Sanford Burnham Prebys Metabolomics Core: Data Report [SECIM SUBMISSION]

Date: 3/11/2016	**SBP Project #**: 16-006	**Researcher:** Emily Hardin	**PI:** Jose Lemos

Biological Samples Submitted

Species: Streptococcus mutans
Samples Provided: Cell pellet
Variables: UA159 Cells (Control Strep)
ΔspxA1 Mutant (One copy of *spxA1* gene deleted)
Exposure to H_2O_2 (time-course)

N: UA159 (4 time points) and ΔspxA1 (2 time points); **Total N = 6**

Assay Modules Performed

Amino Acids (AAs)
Organic Acids (OAs)

Sample Preparation and Data Processing: JAC and AZ
Reporting: JAC, AZ, and SJG
Instrumentation:
-AAs: Agilent 1290/6490 LC/MS/MS
-OAs: Thermo Quantiva LC/MS/MS

Background

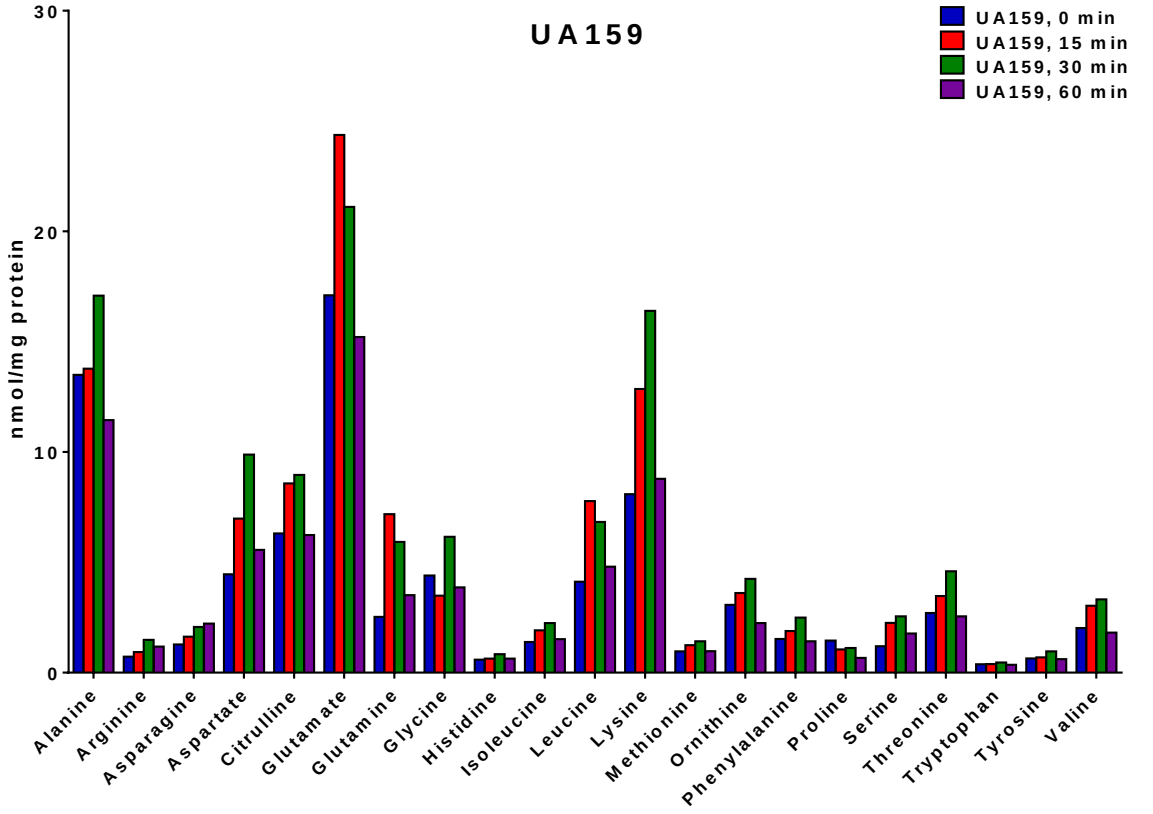
In order to successfully colonize the oral cavity, Streptococcus mutans must be capable of withstanding a variety of stressors, including oxidative stress. High oxygen concentrations disfavor growth of S. mutans and other oral bacteria. Previous work has shown that two regulatory proteins, SpxA1 and SpxA2, are key activators of oxidative stress genes and are critical to the survival of S. mutans in the oral cavity.

