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The SOS Response Mediates Sustained Colonization of the Mammalian Gut

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ABSTRACT Bacteria have a remarkable ability to survive, persist, and ultimately adapt to environmental challenges. A ubiquitous environmental hazard is DNA damage, and most bacteria have evolved a network of genes to combat genotoxic stress. This network is known as the SOS response and aids in bacterial survival by regulating genes involved in DNA repair and damage tolerance. Recently, the SOS response has been shown to play an important role in bacterial pathogenesis, and yet the role of the SOS response in nonpathogenic organisms and in physiological settings remains underexplored. Using a commensal *Escherichia coli* strain, MP1, we showed that the SOS response plays a vital role during colonization of the murine gut. In an unperturbed environment, the SOS-off mutant is impaired for stable colonization relative to a wild-type strain, suggesting the presence of genotoxic stress in the mouse gut. We evaluated the possible origins of genotoxic stress in the mouse gut by examining factors associated with the host versus the competing commensal organisms. In a dextran sulfate sodium (DSS) colitis model, the SOS-off colonization defect persisted but was not exacerbated. In contrast, in a germ-free model, the SOS-off mutant colonized with efficiency equal to that seen with the wild-type strain, suggesting that competing commensal organisms might be a significant source of genotoxic stress. This report extends our understanding of the importance of a functional SOS response for bacterial fitness in the context of a complex physiological environment and highlights the SOS response as a possible mechanism that contributes to ongoing genomic changes, including potential antibiotic resistance, in the microbiome of healthy hosts.

KEYWORDS DNA damage, bacterial stress response, intestinal colonization, microbe-host interaction, microbe-microbe interactions

Bacteria have a plethora of stress response pathways that enable them to rapidly and effectively respond to changes in their environment. One such stress response pathway, the SOS response, is activated by the presence of DNA damage and mediates bacterial survival by promoting repair of the damaged DNA (1). The transcriptional repressor of the SOS response is the dual-functional repressor-protease LexA (Fig. 1A). In the absence of DNA damage, LexA binds to promoters upstream of SOS-regulated genes preventing their transcription. When DNA is damaged, RecA, the sensor of the DNA damage, is activated and stimulates LexA to undergo self-cleavage, relieving its transcriptional repression of SOS-controlled genes (2, 3). The SOS regulons differ across bacterial species, but core genes typically include those responsible for repairing or tolerating DNA damage (4). Importantly, DNA repair can occur in phases, with early high-fidelity repair followed by the activity of lower-fidelity damage tolerance pathways involving translesion DNA polymerases (5, 6). The SOS response can also impact larger

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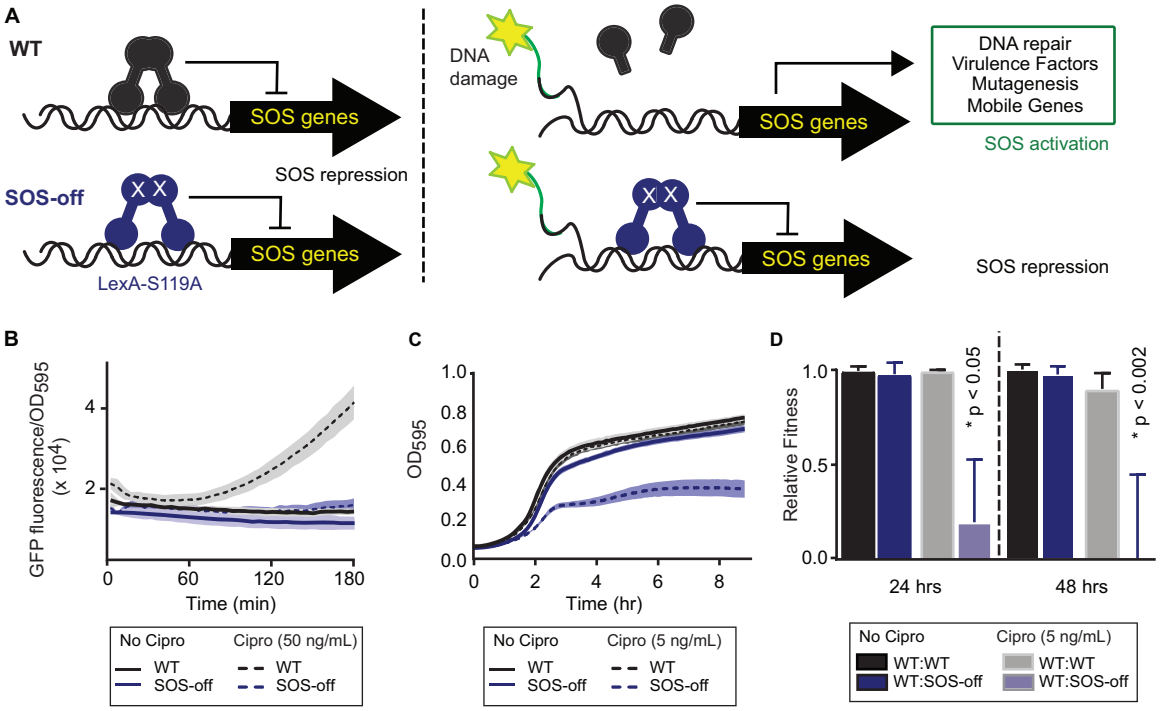

FIG 1 The SOS pathway contributes to survival in the presence of DNA damage. (A) Schematic of the SOS response. In the absence of DNA damage, LexA acts as a repressor for the SOS response. DNA damage leads to self-cleavage of LexA, activating the response in wild-type strains but not in an SOS-off mutant with a catalytically inactive LexA. (B) SOS reporter assay. The WT or SOS-off strains with a SOS reporter plasmid, containing GFP under the control of the *recA* promoter, were examined in the presence or absence of ciprofloxacin (Cipro). The time-dependent induction of GFP is represented as the fluorescence intensity normalized to the optical density at 595 nm (OD_{595}), with error bands showing the standard deviations of results from three independent biological replicates for each condition. (C) Growth of cells in the absence or presence of subtherapeutic levels of ciprofloxacin. Optical density at 595 nm was measured at 10-min intervals at 37°C, with error bands showing the standard deviations of results from four independent biological replicates for each condition. (D) Relative fitness levels were evaluated in competition experiments between GFP or mCherry-labeled WT:WT strains and WT:SOS-off strains in the presence or absence of ciprofloxacin at 24 h and 48 h. The mean fitness level of each strain was calculated from two independent competition experiments. No colonies were detected in the SOS-off mutant at 48 h with ciprofloxacin treatment, with the top of the error bar representing the limit of detection. The *P* values reported for the WT:SOS-off competition are based on a two-tailed unpaired Student's *t* test.

genomic changes through its regulation of conjugative elements (7, 8). These DNA repair and diversifying functions likely contribute to the role of the SOS response in bacterial adaption to external stressors, including resistance to antibiotics (9–12).

