Report on the faculty development grant, Summer 2022:

"In Vitro Investigation of Bacterial Growth and Functionality in Simulated Respiratory Tract Lining Fluid"

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The goals of the faculty development were:

[1] Purchase and cultivate respiratory strains of lung commensal bacteria

[2] Generate long-term glycerol stocks of bacterial strains

[3] Ascertain a range of RTLF concentrations that:

- [a] supports growth of each bacterial strain
- [b] inhibits biofilm production or activity

[4] Correlate metabolic activity to cell viability and biofilm production

[5] Collect and store DNA and protein samples

[6] Present data at biology department meeting

These goals were met as described below:

[1] [2]

Acquired laboratory strains of *S. aureus* (SA)*, S. pneumoniae* (SP), and *M. catarrhalis* (MC); and cultivated the mircobes in brain-heart-infusion (BHI) media for either 1 day (SA), or 2 days (SP and MC). Overnight cultures were prepared for both experiments and long-term glycerol stocks, which would last several years at -80°C. For each microbe, an overnight culture was diluted 1:1 with 50% glycerol; microcentrifuges were then filled with 1 ml of this solution. For each organism, there are a dozen glycerol stocks.

[3a]

To determine if the bacterial strains could survive in simulated respiratory tract lining fluid (sRTLF), bacterial cultures were inoculated in a range of sRTLF dilutions (0, 50, 75, 90, 95, 100% in TSB) and incubated for either 1 day (SA), or 2 days (SP and MC). Cell density of the inoculated sRTLF dilutions was determined by measuring absorbance at 600nm (OD600). As can be seen in FIGURE 1, the SRTLF, at any concentration, decreased the bacterial cell density for SA and SP when normalized to the 0% sRTLF solution (i.e, 100% TSB; positive control); the bacterial cell density of MC only slightly decreased. Of interest, when the inoculated sRTLF dilutions for SA were plated on nutrient agar, growth was observed for all solutions, though the growth of the 100% sRTLF solution was substantially less than for the other dilutions. This is indicative of the sRTLF, when diluted, being bacteriostatic (i.e., inhibits growth, doesn't kill) for the tested incubation time (FIGURE 2). This result prompted characterization of the impact of sRTLF on growth rate. SA was inoculated in a range of sRTLF dilutions (0, 50, 75, 90, 95, 100%) and OD600 was measured every hour for 6 hours (FIGURE 3). Unsurprisingly, 0% sRTLF resulted in the characteristic growth curve of bacteria, with a steady increase in absorbance seen ~2 hours after inoculation (indicative of exponential growth). However, for the remaining inoculated sRTLF dilutions, growth was far more muted, with little to no increase in absorbance seen after 2 hours.

[3b]

To determine biofilm production of the individual bacterial species in sRTLF, bacterial cultures were inoculated in the sRTLF dilutions on 12-well plates, and incubated for either 1 day (SA), or 2 days (SP and MC). Total biofilm biomass of the inoculated sRTLF dilutions was determined by performing the crystal violet assay and measuring absorbance at 590nm. For each bacterial species, the sRTLF, at any concentration, decreased the biofilm biomass (when normalized to the 0% sRTLF solution), with substantial decreases seen at 75% sRTLF (FIGURE 4). To determine if the measured biofilm biomass possessed viable bacterial cells, inoculated sRTLF dilutions for SA were plated for 24 h before an AlamarBlue assay was performed and absorbance measured at 570nm. As the sRTLF concentration increased, there was a steady decrease in the metabolic activity when normalized to the 0% sRTLF solution (FIGURE 5).

[4]

To determine metabolic activity in the presence of sRTLF, SA was inoculated in a range of sRTLF dilutions for 1 day before the MTT assay was performed and absorbance measured at 570nm. Absorbance was significantly reduced for all sRTLF dilutions, when normalized to the 0% sRTLF solution, indicative of reduced metabolic activity (FIGURE 6).

[5]

To determine the impact of sRTLF exposure on the bacterial species at a later time, DNA and protein samples were collected. Bacterial cultures were inoculated in the sRTLF dilutions and incubated for either 1 day (SA), or 2 days (SP and MC). For DNA collection, cultures were centrifuged; re-suspended in a lysozyme/proteinase K solution; purified with phenol/chloroform; and placed at -80°C. There is one set of the conditions for each bacterial species. For protein collection, cultures were centrifuged and re-suspended in lysis buffer, and placed at -80°C. There is one set of the conditions for each bacterial species.

