the indicated concentrations of H_2O_2. Data are expressed as percent survival compared to cultures without added H_2O_2 and are presented as means ± SEM (n=3 cultures per strain per condition, *p<0.05 relative to NC101Δ*oxyS*). c) Motility in soft agar over 12 hours. Data are expressed as the diameter of the growth ring (mm) and are presented as means ± SEM (n=3 assays per strain per condition, *p<0.05 relative to NC101. d and e) Flagellar gene expression during growth. Data are expressed as the abundance of *flhD* and *fliC* transcripts relative to bacterial 16S at the indicated times of growth in liquid LB and are presented as the means ± SEM (n=3 assays per strain per condition, *p<0.05 relative to NC101).

previously shown that IFNγ secretion by stimulated mesenteric lymph node cells from germ-free *Il10*$^{-/-}$ mice is negligible.[14] The decreased inflammation was not due to reduced number of bacteria in luminal contents as colony forming units in cecal contents of the two groups of mice were similar (Figure 3(a)). These data suggest that the presence of *oxyS* attenuates chronic immune-mediated colitis by affecting other bacterial properties than growth.

E. coli oxyS *reduces bacterial translocation in the mouse intestine*

We next aimed to determine mechanisms by which *oxyS* reduces experimental colitis. Increased bacterial biofilms are associated with human IBDs and bacterial flagella promote increased binding of *E. coli* to epithelial cells and intestinal mucus.[25–28,31–34] Because *oxyS* is associated with impaired expression of *flhD* and *fliC* and decreased bacterial motility, we predicted that *oxyS* expression also decreases biofilm formation and adherence to intestinal epithelial cells.

To test this, we first performed *in vitro* biofilm assays by growing *E. coli* NC101 on polystyrene surfaces and quantifying biofilm using crystal violet staining. We found that *E. coli* NC101 formed less biofilm on plastic surfaces compared with NC101Δ*oxyS* (Figure 4(a)). However, this was not due to differences in curli fiber expression as colony colors and morphologies of NC101 and NC101Δ*oxyS* grown on Congo red agar plates were similar (data not shown). We next measured adherence to colonic mucus *in vivo* by monocolonizing germ-free mice with *E. coli* NC101 or NC101Δ*oxyS* for 1 week and then quantifying

