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Resistance to sucralose in *Escherichia coli* is not conferred by mutations in the quinolone resistance determining regions of *gyrA*

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SUMMARY Sucralose, a synthetic non-caloric sweetener (NCS) commonly consumed in North America, is associated with an increasing number of metabolic disorders that may be result from NCS-microbiome interactions and the bacteriostatic action of sucralose on various species including *Escherichia coli*. A mechanism of action for the bacteriostatic effects of sucralose in the absence of sucrose has not been characterized. However, studies have revealed increased resistance to quinolone antibiotics, inhibitors of DNA gyrase, in bacterial strains grown in sub-inhibitory concentrations of sucralose. Here, we aim to investigate the mechanism of action of sucralose resistance in *E. coli* as it relates to quinolone resistance, by targeting the quinolone resistance-determining region (QRDR) of the *gyrA* gene, the DNA-binding component of DNA gyrase. Our results reveal distinct phenotypic differences between non-sucralose resistant (NS) and high sucralose resistant (HS) strains, and that the HS strain phenotype is maintained in the absence of sucralose. Additionally, no mutations were found in the QRDR of the *gyrA* gene. Despite a lack of direct association between sucralose resistance and quinolone resistance in *E. coli*, our study provides insight into alternative mechanisms of action for sucralose resistance.

INTRODUCTION

Non-caloric sweeteners (NCS) are natural or synthetic compounds with structural similarities to nutritive mono- and disaccharides such as dextrose and sucrose, thus retaining a sweet taste due to their reactivity with the same sweet taste receptors (1,2). NCS cannot be metabolized by mammalian cells to yield energy. The deviations in chemical structure of NCS prevent glycolysis and/or earlier stages of carbohydrate metabolism through the inhibition of metabolic enzymes (1,2). NCS have become ubiquitous in modern Western diets, often used for the management of metabolic disorders such as diabetes, and are frequently added into “low calorie” versions of foods (3). Consumption of NCS by the general public is a rising trend both in North America and globally, led primarily by the artificial sweeteners saccharin and sucralose (3).

The rise in NCS consumption has broad health implications. Although consumed by many diabetics and other patients with metabolic disorders, a steadily growing body of research associates NCS consumption by the general public with various metabolic disorders including Type 2 diabetes (4-8). Despite not being metabolized by mammalian cells (1), more recent evidence implicates the association of NSC and metabolic disorders through interactions between these compounds and the gut microbiome (2, 4-8). A suggested mechanism for the association between NCS and metabolic disorders via the microbiome is the differential inhibition of commensal species, wherein some organisms are inhibited to a greater degree than others and thus the overall composition of the microbiome is altered (4-8).

Sucralose is a synthetic NCS and one of the most commonly consumed NCS in North America, where it is marketed to the general public under the brand name Splenda (3). Structurally, sucralose mimics the disaccharide sucrose, with hydroxyl groups on carbons 4, 1’, and 6’ replaced by chlorides thus preventing its metabolism by mammalian cells (1). Sucralose has been shown to exhibit a bacteriostatic effect on *Escherichia coli*, a model organism used for *in vitro* studies on the microbiome (8-9). In *in vitro* co-cultures of *E. coli*

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and *Enterobacter aerogenes*, another ubiquitous organism in human gut commensal communities, the presence of sucralose allowed for *E. aerogenes* to outcompete *E. coli* through differential levels of growth inhibition (8). In addition to *E. coli* and *E. aerogenes*, sucralose inhibits growth for a variety of both commensal organisms in mammalian microbiomes and environmental bacteria, indicating a broad range of action (8-13). The bacteriostatic effects of sucralose had previously been attributed to the inhibition of the symporter sucrose permease, which is necessary for the uptake of the nutritive disaccharide sucrose into bacterial cells, and the enzyme invertase, which is necessary for hydrolyzing this disaccharide into fructose and glucose (10). However, bacteriostatic effects of sucralose have also been observed in the absence of sucrose in media, and with bacteria that do not readily metabolize sucrose in the presence of other carbon sources such as glucose (8), suggesting that sucralose may have an alternative mechanism of action as a bacteriostatic agent other than the inhibition of sucrose metabolism.