Outside its canonical function, recent research has demonstrated for a wide range of pathogens that the SOS response may play broader roles in bacterial pathogenesis and virulence. For example, SOS induction enhances expression of fibronectin binding protein in *Staphylococcus aureus* (13), contributes to biofilm production in *Pseudomonas aeruginosa* (14), and regulates Shiga toxin (Stx) and cholera toxin in *Escherichia coli* O157:H7 (15–17) and *Vibrio cholerae*, respectively (18, 19). Further, SOS induction may play an important role in interspecies competition that predominates in the natural environment of the host. For example, colicins are bacteriocins produced by *Enterobacteriaceae* that kill phylogenetically similar relatives and many colicins are intimately connected with the SOS response (20–22). Among the sequenced enteric bacteriocin promoters, over 75% of colicins are regulated by the SOS response (21) and many are induced by DNA damaging antibiotics (22). While much of the work has focused on pathogens, our understanding of the SOS response in commensal bacteria remains limited and there is an increasing need to fill this gap given the integral role commensals play in health and disease.

Although there is growing recognition of more-diverse roles for the SOS response, how the SOS response is induced inside a host organism remains an open area of investigation. Largely, the historical focus on this pathway has related to single and

often synthetic exogenous stressors, such as UV light or antibiotics, even though the pathway has likely evolved to deal with the multiple stressors naturally found in a living host environment. Studies highlighting a role for the SOS response in pathogenesis have shown that host environments can be relevant stressors for some model pathogens. SOS activation increased tissue adhesin and biofilm formation in a uropathogenic *E. coli* (UPEC) virulence model (23), and inactivation of the SOS response or specific SOS effectors decreased bladder colonization in a mouse model (24, 25). Interestingly, while SOS activation is required for cholera toxin prophage induction *in vitro* (19), induction of cholera toxin prophage was not observed in an infant mouse model of *V. cholerae* infection. Additionally, equal numbers of a wild-type (WT) strain and SOS-inactive strains were recovered 24 h postinfection, thus leading the authors to conclude that the infant mouse gut is not a potent stimulus for the SOS response (26).

The mammalian gut has been posited as an environment that might induce the SOS response. In the gut, both host factors and competing microbes are potential sources of DNA damage. On the host side, for example, various bile salts increase the expression of SOS induced genes *ex vivo* (27). Additionally, pathogen-associated gut inflammation in a germfree mouse model provided a DNA damaging stimulus that induced the prophage associated with Stx production in *E. coli* O157:H7 (28). On the microbial side, Stx production is increased in the presence of colicin-producing bacteria (29).

While studies performed with pathogens have suggested that the SOS response mediates some of the interplay between the host and bacteria, the role of the SOS response in colonization remains unknown. The mediators of colonization are particularly important to decipher, as the mammalian gut is a rich environment for microbial interactions and the colonizing microbiome has been linked to both bacterial and nonbacterial diseases. To address this gap, we utilized a natural *E. coli* isolate, MP1 (30), to directly examine the role of the SOS response during sustained colonization of the murine gut in the absence of exogenous factors that could perturb host responses and microbial diversity.

We demonstrate that an SOS-off mutant is compromised relative to the wild-type strain in sustained colonization using a competitive cocolonization model. Additionally, we addressed the relative contributions of host inflammatory responses versus the competing microbial communities in explaining the impact on colonization observed with the SOS-off mutant. Our results demonstrate the importance of the SOS response for maintenance of colonization of nonpathogenic *E. coli*. This conclusion implies that genotoxic stressors are likely continually at play in the gut and that the SOS response could contribute to genomic and population plasticity even in a healthy microbiome.

RESULTS

SOS deficiency compromises *in vitro* fitness of MP1 in the presence of DNA damaging agents. The role of the SOS response in complex host environments is not well established. Here, we focused on the murine gut and MP1, an *E. coli* strain that is a natural mouse colonizer and is amenable to genetic manipulation. This strain can also achieve stable colonization in the absence of continuous antibiotic treatment, a departure from the standard colonization model (31, 32). This MP1 model has been used previously to establish the importance of selected two-component signaling systems in sustained colonization and to demonstrate a critical role for bacterial nitrogen production in modifying the gut microbiome (30, 33).

To decipher the impact of the SOS pathway, we engineered an MP1 derivative with its native *lexA* locus replaced with an inactive *lexA*S119A allele. LexA-S119A has a point mutation in the catalytic residue of the serine protease domain that prevents self-cleavage (34), keeping LexA in the DNA-bound state and rendering the SOS response constitutively off (Fig. 1A). To confirm this phenotype, we first examined SOS induction using a reporter plasmid which places the green fluorescent protein gene (*gfp*) under the control of the SOS-inducible *recA* promoter (35). Using this system, in the presence of ciprofloxacin as the DNA damaging agent, the wild-type strain showed inducible expression of GFP whereas the SOS-off mutant showed no response (Fig. 1B).