[6]

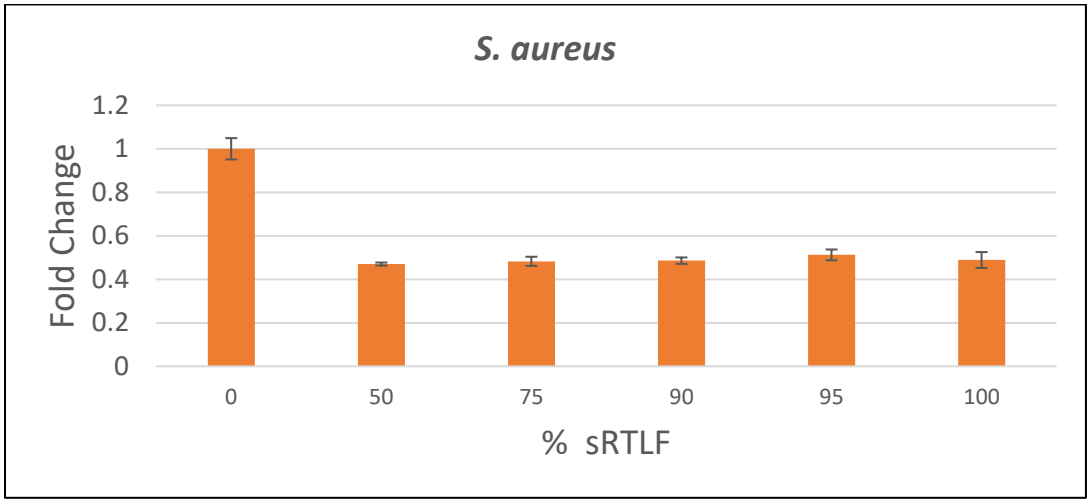
The above mentioned data was prepared as an oral presentation that was part of the Faculty development grant presentation series.

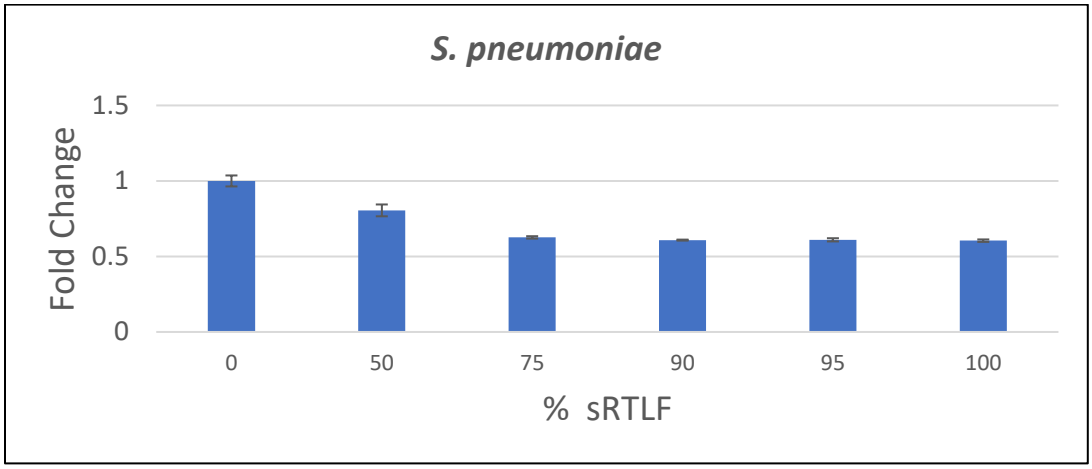
Conclusions:

The faculty development grant permitted me to acquire and cultivate bacterial species that are crucial to my research endeavors, and will enhance the laboratory exercises in BIO250L, BIO401L, and BIO402L. Some of the experiments were only conducted with one bacterial species (SA) due to supplies arriving late as well as due to the need (and time) to get an appropriate number of repeats (biological replicates, n, = 3; experiments done in triplicate) for the results to be meaningful. Nevertheless, my results provide preliminary insight into the ability of commensal bacteria to survive within the respiratory tract. Individually, SA and SP struggled to grow and produce biofilm in the sRTLF dilutions, while MC could grow but not produce biofilm. However, once diluted, the sRTLF appears to become more bacteriostatic (at least in the case of SA*). In vivo*, such a scenario would likely occur when the respiratory epithelia sustain an acute injury; and cell debris, blood, matrix proteins, etc. mix with the lining fluid. Such contaminants would also serve as a substrate for bacteria to attach to, increasing the likelihood of survival. The bacteria also have an opportunity to interact with epithelial cells more so. These intriguing prospects, along with the results from this project, will guide future research endeavors.