Though mechanisms of action—independent of permease and invertase inhibition—for the bacteriostatic effects of sucralose are yet to be formally elucidated and described, some studies have sought to further characterize the operational nature of sucralose resistance. Thus far, an association has been made between sucralose resistance and resistance to quinolones: a class of antibiotics that inhibit DNA gyrase, a widely conserved enzyme responsible for supercoiling DNA and thus essential for processes such as transcription and DNA replication (14, 15). Pre-treatment of wild-type *E. coli* with sub-inhibitory concentrations of sucralose in growth medium yielded greater numbers of resistant mutants to inhibitory doses of quinolone antibiotics oxolinic acid and moxifloxacin, compared to *E. coli* cultures grown in the absence of sucralose (16). A similar effect was not observed for other classes of antimicrobials. The increase in quinolone-resistant mutant count following sub-inhibitory sucralose exposure was not attributed to a direct mutagen effect of sucralose, as previous genotoxicity testing and DNA damage assays have shown that sucralose does not induce random mutations in genomes and that it does not directly damage DNA in *E. coli* (17). The broad—yet differential—action of sucralose across many species of microorganisms, the lack of sucralose genotoxicity as a direct chemical mutagen or DNA damaging agent, and the association of sucralose with increased resistance specifically to quinolone antibiotics, suggest that sucralose may act as an inhibitor of DNA gyrase.

In this study we sought to identify a molecular mechanism for a sucrose-metabolism independent bacteriostatic effect of sucralose on *E. coli*, with a focus on the association of sucralose treatment with quinolone resistance phenotypes. As quinolone resistance in *E. coli* is conferred by specific mutations in the *gyrA* gene encoding the DNA-binding component of DNA gyrase (15), we focused our investigations on the same portion of the *E. coli* genome, the quinolone resistance-determining region (QRDR) of *gyrA.* A strain of sucralose-resistant *E. coli* was obtained by subculturing wildtype *E. coli* DH5α on increasing concentrations of sucralose, and its *gyrA* gene was sequenced to determine if any mutations occurred in our region of interest. As well, the stability of the sucralose resistance was tested and the growth of the strain under sucralose in both complete and minimal media was characterized and compared to sucralose susceptible *E. coli*. Although a mutation was not detected in the QRDR of *gyrA*, the sucralose resistance phenotype was found to be stable, suggesting that there is a genomic mutation that confers this profile. Furthermore, growth curve experiments suggested that sucralose acted in the absence of sucrose to enact bacteriostatic effects on sucralose-susceptible *E. coli*.

METHODS AND MATERIALS

Bacterial strains, media, and growth conditions in sucralose. Wildtype *E. coli* DH5α strains were grown in an overnight culture of trypticase soy broth (TSB) in 37ºC. TSB was purchased from (Becton Dickinson 9003524). Sucralose was purchased from TCI America (S0839 TUVTA-NO). TSB with added sucralose were made for 1mM, 10mM, 50mM, 100mM concentration of sucralose. *E. coli* DH5α strains were then cultivated in these conditions to create low-sucralose resistance (LS, resistant to 1mM sucralose), medium-sucralose resistance (MS, resistant to 10mM sucralose), intermediate-sucralose resistance (IS, resistant to 50mM sucralose), and high-sucralose resistance (HS, resistant to 100mM

TABLE. 1 Primer Sequence Used in Amplifying *gyrA* QRDR (19)

Target gene	Primer	Sequence 5'-3'	Product size (bp)
gyrA	gyrA F gyrA R	5'-TACACCGGTCAACATTGAGG-3' 5'-TTAATGATTGCCGCCGTCGG-3'	586

sucralose) strains. Each strain was grown in media supplemented with the respective concentration of sucralose until it reached the same OD as the subcultured wildtype control (cultivated in 0mM sucralose), obtaining a stable strain after maintaining the OD for three days. The strain was then subcultured in progressively higher sucralose concentrations to a final concentration of 100mM. Each stable strain was maintained by refreshing the media with respective concentrations of sucralose until a stable HS strain was obtained after 21 days. Minimal media for growth curve experiments containing glucose as the viable carbon source (18) was made for 1mM, 10mM, 50mM, 100mM concentrations of sucralose. For all experiments using solid media, trypticase soy agar (TSA) was prepared by supplementing TSB with 1.5% Select Agar (InVitrogen 1004793).