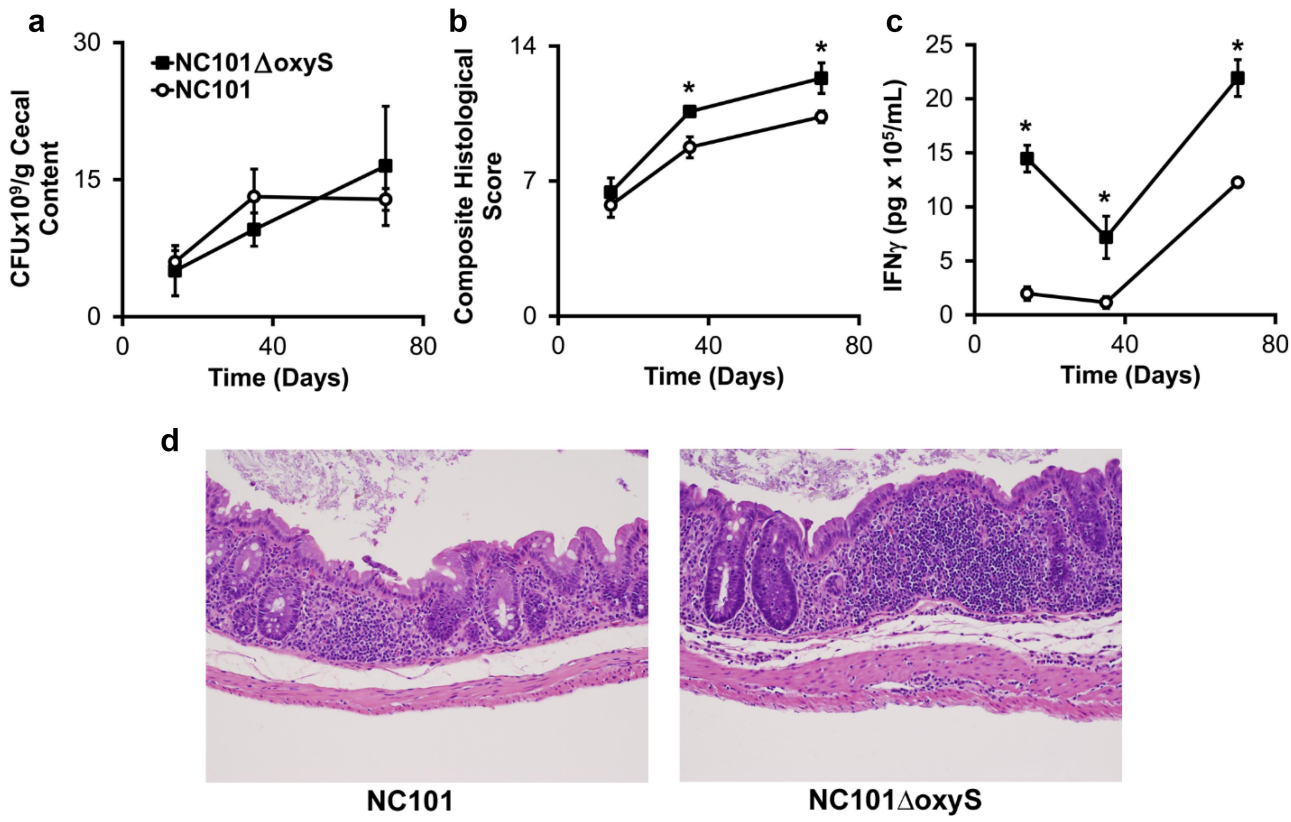

Figure 3. *E. coli oxyS* attenuates experimental colitis in monocolonized *Il10*$^{-/-}$ mice. a) Colony forming units of *E. coli* in cecal contents, b) Composite histological inflammation scores (range 0–16), and c) IFNγ secretion by mesenteric lymph node cells stimulated *ex vivo* with sterile NC101 bacterial lysate. Data are presented as means ± SEM (n=6 mice/group/timepoint, *p<0.05 relative to NC101-colonized mice. d) Representative photomicrographs of cecal tissue from mice monocolonized for 80 days (40x).

bacteria in dithiothreitol washings of cecum and distal colon tissue. We found no statistically significant difference in numbers of mucus-associated bacteria in the two groups of mice though there appears to be a trend toward decreased numbers in mice monocolonized with NC101 v. NC101Δ*oxyS* (Figure 4(b)). We next measured bacterial numbers in intestinal epithelial cell preparations as well as homogenized intestinal tissue depleted of mucus and epithelial cells (i.e subepithelial tissue). We detected significantly fewer bacteria in subepithelial cecal tissue from mice monocolonized with NC101 v. NC101Δ*oxyS* (Figure 4(d)). There were statistically insignificant trends toward lower numbers of bacteria in intra-epithelial and subepithelial distal colon tissues from mice monocolonized with NC101 v NC101Δ*oxyS* (Figure 4 (c-d)). We could not detect significant numbers of *E. coli* in the subepithelial compartment of colon tissue from NC101 or NC101Δ*oxyS* monocolonized mice using immunofluorescence microscopy, likely due to the rarity of bacterial translocatoin events or short-lived nature of subepithelial bacteria (data not shown). However, the culture data shown in Figure 4 suggest that the presence of *oxyS* might impair *E. coli* adherence to intestinal mucus, invasion into epithelial cells and translocation to subepithelial tissues where the majority of the intestinal immune cells typically reside. We speculate that the possible decreased translocation of bacteria into subepithelial tissues may contribute to the decreased colitis observed in NC101 vs. NC101Δ*oxyS* monocolonized mice.

