Preventing the Activation of a Stress Gene Response in *Escherichia coli* Using Acetate, Butyrate, and Propionate

Kaylee M. Weigel, Kathleen M Ruff-Schmidt*, Birgit M. Prüß, & Danielle L J Condry**

Department of Microbiological Sciences, North Dakota State University, Fargo, ND

https://doi.org/10.33697/ajur.2022.065

Student email: kaylee.weigel@ndsu.edu, kathleen.schmidt@ndsu.edu
*Mentor email: birgit.pruess@ndsu.edu, danielle.condry@ndsu.edu**

**Authors contributed equally*

ABSTRACT
Regulation of microbial symbiosis in the human intestinal tract is imperative to maintain overall human health and prevent dysbiosis-related diseases, such as inflammatory bowel disease and obesity. Short-chain fatty acids (SCFA) in the intestine are produced by bacterial fermentation and aid in inflammation reduction, dietary fiber digestion, and metabolizing nutrients for the colon. SCFA, notably acetate, butyrate, and propionate, are starting to be used in clinical interventions for GI diseases. While acetate has been shown to mitigate a stress response in the proteome of *Escherichia coli* cells, little is known about the effects of butyrate and propionate on the same cells. This study aims to evaluate the effects that butyrate and propionate have on the activation of stress promoters in *E. coli* when induced with a known stressor. Three different strains of *E. coli* containing the pUCD615 plasmid were used, each with a different promoter fused to the structural genes of the *lac* operon on the plasmid. Each promoter detected a unique stress response: *grpE'::lux* fusion (heat shock), *recA'::lux* fusion (SOS response), and *katG'::lux* fusion (oxidative damage). Activation of these stress promoters by treatment groups resulted in the emission of bioluminescence which was quantified and compared across treatment groups. All three SCFAs at 25 mM added to cultures prior to stressing the bacteria caused significantly lower bioluminescence levels when compared to the stressed culture without prior addition of SCFA. This indicates that these SCFAs may reduce the stress response in *E. coli*.

KEYWORDS
Short-chain fatty acids; acetate; butyrate; propionate; *Escherichia coli;* stress response; *Vibrio fischeri luxCDABE; grpE; katG; recA*

INTRODUCTION
Metabolic syndromes, including obesity and type 2 diabetes, are 21st-century epidemics, and their etiologies have been linked to the gut microbiota.[1] The gastrointestinal tract harbors approximately 40 trillion bacteria that metabolize short-chain fatty acids (SCFA) from the fermentation of indigestible foods— particularly the metabolic breakdown of complex sugars.[2] The chemical structures of SCFAs are organic compounds with carboxylic acid functional groups attached. SCFAs have been shown to have many beneficial effects on the host: being used as fuel for epithelial cells in the colon, protection against metabolic control deterioration and inflammation, increasing colonic and hepatic portal venous blood flow, and maintenance of mucosal integrity.[3–5]

SCFAs are important to human digestive health and maintenance, produced as fermentation byproducts by bacteria of the gut microbiota.[6] They are critical in overall metabolic integrity and are large energy producers for intestinal epithelial cells (IECs); their energy supplement ranking being butyrate > propionate > acetate.[1,7] SCFAs that escape from colon cells enter the portal vein of the liver and can be converted into acetyl coenzyme A, which serves a key role in cellular metabolism and is a fundamental indicator of cellular metabolic state.[8] This class of nutrients is sensed by unique gut receptors FFA2, FFA3, GPR109a, and Olfr78– the role of these receptors is regulation of intestinal motility and maintenance of both the epithelial barrier and immune cell function.[9] While there are seven common SCFAs present in the gastrointestinal tract (GIT), the three most common occur in ratios between 40:20:20 to 75:15:10 as acetate, propionate, and butyrate, respectively.[9,10] Acetate, propionate, and butyrate are closely related in structure, only differing in the number of carbons. **Figure 1** depicts these structural differences. SCFAs butyrate and propionate activate a neural circuit between the gut and the brain, which induces intestinal gluconeogenesis—a metabolic process that has several metabolic benefits.[11] A significant role of these acids is their assets as an energy source and their ability to induce positive metabolic outcomes, such as insulin sensitivity.[1,11] Specifically, propionate acts as a glucose precursor in the gut, which activates a portal glucose sensor, which leads to improved glucose tolerance and insulin sensitivity.[10,11]

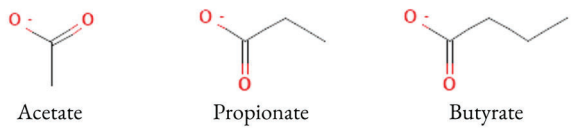

Figure 1. Chemical structure of acetate,[12] propionate,[13] and butyrate,[14] respectively.

Although naturally produced SCFAs have positive effects on the microbiome, there is evidence to suggest that acetate, when externally added in the acidic form, induced a stress response in *Escherichia coli*.[15] Kirkpatrick et al found that acetic acid at 50 mM induced multiple members of the RpoS regulon; Dps (a DNA-binding protein) was the most strongly induced, while seventeen proteins were repressed.[15] RpoS is a sigma factor responsible for the general stress response and regulating stationary phase in bacterial cells.[16] Kirkpatrick et al also found that formic acid prompted a stress response in *E. coli* like that of acetic acid. The addition of acetic acid after the stress response mitigated the activation of those proteins.[15] Further studies have shown that sodium acetate enhanced the resistance of *E. coli* to oxidative stress and heat killing, and all three SCFAs (acetate, butyrate, and propionate) at neutral pH increased the acid survival of *E. coli*.[6] Although there is substantive research exploring the effects of acetate on stress response in *E. coli*, there is a current gap in understanding the same effects of butyrate and propionate.

Although *E. coli* represents less than 1% of intestinal microbiota, it is the predominant facultative aerobic species in the gut.[17] As a commensal organism, *E. coli* promotes normal gut homeostasis.[17] In this study, *E. coli* is the model organism for the human microbiota to demonstrate the absence or presence of an oxidative stress response, a DNA damage response, and the heat shock response when the bacteria are exposed to acetate, butyrate, and propionate.