It has previously been established that inactivation (S119A) of LexA in *E. coli* MG1655 was not associated with a measurable defect in cell growth or fitness in the absence of DNA damage (11). There was, however, a notable growth defect in the presence of DNA damage. We compared the wild-type strain and the corresponding SOS-off derivative in similar settings to determine if they demonstrated similar phenotypes in MP1. In the absence of DNA damaging stress, the two strains had similar growth kinetics (Fig. 1C). However, in the presence of subtherapeutic ciprofloxacin levels, growth was stunted in the SOS-off mutant but not in the wild-type strain (Fig. 1C). To more rigorously compare the strains, we performed fitness competition experiments (36). We introduced the SOS-off allele into a MP1 derivative marked with a tetracycline resistance cassette and mCherry under the tight control of a *tet* promoter (30). We partnered this SOS-off strain with a WT MP1 derivative containing GFP under the control of the *tet* promoter to allow facile discrimination of the WT and SOS-off strains in a competition experiment using these fluorescent markers. In the absence of DNA-damaging stress, no fitness defect was observed for the SOS-off mutant relative to wild-type strain at either 24 or 48 h (Fig. 1D). In the presence of sublethal DNA-damaging stress, the SOS-off mutant was significantly defective at 24 h and completely outcompeted by the wild-type strain, with no detectable colonies, by 48 h. Thus, as anticipated, the SOS-off mutant has fitness comparable to that of the wild-type strain in the absence of DNA damaging stress *in vitro* but is compromised in the presence of DNA damaging stress.

The SOS response is important for robust growth in the mouse gut. Establishing the reliability of the strains *in vitro* allowed us to perform competition experiments in the murine gut. Most mouse colonization studies have employed streptomycin-resistant strains of bacteria, with mice being fed streptomycin continually in their drinking water. In those experiments, streptomycin aided in overcoming colonization resistance (37, 38) and allowed for sustained expansion of strains not typically found in the mouse gut because a significant portion of the competing flora was eliminated (38, 39). The MP1 colonization model, by contrast, employs a brief (72-h) pretreatment with streptomycin followed by a 24-h washout period. Previous work has shown that the normal flora rebounds within 5 to 6 days following streptomycin pretreatment (40), allowing us to use this system to address whether sustained colonization in the gut depends upon the SOS response. Further, eliminating continual streptomycin treatment allowed us to understand the contribution of the SOS response to colonization in the absence of a confounding antibiotic stressor during the course of the experiment (41).

We first orally inoculated groups of CD-1 female mice with equal mixtures of a SOS-off mutant *mcherry*-marked strain and a wild-type *gfp*-marked strain. In a control cohort, we performed parallel experiments with equal mixtures of two different wild-type strains marked either with *mcherry* or with *gfp*. To understand the colonization dynamics, we quantified the initial inoculum and subsequently collected fecal samples at the start and at various days postinoculation until a set endpoint was reached. At each time point, mass-normalized fecal samples were serially diluted and plated onto LB media containing tetracycline (Fig. 2A). Tetracycline permits the selection of MP1 strains from the colonizing milieu of competing bacteria in feces and induces the expression of the fluorescent markers, permitting us to measure the total CFU levels and the relative distributions of the GFP-marked versus mCherry-marked strains. To quantify the fitness of the strains, we calculated the competitive index (CI) by taking the ratio of input CFU counts to output CFU counts. In the control cohort, the total CFU count reached levels of $\sim 10^{12}$ per g stool in the day immediately following inoculation (see Fig. S1 in the supplemental material). After 1 week, the total CFU count stabilized at $\sim 10^{5}$, representing the establishment and maintenance of the population. These levels were maintained for at least 4 weeks, demonstrating the strength of the MP1 model system for evaluating sustained colonization. Throughout the experimental time points examined, as expected, the GFP-marked wild-type versus mCherry-marked wild-type strains displayed equal fitness levels (Fig. 2B). However, for the wild-type versus SOS-off mutant, significant differences emerged. The results of comparisons of