Discussion

Microbial-host interactions play key roles in the pathogenesis of inflammatory bowel diseases and experimental colitis. We have shown in the current studies that a global *E. coli* stress response regulator, *oxyS*, is upregulated during experimental colitis and attenuates bacterial motility, biofilm

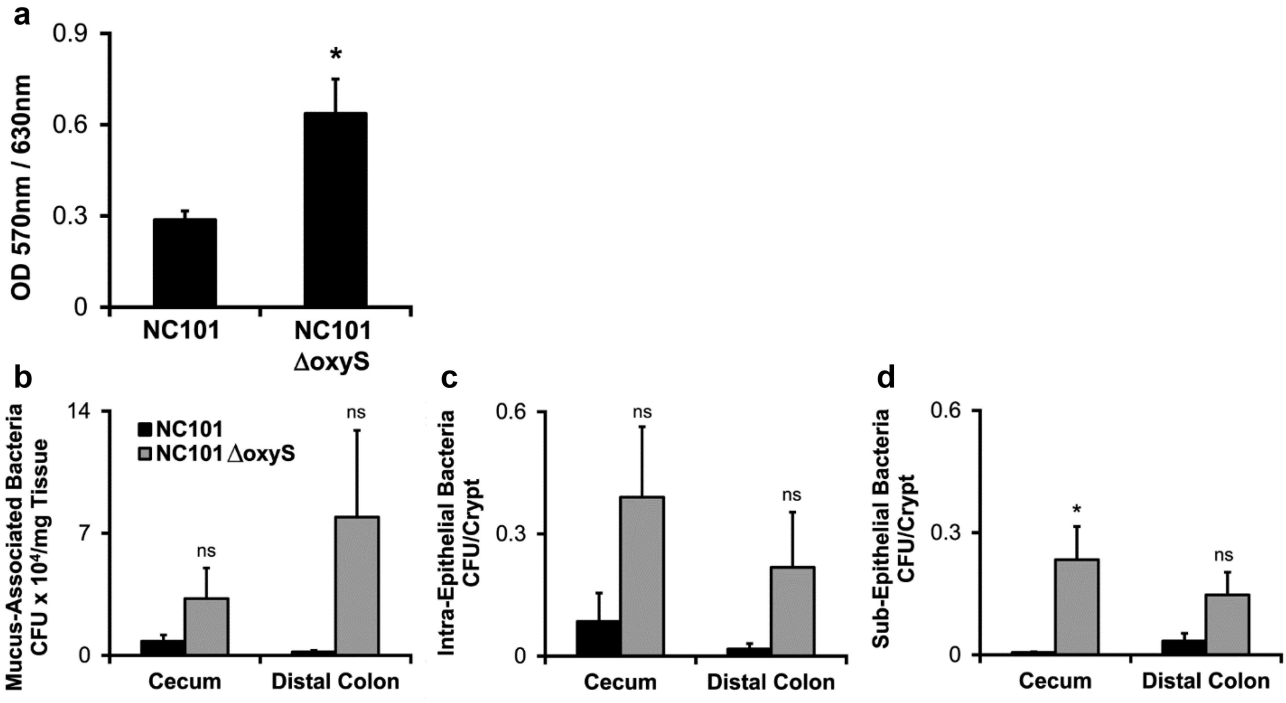

Figure 4. *E. coli oxyS* decreases biofilm formation *in vitro* and bacterial translocation in the cecum of monocolonized Il0$^{-/-}$ mice. a) Quantity of bacterial biofilm on a polystyrene surface as measured by the optical density of crystal violet-stained bacterial biofilms (570nm)/optical density of bacterial cultures prior to biofilm staining (630nm). Data are presented as the means ± SEM (n=16 cultures per strain). b-d) Colony forming units of tissue associated *E. coli* in the cecum and distal colon from *Il10*$^{-/-}$ mice monocolonized with *E. coli* for 1 week. Data are presented as the means ± SEM (n=5 mice/group, *p<0.05 relative to NC101).

formation, epithelial translocation, and intestinal inflammation.