E. coli harbors many proteins that are essential for cell survival and adaptation to stressors. RecA is a protein of *E. coli* induced in response to DNA damage, in addition to homologous recombination.[18] In the SOS response, the RecA protein is converted into a specific and active protease and cleaves the LexA repressor.[19] GrpE is one of three proteins that constitute the *E. coli* DnaK chaperone machine, belonging to the heat shock protein family.[20] The expression of this protein is transiently induced under stress conditions, specifically regarding heat or ethanol treatment.[21] GrpE, along with DnaK and DnaJ, is responsible for reactivating damaged proteins under stress, proteolysis, and autoregulation of the heat shock response.[21] The *katG* gene is a catalase gene in *E. coli* that encodes for hyperperoxidase I (HPI) synthesis in the presence of H_2O_2 and is regulated by OxyR.[22,23] KatG participates in antioxidant defense mechanisms regarding oxidative damage and hydrogen peroxide-induced stress.[24]

For this study, promoter fusions to the *luxCDABE* operon from *Vibrio fischeri* were used in *E. coli* W3110. These fusions had been constructed and used previously to detect stress.[25,26] The plasmid used for testing was pUCD615 which is a broad-host-range plasmid containing promoterless *luxCDABE*.[25,26] Stress promoters are added to the 5' end of the *luxCDABE* operon to detect the stress response with bioluminescence output. The heat shock response is determined by the *grpE'::lux* fusion, the *katG'::lux* fusion shows the oxidative damage response, and the SOS response is tested using the *recA'::lux* fusion.[25,27] Activation of these stress promoters is determined and quantified via the emission of bioluminescence.[28] Kanamycin resistance was used as a selective marker, and the map of the plasmid used is shown in **Figure 2**.

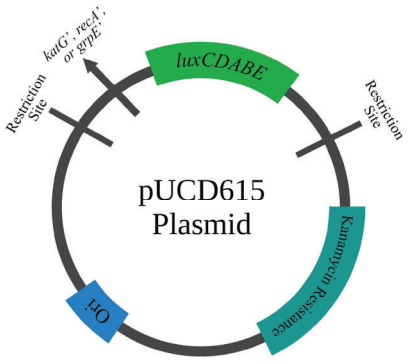

Figure 2. Map of the pUCD615 plasmid. Ori is the origin of replication. Each fusion plasmid contains one of the promoters of the indicated genes: *katG', recA',* or *grpE'*. The *luxCDABE* operon is from *V. fischeri*.

Based on previous research that established a protective effect of SCFAs on the intestinal microbiota,[5,29,30] we hypothesize that the addition of acetate, butyrate, and propionate would prevent the activation of the stress genes in *E. coli* in the presence of the respective known stressor.

MATERIALS AND METHODS

Short Chain Fatty Acid Compounds

All SCFAs used were analytical grade (> 98% purity) and were in their sodium salt form (sodium propionate, sodium butyrate, and sodium acetate). Sodium propionate and sodium butyrate were purchased from Tokyo Chemical Industry (TCI) Co Chemicals: sodium propionate CAS RN: 137-40-6, > 98% purity; sodium butyrate CAS RN: 156-54-7, > 98% purity. Sodium acetate was purchased from BeanTown Chemical, CAS number 127-09-3, > 99.995% trace metal basis. All SCFA were used at three concentrations: 12.5 mM, 25 mM, and 50 mM. The concentration of 25 mM was selected based on similar concentrations of SCFAs (specifically butyrate and propionate) found naturally in the human gut.[31,32] The concentration of 50 mM was chosen based on previous research regarding acetic acid at 50 mM inducing the RpoS regulon in *E. coli*.[15] The pH of the LB media containing SCFA at these three concentrations was nearly identical to the pH of the LB media alone.

Bacterial Strain and Plasmids

All *E. coli* strains and plasmids were kindly provided by Dr. Amy Vollmer (Swathmore College, PA). **Table 1** summarizes strains used containing all *lux* fusion plasmids and all detailed descriptions are cited in the references provided.

Strains/Plasmid	Description	Reference(s)
Strains		
W3110	F-, λ-, IN(*rrnD-rrnE*)1, *rph*-1	33
DPD2511	pKatGLux2/RFM443	27
TV1061	pGrpELux5/RFM443	34
DPD2794	pRecALux3/RFM443	26
Plasmids		
pUCD615	Amp^r Kan^r multiple cloning site upstream of *luxCDABE*	35
pKatGLux2	Same as pUCD615, but *katG'::luxCDABE*	27
pGrpELux5	Same as pUCD615, but *grpE'::luxCDABE*	34
pRecALux3	Same as pUCD615, but *recA'::luxCDABE*	26

Table 1. *E. coli* strains and plasmids.

Growth of the Bacteria

All *E. coli* strains were grown on Luria-Bertani (LB) agar plates containing 50 μg/mL kanamycin sulfate and were incubated at 37 °C. Experimental cultures were inoculated into 5 mL tubes containing LB and 25 μg/mL kanamycin sulfate overnight prior to each experiment. The overnight cultures were diluted into 5mL tubes containing fresh LB at an OD_{600} of 0.05 and incubated at 37 °C for four hours prior to being added into the plate. These *E. coli* subcultures were added to the 96 well plates with LB media and incubated at 37 °C for an additional two hours prior to adding treatments. **Figure 3** depicts a timeline of the complete process.

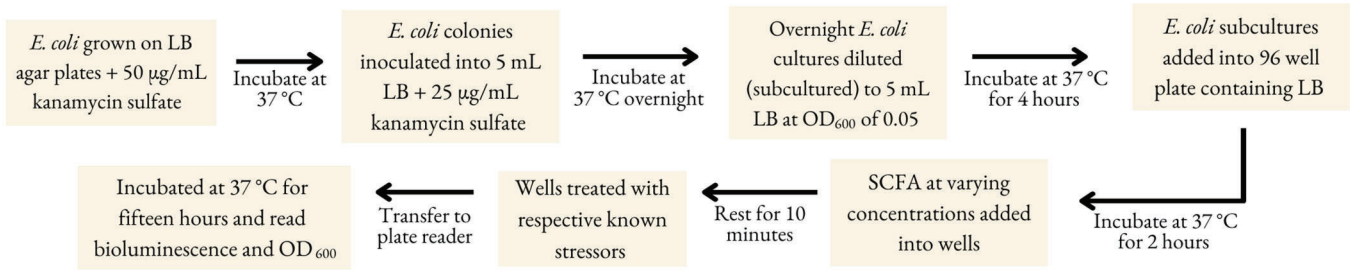

Figure 3. Timeline of experiments. For combinations of SCFAs and stressors, see Table 2.