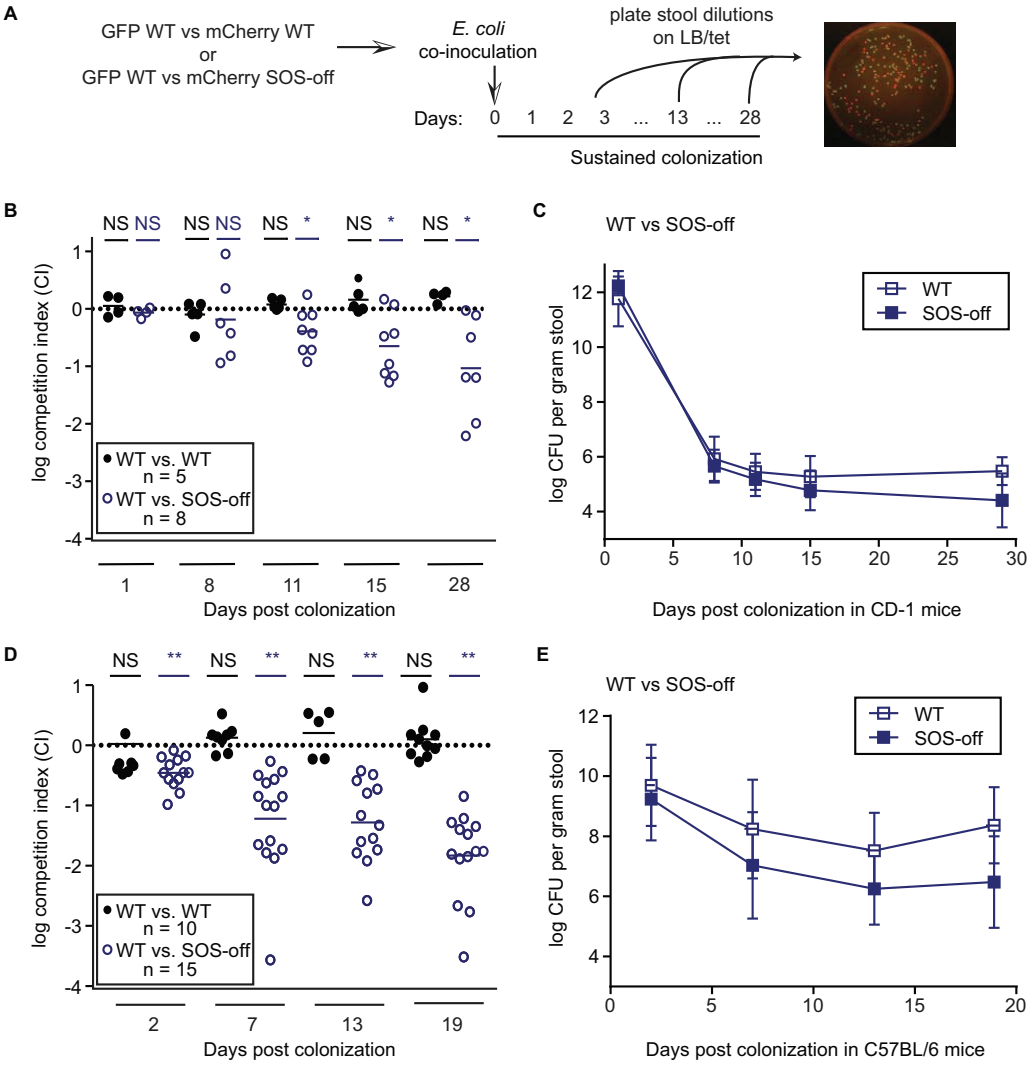

FIG 2 Colonization of the adult mouse gut. (A) Schematic of colonization protocol. Mice were inoculated with equal mixtures of *E. coli* strains, and fecal samples were collected at various days postinoculation. A representative image with colonies marked with GFP or mCherry is shown. tet, tetracycline. (B to E) Groups of 6-to-8-week-old CD-1 female mice (B and C) or 6-to-8-week-old C57BL/6 male mice (D and E) were coinoculated with equal mixtures of either WT:WT strains (black filled circles) or WT:SOS-off strains (blue open circles). (B and D) Log competitive index (CI) values were calculated as the ratio of output colonies normalized to the input ratio. Each circle indicates a specific animal. Significant *P* values are noted (NS, not significant; *, <0.01; **, <0.001) and were calculated using a one-sample *t* test. If colonies were too numerous to count on the plate, the corresponding animal was excluded from the data for that day. (C and E) CFU counts per gram of stool for the WT:SOS-off competition only. The analogous data from the WT:WT competition are shown in Fig. S2. Each square represents the mean and standard deviation for results from all individual mice. The limits of detection were 10^2 for the CD-1 mice and 10^3 for C57BL/6 mice.

the proportion of WT versus the proportion of SOS-off remained nonsignificant (ns) up to day 8 (log CI, −0.17, ns). By day 11, however, the SOS-off mutant had a significant colonization defect (log CI, −0.4, $P < 0.01$), which increased with each subsequent time point. At day 28, the SOS-off mutant was outcompeted by the wild-type strain by over 10-fold (log CI, −1.2, $P < 0.001$), reflecting a progressive decrease in the CFU level of the SOS-off strain, while the wild-type strain level was sustained (Fig. 2C). Collectively, these experiments suggest that although the SOS-off mutant can establish colonization, it has reduced sustained colonization capacity relative to the wild type.

Immune responses and microbiota composition can vary with mouse models and mouse gender. To explore the generality of our result, we next examined competition between wild-type and SOS-off mutant strains in a C57BL/6 male mouse model. In the control experiments with WT:WT strain competition, we observed a more modest initial

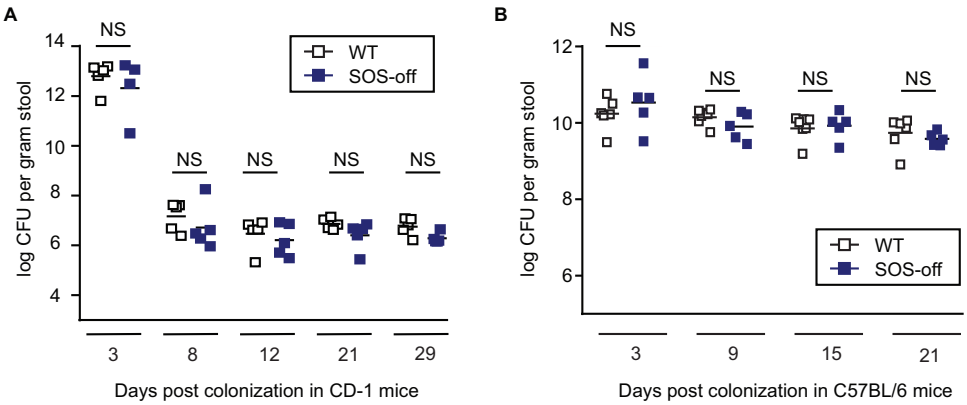

FIG 3 Solo colonization of the adult mouse gut. Groups of 6-to-8-week-old CD-1 female mice (A) or 6-to-8-week-old C57BL/6 male mice (B) were orally inoculated with either the wild-type strain (black open squares) or the SOS-off mutant (blue filled squares). Fecal samples were collected on various days postinoculation, and the bacterial CFU counts per gram of stool for each strain on a given day are shown. Each symbol represents one animal, and the limit of detection was 10^2 for CD-1 mice and 10^3 for C57BL/6 mice. NS, statistically insignificant difference as determined by two-tailed unpaired Student's *t* test.

drop in CFU and higher sustained colonization levels than were seen with the CD-1 model. The initial CFU was $\sim10^9$ per g stool, and the CFU dropped to $\sim10^7$ but maintained those levels throughout the duration of the experiment, representing an $\sim10^2$-higher level of colonization than was observed in CD-1. As with the experiments in CD-1 mice, we captured the colonization kinetics by taking fecal samples throughout the experiment, and we determined the bacterial load and calculated the CI for each time point (Fig. 2D). In C57BL/6 mice, by day 2 the SOS-off mutant was outcompeted by the wild-type strain (log CI, -0.45, $P < 0.001$). Further, by day 7 it was outcompeted by >10-fold (log CI, -1.2, $P < 0.001$). By the endpoint evaluated (day 19), the SOS-off mutant was outcompeted by >65-fold (log CI, -1.9, $P < 0.001$), suggesting a greater overall impact on sustained colonization in the C57BL/6 model than in the CD-1 model. As with the CD-1 colonization model, the CI change was a result of decreasing CFU with the SOS-off mutant rather than of increasing CFU with the wild-type strain (Fig. 2E). Although there were differences regarding total bacterial burden and the extent of competition defect, the results were similar in the two models, suggesting that the reduced colonization capacity of the SOS-off mutant may not be specific to one strain or gender of mouse.

