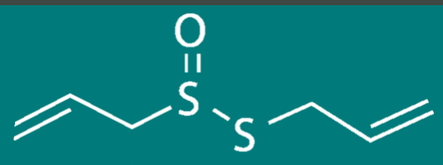
Mechanisms of *Haemophilus Influenzae* Response Against Allicin Damage

Tye Gonzalez, Ulrike Kappler
School of Chemistry and Molecular Bioscience
The University of Queensland AUSTRALIA

Background

Allicin



- Derived from garlic.
- Natural antimicrobial.
- Thought to induce oxidative stress response in *E. Coli* as well as other stress responses (I.e. heat shock). [2]
- *S*-thioallylates accessible cysteine residues in proteins.[1]
- Potential treatment for *Haemophilus Influenzae* infection.

Haemophilus Influenzae

- Non-typeable strain (NTHi) most common clinical strain.
- NTHi is hard to vaccinate against due to lack of surface conservation.
- Found in upper and lower respiratory tract infection and can exacerbate disease such as COPD or cystic fibrosis.

Aims

- Assess quality and coverage of proteome and transcriptome datasets.
- Identify the magnitude of proteome and transcriptome regulation in response to allicin damage.
- Identify key proteins/genes that respond to allicin damage and, in turn, biological mechanisms for response to allicin damage.

Methods

Sample Collection

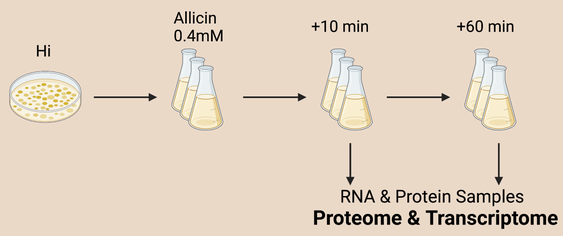


Figure 1. Methods used for allicin treatment and sample sequencing. Samples were collected and sequenced prior to project.

Data Preparation

Transcriptome Data

- Illumina RNA-seq was used for sequencing.
- Quality control, trimming and alignment performed in CLC Genomics Workbench.

Proteome Data

- Quantitative mass spectrometry data collected and processed by Metabolomics Australia UQ.

Data Analysis

- Automated pipeline was created using python to sort and filter proteome and transcriptome data as well as generating volcano plots, tables and gene ontology annotations.

Proteome and transcriptome data is high quality

- In order to make biologically relevant conclusions, we must first assess whether the datasets are of high quality.

Transcriptome

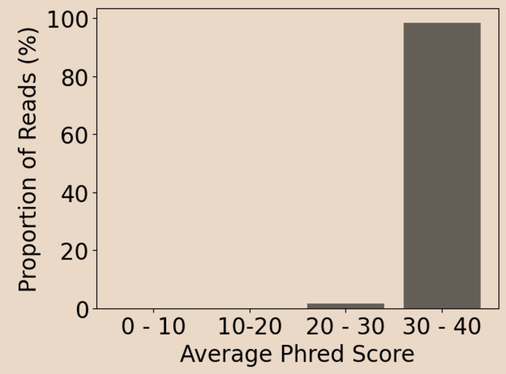


Figure 2. Average Phred scores for RNA-seq data.

Proteome

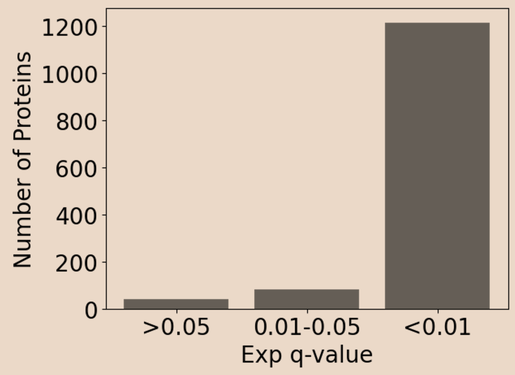


Figure 3. Experimental q-values for proteins detected.

- Majority of gene transcripts expressed with high Phred score of 30+ (0.001% chance of error)
- Majority of proteins detected with high confidence based on experimental q-value.