SCFA Treatment of the Bacteria

This experiment had two treatment groups in addition to positive and negative controls for the *E. coli* strains: a baseline SCFA treatment and stress reduction treatment. The baseline SCFA treatment group involved each strain treated with one SCFA at concentrations of 12.5 mM, 25 mM, or 50 mM. The stress reduction group involved each strain treated with each SCFA at all three concentrations and adding the known stressor ten minutes after SCFA addition. **Table 2** shows the treatment groups used.

Treatment Group	Well Composition
Negative Control	*E. coli* strain + LB media
Positive Control	*E. coli* strain + LB media + known stressor
Baseline SCFA	*E. coli* strain + LB media + each SCFA
Reduced Stress Expression	*E. coli* strain + LB media + each SCFA + known stressor

Table 2. Treatment Group Descriptions.

Known Stressors of Bacterial Genes

All strains of *E. coli* were exposed to known stressors in the 96 well plate to induce the respective stress genes and serve as a baseline to compare our treatment groups to. The *recA'* wells were exposed to UV light (MODEL UVL-56 BLAK-RAY LAMP, UVP Inc. San Gabriel, CA) three inches above the plate for fifteen seconds to positively test for the SOS response mechanism. All *grpE'* wells were treated with 40% EtOH to positively test for the heat shock response, and *katG'* was treated with 0.04% H_2O_2 as a positive control to test for oxidative damage.[25,26] All stressors were added to wells that already contained bacteria. Both EtOH and H_2O_2 solutions were wrapped in aluminum foil and stored at -18 °C. Each plasmid containing a promoter had the ability to emit bioluminescence when evoking a stress response, and bioluminescence was measured in relative light units (RLU) every twenty minutes for fifteen hours. Biomass was measured simultaneously with OD_{600}.

96-well Plate Design

A 96-well plate was used to perform the experiment. Total amounts in each well were 200 µl. The volume of LB was adjusted to accommodate for the additional volume of SCFAs and stressors. Plate stickers were used to prevent evaporation over the 15-hour period. All experiments were performed in three technical replicates on one plate. One representative plate was used for analysis. In addition, five biological (different overnight cultures) replicates were done to verify trends in the data. Bioluminescence and biomass were read by a BioTek Synergy H1 Reader (Agilent, Santa Clara, CA).

Data Analysis

Bioluminescence data obtained as RLU was divided by the biomass data obtained as OD_{600} to obtain values that were normalized to the biomass. This value served as the mean of the replicates of the respective combinations of promoter fusion, stress, and SCFA. The average and standard deviations were determined across the three replicates from a representative plate and expressed as RLU/OD_{600}. Statistical analysis was done using the Graphpad Prism8 software. A one-way ANOVA with a post-Dunnett's multiple comparison test was performed to compare the ratios of biomass and bioluminescence to the positive control at the 20-minute time point. All statistics were analyzed with a 95% confidence interval (p-value <0.05) as a cut-off for statistical significance between the groups.

RESULTS

Biomass was not affected by the addition of SCFA

The biomass (OD_{600}) and bioluminescence (RLU) curves of *E. coli* DPD2511, TV1061, DPD2794, and W3110 containing the plasmids with the *recA'::lux*, *katG'::lux*, and *grpE'::lux* and promoterless *luxCDAB*E operon can be seen in **Figure 4**. Three different SCFAs – acetate, butyrate, and propionate – were used for testing. **Figure 4** depicts the *E. coli* response to propionate; the two remaining SCFAs followed significantly similar trends (data not shown). All *E. coli,* regardless of the promoter fusion, showed stagnant biomass over a 15-hour period. The biomass was independent of the treatments and controls for each strain, and differences were not statistically significant (p-value >0.05) between any of the strains.