Similarly, we have previously reported that specific *E. coli* stress response genes that protect against heat shock, oxidative stress, and acid exposure are upregulated during colitis and decrease the colitogenic potential of *E. coli*.[11,14] *OxyS* is unique among these previously described stress response genes in that it encodes a small regulatory RNA that impacts the expression of several downstream target genes.[15] Unlike *gadAB* and *ibpB*, *oxyS* expression potentially alters the function of multiple bacterial stress response pathways. Therefore, assuming that bacterial stress response pathways in general attenuate experimental colitis and IBDs as has been observed with *gadAB*[14] and *ibpB*,[11] then pharmacologically augmenting *oxyS* expression may more effectively decrease intestinal inflammation. Since the environment of the inflamed colon likely imposes physiological stress on luminal bacteria, it is not surprising that they respond by upregulating pathways that counteract the stressful stimuli. However, the evolutionary reasons why the presence of intact bacterial stress responses such as *oxyS* attenuates colon inflammation and yet does not enhance bacterial survival are still enigmatic.

We show that the presence of the native *oxyS* gene decreases transcription of native flagellar genes and bacterial motility. Our findings are consistent with those of others who have previously shown that plasmid based overexpression of *oxyS* impairs expression of an *flhDC* reporter construct by binding to the 5' UTR of *flhDC* mRNA and that *oxyS* overexpression decreases *E. coli* motility.[24] However, our finding that *oxyS* also reduces transcription of *fliC*, the major subunit of the flagellar filament that is expressed late in the flagellar assembly process, has not been previously reported as far as we are aware. There are no known *oxyS* binding sites in the regulatory regions of *fliC* or the sigma 28 factor responsible for *fliC* transcription,[35] so the mechanisms by which *oxyS* regulates *fliC* expression remain unclear.

We speculate that inflammatory bowel diseases and experimental colitis are driven in part by translocation of resident intestinal bacteria across the intestinal epithelial cell layer and subsequent activation of the subepithelial mucosal immune system. Indeed, in studies of patients with Crohn's disease, others have shown that unique types of bacteria translocate to mesenteric fat[36] and still other types of bacteria induce antigen specific antibody responses.[37] Our data demonstrate that the absence of *oxyS* in *E. coli* NC101 is associated with increased inflammation in the *Il10*$^{-/-}$ model of experimental colitis and increased numbers of bacteria in subepithelial cecal tissues and perhaps increased adherence to mucus and invasion into intestinal epithelial cells. It is unknown whether the decreased intestinal bacterial translocation is the cause of attenuated colitis in these mice, but the decreased IFNγ production by *E. coli* antigen stimulated mesenteric lymph node cells supports this hypothesis.

These studies have focused solely on a single murine *E. coli* isolate, NC101. We chose to study *E. coli* NC101 because it reproducibly and reliably causes severe, rapid onset colitis in our gnotobiotic monocolonized *Il10*$^{-/-}$ mice and has an annotated genome sequence. While this isolate shares some properties with adherent-invasive, human-derived, IBD-relevant *E. coli* strains,[14,38] it is uncertain whether the findings presented here using NC101 can be extrapolated to these other adherent-invasive *E. coli* strains. Therefore, further investigation of the role of *oxyS* in human-derived, IBD-relevant *E. coli* strains during experimental colitis is warranted.