The SOS-off mutant can independently colonize the murine gut. In the experimental design, it is believed that the streptomycin pretreatment opens a niche to allow the MP1 strains to overcome colonization resistance. We considered whether the competition defect seen in comparisons between the wild-type and SOS-off strains was a product of direct competition between the two strains in the same niches or whether the SOS-off mutant is defective for sustained colonization of the gut in isolation. To distinguish between these possibilities, two separate groups of CD-1 female mice or C57BL/6 male mice were orally inoculated with either the wild-type strain alone or the SOS-off mutant alone. In each system, the initial kinetics after inoculation were similar to those observed in the competition experiment, and CFU counts subsequently stabilized in the gut within 1 week. Notably, however, in both CD-1 and C57BL/6 mice, colony counts for both the wild-type strain and the SOS-off mutant remained stable at >4 weeks and there was no statistical difference between the colonization levels of the SOS-off mutant and the wild-type strain (Fig. 3). Stable colonization of the SOS-off mutant alone suggests that direct competition with the wild-type strain occurred in the coinoculation model and that this direct competition reduced the colonization capacity of the SOS-off mutant.

Acute inflammation does not exacerbate the colonization defect. In the *in vitro* competition experiments, in the absence of DNA damaging stress, the SOS-off mutant did

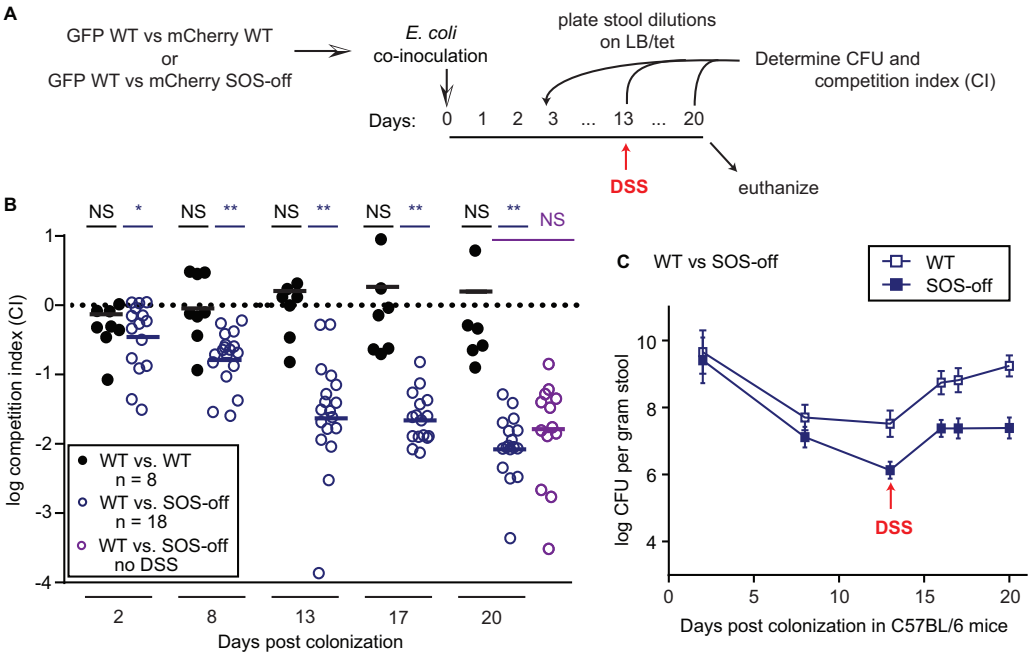

FIG 4 Effect of host inflammation on colonization. (A) Schematic of colonization protocol with DSS. Mice were inoculated with equal mixtures of either WT:WT or WT:SOS-off strains. At 13 days postinoculation, 4% DSS was administered in the water. Mice were allowed to drink *ad libitum*. Fecal samples were collected on various days until the mice were euthanized. (B) Groups of C57BL/6 male mice were inoculated with WT:WT strains (black filled circles) or WT:SOS-off strains (blue open circles). Log competition index data are shown for each competition. The purple circles represent mouse data replicated from Fig. 2 (from mice that did not receive DSS treatment) for ease of comparison. Each circle indicates a specific animal. Significant *P* values are noted (NS, not significant; *, <0.01; **, <0.001), calculated using a one-sample *t* test. (C) CFU counts per gram of stool from the WT:SOS-off competition only. The arrow indicates the start of continuous DSS administration. The analogous data from the WT:WT competition are shown in Fig. S3. Each square represents the mean and standard deviation of the results from all individual mice.

not have a fitness defect relative to the wild-type strain, but in the presence of DNA damage a fitness defect manifested (Fig. 1D). This implies that the SOS-off strain was subject to a source of genotoxic stress in the mouse gut that impacted sustained colonization. To examine the source of the environmental stress, we next explored two sources of genotoxic stress: host inflammation and competition between commensal microbes.

We reasoned that one way to assess the impact of host inflammation would be to chemically induce acute gut inflammation using dextran sulfate sodium (DSS). This well-established model promotes acute colitis with an increase in levels of cytokines, chemokines, and nitric oxide, all of which could be a source of genotoxic stress for bacteria in the gut (42, 43). A major characteristic of DSS-induced inflammation is an outgrowth of *Enterobacteriaceae*, presumably occurring because *E. coli* can efficiently utilize nitrates and formate to outcompete other bacteria in the gut (44, 45). To examine the impact of DSS, we first precolonized C57BL/6 male mice with equal mixtures of either the GFP-marked: mCherry-marked WT:WT strains or the WT:SOS-off strains. Colonization was sustained for 13 days, at which point we administered 4% DSS in the drinking water. The mice were allowed to drink the water *ad libitum*. We collected fecal samples (Fig. 4A) until the experiment was terminated at the disease activity endpoint as determined by clinical criteria (46). The presence of significant DSS-induced inflammation was confirmed by disease activity index, gross colon examination, and histology (Fig. S2).