Bacterial Genomic DNA Extraction. Bacterial DNA extracts used as PCR templates for this study were prepared by column extraction using PureLink™ Genomic DNA Mini Kit K182001 (Invitrogen 2132086). DNA was isolated from 3 separate colonies each of NS and HS grown on TSA and 100mM sucralose TSA plates respectively. Prior to beginning extraction, HS samples were washed with TSB, then all samples were washed with DNase free sterile distilled water to remove excess sucralose. DNA concentration in these extracts was measured using NanoDrop 2000 (Invitrogen); samples were then aliquoted and stored at –20°C for long-term storage or kept on ice for immediate use.

QRDR *gyrA* Gene amplification. The *gyrA* QRDR of all isolates were amplified by PCR using gene-specific primers as shown in Table 1. 250 µL of PCR master mix, as shown in Table 2, was prepared for use in 7 PCR reactions with the primer sequence. Genomic DNA from 3 samples each of HS and NS replicates were added ranging from 5-10 µL to each tube according to their different extract concentrations. Additional water was added if needed for a total volume 25 µL per reaction tube. Table 2 also shows the cycling conditions used for amplification.

Agarose gel electrophoresis. 5 µL of PCR products were prepared with 1 µL 6X Orange DNA Loading Dye (ThermoFisher R0631) for each tube. The samples and 10 µL of 1 Kb Plus DNA ladder (Invitrogen 10787026) were loaded onto a 1% (w/v) agarose gel made with SYBR Safe DNA Gel Stain in 0.5 X TBE (Invitrogen S33101). Gel was run for 1 hour at 90-100 mV in 1X TBE. Samples were visualized using the ChemiDoc MP Imaging System (Bio-Rad 12003154).

PCR purification and sequencing. PCR products were purified using PureLink™ PCR Purification Kit K3100001 (Invitrogen 426.09.31.02). PCR samples were stored in 10 mM Tris-HCl and sent in 10 µL samples with 50 µL of forward *gyrA* primer to GENEWIZ for Sanger sequencing.

Growth curves. *E. coli* cultures were grown overnight in TSB at 37ºC. 96-well flat bottom plates were loaded with 5 µL of each of the strains (wildtype control, LS, MS, IS, HS), in 195 µL of TSB or minimal media containing sucralose at each concentration used in the study (0mM, 1mM, 10mM, 50mM, 100mM) such that each strain was grown in duplicate wells at all concentrations of sucralose. Well plates were incubated in an EPOCH2 Microplate Reader (BioTek) for 16 hours at 37ºC and linearly shaken at 6rpm. OD_{600} readings from each well were automatically taken at 10-minute intervals, and wells containing sterile TSB without sucralose were used to blank all readings.

TABLE. 2 PCR Master Mix and Cycling Conditions for *gyrA* QRDR Amplification (20). (*) template DNA volume dependent on concentration; volume should be selected to add 1ng-1µg of DNA. (**) DNAse free water volume dependent on volume of template DNA used

PCR Master Mix Reagents	Volumes (µL)	Cycle Stage		Cycling Conditions
10x buffer -Mg	2.5	Prenaturation		94°C - 5 min
50 mM MgCl2	0.75	Denaturation		92°C - 25 seconds
10 mM dNTP	0.5	Annealing	30 cycles of amplification	55°C - 1 minute
Platinum taq pol	0.15	Elongation		74°C - 2.5 minute
100 uM forward primer 100 uM reverse primer	0.05 0.05	Final extension		74°C - 10 minutes
Template DNA	1 ng - 1 µg*			
DNase free water	Variable**	Hold		4°C
Total volume	25			

Sucralose Resistance Phenotype Stability Assay. HS strain was subcultured in TSB with 0mM and 100mM sucralose for 48 hours. Both cultures, and an NS culture as a control, were plated on TSA plates with 0mM sucralose and 100mM sucralose. Plates were incubated at 37°C overnight and observed the next day for growth.