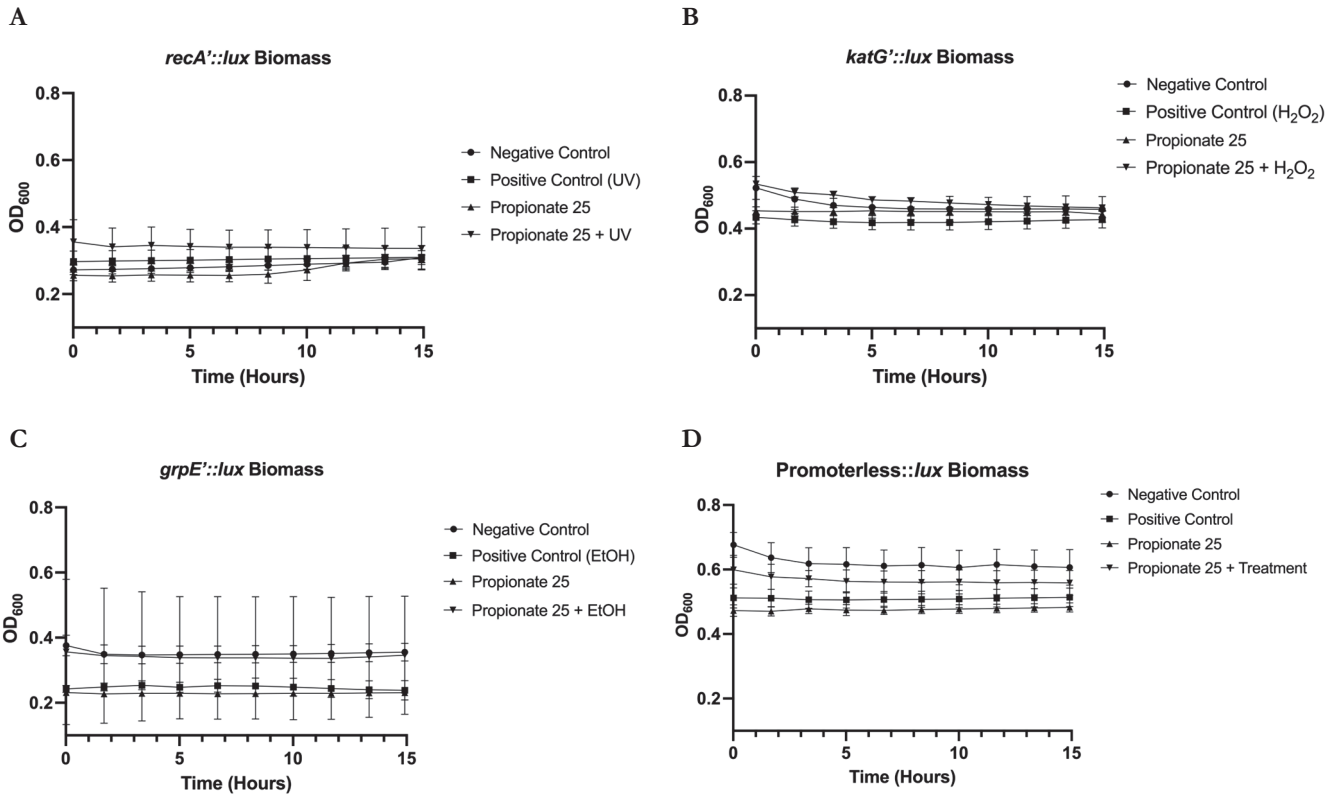

Figure 4. *E. coli* biomass for all four treatments. A) Shows the biomass of DPD2794 (*recA'*). B) Shows the biomass of DPD2511 (*katG'*). C) Shows the biomass of TV1061 *(grpE')*. D) Shows the biomass of W3110 with promoterless plasmid. Treatment groups consisted of negative control (•), positive control (▪) (known stressors UV light, H_2O_2, and EtOH, respectively), SCFA at 25 mM concentration (▲), and an SCFA at 25 mM in addition to a known stressor (▼).

Bioluminescence response to SCFAs

The stress response for the first three hours in *E. coli* strains DPD2794 *(recA')*, DPD2511 (*katG')*, TV1061 (*grpE')*, and W3110 (promoterless) can be seen in **Figure 5**. Propionate data can be seen in **Figure 5** as a representative; acetate and butyrate followed similar patterns (data not shown).

The stress caused an almost immediate bioluminescence response, measured in relative light units (RLU) in the positive control treatments, which lasted for about two hours, then dramatically decreased. The *recA'::lux* fusion (**Figure 5A**) was stressed with UV as a positive control and exhibited peak bioluminescence at 20 minutes. All other treatment groups were significantly different (p-value <0.0001) from the positive control (UV). The *katG'::lux* (**Figure 5B**), which was stressed with H_2O_2, and the *grpE'::lux* (**Figure 5C**) which was stressed with EtOH followed the same pattern, and both exhibited peak bioluminescence at 20 minutes. The promoterless plasmid (**Figure 5D**) showed no bioluminescence throughout the entire 15-hour period and was used to demonstrate that the responses measured were not specific to the *lux* operon reporter genes.

