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Temporal changes in Shiga-Toxin producing *Escherichia coli* (STEC) O121 transcriptome during storage in bleached flour

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Abstract

Introduction: Persistence of Shiga-toxin producing *Escherichia coli* (STEC) within low-moisture foods, such as flour, has led to several STEC-related outbreaks associated with consumption of raw or undercooked flour products once considered microbiologically safe. The molecular mechanisms by which STEC can survive in flour are not well understood.

Purpose: This study sought to develop an effective sample treatment and RNA extraction method for pathogen contaminated polysaccharide-based matrices like flour; and to profile STEC transcriptome changes during desiccation and storage in flour.

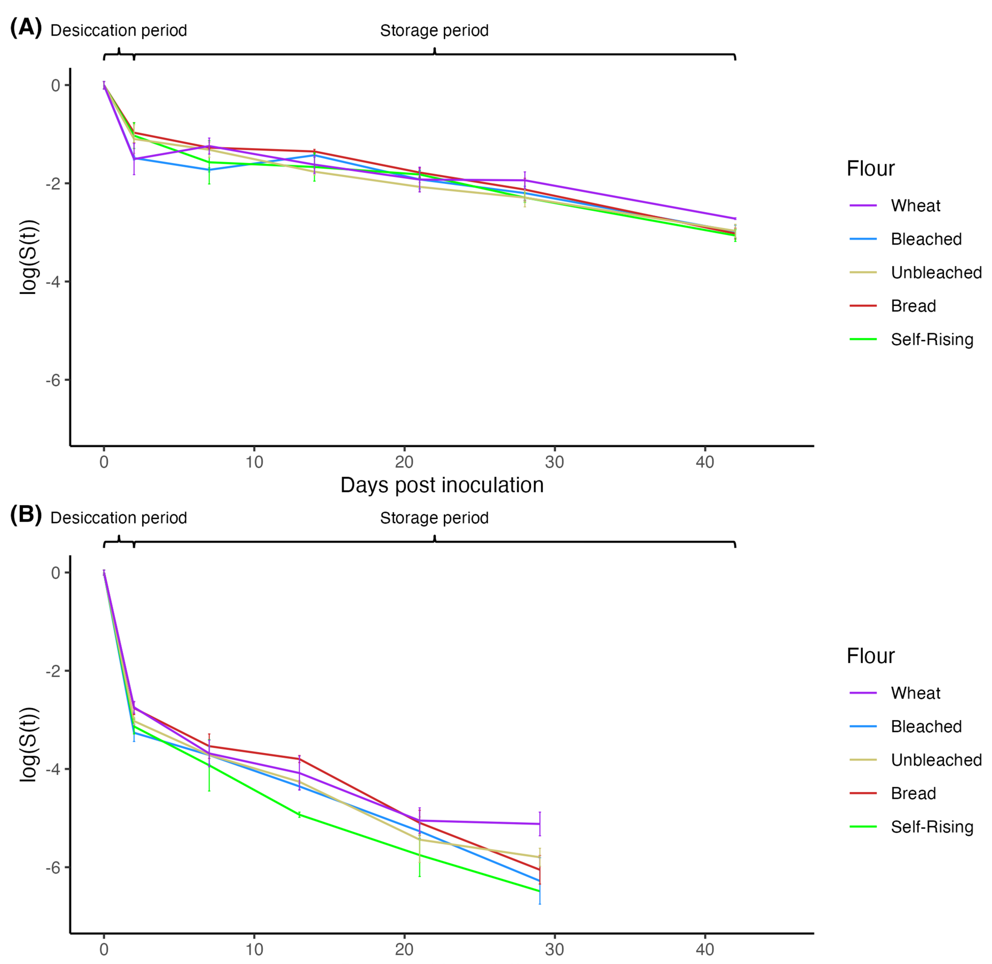
Methods: All-purpose bleached flour was inoculated with STEC O121 (CFSAN051458) at 10^{10} CFU/g flour. Flour samples were collected at nine time points within the first 48 hours post inoculation and treated with different centrifugation-filtration schemes. The RNA extraction method was optimized, and, after total RNA extraction of all samples, libraries were prepared using the Illumina Stranded Total RNA Prep kit and sequenced on the Illumina NextSeq2000 platform. The raw reads were quantified using salmon and analyzed with DESeq2 to identify temporal changes in the transcriptome profile upon flour inoculation and storage.