RESULTS

Growth rate and maximum growth at stationary phase are distinct between non-sucralose resistant and high sucralose resistant strains of *E. coli*. We aimed to characterize the growth phenotypes of the strains obtained subculturing in different media and sucralose stress conditions. We obtained growth curves of HS and NS strains subjected to both 0mM and 100mM sucralose conditions in TSB as complete media, and minimal media with glucose as the viable carbon source using OD_{600} readings; 0mM media negative controls were used as blanks. In TSB, the NS strain displayed a prominent decrease in both growth rate and maximum growth achieved under 100mM sucralose compared to 0mM sucralose, as indicated by lower OD_{600} values during both exponential and stationary phases (Fig. 1A). The NS strain in 0mM sucralose also appeared to exhibit a diauxic growth curve (Fig. 1A). In minimal media, the NS strain grew slower in both 0mM and 100mM sucralose (10 hours to reach stationary phase) than NS strains grown in TSB (4 hours and 7 hours to reach stationary phase for 0mM and 100mM respectively), with notable absence of any diauxic growth in minimal media (Fig. 1A and 1C). Sucralose presence still reduced the growth rate in the exponential phase and the maximum growth yield in stationary phase of the NS strain in minimal media (Fig. 1C).

The HS strains incubated with 0mM and 100mM sucralose in TSB had similar growth curves and OD_{600} values during exponential and stationary phases (Fig. 1B). The HS strain in 100mM sucralose (OD_{600} of 0.50 at 16 hours) had a higher maximum growth in stationary phase than the NS strain in 100mM sucralose (OD_{600} of 0.35 at 16 hours) (Fig. 1A-B). Diauxic growth was not observed in the HS strains grown in TSB; the maximum growth of the HS strain under 0mM sucralose (OD_{600} of 0.53 at 16 hours) was lower than

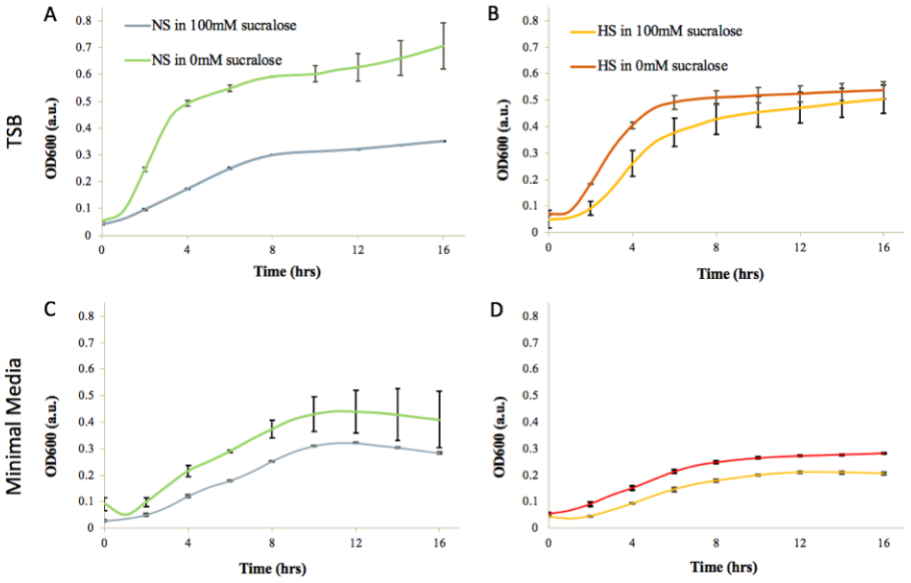

FIG. 1 Growth curves of *E. coli* DH5α measured over 16 hours at 37˚C displays inhibited growth of non-sucralose resistant strain in the presence of high sucralose. (A, C) Non-sucralose resistant strains grown in TSB media or minimal media supplemented with either the absence of sucralose, 0mM, or high concentration of sucralose, 100mM. (B, D) High sucralose resistant strains grown in the absence of sucralose and in high sucralose conditions as per A and C. Error bars represent ± range measured every two hours. Treatments were conducted in duplicate (n=2). OD_{600} values are following blanking with media containing 0mM of sucralose.