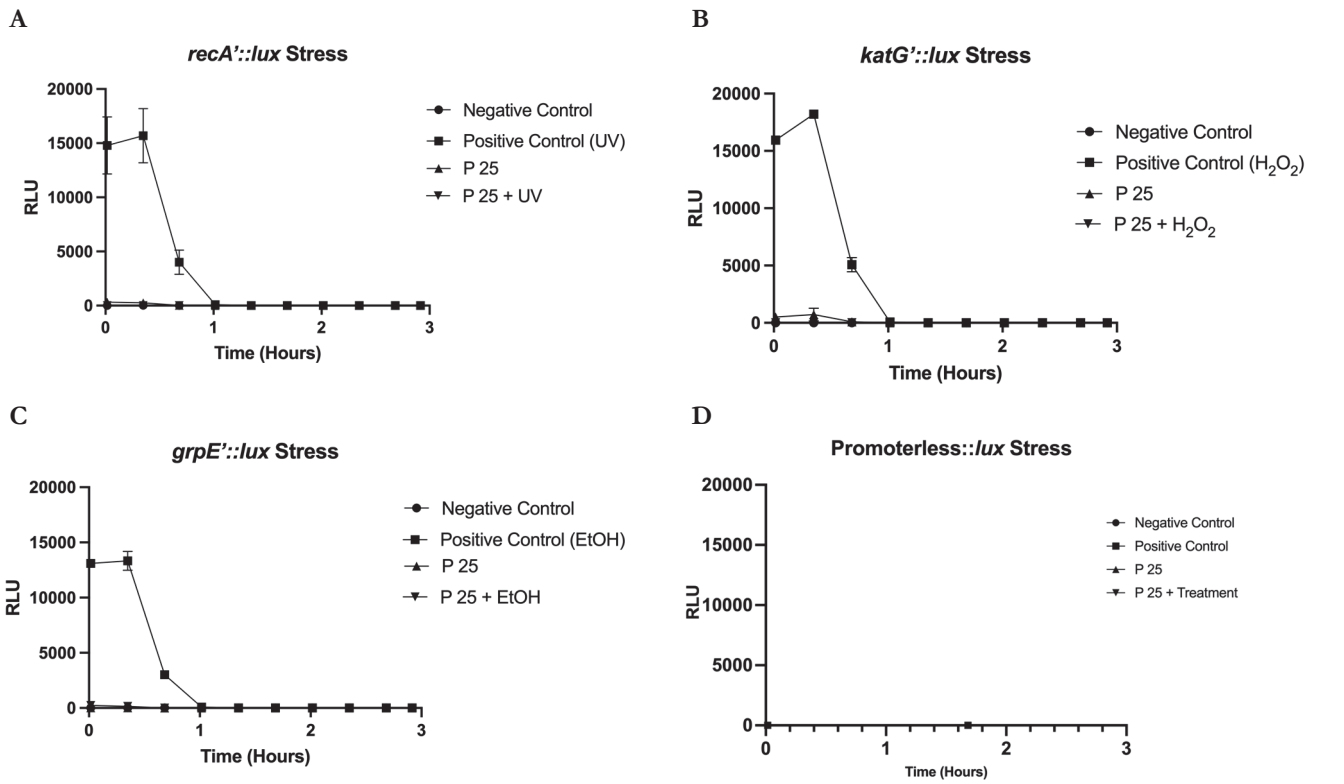

Figure 5. *E. coli* bioluminescence for all four treatments. A) Shows the bioluminescence of DPD2794 *(recA').* B) Shows the bioluminescence of DPD2511 (*katG').* C) Shows the bioluminescence of TV1061 *(grpE').* D) Shows the bioluminescence of W3110 with promoterless plasmid. Treatment groups consisted of negative control (•), positive control (▪) (known stressors UV light, H_2O_2, and EtOH, respectively), SCFA at 25 mM concentration (▲), and an SCFA at 25 mM in addition to a known stressor (▼).

SCFAs mitigate activation of stress promoters in the presence of known stressors

Figure 6 shows the normalized data from the 20-minute time points in **Figure 4** and **5**, when bioluminescence data in RLU were divided by the OD_{600} values for each of the treatments and promoter fusions. The negative control was statistically significant from the positive control *(p*-value <0.0001), but not statistically significant from the SCFA treatment groups at all concentrations. This trend was observed for all three promoters (data not shown).

Normalized bioluminescence was compared to the positive control in the *recA'* promoter across treatment groups for acetate, butyrate, and propionate at 25 mM and 25 mM + a known stressor (UV) (**Figure 6A**). The positive control (UV) was significantly different *(p*-value <0.0001) from the negative control and all other treatments as shown by a one-way ANOVA with a Dunnett's post-hoc test.

The SCFAs acetate, butyrate, and propionate at 25mM concentration and with the addition of a known stressor in cells containing the *katG'* promoter followed a similar trend to the *recA'* data as shown in **Figure 6B** at 20 minutes. All SCFA treatments, in addition to the negative control, displayed a statistically significant difference *(p*-value <0.0001) from the positive control (H_2O_2).

Different treatment groups of SCFAs with the same concentrations as described in **Figure 6A** and **6B** were utilized to test *grpE'* as shown in **Figure 6C.** Similar to the other promoters, all SCFA treatments, in addition to the negative control, displayed a statistically significant difference *(p*-value <0.0001) from the positive control (EtOH).

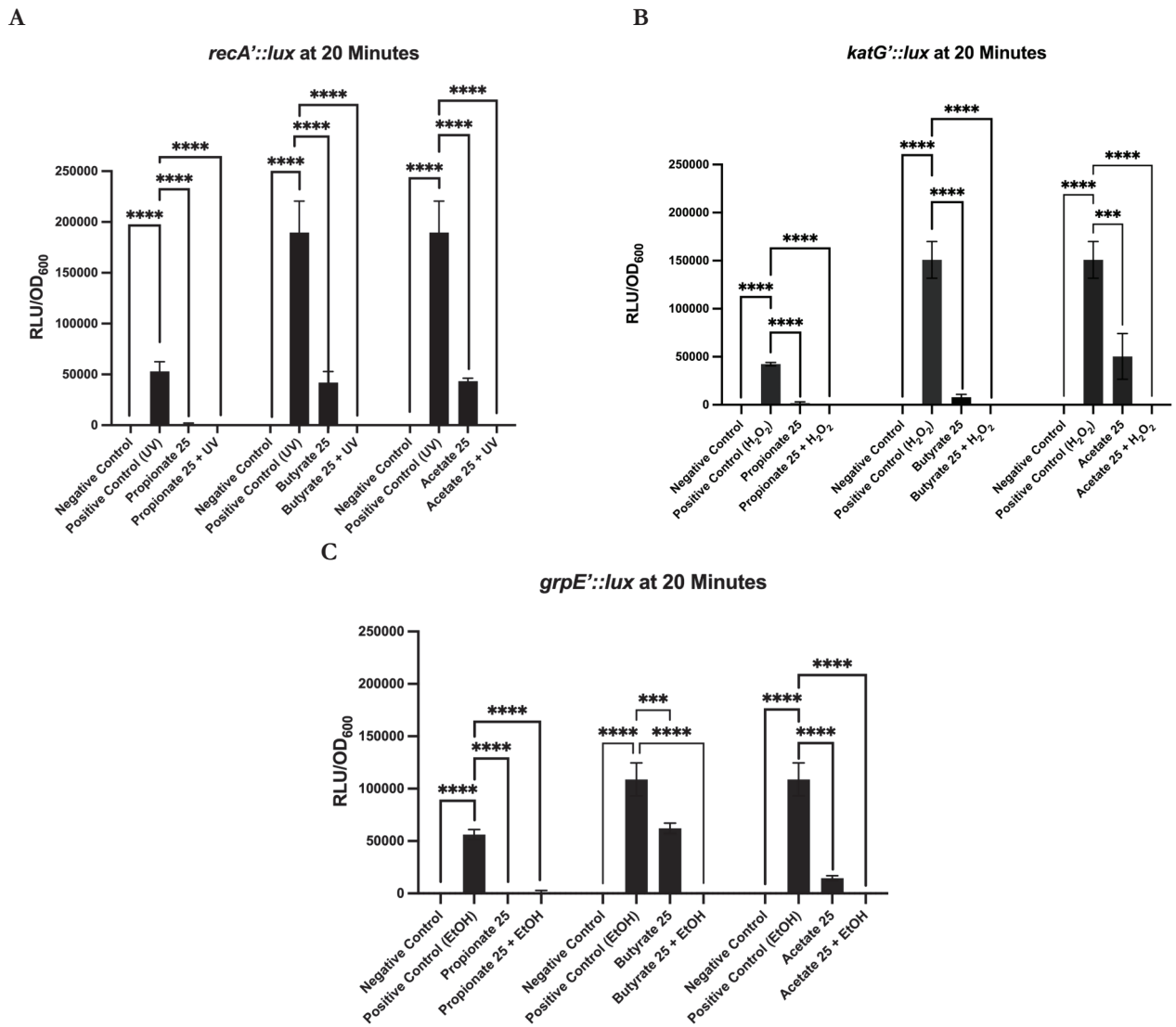

Figure 6. Maximum bioluminescence/OD_{600} at the 20-minute time point. A) DPD2794 (*recA'*). B) DPD2511 (*katG'*). C) TV1061 *(grpE').* **** indicates a statistically significant difference (p<0.0001*)* from the positive control as determined by a one-way ANOVA and a Dunnett's *post hoc* test.