For instance, this project has already resulted in a research opportunity for a Lincoln student this spring term. That project focuses on the prospect of commensal bacteria working together to make biofilms in the presence of sRTLF. With the data that will be collected from that research, there is potential for participation (poster) in the American Society for Microbiology meeting, ASM Microbe, in the summer 2023 (in addition to being presented at Lincoln's research symposium by the student).

FIGURE 1: sRTLF decreases bacterial cell density.





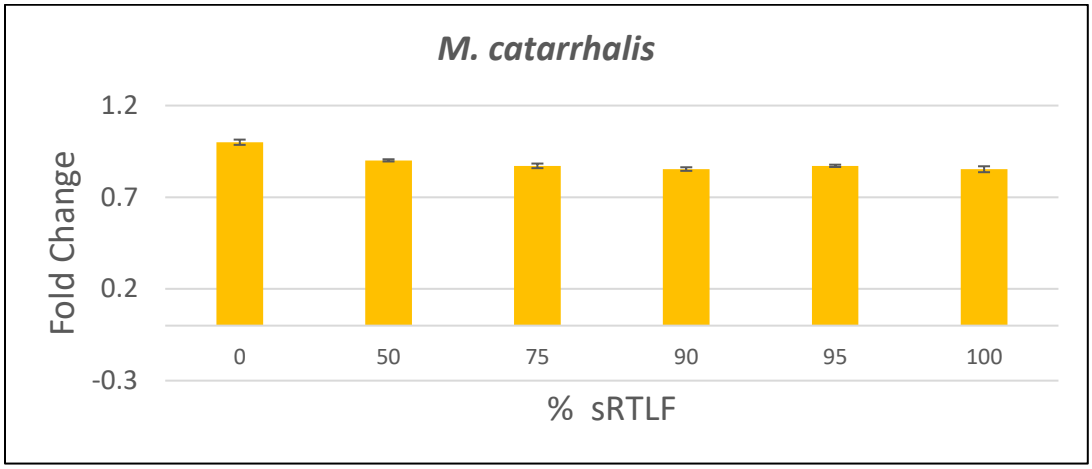


FIGURE 2: sRTLF inhibits bacterial growth (*S. aureus*)