In the WT:WT control experiment, no competition was observed out to day 13 (log CI, 0.2, ns) (Fig. 4B). Administration of DSS resulted in a bloom of bacterial counts, with a 2-log increase in the CFU 3 days after DSS administration (Fig. S3) and no significant difference in the CI (log CI, 0.12, ns) (Fig. 4B). In the WT:SOS-off competition, the strains tracked as expected, and by day 13 there was an ~10-fold difference in CFU (10^7 versus 10^8 log CFU/g stool) and the associated log CI value (log CI, −1.5, $P > 0.001$). Impor-

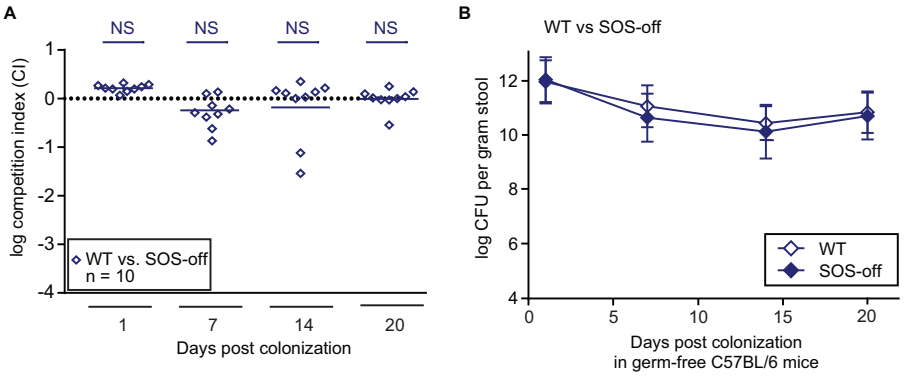

FIG 5 Colonization of the adult mouse gut in a germfree setting. Groups of 6-to-8-week-old germfree C57BL/6 mice were orally inoculated with equal mixtures of WT:SOS-off strains. Fecal samples were acquired on various days postinoculation and at various days the (A) log competitive index and (B) CFU per g stool were determined. NS, not statistically significant as determined by one-sample *t* test.

tantly, after DSS administration blooms were also observed in both strains: by 3 days after DSS administration, the wild-type strain CFU had increased by ~2 logs and the SOS-off mutant CFU had also increased by ~2 logs (averaged across the observed mice). Thus, despite the induction of acute inflammation by DSS and the associated inflammatory mediators, the SOS-off mutant CFU was able to expand *in vivo* to a degree comparable to that seen with the wild type. When the experiment was terminated due to disease burden, the wild-type strain was found to have outcompeted the SOS-off mutant to a greater extent than prior to DSS treatment (log CI, −2.0, $P >$ 0.001) (Fig. 4B); however, the fitness defect in the inflamed gut was not statistically different from the fitness defect we had observed in the healthy gut as described above (log CI of −2.0 and −1.9, respectively) (Fig. 4B). Notably, in this experimental setup, we also aimed to confirm that the feces samples represented an accurate reflection of colonization in the tissues. We collected cecal contents from the mice at the time of sacrifice and plated them for bacterial counts. The cecal CFU patterns observed were consistent with those determined with the feces samples, confirming that feces samples represent a reliable surrogate of the gut colonization (Fig. S4). Taking the results together, DSS-mediated inflammation did not amplify the defects in colonization by the SOS-off mutant to a significant extent.

Eliminating endogenous microbes enhances fitness of SOS-off strain. The microbiome is able to dynamically shift based on metabolic conditions or disease states, suggesting that active competition between microbes is an ongoing phenomenon. As such, we hypothesized that the gut microflora could be contributing to the reduction in the sustained colonization capacity of the SOS-off mutant relative to the wild type. To test this possibility, we orally inoculated germfree C57BL/6 mice with equal mixtures of the SOS-off mutant and the wild-type strain and analyzed feces over a ~3-week period. In this setting, colonization differs from that in the standard C57BL/6 model in that there is no need for streptomycin pretreatment. After inoculation, the total CFU reached levels of ~10^{12} and declined only to levels of ~10^{10} to 10^{11} over the course of the experiment (Fig. 5B). In contrast to the standard model, where a time-dependent decline in the CFU counts of the SOS-off strain was observed, in this germfree setting, the wild-type and SOS-off strains had similar CFU counts throughout the experimental time course, and the log CI values did not differ significantly from zero, indicating that there was no competition defect. To confirm that the absence of a competition defect was not due to differential rates of strain shedding from the colon, we determined the CFU of the wild-type strain and the SOS-off mutant directly from cecal contents at the last time point. Once again, the differences were not statistically significant (Fig. S4). Thus, while the wild-type strain outcompeted the SOS-off mutant in a standard colonization model, having a functional SOS response was not required for sustained

colonization in the germfree model, suggesting that the source of genotoxic stress requiring a functional SOS response was associated with an intact gut microbiome.

DISCUSSION

The SOS response is a vital stress response pathway that has long been studied in well-defined laboratory settings. Those studies have been invaluable with respect to our understanding of the molecular basis of the SOS response and its role in regulating DNA repair and genomic diversity. However, they offer only limited insight into the relationship between the SOS response and complex physiological environments. Attempts to examine the SOS response in a natural host have mainly focused on pathogens (24, 26) or specific gene products of the SOS regulon (25, 47–49). Collectively, those studies have suggested that the SOS response is an important component for successful bacterial interaction with the surrounding environment. However, knowledge of the broader role of the SOS response in a commensal strain in a host environment was lacking. Significantly, our results demonstrate that the SOS response is important for sustained colonization of a commensal *E. coli* strain in the murine gut. In two different mouse models (CD-1 and C57BL/6), the wild-type strain outcompeted the SOS-off mutant in colonization. Interestingly, in solo colonization experiments the SOS-off mutant was recovered at counts statistically similar to those seen with the wild type, suggesting that the competing strains occupied the same niche in this model and that competition was important to elicit the quantitative differences between strains. This is the first report demonstrating that the SOS response is important for sustained colonization of a commensal *E. coli* strain in the murine gut.