DISCUSSION

The goal of this study was to test the hypothesis that SCFAs acetate, butyrate, and propionate would prevent the activation of stress promoters in the presence of their respective known stressors. The results demonstrate that acetate, butyrate, and propionate at a concentration of 25 mM does not appear to activate stress promotors in *E. coli* as shown in the DPD2794 *recA'::lux,* DPD2511 *katG'::lux,* and TV1061 *grpE'::lux.* Additionally, acetate, butyrate, and propionate at 25 mM prevented the activation of stress promoters even in the presence of known stressors. This may indicate that the presence of these SCFAs protected the bacteria from activating a respective stress response.

In addition to bioluminescence, biomass was measured every 20 minutes for 15 hours. Biomass in all the strains used for testing – DPD2794 *recA'::lux,* DPD2511 *katG'::lux,* TV1061 *grpE'::lux,* and the W3110 promoterless::*lux* – was consistent over the entire 15 hours with no statistically significant differences between treatments. The positive controls and concentrations were chosen due to their ability to cause stress, but not kill the bacteria. Biomass of *E. coli* did not produce a standard growth curve throughout experimentation, possibly due to high OD readings at the point of inoculation. This was intended to show that the induced stress did not impact growth. The stationary phase biomass levels also helped maintain a steady state of ATP that is required to maximize bioluminescence from the *luxCDABE* operon.[36] In the *lux* operon, especially the *lux* C, D, and E genes, fatty-acid reductase is used for light emission due to the aldehyde-independent genes that flank luciferase genes.[36]

Normalized stress gene expression, measured as RLU/OD_{600} over a 15-hour period, peaked at 20 minutes for the positive control treatments. The positive control was the only treatment option that showed a significant difference from the negative control. There was no statistically significant difference between the negative control and the SCFA treatment groups, which indicates that the SCFAs in their sodium form do not cause any of the three tested stresses in *E. coli* at the mM levels used in this study. The SCFAs at 25 mM in addition to a known stressor also did not elicit a stress response in the gene, a response that was observed in the known stressors alone, which shows a lack of activation of the *lux* operon from the respective stress promoter. This could be indicative of a protective effect of SCFAs on the *E. coli* stress response, preventing the activation of a stress response gene in the presence of stressors.

Prior to this study, there was limited knowledge on how SCFAs acetate, butyrate, and propionate in their sodium forms affect the *E. coli* stress response. One study was done by Kirkpatrick to determine how acetate in its acidic form causes stress to *E. coli* when combined with formic acid.[15] Kirkpatrick *et al.* determined that acetic acid at 50 mM induced the RpoS regulon. They also found that acetic acid induced metabolic enzymes GatY and YfiD, which are part of stress response to acid (low pH), indicating that the stress activation response may be due to the acidic nature of acetic acid and not based on the SCFA itself.[15,37] Further, formic acid was found to induce a stress response in a similar fashion to acetic acid, but *E. coli* stress responses were reduced when formic acid was added in addition to acetic acid.[15] However, in our study, the starting pH of the culture did not change due to the presence of the SCFAs. This could explain the contradiction between the above studies and ours. SCFAs used for the purpose of this study were in their sodium form to limit the effect that pH changes had on the bacteria. Another study showed that pretreatment with sodium acetate exhibited a protective effect against oxidative stress and increased acidic survival in *E. coli* O157:H7.[6] However, there was a lack of research on whether SCFAs butyrate and propionate have the same effects on *E. coli,* which our study addressed. The mechanism of action for how these SCFAs prevent the activation of stress promoters in *E. coli* is unknown. It has been hypothesized that their effect is dependent on oxygen availability and environment pH.[32]

Future directions for this experiment would seek to test whether the SCFAs butyrate and propionate in their sodium form specifically caused a protective effect in *E. coli.* Due to the results of previous research on acetate and based on the patterns observed in **Figure 6**, there is the possibility that the SCFAs are protective against stress. To further examine this effect, it would need to be determined that SCFAs at 25 mM directly increase the survival rate of *E. coli.* Additionally, a broader effect of SCFA stress in the gut microbiome could be investigated by performing similar experiments in other bacterial species, such as *Listeria monocytogenes*.[38] Further research could explore other stress genes, such as the *lexA'::lux* to determine if SCFAs have a protective effect against low pH.[39]

Currently, medical treatments are being explored by artificially using SCFAs – specifically butyrate—to rescue the gut microbiota and restore normal functions in those who experience metabolic syndromes that correlate with the depletion of normal levels of these fatty acids.[40,41] Various ailments and conditions such as ulcerative colitis, type 1 and type 2 diabetes, obesity, and other forms of gut dysbiosis are improved by dietary supplementation of SCFAs, which restore homeostasis in the gut microbiome.[1,9,40,41] Understanding the relationship between SCFAs and survival rates of *E. coli* could allow for a better understanding of how acetate, butyrate, and propionate affect the gut microbiome and host homeostasis, with the ultimate goal of using them as a mitigation technique for gut dysbiosis.

ACKNOWLEDGEMENTS

The authors thank Dr. Amy Vollmer for providing the strains used in this study, Dr. Glenn Dorsam for providing acetate, butyrate, and propionate, Dr. Barney Geddes for helpful insight and discussions, and the Department of Microbiological Sciences at North Dakota State University for their financial support.