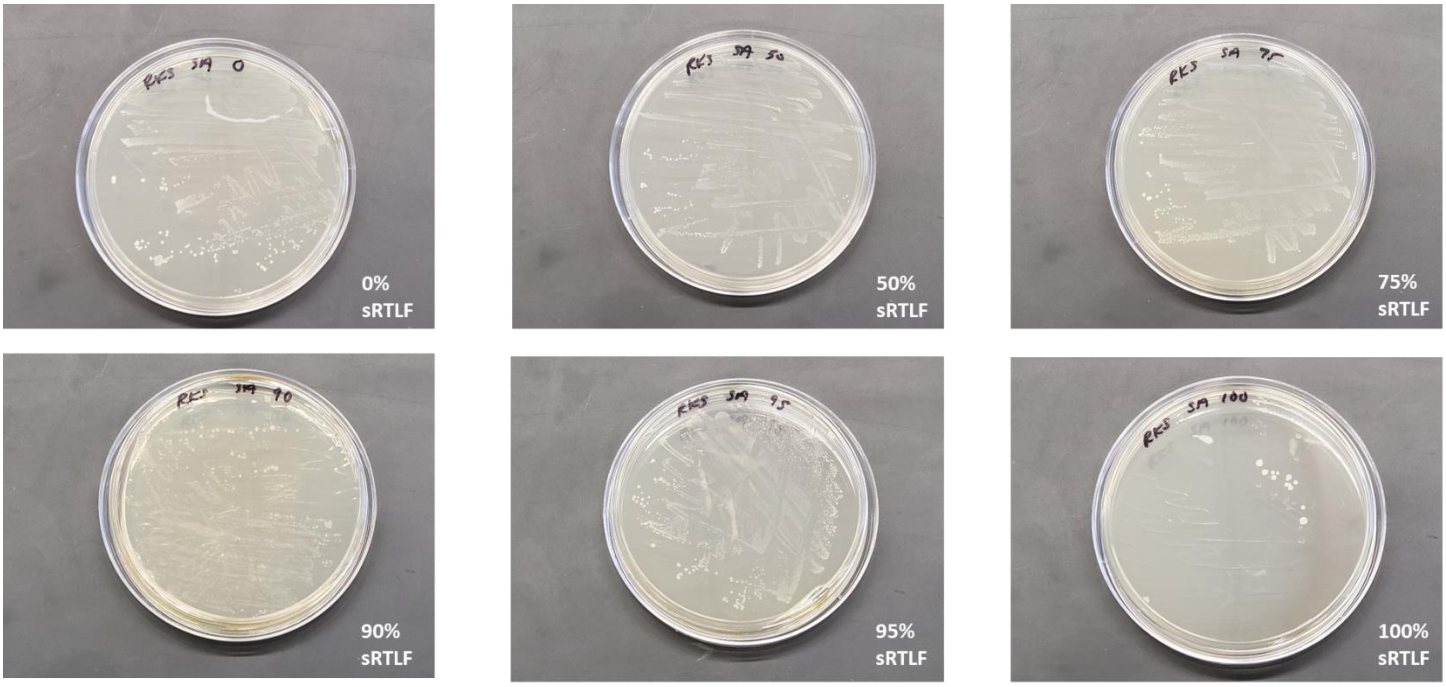


FIGURE 3: sRTLF substantially reduces growth rate (*S. aureus*)