Results: Cellular loss and residual flour in the filtrates were successfully minimized by electing to utilize a 5 μm pore-size membrane filter following quick centrifugation, which consistently led to isolation of high-quality RNA. Preliminary analysis revealed several genes were differentially expressed in all flour samples relative to a pure culture control. Approximately 1700 genes were expressed significantly differently ($P_{adj} < 0.05$) between culture control and flour inoculation. Expression levels for temporally associated genes, mainly stress response and metabolic genes, had a major spike in the rate of expression change at 30 minutes post inoculation. However, the levels appeared to stabilize within an hour of storage at which point few genes were differentially expressed relative to the prior timepoint.

Significance: Transcriptomic profiling of STEC persistence within flour will increase the understanding of STEC survival in low-moisture environments and help develop new mitigation strategies.

Background

Figure 1. Image of raw flour formed into dough



https://my.clevelandclinic.org/health/diseases/16638-e-coli-infection

Figure 2. Artist rendering of pathogenic *E. coli*

Figure 3. STEC survival in different flours during long-term storage. Three samples of each flour (differentiated by color as per the figure legend), inoculated with either (A) STEC O121 or (B) STEC O157, were sampled and enumerated. The log reduction of STEC populations (logS(t), wherein $S(t) = N_i/N_0$) is plotted against the time. Error represents the mean ± standard deviation of three samples.

- Raw/undercooked wheat flour consumption associated with multiple outbreaks
- Shiga toxin-producing *Escherichia coli* (STEC) O121:H19 outperforms O157:H7 during desiccation in wheat flour
- O121 isolated from 2016 all-purpose flour-associated outbreak
- Functional genomics studies improve understanding of survival mechanisms

Hypothesis: Which genetic networks allow O121 to persist in the desiccated flour environment?

Methods

Flour maintenance

- All-purpose bleached flour used as food matrix
- Flour acclimation in a glove box set to 50% relative humidity
- *E. coli* O121:H19 used to inoculate flour at 10 log CFU/g flour

Sampling plan

- 3 trials (1 week each)
- Pure inoculum and pure inoculum subjected to filtration
- Point of inoculation (0 hr)
- 0.5-, 1-, 2-, 3-, 4-, 8-, and 48-hour post inoculation
- 60 samples total (n = 6)

Filtration methods

- Flour resuspension in cold (4°C) 15% EtOH
- Spin resuspension at 5,000 x g for 1 min at 4 °C
- Resuspend pellet in dH_2O and pellet cells for RNA isolation

Extract total RNA
- 1 M DTT to prevent RNase
- NorGen Biotek total RNA

Sequence
- Illumina NextSeq 2000
- P3 reagents

RNA-Seq analysis
- Salmon quantification
- DESeq2 expression analysis

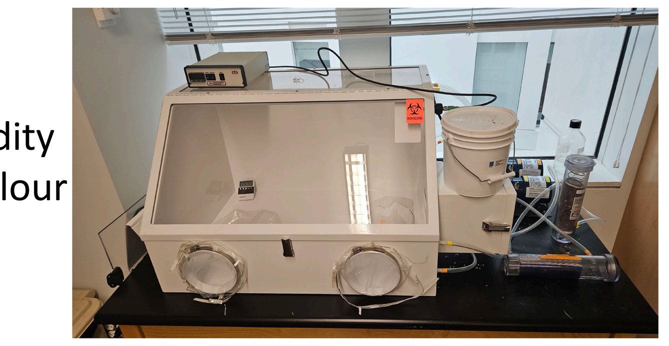


Figure 4. Image of humidity-controlled glovebox. Desiccation beads frequently exchanged to maintain humidity.

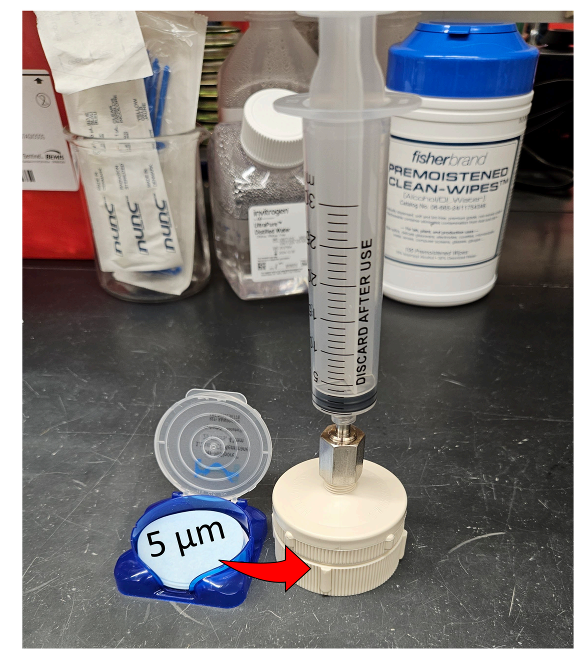


Figure 5. Image of filtration system. 5 μm PVDF filter added within Swinnex holder and autoclave-sterilized prior to use.