Transcriptome and proteome data displayed high coverage of genome

- Coverage of *Haemophilus Influenzae* genome was examined in order to assess sensitivity.

Data Source	Sum of Proteins/ Transcripts Detected	Total Genome No.	Genome Coverage (%)
Aggregate Proteome	1339	1872	71.5%
Aggregate Transcriptome	1983	2002	99.1%
Average Proteome Sample	1221 ± 33	1872	65.2 ± 1.77 %
Average Transcriptome Sample	1928 ± 2	2002	96.3 ± 0.11 %

Table 1. Coverage of proteome and transcriptome across all 12 samples (aggregate) as well as the average coverage in each sample.

- High proportion of genome coverage coupled with low variance indicates data was of high quality and sufficient for making biological conclusions

Initial response shows differential regulation between proteome and transcriptome

- In order to gauge the response profile to allicin damage, we first examine the regulation of the proteome and transcriptome at both time points.

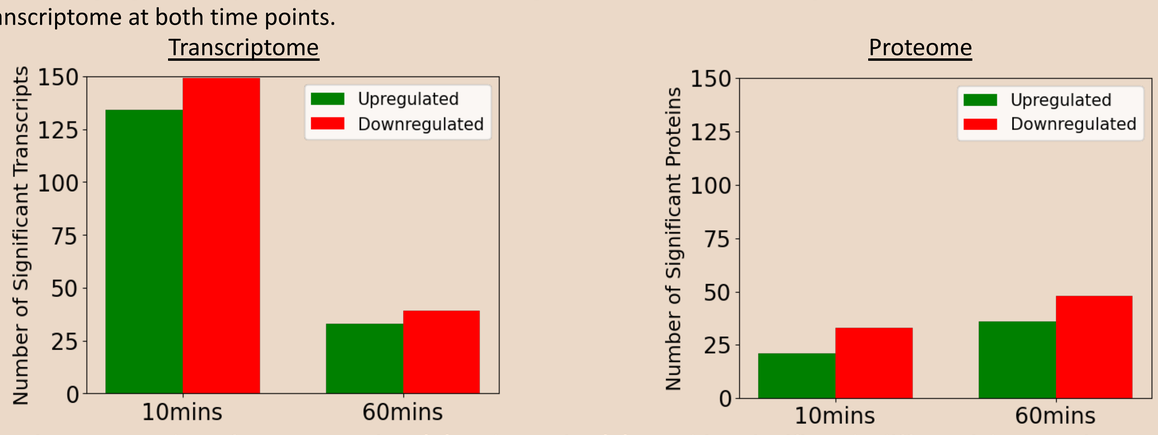


Figure 4. Number of gene transcripts (A) and proteins (B) matching significantly differential expression criteria. Differential expression was deemed significant if abundance ratio was greater than 2-fold and p-value was <0.05 (Bonferroni correction was used in transcriptome data).

- Gene expression responds quickly to allicin stress and leads change in protein expression.