REFERENCES

1. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. (2014) The Role of Short-Chain Fatty Acids in Health and Disease. *Adv Immunol* 91-119. *doi:10.1016/B978-0-12-800100-4.00003-9*
2. Sender R, Fuchs S, Milo R. (2016) Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLOS Biology* 1-14. *doi:10.1371/journal.pbio.1002533*
3. Topping DL. (1996) Short-chain fatty acids produced by intestinal bacteria. *Asia Pacific Journal of Clinical Nutrition* 15-19.
4. Cook SI, Sellin JH. (1998) Review article: Short chain fatty acids in health and disease. *Alimentary Pharmacology and Therapeutics* 499-507. *doi:10.1046/j.1365-2036.1998.00337*
5. Morrison DJ, Preston T. (2016) Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut microbes* 189-200. *doi:10.1080/19490976.2015.1134082*
6. Arnold CN, McElhanon J, Lee A, Leonhart R, Siegele DA. (2001) Global Analysis of *Escherichia coli* Gene Expression during the Acetate-Induced Acid Tolerance Response. *Journal of Bacteriology* 2178-2186. *doi:10.1128/JB.183.7.2178-2186.2001*

7. Lin Y, Fang ZF, Che LQ, Xu SY, Wu D, Wu CM, Wu XQ. (2014) Use of Sodium Butyrate as an Alternative to Dietary Fiber: Effects on the Embryonic Development and Anti-Oxidative Capacity of Rats. *PLOS One.* *doi:10.1371/journal.pone.0097838*
8. Shi L, Tu BP. (2015) Acetyl-CoA and the regulation of metabolism: Mechanisms and consequences. *Current Opinion in Cell Biology* 125-131. *doi:10.1016/j.ceb.2015.02.003*
9. Priyadarshini M, Kotlo KU, Dudeja PK, Layden BT. (2018) Role of Short Chain Fatty Acid Receptors in Intestinal Physiology and Pathophysiology. *Comprehensive Physiology* 1091-1115. *doi:10.1002/cphy.c170050*
10. Wong JMW, de Souza R, Kendall CWC, Emam A, Jenkins DJA. (2006) Colonic Health: Fermentation and Short Chain Fatty Acids. *Journal of Clinical Gasterenterology* 235-243. *doi:10.1097/00004836-200603000-00015*
11. De Vadder F, Kovatcheva-Datchary P, Zitoun C, Duchampt A, Bäckhed F, Mithieux G. (2016) Microbiota-Produced Succinate Improves Glucose Homeostasis via Intestinal Gluconeogenesis. *Cell Metabolism* 151-157. *doi:10.1016/j.cmet.2016.06.013*
12. National Center for Biotechnology Information. PubChem Compound Summary for CID 175, Acetate, *https://pubchem.ncbi.nlm.nih.gov/compound/Acetate*
13. National Center for Biotechnology Information. PubChem Compound Summary for CID 104745, Propionate, *https://pubchem.ncbi.nlm.nih.gov/compound/Propionate*
14. National Center for Biotechnology Information. PubChem Compound Summary for CID 104775, Butyrate, *https://pubchem.ncbi.nlm.nih.gov/compound/Butyrate*
15. Kirkpatrick C, Maurer LM, Oyelakin NE, Yoncheva YN, Maurer R, Slonczewski JL. (2001) Acetate and Formate Stress: Opposite Responses in the Proteome of *Escherichia Coli. Journal of Bacteriology* 6466-6477. *doi:10.1128/JB.183.21.6466-6477.2001*
16. Battesti A, Majdalani N, Gottesman S. (2011) The RpoS-Mediated General Stress Response in *Escherichia coli. Annual Review of Microbiology* 189-213. *doi:10.1146/annurev-micro-090110-10294*
17. Delmas J, Dalmasso G, Bonnet R. (2015) *Escherichia coli*: The Good, the Bad and the Ugly. *Clinical Microbiology.* *doi:10.4172/2327-5073.1000195*
18. Kowalczykowski SC. (1991) Biochemical and biological function of *Escherichia coli* RecA protein: behavior of mutant RecA proteins. *Biochimie* 289-304. *doi:10.1016/0300-9084(91)90216-N*
19. Huisman O, D'Ari R, Gottesman S. (1984) Cell-division control in *Escherichia coli*: specific induction of the SOS function SfiA protein is sufficient to block septation. *Proceedings of the National Academy of Sciences* 4490-4494. *doi:10.1073/pnas.81.14.4490*
20. Ang D, Chandrasekhar GN, Zylicz M, Georgopoulos C. (1986) *Escherichia coli* grpE gene codes for heat shock protein B25.3, essential for both lambda DNA replication at all temperatures and host growth at high temperature. *Journal of Bacteriology* 25-29. *doi:10.1128/jb.167.1.25-29.1986*
21. Wu B, Ang D, Snavely M, Georgopoulos C. (1994) Isolation and characterization of point mutations in the *Escherichia coli* grpE heat shock gene. *Journal of Bacteriology* 6965-6973. *Doi:10.1128/jb.176.22.6965-6973.1994*
22. Zhang L, Alfano JR, Becker DF. (2015) Proline Metabolism Increases *katG* Expression and Oxidative Stress Resistance in *Escherichia coli. Journal of Bacteriology* 431-440. *doi:10.1128/JB.02282-14*
23. Panek HR, O'Brian MR. (2004) KatG Is the Primary Detoxifier of Hydrogen Peroxide Produced by Aerobic Metabolism in *Bradyrhizobium japonicum. Journal of Bacteriology* 7874-7880. *doi:10.1128/JB.186.23.7874-7880.2004*
24. Doukyu N, Taguchi K. (2021) Involvement of catalase and superoxide dismutase in hydrophobic organic solvent tolerance of *Escherichia coli. AMB Express* 97. *doi:10.1186/s13568-021-01258-w*
25. Vollmer AC, Kwakye S, Halpern M, Everbach EC. (1998) Bacterial Stress Responses to 1-Megahertz Pulsed Ultrasound in the Presence of Microbubbles. *Applied and Environmental Microbiology* 3927-3931. *doi:10.1128/aem.64.10.3927-3931.1998*
26. Vollmer AC, Belkin S, Smulski DR, van Dyk TK, Larossa RA. (1997) Detection of DNA Damage by Use of *Escherichia Coli* Carrying RecA::Lux, UvrA::Lux, or AlkA::Lux Reporter Plasmids. *Applied and Environmental Microbiology* 2566-2571. *doi: 10.1128/aem.63.7.2566-2571.1997*
27. Belkin S, Smulski DR, Vollmer AC, van Dyk TK, Larossa RA. (1996) Oxidative Stress Detection with *Escherichia Coli* Harboring a KatG::Lux Fusion. *Applied and Environmental Microbiology* 2252-2256. *doi: 10.1128/aem.62.7.2252-2256.1996*
28. van Dyk TK, Majarian WR, Konstantinov KB, Young RM, Dhurjati PS, LaRossa RA. (1994) Rapid and sensitive pollutant detection by induction of heat shock gene-bioluminescence gene fusions. *Applied and Environmental Microbiology* 1414-1420. *doi:10.1128/aem.60.5.1414-1420.1994*
29. Chambers ES, Preston T, Frost G, Morrison DJ. (2018) Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health. *Current Nutrition Reports* 198-206. *doi:10.1007/s13668-018-0248-8*
30. Silva YP, Bernardi A, Frozza RL. (2020) The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Frontiers in Endocrinology. doi:10.3389/fendo.2020.00025*
31. Lin J, Smith MP, Chapin KC, Baik HS, Bennett GN, Foster JW. (1996) Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli. Applied and Environmental Microbiology* 3094-3100. *doi:10.1128/aem.62.9.3094-3100.1996*