To more thoroughly capture the dynamic process of colonization, we took a kinetic approach by monitoring bacterial burden and calculating CI continuously throughout the experiment. Taking this kinetic approach, we drew two major conclusions. First, our data imply that the bacteria were subject to low levels of DNA-damaging stress as they interacted in the gut microenvironment. Data from the *in vitro* experiments suggest that the growth and fitness defect of the SOS-off mutant was apparent only in the presence of a DNA damaging agent. Notably, prior work in MG1655 also demonstrated that for stressors not associated with DNA damage, the SOS-off strain had fitness comparable to that of a wild-type strain (11). In our experiments, in contrast to results with competition, during solo colonization the SOS-off mutant was recovered at a CFU level statistically similar to that of the wild type. We posit that the fitness burden was enhanced in the presence of a wild-type competitor because the wild-type strain was able to respond more effectively to the low-level DNA damage. Thus, in the competition setting, the SOS-off mutant can initiate colonization but cannot maintain it relative to the wild-type strain. It is possible that if the time frame of the solo colonization experiment had been extended there would have been an eventual decline in the SOS-off colony counts in the absence of competition.

Second, the colonization kinetics suggest that the SOS response might be more important for maintaining colonization than for initiating colonization. In the competition experiments, although the kinetics differed slightly between the CD-1 and C57BL/6 mice, both the wild-type strain and the SOS-off mutant were recovered throughout the experiments, but there was a subsequent time-dependent decline of the SOS-off mutant CFU. The more limited effect during the first 24 h aligns with prior results in *Vibrio cholerae* (26), where wild-type *V. cholerae* and a SOS-off mutant strain were recovered in equal numbers at 24 h. Such limitations in distinguishing initiation effects versus maintenance effects have previously also been observed in the study of nitric oxide-associated stress in a *V. cholerae* infant mouse model (50). The results of short-term colonization with *V. cholerae* and in our study, however, differ from those observed with UPEC, where initial colonization of the urinary tract was compromised in an SOS-deficient UPEC strain (24, 25). These distinct findings illustrate that different host environments may have different SOS response requirements. Equally importantly, these findings highlight potential differences in mechanisms that might be at play in pathogens versus commensal strains.

For a resident microbe, the gut is a complex environment where there is an interplay between host factors and competing microbes. To examine the effect of perturbing the host environment, we used DSS to induce inflammation. Inflammation is often associated with an increase in levels of DNA damaging stressors such as reactive nitrogen, oxygen, and hypochlorite, and DSS has been linked to the induction of nitric oxide synthetase activity as bacteria breach the mucus layer (51, 52). However, DSS-induced inflammation had no effect on competition. We speculate that either the strains were not directly subjected to this associated inflammatory response or, as host-adapted strains, they had evolved means to adequately handle these inflammatory responses independently of the SOS response. In this regard, prior work presented some stimulating results for further consideration in the context of our studies. In *Salmonella*, an increase in phage transfer was noted to be dependent upon an inflammation-induced SOS response (53, 54). Interestingly, when mice defective in either NO synthase-NADPH oxidase or myeloperoxidase were examined, levels of reactive oxygen species, nitrogen species, and hypochlorite production were found to have decreased, and yet phage transfer occurred at levels similar to those observed with the wild-type mice. These results suggest that SOS-inducing stress associated with inflammation could have been coming from alternative sources, including competing microbes, rather than the host itself.

In germfree mice, no competitive advantage was observed for the wild-type strain relative to the SOS-off mutant. We envision at least two scenarios that can explain this result which are not mutually exclusive. First, given the absence of established competing flora in the germfree model, the *E. coli* strains expanded into niches where host sources of DNA damage are minimal. In line with this possibility, the higher levels of colonization suggest the possibility that alternative niches not present in the standard model could dominate the population counts in the germfree model, making the fitness defect undetectable. Second, it is possible that competing gut microbes are a potential source of genotoxic stress in the standard model and absent in the germfree model. This explanation aligns with prior literature where SOS-controlled effectors, such as Shiga toxin, were reported to show increased production in the presence of other microbes (29). The fact that the defect in the SOS-off mutant was apparent only in the setting of competition experiments in the intact microbiome model suggests that different factors may be at play when a narrow niche is opened by streptomycin pretreatment in the standard model versus the wide-open sterile gut in gnotobiotic experiments. These possible differences have implications for the streptomycin-treated mouse model, where streptomycin is maintained throughout the duration of the experiment to sustain a colonizing strain. As streptomycin eliminates a significant portion of the microbial diversity in the gut (38), this model risks masking possible contributions from microbes that are eliminated by this sustained selection.

The SOS response provides many diverse functions for the bacteria and, consequently, multiple effectors could be contributing to sustained colonization of the gut. Given its canonical function in DNA damage repair and tolerance, the inability to rapidly repair the damage could contribute to the fitness defect of the SOS-off mutant. However, noncanonical functions could also be relevant in the colonization model. Interestingly, prior work in the streptomycin-treated model has suggested that colicin-producing bacteria better sustain long-term colonization of the mouse gut (55). MP1 harbors a plasmid with a colicin gene that contains LexA-binding motifs in its promoter. While we did not aim to isolate the key SOS effectors that mediate sustained colonization, it is conceivable that colicins may play a role. Moreover, given the diverse and complex nature of the SOS regulon, it is probable that multiple SOS-controlled effector proteins are responsible for the colonization defect of the SOS-off mutant.

Our results indicate that the SOS response contributes to the full fitness of a commensal bacterium in the context of a natural gut environment. Beyond its implications in colonization, this finding has added significance with regard to the generation of genomic diversity in the gut. The SOS response is known to be a major driver of mutagenesis via its DNA damage tolerance mechanisms and is associated with the movement of larger blocks of DNA via control over phage induction and integrons (8,

56). Our results imply that commensal bacteria are subject to genotoxic stress that requires activation of the SOS response, even in the healthy microbiome. In the absence of external stressors such as antibiotics, these commensal organisms may still trigger genome-diversifying activities that could promote the acquisition of mutations or genes associated with antibiotic resistance. Thus, the relevance of stress responses and potential opportunities for targeting these responses clinically could extend beyond acute infections to chronic settings that may be predisposed for infections to occur.