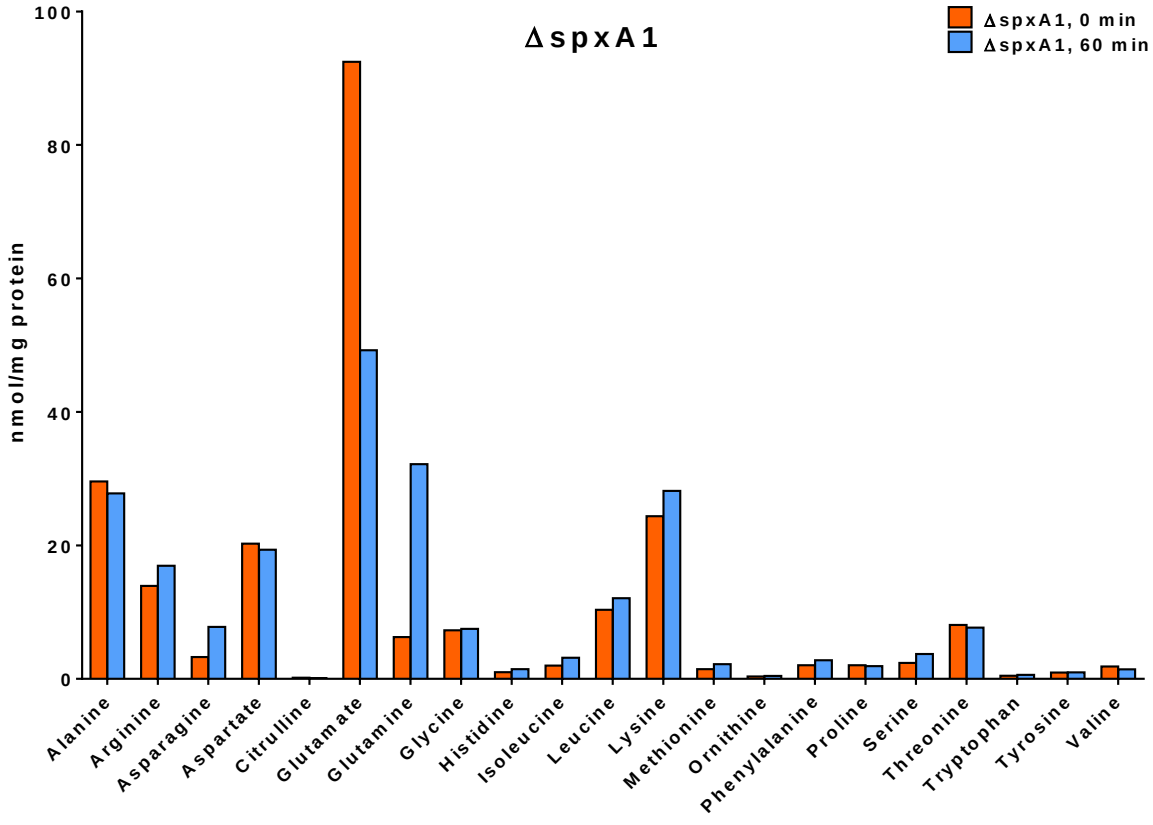
Study Overview

Frozen Strep (as a cell pellet) samples were received on dry ice and stored at -80° C. Before any assays were carried out, the pellet was lyophilized to dryness. The lyophilized pellet was homogenized in 300 µL of 50/50 acetonitrile/0.3% formic acid using a Precellys (bead beating) system. Data were normalized to protein content (provided by Researcher).