32. Bushell F, Herbert JMJ, Sannasiddappa TH, Warren D, Turner AK, Falciani F, Lund PA. (2021) Mapping the transcriptional and fitness landscapes of a pathogenic *E. coli* strain: The effects of organic acid stress under aerobic and anaerobic conditions. *Genes* 1-25. *doi:10.3390/genes12010053*

33. Ernsting BR, Atkinson MR, Ninfa AJ, Matthews RG. (1992) Characterization of the regulon controlled by the leucine-responsive regulatory protein in *Escherichia coli*. *Journal of Bacteriology* 1109-1118. *doi:10.1128/jb.174.4.1109-1118.1992*

34. van Dyk TK, Smulski DR, Reed TR, Belkin S, Vollmer AC, LaRossa RA. (1995) Responses to toxicants of an *Escherichia coli* strain carrying a uspA'::lux genetic fusion and an *E. coli* strain carrying a grpE'::lux fusion are similar. *Applied and Environmental Microbiology* 4124-4127. *doi:10.1128/aem.61.11.4124-4127.1995*

35. Rogowsky PM, Close TJ, Chimera JA, Shaw JJ, Kado CI. (1987) Regulation of the vir genes of *Agrobacterium tumefaciens* plasmid pTiC58. *Journal of Bacteriology* 5101-5112. *doi:10.1128/jb.169.11.5101-5112.1987*

36. Meighen EA. (1988) Enzymes and Genes from the lux Operons of Bioluminescent Bacteria. *Annual Review of Microbiology* 151-176. *doi:10.1146/annurev.mi.42.100188.001055*

37. Blankenhorn D, Phillips J, Slonczewski JL. (1999) Acid- and Base-Induced Proteins during Aerobic and Anaerobic Growth of *Escherichia coli* Revealed by Two-Dimensional Gel Electrophoresis. *Journal of Bacteriology* 2209-2216. *doi:10.1128/JB.181.7.2209-2216.1999*

38. Quereda JJ, Dussurget O, Nahori MA, Ghozlane A, Volant S, Dillies MA, Regnault B, Kennedy S, Mondot S, Villoing B, Cossart P, Pizarro-Cerda J. (2016) Bacteriocin from epidemic *Listeria* strains alters the host intestinal microbiota to favor infection. *Proceedings of the National Academy of Sciences* 5706-5711. *doi:10.1073/pnas.1523899113*

39. Dri AM, Moreau PL. (1994) Control of the LexA regulon by pH: evidence for a reversible inactivation of the LexA repressor during the growth cycle of *Escherichia coli*. *Molecular Microbiology* 621-629. *doi:10.1111/j.1365-2958.1994.tb01049.x*

40. Chapman MAS, Grahn MF, Hutton M, Williams NS. (2005) Butyrate metabolism in the terminal ileal mucosa of patients with ulcerative colitis. *British Journal of Surgery* 36-38. *doi:10.1002/bjs.1800820115*

41. Scheppach W, Sommer H, Kirchner T, Paganelli GM, Bartram P, Christl S, Richter F, Dusel G, Kasper H. (1992) Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. *Gastroenterology* 51-56. *doi:10.1016/0016-5085(92)91094-K*

ABOUT STUDENT AUTHORS

Kathleen Ruff-Schmidt graduated from North Dakota State University with a B.S. in Microbiology in May of 2022. She is currently attending medical at the University of North Dakota and plans to obtain her MD. Kaylee Weigel will graduate from North Dakota State University in May of 2023 with a B.S. in Microbiology and in May of 2024 with an M.S. in Microbiology. She plans to attend medical school to obtain both an MD and a Ph.D.

PRESS SUMMARY

Regulation of microbes in the human intestinal tract maintains overall human health and prevents diseases, such as inflammatory bowel disease and obesity. Short-chain fatty acids (SCFAs) in the intestine are produced by bacterial fermentation and aid in inflammation reduction, dietary fiber digestion, and metabolizing nutrients for the colon. SCFAs are starting to be used in clinical interventions for inflammatory diseases. This study aims to evaluate the effects of SCFA on the activation of the stress response in *E. coli,* which is a representative bacterium of the intestine. Three different SCFAs were added to cultures prior to stressing the bacteria causing a lower level of stress activation when compared to bacteria that were stressed in the absence of SCFAs. Our data is consistent with the idea that SCFAs reduce stress in bacteria and consequently the inflammatory response that contributes to disease.