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Global transcriptomes of *Salmonella enterica* serovar Agona reveals the mechanism of survival on low moisture food

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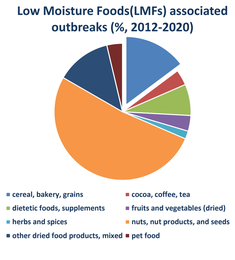
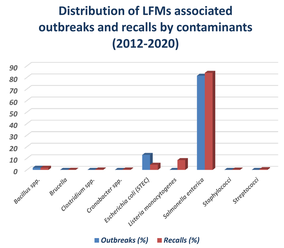
Abstract

Salmonella enterica is one of the major foodborne pathogen known for its ability to survive in a variety of environmental conditions. One of the serovar *S*. Agona's ability to survive on extremely low-moisture foods, such as cereals, for extended periods is an example of its remarkable adaptability and persistence in extreme low moisture environments. This characteristic is of significant concern in terms of food safety, as it highlights the challenges in preventing and controlling pathogen contamination in dry or low-moisture food products. The aim of this study was to compare the global transcriptomes of *S*. Agona cells grown in liquid media and post drying condition, to identify genes that impact their survival on extreme low moisture food (LMF).

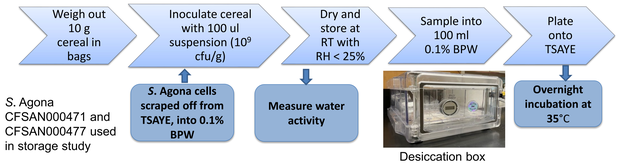
Introduction



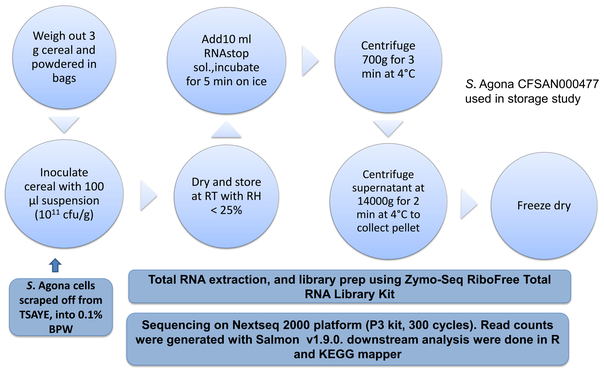


- ❑ *Salmonella enterica* serovar Agona caused recurrent multistate outbreaks associated with cereal between 1998 and 2008, highlighting the persistence of *Salmonella* over time in food processing facilities.
- ❑ There is a knowledge gaps in the molecular mechanisms of their survival. However, understanding the survivability and physiology of this pathogen in low moisture food (LMF) and low-moisture environments is necessary for developing future intervention strategies.

Materials and Methods



Flow Diagram 1. Survival of *S*. Agona on cereal for 90 days on low moisture food (experiment was done in desiccation box to maintain RH < 25%)



Flow Diagram 2. Transcriptome study of *S*. Agona on low moisture food (experiment was done in desiccation box to maintain RH < 25%)

Results and Discussion

Population levels of *Salmonella* were examined from inoculated cereals that underwent desiccation stress for 90 days. Right after inoculation, approximately 9 log cfu/g *Salmonella* cells were recovered. The level was then gradually decreased by 2 log cfu/g within 8 hours of drying ($P<0.05$). However, after initial reduction, *Salmonella* population remained at ~7-8 log cfu/g for 90 days. A tailing effect in the survival curve of *S*. Agona was observed indicating the existence of a resistant subpopulation within the main population.

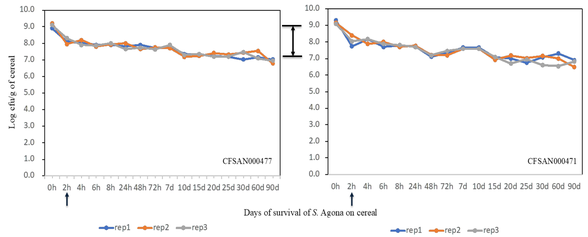


Figure 1. The line graph shows *S*. Agona survival/persistence on cereal over the period of 90 days which was following Weibull distribution

There were 1120 differentially expressed genes (DEGs) of *S*. Agona in response to desiccation stress (Padj < 0.01, |log2FoldChange| >1) where 647 were downregulated and 473 were upregulated. Functional analysis of downregulated DEGs showed most of the genes were associated with metabolic pathways followed by translation, suggesting slower growth in the population.

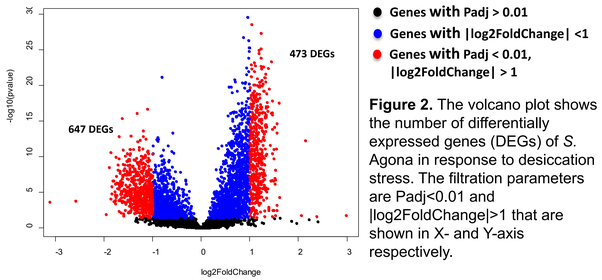


Figure 2. The volcano plot shows the number of differentially expressed genes (DEGs) of *S*. Agona in response to desiccation stress. The filtration parameters are Padj<0.01 and |log2FoldChange|>1 that are shown in X- and Y-axis respectively.