that of the NS strain (OD_{600} of 0.71 at 16 hours) (Fig. 1A-B). In minimal media, the HS strain under 100mM sucralose had a lower maximum growth in stationary phase than that of the HS strain grown in 0mM sucralose, and lower HS growth in minimal media for both conditions was observed compared to NS (Fig. 1B and 1D). The HS strains grown in minimal media, similar to the NS strains, grew slower and had reduced growth at stationary phase than the strains grown in TSB (Fig. 1B and 1D). Altogether the growth curves suggest that the HS and NS strains exhibit phenotypic differences with regards to growth rates and yields under different media conditions and sucralose concentrations. The HS strain shows the highest growth rate and yield in complete media supplemented with sucralose, whereas the NS strain's growth is inhibited by sucralose in both minimal and complete media.

Sucralose resistance phenotype is maintained in the absence of sucralose. Having characterized the HS strain relative to the NS control, we aimed to establish whether these phenotypic differences were stable in the absence of growth stress, and validate the possibility of a genetic mutation conferring resistance as opposed to differential gene expression using a resistance phenotype stability assay. The HS strain was maintained for 48 hours under 0mM and 100mM sucralose in TSB, after which both cultures as well as an NS culture grown in 0mM sucralose were plated out onto TSA containing either 0mM or 100mM sucralose for 24 hours. Colony growth was observed in all samples grown at 0mM sucralose, with visibly greater density of growth from the NS strain compared to the HS strain (Fig. 2A-C). On TSA with 100mM sucralose, growth was generally sparser compared to TSA with 0mM sucralose (Fig. 2A-F). No growth of the NS strain was observed on TSA containing 100mM sucralose (Fig. 2D-F). In contrast, the HS strains displayed colony growth on 100mM sucralose TSA following 48 hours of maintenance with both 0mM and 100mM sucralose, consistent across all replicates (Fig. 2D-F). As the HS strain was maintained at 0mM sucralose for 48 hours, resistance phenotypes emerging from differential gene expression in response to sucralose presence, rather than a permanent mutation conferring resistance, would have been lost in this sustained absence of sucralose, and thus growth would not have been observed on 100mM TSA once the HS —> NS culture was plated out. Growth of HS strains on solid media containing 100mM sucralose, following transfer into broth without sucralose, suggests that the HS strain resistance is stable in the absence of sucralose and further supports the idea that sucralose resistance in this strain is conferred by a genetic mutation that can be identified via sequencing.

The sucralose resistance phenotype of HS is not conferred by a mutation in the *gryA* QRDR sequence. To determine if the HS strain resistance phenotype was conferred by a

gyrA mutation, we chose to analyze the 647bp QRDR sequence of *gyrA* that is notably involved in quinolone resistance (15). DNA isolation and PCR amplification were conducted to obtain the desired QRDR sequence of the DNA gyrase subunit gene *gyrA*. We were able to successfully isolate and amplify the segment of *gyrA* in HS1-3 and NS1-3 strains, which represent samples of different isolated from each strain, as qualitatively determined by agarose gel electrophoresis (Fig. S1). DNA bands matched the band corresponding to 700bp on the DNA ladder which is the desired length of the *gyrA* segment (Fig. S1). Sequences for samples were obtained via Sanger sequencing. Multiple sequence alignment displayed no mutations in *gyrA* QRDR sequence in any of the HS or NS samples (Fig. 3). Both HS and NS strains matched 99-100% in identity to the wildtype *E. coli* reference sequence, with differences in non-specific nucleotides (Fig. 3). The comparative analysis suggests that the *gyrA* QRDR sequence under observation from our selected samples is not responsible for conferring sucralose resistance in the HS strain.