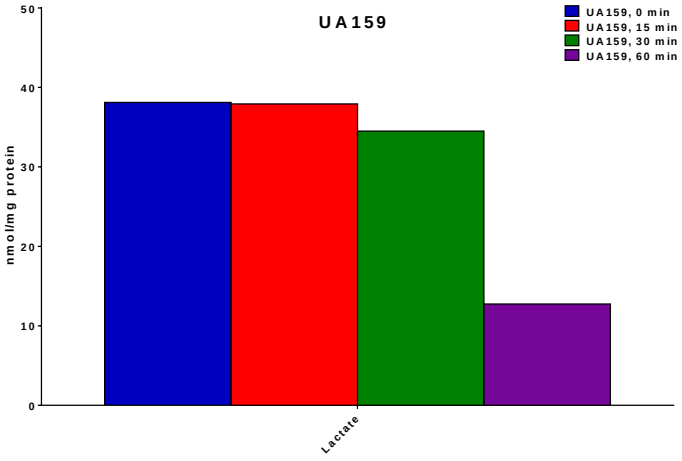
Amino Acids

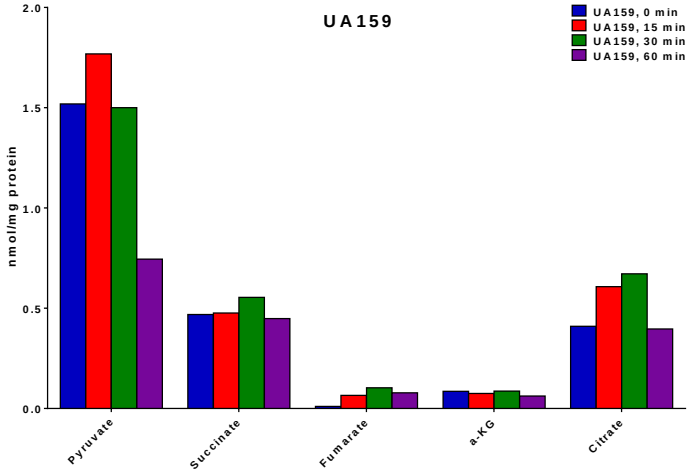
Effects of H_2O_2 Exposure on Metabolite Levels in S. Mutans

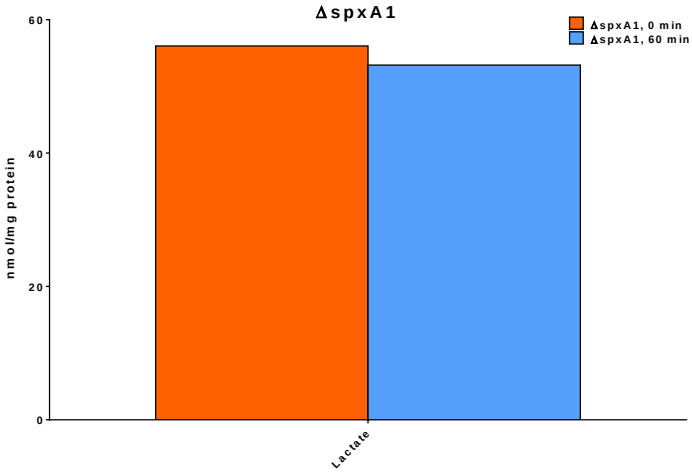


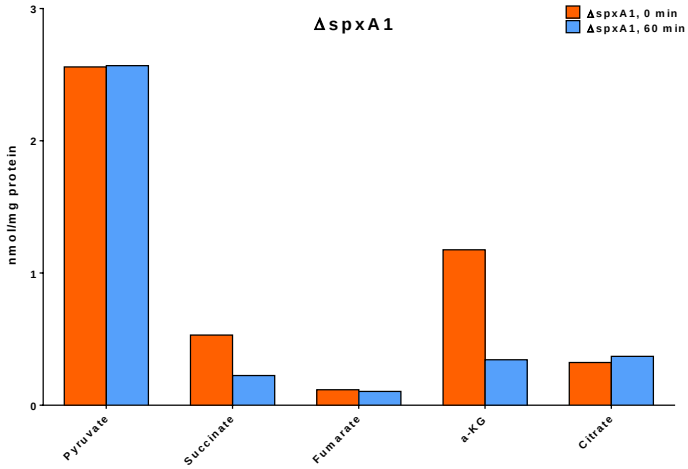


Organic Acids
Effects of H_2O_2 Exposure on Metabolite Levels in S. Mutans









COMMENTS

Our ability to quantify all amino acids and organic acids (except for malate) with the provided number of cells is noteworthy. The reported values are sound and reliable.

While it is difficult to draw any strong conclusions from this study (no replicates provided for any sample), it is interesting to observe some patterns in the data:

Amino acids:

In UA159 (control) Strep, H_2O_2 exposure resulted in a general increase in the levels of multiple free AAs at both the 15- and 30-minute time-points. Interestingly, at the 60-minute time-point, this trend is reversed, with the levels of many AAs returning to the levels observed in the t = 0 sample (no exposure to H_2O_2).

In ΔspxA1 mutant Strep, samples were only exposed to H_2O_2 for either 0 or 60 minutes. The most striking change after the 60-minute exposure occurred in the levels of glutamate and glutamine, which display a 50% decrease and 414% increase, respectively.

Organic acids:

In UA159 Strep, both lactate and pyruvate display > 50% reductions in their levels after 60 minutes of exposure to H_2O_2. Shorter exposure times appear to have minimal effect on these OAs.

In ΔspxA1 mutant Strep, the 60-minute exposure to H_2O_2 resulted in no observable change to the levels of either lactate or pyruvate. Interestingly, both succinate and α-KG display marked decreases in bacteria exposed to H_2O_2 for 60 minutes.