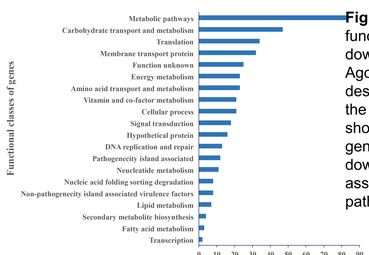


Figure 3. The bar graph shows functional analysis of downregulated DEGs of *S*. Agona in response to desiccation stress. X-axis shows the number of genes and Y-axis shows the functional classes of genes. Most of the downregulated genes were associated with metabolic pathways

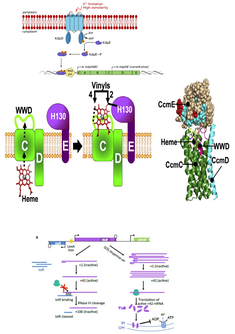
In top 50 DEGs, Specific operons involved in translation (*rps* and *rpl*) were down-regulated in the desiccated cells, suggesting slower growth in the population.

Only three genes were upregulated.

1. *kdp* operon ↑ e.g. *kdpF*, membrane protein, regulatory function and reduce replication

2. *ccm* operon ↑: involved in cytochrome c biogenesis →reduces molecular oxygen to form water in a reaction coupled to energy conservation

3. *tisB*↑: directly associated with persister formation. Activated upon DNA damage as part of the SOS response.



A significant downregulation of genes were observed shortly after the drying treatment (30 minutes and 1 hour post-drying). However, the extent of downregulation decreased as time passed, with fewer genes being downregulated at the 2-hour and 4-hour time points. Additionally, at the 4-hour time point, there was an upregulation of genes, indicating a shift in gene expression patterns.

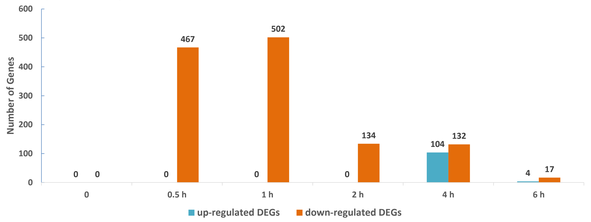


Figure 4. The column graph shows the temporal changes of number of upregulated and downregulated genes. Blue column shows the upregulated and orange column shows downregulated DEGs.

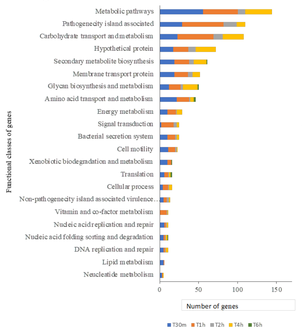


Figure 5. The bar graph shows temporal changes in functions with KEGG Pathway enrichment analysis on down-regulated DEGs. Blue, orange, gray, yellow and green color represent 30mins, 1h, 2h, 4h and 6h post-drying timepoint respectively.

The pathway enrichment analysis suggests a notable shift in the cellular response to desiccation stress. Cells were more prone in conserving energy, altering metabolic processes or adjusting its virulence factors to adapt to the new environment.

An initial downregulation of genes associated pathogenicity island with subsequent upregulation of small number of genes indicates after initial adaptation, *S*. Agona started resuming normal activities as pathogen.

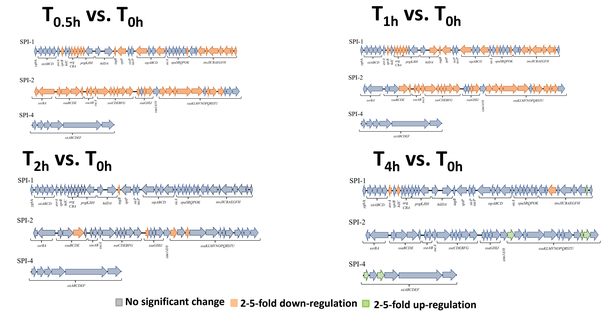


Figure 6. The arrows in the above figure depicting the location, size and arrangement of genes of *Salmonella* pathogenicity island (SPs). Gray, orange and green depicting no significant change, 2-5 fold downregulation and 2-5 fold upregulation respectively. Major virulence genes showing down-regulation during initial phase of desiccation

Conclusion

1. This transcriptome study identified *S*. Agona genes that may be influence their growth and survival on low moisture food.
2. After initial desiccation, significant downregulation of regulatory genes such as metabolic genes, virulence factors might indicate their prioritization for essential functions and energy conservation during desiccation.
3. Among few upregulated genes, *tisB* that is associated with persister formation may be a key factor contributing to recurrent outbreaks from the same industrial facility.
4. Understanding the mechanism of *S*. Agona's response to the environmental stresses like desiccation is crucial for developing strategies to control and mitigate the spread of pathogens in various industrial settings.

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