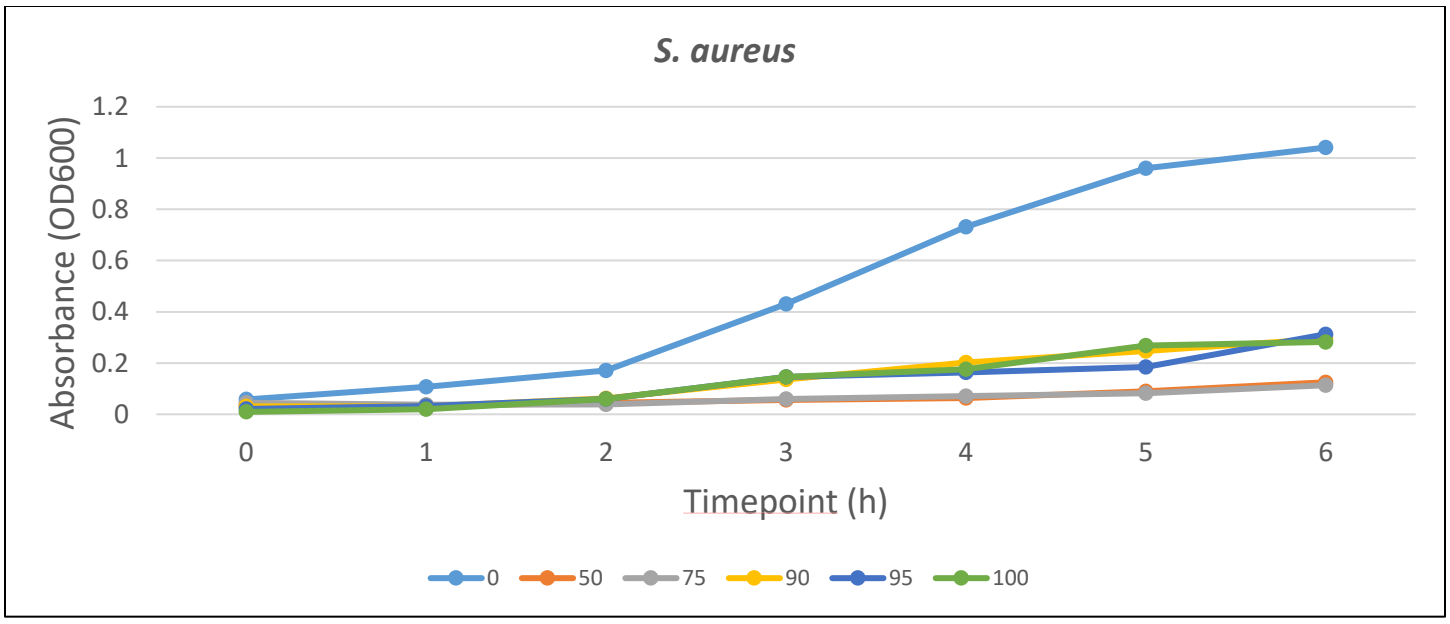
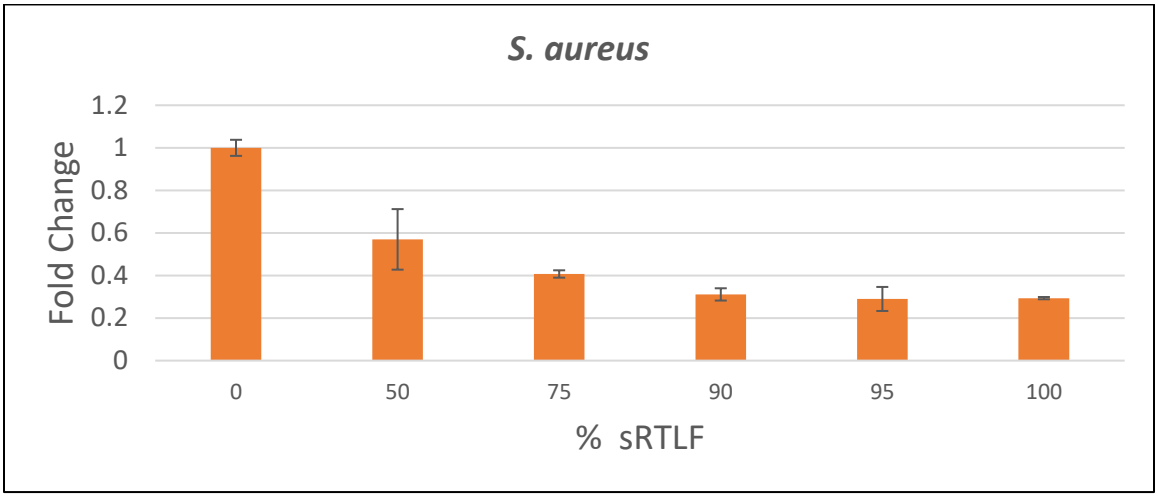
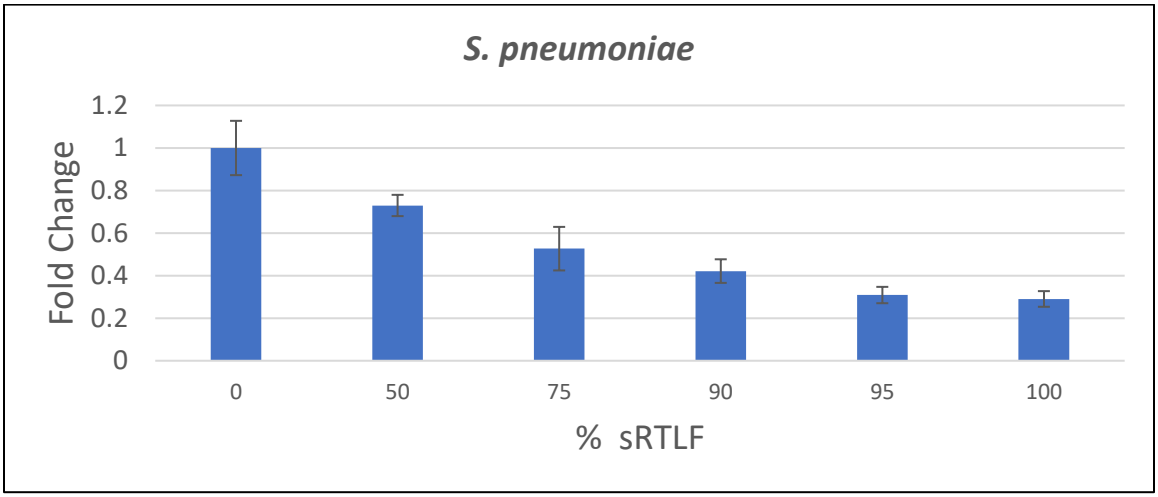