In summary, we show that the *E. coli* stress response regulator, *oxyS*, is significantly upregulated in luminal *E. coli* during experimental colitis and that the presence of *oxyS* attenuates bacterial motility, biofilm formation, and translocation as well as colitis severity. Further studies of bacterial stress responses to intestinal inflammation may lead to new understanding of the pathogenesis and optimal treatment of inflammatory bowel diseases.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was funded by grants from [NIH K08 DK087896] (J.J.H.), a Career Development Award from the Crohn's and Colitis Foundation #2662 (J.J.H.), a Research Fellowship Award from the Crohn's and Colitis Foundation #3623 (S.Y. T.), a Gastroenterology Research Training Award to D.A. [USPHS T32 DK07737, R. Balfour Sartor, PI]. We acknowledge the Gnotobiotic, Histology, and Immunotechnology Cores at the UNC Center for Gastrointestinal Biology and Disease [supported by NIH P30DK34987, NIH P40OD 010995, and the Crohn's and Colitis Foundation #2434].

Data availability statement

The data that support the findings of this study are available from the corresponding author, JH, upon reasonable request.

References

1. Sartor RB, Wu GD. Roles for intestinal bacteria, viruses, and fungi in pathogenesis of inflammatory bowel diseases and therapeutic approaches. Gastroenterology. 2017;152(2):327–339.e4. doi:10.1053/j.gastro.2016.10.012.
2. Hansen J, Gulati A, Sartor RB. The role of mucosal immunity and host genetics in defining intestinal commensal bacteria. Curr Opin Gastroenterol. 2010;26(6):564–571.
3. Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, Bringer MA, Swidsinski A, Beaugerie L, Colombel JF. High prevalence of adherent-invasive Escherichia coli associated with ileal mucosa in Crohn's disease. Gastroenterology. 2004;127(2):412–421. doi:10.1053/j.gastro.2004.04.061.
4. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, Andrews E, Ajami NJ, Bonham KS, Brislawn CJ, et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature. 2019;569(7758):655–662. doi:10.1038/s41586-019-1237-9.
5. Hansen JJ, Sartor RB. Insights provided by animal models into the pathogenesis and treatment of IBD. In: Bernstein C, editor. The inflammatory bowel disease yearbook. London: Remedica; 2007. p. 19–55.
6. Khanna PV, Shih DQ, Haritunians T, McGovern DP, Targan S. Use of animal models in elucidating disease pathogenesis in IBD. Semin Immunopathol. 2014;36(5):541–551. doi:10.1007/s00281-014-0444-6.
7. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993;75(2):263–274. doi:10.1016/0092-8674(93)80068-P.
8. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB.

Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. Infect Immun. 1998;66 (11):5224–5231. doi:10.1128/IAI.66.11.5224-5231.1998 .
9. Kim SC, Tonkonogy SL, Albright CA, Tsang J, Balish EJ, Braun J, Huycke MM, Sartor RB. Variable phenotypes of enterocolitis in interleukin 10-deficient mice monoassociated with two different commensal bacteria. Gastroenterology. 2005;128(4):891–906. doi:10.1053/j.gastro.2005.02.009 .
10. Kim SC, Tonkonogy SL, Karrasch T, Jobin C, Sartor RB. Dual-association of gnotobiotic il-10−/− mice with 2 nonpathogenic commensal bacteria induces aggressive pancolitis. Inflamm Bowel Dis. 2007;13(12):1457–1466. doi:10.1002/ibd.20246.
11. Patwa LG, Fan TJ, Tchaptchet S, Liu Y, Lussier YA, Sartor RB, Hansen JJ. Chronic intestinal inflammation induces stress-response genes in commensal Escherichia coli. Gastroenterology. 2011;141(5):1842–1851.e10. doi:10.1053/j.gastro.2011.06.064.
12. Sivignon A, Chervy M, Chevarin C, Ragot E, Billard E, Denizot J, Barnich N. An adherent-invasive Escherichia coli -colonized mouse model to evaluate microbiota-targeting strategies in Crohn's disease. Dis Model Mech. 2022;15(10). doi:10.1242/dmm.049707.
13. Kotlarz D, Beier R, Murugan D, Diestelhorst J, Jensen O, Boztug K, Pfeifer D, Kreipe H, Pfister ED, Baumann U, et al. Loss of interleukin-10 signaling and infantile inflammatory bowel disease: implications for diagnosis and therapy. Gastroenterology. 2012;143 (2):347–355. doi:10.1053/j.gastro.2012.04.045.
14. Tchaptchet S, Fan TJ, Goeser L, Schoenborn A, Gulati AS, Sartor RB, Hansen JJ, Bäumler AJ. Inflammation-induced acid tolerance genes gadAB in luminal commensal Escherichia coli attenuate experimental colitis. Infect Immun. 2013;81(10):3662–3671. doi:10.1128/IAI.00355-13.
15. Altuvia S, Weinstein-Fischer D, Zhang A, Postow L, Storz G. A small, stable RNA induced by oxidative stress: role as a pleiotropic regulator and antimutator. Cell. 1997;90(1):43–53. doi:10.1016/S0092-8674(00)80312-8.
16. Barshishat S, Elgrably-Weiss M, Edelstein J, Georg J, Govindarajan S, Haviv M, Wright PR, Hess WR, Altuvia S. OxyS small RNA induces cell cycle arrest to allow DNA damage repair. Embo J. 2018;37 (3):413–426. doi:10.15252/embj.201797651.
17. Elgrably-Weiss M, Hussain F, Georg J, Shraiteh B, Altuvia S. Balanced cell division is secured by two different regulatory sites in OxyS RNA. RNA. 2024;30 (2):124–135. doi:10.1261/rna.079836.123.
18. Doi H, Hoshino Y, Nakase K, Usuda Y. Reduction of hydrogen peroxide stress derived from fatty acid beta-oxidation improves fatty acid utilization in Escherichia coli. Appl Microbiol Biotechnol. 2014;98 (2):629–639. doi:10.1007/s00253-013-5327-6.
19. Gonzalez-Flecha B, Demple B. Role for the oxyS gene in regulation of intracellular hydrogen peroxide in Escherichia coli. J Bacteriol. 1999;181(12):3833–3836. doi:10.1128/JB.181.12.3833-3836.1999.
20. Henderson CA, Vincent HA, Casamento A, Stone CM, Phillips JO, Cary PD, Sobott F, Gowers DM, Taylor JEN, Callaghan AJ. Hfq binding changes the structure of Escherichia coli small noncoding RNAs OxyS and RprA, which are involved in the riboregulation of rpoS. RNA. 2013;19(8):1089–1104. doi:10.1261/rna.034595.112.
21. Salim NN, Feig AL. An upstream hfq binding site in the fhlA mRNA leader region facilitates the OxyS-fhlA interaction. PLoS One. 2010;5(9):e13028. doi:10.1371/journal.pone.0013028.
22. Johnson JR, Clabots C, Rosen H. Effect of inactivation of the global oxidative stress regulator oxyR on the colonization ability of Escherichia coli O1: K1: H7 in a mouse model of ascending urinary tract infection. Infect Immun. 2006;74(1):461–468. doi:10.1128/IAI.74.1.461-468.2006.
23. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci USA. 2000;97 (12):6640–6645. doi:10.1073/pnas.120163297.
24. De Lay N, Gottesman S. A complex network of small non-coding RNAs regulate motility in Escherichia coli. Mol Microbiol. 2012;86(3):524–538. doi:10.1111/j.1365-2958.2012.08209.x.
25. Giron JA, Torres AG, Freer E, Kaper JB. The flagella of enteropathogenic Escherichia coli mediate adherence to epithelial cells. Mol Microbiol. 2002;44(2):361–379. doi:10.1046/j.1365-2958.2002.02899.x.
26. Erdem AL, Avelino F, Xicohtencatl-Cortes J, Giron JA. Host protein binding and adhesive properties of H6 and H7 flagella of attaching and effacing Escherichia coli. J Bacteriol. 2007;189(20):7426–7435. doi:10.1128/JB.00464-07.
27. Eaves-Pyles T, Allen CA, Taormina J, Swidsinski A, Tutt CB, Jezek GE, Islas-Islas M, Torres AG. Escherichia coli isolated from a Crohn's disease patient adheres, invades, and induces inflammatory responses in polarized intestinal epithelial cells. Int J Med Microbiol. 2008;298(5–6):397–409. doi:10.1016/j.ijmm.2007.05.011.
28. Barnich N, Boudeau J, Claret L, Darfeuille-Michaud A. Regulatory and functional co-operation of flagella and type 1 pili in adhesive and invasive abilities of AIEC strain LF82 isolated from a patient with Crohn's disease. Mol Microbiol. 2003;48(3):781–794. doi:10.1046/j.1365-2958.2003.03468.x.
29. Carvalho FA, Barnich N, Sauvanet P, Darcha C, Gelot A, Darfeuille-Michaud A. Crohn's disease-associated Escherichia coli LF82 aggravates colitis in injured mouse colon via signaling by flagellin. Inflamm Bowel Dis. 2008;14(8):1051–1060. doi:10.1002/ibd.20423.
30. Troge A, Scheppach W, Schroeder BO, Rund SA, Heuner K, Wehkamp J, Stange EF, Oelschlaeger TA. More than a marine propeller–the flagellum of the probiotic Escherichia coli strain Nissle 1917 is the major