Figure 6. Image of Illumina NextSeq2000 platform. Total RNA library prepared using the Illumina Stranded Total RNA Library Preparation Kit.

Results – Temporal DEG

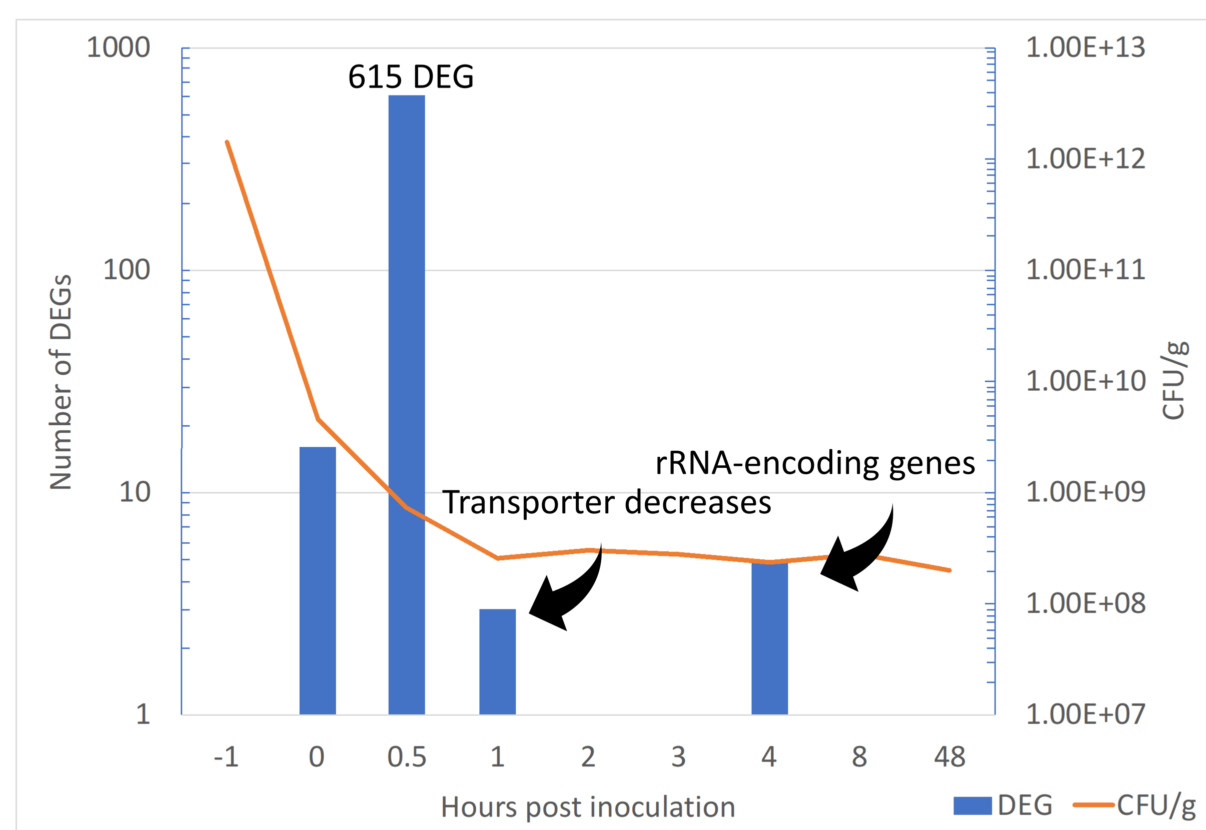


Figure 7. Temporally-associated transcriptome of STEC O121 in bleached flour. All-purpose bleached flour was inoculated with STEC O121 and sampled at several time points within 48 hours. Following DESeq2 analysis between each time point relative to the prior one, the number of differentially expressed genes (DEG) were calculated and represented above. The number of significant (P < 0.05) DEG between the timepoint on the x-axis relative to the prior timepoint are represented by the blue columns and represented on the left side. The average population of O121 within the flour is represented by the orange line and indicated on the right side.