Proteome and Transcriptome show different functional response to allicin damage

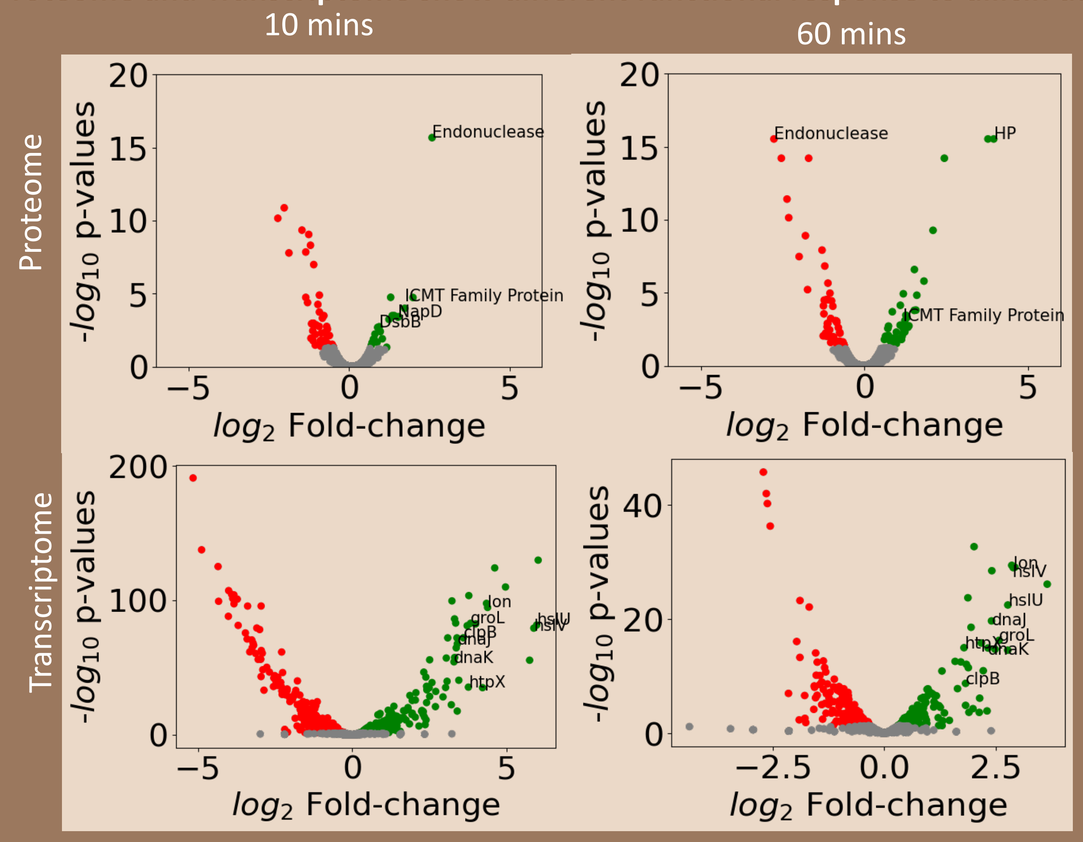


Figure 5. Volcano plots depicting the differential expression of proteome and transcriptome response.

- Volcano plots in figure 5 suggest protein repair and digestion to be upregulated in response to allicin damage.

Protein/Gene	Function	Proteome 10 min (Fold-Change)	Proteome 60 min (Fold-Change)	Transcriptome 10 min (Fold-Change)	Transcriptome 60 min (Fold-Change)
Endonuclease	DNA Repair	13.227	-15.873	1.4965	1.4566
ICMT Family Protein	Cysteine Modification	5.65	3.242	1.3160	1.3385
DnaJ	Protein Chaperone	1.227	1.012	10.6105	5.2990
DnaK	Protein Chaperone	1.401	1.477	9.8469	5.0206
GroL	Protein Chaperone	1.254	1.136	14.2950	5.9565
DsbB	Protein Repair	2.557	-1.3	-1.6247	1.0628
HslU	ATP-dependent protease	1.193	1.212	63.1748	6.8296
HslV				59.2149	7.2579

Table 2. Isolated proteins/genes of interest and their fold changes in the transcriptome and proteome data sets.

- Protein repair, folding and digestion functional proteins are upregulated.
- Response to protein damage upregulated.
- Possible evidence for an initial response to DNA damage with upregulation of HNH endonuclease followed by downregulation.

Conclusions

- *Haemophilus Influenzae* responds to allicin by upregulating proteins involved in folding and repair in order to salvage damaged proteins as well as proteases for degradation.
- Further studies may investigate these effects across more frequent time points in order to generate a more continuous model of allicin damage response.

References

1. Müller A, Eller J, Albrecht F, Prochnow P, Kuhlmann K, Bandow JE, et al. Allicin Induces Thiol Stress in Bacteria through S-Allylmercapto Modification of Protein Cysteines. J Biol Chem. 2016;291(22):11477-90.
2. Borlinghaus J, Foerster Née Reiter J, Kappler U, Antelmann H, Noll U, Gruhlke MCH, et al. Allicin, the Odor of Freshly Crushed Garlic: A Review of Recent Progress in Understanding Allicin's Effects on Cells. Molecules. 2021;26(6):1505.