adhesin mediating binding to human mucus. Int J Med Microbiol. 2012;302(7–8):304–314. doi:10.1016/j.ijmm.2012.09.004.
31. Swidsinski A, Weber J, Loening-Baucke V, Hale LP, Lochs H. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. J Clin Microbiol. 2005;43(7):3380–3389. doi:10.1128/JCM.43.7.3380-3389.2005.
32. Golinska E, Tomusiak A, Gosiewski T, Wiecek G, Machul A, Mikolajczyk D, Bulanda M, Heczko PB, Strus M. Virulence factors of enterococcus strains isolated from patients with inflammatory bowel disease. World J Gastroenterol. 2013;19(23):3562–3572. doi:10.3748/wjg.v19.i23.3562.
33. Martinez-Medina M, Naves P, Blanco J, Aldeguer X, Blanco JE, Blanco M, Ponte C, Soriano F, Darfeuille-Michaud A, Garcia-Gil LJ. Biofilm formation as a novel phenotypic feature of adherent-invasive Escherichia coli (AIEC). BMC Microbiol. 2009;9(1):202. doi:10.1186/1471-2180-9-202.
34. Baumgartner M, Lang M, Holley H, Crepaz D, Hausmann B, Pjevac P, Moser D, Haller F, Hof F, Beer A, et al. Mucosal biofilms are an endoscopic feature of irritable bowel syndrome and ulcerative colitis. Gastroenterology. 2021;161(4):1245–1256.e20. doi:10.1053/j.gastro.2021.06.024.
35. Tjaden B, Goodwin SS, Opdyke JA, Guillier M, Fu DX, Gottesman S, Storz G. Target prediction for small, noncoding RNAs in bacteria. Nucleic Acids Res. 2006;34(9):2791–2802. doi:10.1093/nar/gkl356.
36. Ha CWY, Martin A, Sepich-Poore GD, Shi B, Wang Y, Gouin K, Humphrey G, Sanders K, Ratnayake Y, Chan KSL, et al. Translocation of viable gut microbiota to mesenteric adipose drives formation of creeping fat in humans. Cell. 2020;183(3):666–683.e17. doi:10.1016/j.cell.2020.09.009.
37. Ahmed Z, Lysek M, Zhang N, Malik TA. Association between serological markers and Crohn's disease activity. J Clin Med Res. 2020;12(1):6–12. doi:10.14740/jocmr4016.
38. Fang X, Monk JM, Nurk S, Akseshina M, Zhu Q, Gemmell C, Gianetto-Hill C, Leung N, Szubin R, Sanders J, et al. Strain-level analysis of Escherichia coli from a time-series of microbiome samples from a Crohn's disease patient. Front Microbiol. 2018;9:2559. doi:10.3389/fmicb.2018.02559.