DISCUSSION

In this study, we aimed to study a possible link between the molecular mechanism behind sucralose resistance in *E. coli* and resistance to quinolone antibiotics, as suggested by previous research (15). A strain of *E. coli* resistant to 100mM sucralose was obtained via repeated subculturing in progressively higher concentrations of sucralose. The stability of sucralose resistance in the strain was confirmed using a resistance phenotype stability assay, and the *gyrA* sequences of this strain and a control strain subcultured in 0mM sucralose were sequenced and compared using Multiple Sequence Alignment. The established sucralose-resistant strain (HS) was shown to have a stable resistance phenotype that was not reversed by growth in the absence of sucralose, suggesting that this resistance is conferred by a genetic mutation rather than by differential gene expression. However, sequence analysis showed no mutations in the *gyrA* QRDR of this strain.

A growing body of evidence shows the negative health effects of consuming NSC and their association with the gut microbiome and bacteriostatic experiments on commensal organisms, resulting in dysbiosis (4, 13). Sucralose is implicated strongly, both as a commonly consumed NCS and a broadly bacteriostatic agent that can potentially alter the compositions of complex bacterial communities (4, 10). Additionally, Onseedaeng and Ratthawongjirakul have found greater numbers of quinolone antibiotic resistant *E. coli* mutants grown in the presence of sucralose, suggesting that there may be a link between sucralose and quinolone resistance in *E. coli* (15). As quinolone resistance is commonly conferred by specific mutations in the QRDR of *gyrA* (15), this specific region was investigated for potential relevance to sucralose resistance.

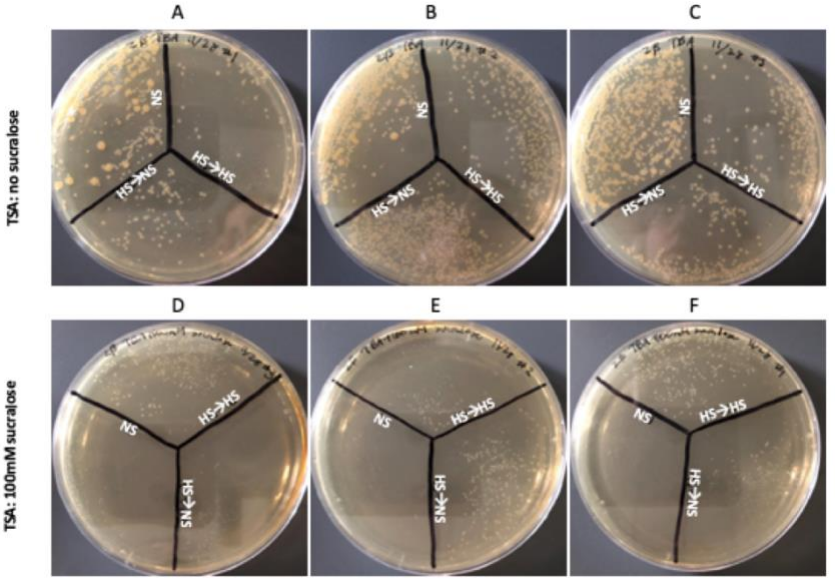

FIG. 2 Sucralose Resistance Phenotype Stability Assay displays sustained sucralose resistance of the HS strain when grown in the absence of sucralose. Plates are divided by treatment conditions grown overnight at 37˚C, in which NS represents the plating of a non-sucralose resistant strain from a 0mM sucralose subculture, HS → HS represents plating of a high sucralose resistant strain suspended in a 100mM sucralose subculture, and HS → NS represents plating of a high sucralose resistant strain suspended in a 0mM sucralose subculture (A-F). Subcultures are grown in either TSA without sucralose (A-C) or TSA containing 100mM of sucralose (D-F) and were done in triplicates (n=3).