FIGURE 4: sRTLF reduces biofilm biomass





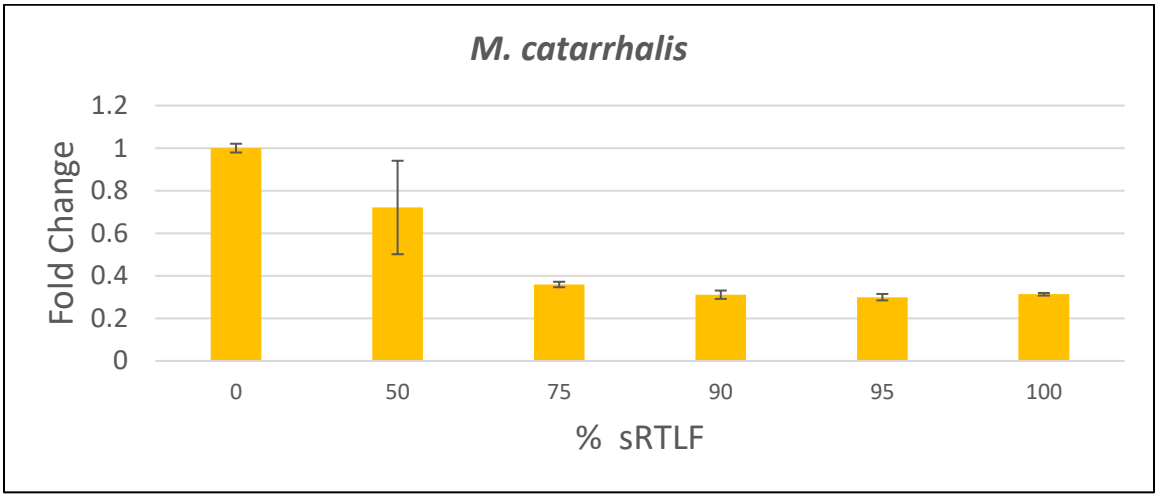


FIGURE 5: sRTLF reduces biofilm metabolic activity (*S. aureus*)

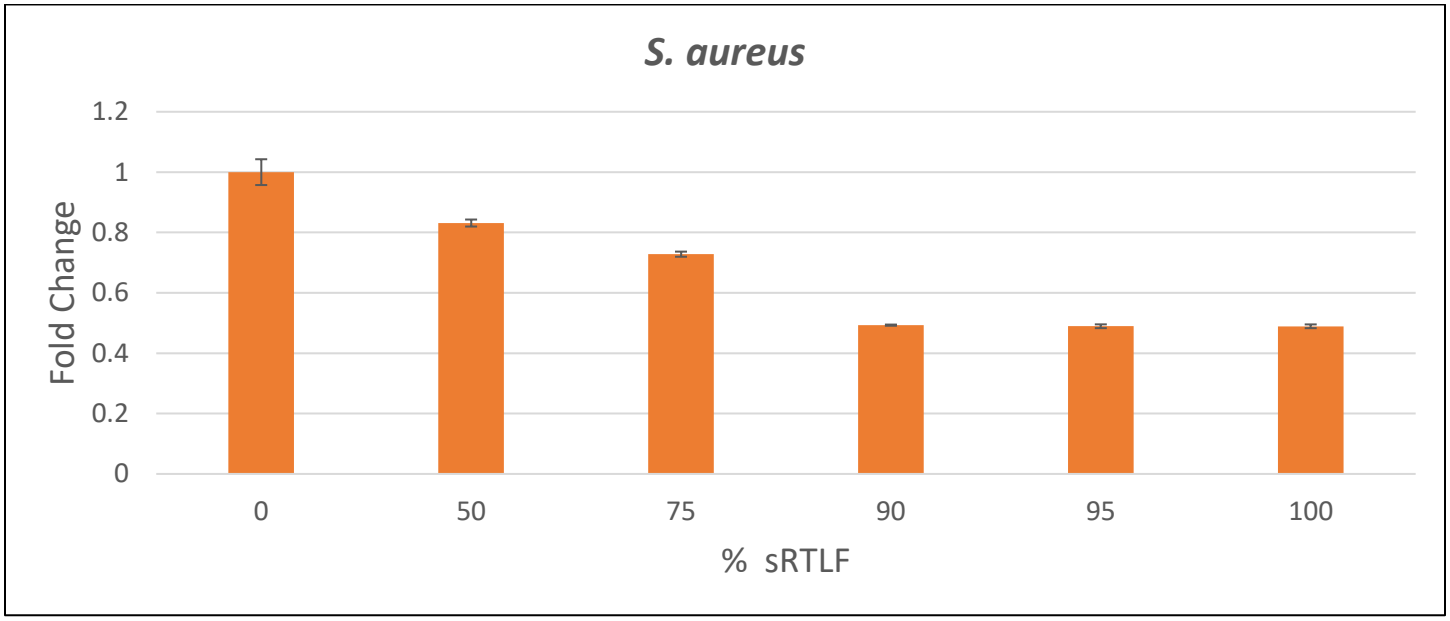


FIGURE 6: sRTLF reduces bacterial metabolic activity (*S. aureus*)