Results – Top 50 DEG

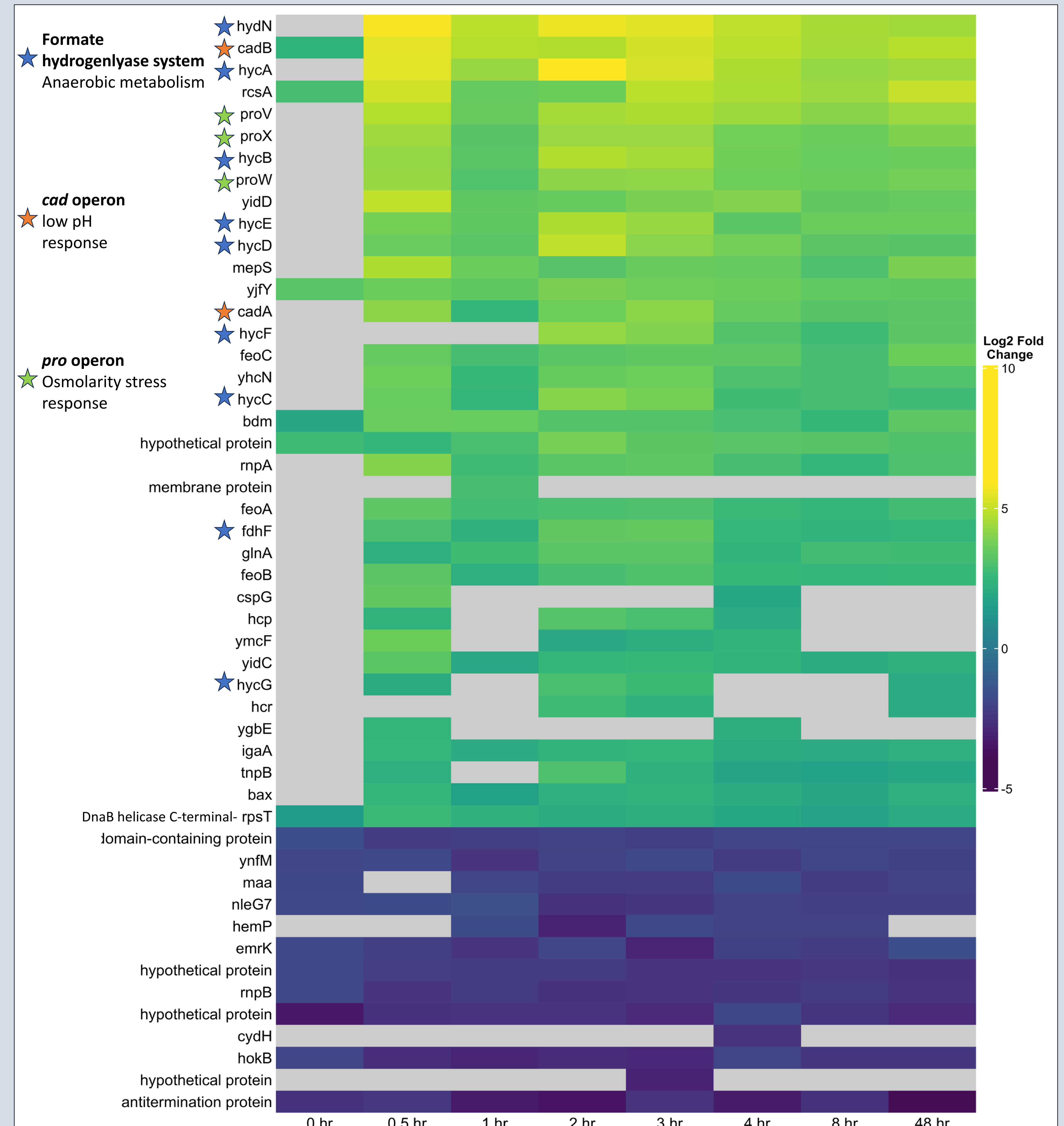


Figure 8. Heatmap representing the top 50 DEG of STEC O121 within flour. All-purpose bleached flour was inoculated with STEC O121 and sampled at several time points within 48 hours. Following DESeq2 analysis, the top 50 most differentially abundant genes relative to a pure culture control were identified and represented as the above heatmap. Brighter yellow color represents higher increased fold changes and darker blue represents higher decreased fold changes.

Conclusions

- Establishment of STEC RNA extraction method within fine, high polysaccharide food matrix
- Acclimation of STEC to flour environment may be within 1 hour
- Several groups of stress-related genes upregulated during storage
- Toxin/virulence factors downregulated during storage

Future Directions

- Examine STEC interaction with flour microbiome
- Evaluation of genes essential for persistence in low moisture foods via integration of genomics and transcriptomics data

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