Using multiple sequence alignment to compare the samples we isolated, we found a 100% match between NS, HS, and the BLAST reference sequence of the QRDR of *gyrA*. Both HS and NS samples that were used for sequencing experiments were obtained after 20 daily subcultures, corresponding with over 1400 divisions given the estimated 20 minute replication time of *E. coli* (21). The lack of mutations in the QRDR of *gyrA* following over 1400 rounds of replication in culture demonstrates the robustness of the DNA gyrase subunit GyrA even in strains adapted to high sucralose concentrations. The lack of mutations in the QRDR of *gyrA* in our samples refutes our hypothesis of a direct link between sucralose and quinolone resistance in *E. coli* involving a quinolone-resistance conferring *gyrA* mutation. However, our sucralose resistance phenotype stability assay (Fig. 2) suggests that the HS strain has a permanent mutation, rather than transient changes in gene expression under stress, as indicated by colony growth on TSA from HS cultures transferred to and maintained in TSB containing no sucralose; had differential gene expression been conferring the observed resistance phenotype, HS strains maintained in absence of sucralose would not have displayed growth once this culture was plated on 100mM sucralose. The sequencing data, in tandem with the resistance phenotype stability assay, demonstrates that sucralose does not have the same mechanism of bacteriostasis as quinolones through the targeting of DNA gyrase genes, and further suggest that a sucralose-resistance conferring mutation could be elsewhere in the genome.

In sequencing the QRDR of HS *gyrA*, the sequences of our samples were between 582-586 bp in length, 594-599 including non-specific nucleotides present at the ends of our sequence, which was 65 bp shorter than expected with our gene-specific primers (15). When performing multiple sequence alignments with our samples of HS and NS, all sequences matched 100% to each other and 99-100% to the reference *gyrA* QRDR with non-specific nucleotides at the ends of the sequence, showing that no mutations were obtained in the region we hypothesized from the association between quinolone and sucralose resistance.

Our original hypothesis on the *gyrA* QRDR and its role in sucralose resistance was a proposed carbon-source independent mechanism of resistance to sucralose, extending from the possibility that sucralose may have carbon-source independent mechanisms of action as a bacteriostatic agent. Inhibition of the sucralose-susceptible NS strain in both TSB, containing a variety of carbon courses as a nutrient-rich complete broth, and minimal media supplemented with glucose as the only viable carbon source, further validate previous findings that sucralose acts in sucrose-independent mechanisms to inhibit bacterial growth (8). As neither TSB nor the prepared minimal media contain sucrose as a carbon source, the observed inhibitory effects of sucralose and the subsequent resistance of the HS strain are unlikely to be due to the previously proposed sucralose resistance mechanism involving sucrose permease and invertase (10). However, a number of phenotypic differences with potential relevance to carbon metabolism were noted between the HS and NS strains. Unlike NS, the HS strain did not present diauxic growth under ideal conditions in TSB.

FIG. 3 No mutation in a segment of gyrA from non-sucralose resistant and high sucralose resistant strains. Multiple sequence alignment of *gyrA* from 3 different colonies (n=3) of sucralose-susceptible (NS) and sucralose-resistant (HS) strains compared to the wildtype BLAST reference sequence of *E. coli* DH5α.

Furthermore, in minimal media, the HS strain in both 0mM and 100mM sucralose grew slower and reached a lower maximum growth yield in stationary phase compared to the NS strain at 0mM. Although sucralose has a bacteriostatic effect on susceptible strains in the absence of sucrose as well as in the presence of glucose alone, the absence of diauxic growth in complete media and low growth in minimal media of the HS strain suggest that the mechanism of resistance to sucralose may still relate to non-sucrose carbon metabolism. The acquisition of a resistance conferring gene relating to the metabolism of non-sucrose carbon sources in the HS strain may have resulted in the observed HS growth phenotypes, including the inhibition of HS by 100mM sucralose in minimal media but not in TSB. As existing literature on sucralose and carbon metabolism in bacteria has focused largely on sucrose metabolism alone, the impact of sucralose on non-sucrose carbon metabolism is a potential area for future investigation.