MATERIALS AND METHODS

Congenic strain generation. The GFP-marked and mCherry-marked MP1 strains, also known as MP7 and MP13, were previously described and used for strain construction and competition experiments (30). The SOS-off mutant was constructed in two steps, using the close linkage of *lexA* with *malE*. First, *malE* was deleted using P1vir transduction from the Keio collection. Strains were confirmed by PCR and by their inability to grow in the presence of maltose as the only carbon source. Second, a previously generated MG1655 *lexA*S119A strain, encoding LexA with a mutation in the catalytic serine that renders it noncleavable, was used to introduce the mutant *lexA* allele (11). The *lexA*S119A allele was introduced into the Δ*malE* strain by P1vir transduction from lysate derived from MG1655 *lexA*S119A. Strains with a restored *malE* gene were verified by their ability to grow on maltose as the only carbon source, and integration of the *lexA*S119A allele was confirmed by PCR and sequencing. The study strains are available upon request.

***In vitro* assays.** In the SOS reporter assay, induction of the SOS response was monitored by reporter plasmids containing GFP under the control of the *recA* promoter as described previously (3). Briefly, bacteria were transformed with GFP-reporter plasmids and cultured in defined media containing 1× M9 salts (Sigma M6030), 0.4% glucose, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.05% Casamino Acids, and 30 μg/ml of kanamycin to maintain the plasmid. Overnight cultures were diluted 1,000-fold into fresh media, incubated with shaking at 37°C, and grown until absorbance at an optical density at 595 nm (OD_{595}) of ~0.3. At that point, 100-μl aliquots were dispended into a 96-well, round-bottom, transparent plate. DNA damage was induced with 50 ng/ml of ciprofloxacin dissolved in phosphate-buffered saline (PBS), and an equivalent amount of PBS was added to control wells. The plates were incubated at 37°C, and GFP relative fluorescence unit (RFU) and OD_{595} data were acquired every 5 min for 180 min on a Tecan Infinite F200 Pro multifunctional plate reader, agitating before every data acquisition cycle.

Growth rates were measured as described previously (11). Briefly, overnight cultures were diluted 1,000-fold into fresh LB in the presence or absence of sublethal concentrations of ciprofloxacin (5 ng/ml) and distributed into 96-well, round-bottom, transparent plates. Cultures were incubated at 37°C with cycled agitation, and OD_{595} measurements were taken continuously.

The competition assay was adapted from established protocols (11). Briefly, overnight cultures of the *gfp*-tagged or *mcherry*-tagged strains were standardized by optical density. The strains were mixed at a 1:1 ratio and diluted 10^6-fold in 3 ml of LB in either the absence or the presence of ciprofloxacin (5 ng/ml). The coculture was incubated overnight at 37°C with aeration for 24 h. The next day, the overnight culture was diluted 10^6-fold and reinoculated into fresh LB and grown for an additional 24 h. To determine the CFU count for each strain, cultures samples were taken at time zero, after 24 h, and after the 48-h growth period; plated onto LB agar–15 μg/ml of tetracycline; and incubated overnight at 37°C. Plates were imaged using a previously described system that permits detection of GFP and mCherry (57). Relative fitness data were then calculated by comparison of the starting population to the population at each time point, according to the formula of Lenski and coworkers (36).

Competition or solo colonization experiments. All animal studies were carried out in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania. Animal protocols followed the guidelines established within the *Guide for the Care and Use of Laboratory Animals* (58).

Experiments were performed with 6-to-8-week-old pathogen-free CD-1 female mice purchased from Jackson Laboratories or pathogen-free C57BL/6 male mice purchased from Charles River Laboratories. Germ-free C57BL/6 mice were maintained in plastic isolator units and fed autoclaved chow and water. Each cage contained 4 to 5 mice. In the standard protocol, to overcome colonization resistance, the mice were provided 5 g/liter streptomycin and glucose in their drinking water for 72 h. Fresh water, without antibiotic and glucose, was then given to the mice for 24 h prior to oral inoculation with *E. coli* strains, and the mice were maintained on antibiotic- and glucose-free water for the remainder of the experiment. For germfree experiments, no streptomycin pretreatment was performed. For the inoculum, bacterial cells were prepared by picking a single colony from an LB agar plate and grown overnight with aeration at 37°C in LB. The following day, OD_{595} was measured using a 1:10 dilution of the overnight culture and the concentration of cells was calculated. Cells were spun down at 3,800 × *g* at 4°C and resuspended in cold phosphate-buffered saline (PBS). Cells were washed twice with PBS, and, after the final wash, cells were resuspended in a volume of PBS that equaled ~10^{10} to 10^{11} cells/ml. To start the competition experiment, the cell suspensions were mixed 1:1 and mice were orally inoculated by gavage with 100 μl of the mixture. Solo colonization experiments were performed by inoculation with 100 μl of the bacterial suspension. A portion of the inoculum was serially diluted and plated on LB agar with 15 μg/ml of tetracycline to determine the input CFU. Throughout the experiments under standard conditions, mice were raised on a standard laboratory rodent diet (LabDiet 5001). For experiments with the colitis model, at day 13 mice were given 4% dextran sulfate sodium (DSS; Affymetrix) (molecular weight, 40 to 50 kDa)

in water and were allowed to drink *ad libitum*. The disease activity score was determined daily as described previously (46). The disease activity score determined when the mice were euthanized. For histopathology performed on the DSS-treated and untreated mice, tissues were fixed in formalin and then processed at the University of Pennsylvania Comparative Pathology Core. Samples were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and reviewed by a veterinary pathologist.

Determination of *E. coli* CFU. At each time point of interest, 3 to 4 fecal pellets were obtained from each mouse. The fresh feces samples were weighed and resuspended in PBS as a slurry to reach a final concentration of roughly 0.5 g of feces per 1 ml PBS. The samples were serially diluted and plated on LB with 15 μg/ml tetracycline. Fluorescence images of plates were obtained as described above. The competitive index (CI) was determined as {[(mCherry fluorescent CFU)/(GFP fluorescent CFU)]/[(input mCherry CFU)/(input GFP CFU)]}, where the input CFU was determined from the inoculum.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/IAI.00711-18.

SUPPLEMENTAL FILE 1, PDF file, 0.5 MB.

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