Finally, it is also important to note that the obtained HS strain was resistant to a relatively high concentration of sucralose that approached but did not meet the MIC90 of sucralose on *E. coli* which is upwards of 157mM (16). Due to time constraints, a strain resistant to this concentration of sucralose was not reliably established to be used in sequencing experiments. Although 100mM appeared to strongly inhibit growth of susceptible strains on solid media (Fig. 2D-F), some growth in broth supplemented with sucralose at this concentration was still observed (Fig. 1A). The concentration of sucralose used in our experiments offer an alternate explanation for the lack of mutations in the QRDR sequence of *gyrA* from our samples. As GyrA is an essential protein involved in both DNA transcription and replication (22), a higher level of environmental stress than the one used in our experiments may be required to induce mutations in *gyrA*. While 100mM of sucralose presented a sufficient degree of environmental pressure to select for a phenotypically distinct strain of *E. coli*, this HS strain may have acquired its resistance through mutations in non-essential or less robustly conserved genes, such as genes involved in non-sucrose carbon metabolism; thus, our HS strain may employ a sucralose resistance mechanism unrelated to the quinolone resistance association. It is possible that a higher concentration of sucralose such as the MIC90 of 157mM may have been required for a *gyrA* mutation—or other quinolone resistance associated mutation—to confer a greater degree of sucralose resistance.

Though there were no mutations in the QRDR sequence of *gyrA* in our HS samples, there are a number of potential explanations that we have aimed to explore in this discussion. One explanation is that a mutation may still be present in the end-regions of the QRDR sequences, which were not detectable in sequencing data or the samples selected. Another explanation is that our strains may specifically be resistant to sucralose through a carbon-metabolism pathway, unrelated to sucrose, and that a higher degree of sucralose-induced stress may be necessary for any associations of sucralose and *gyrA* or quinolone resistance to be observable at a genomic level.

Conclusions Based on previous findings that cultivation of *E. coli* in sucralose resulted in an increase in mutants resistant to quinolones (15), we sought to determine whether mutations in *gyrA* involved in quinolone resistance would be involved in sucralose resistance. For this study, an *E. coli* strain resistant to 100mM sucralose was obtained via subculturing. The strain was characterized as being phenotypically distinct from a sucralose-susceptible strain of *E. coli*, also obtained through subculturing. Namely, in the absence of sucralose in TSB the HS strain grew less than the NS strain, and the HS strain grew similarly in the absence and presence of sucralose in rich media; however, the HS strain grew poorly in minimal media compared to the NS strain. Upon establishing the stability of the resistance phenotype in the absence of sucralose as the selecting condition, the QRDR sequence of *gyrA* between the sucralose-resistant and susceptible strains were compared, revealing that this region of *gyrA* was not mutated in sucralose resistant *E. coli*. Although a direct link between sucralose and quinolone resistances via *gyrA* could not be established in our experiments, the data nonetheless shed further insights into alternate mechanisms of action for sucralose as a bacteriostatic agent.

Future Directions The overall goal of our project was to determine whether mutations in *gyrA* involved in quinolone resistance was a mechanism that conferred a high sucralose-resistance phenotype in *E. coli*. We discovered no mutations in the *gyrA* gene segment QRDR we examined, but that the resistance phenotype was stable from our resistance phenotype stability assay. For future studies, the full gene length of 2625bp (23) and promoter sequence of *gyrA* can be investigated by amplifying the entire gene and sequencing it. Another method is to conduct whole genome sequencing to see if the mutation resides in another part of the genome independent of quinolone resistance, such as the various genes associated with non-sucrose carbon metabolism. Particularly, the Oxford Nanopore MinION is an optimal choice as a fast and efficient portable DNA sequencer that can produce complete reads of circular bacterial genomes. Alternative to sequencing methods, a full quinolone resistance assay can be conducted on the HS strain to further explore the link between sucralose and quinolone resistance and therefore a *gyrA* mutation. Due to time constraints, we were not able to obtain a stable *E. coli* strain under the reported MIC90 value for sucralose, in the 150-157mM range (8). To further strengthen the results from our experiment, the next step would be to subculture for a high-sucralose resistant strain grown in 150mM sucralose media, validate the results of our experiments with this new strain, and conduct further investigations on the potential link between sucralose and quinolone resistance.

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