

A Study for the Chemical Markers of *Pseudomonas punonensis*

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Background, Details, Reasons

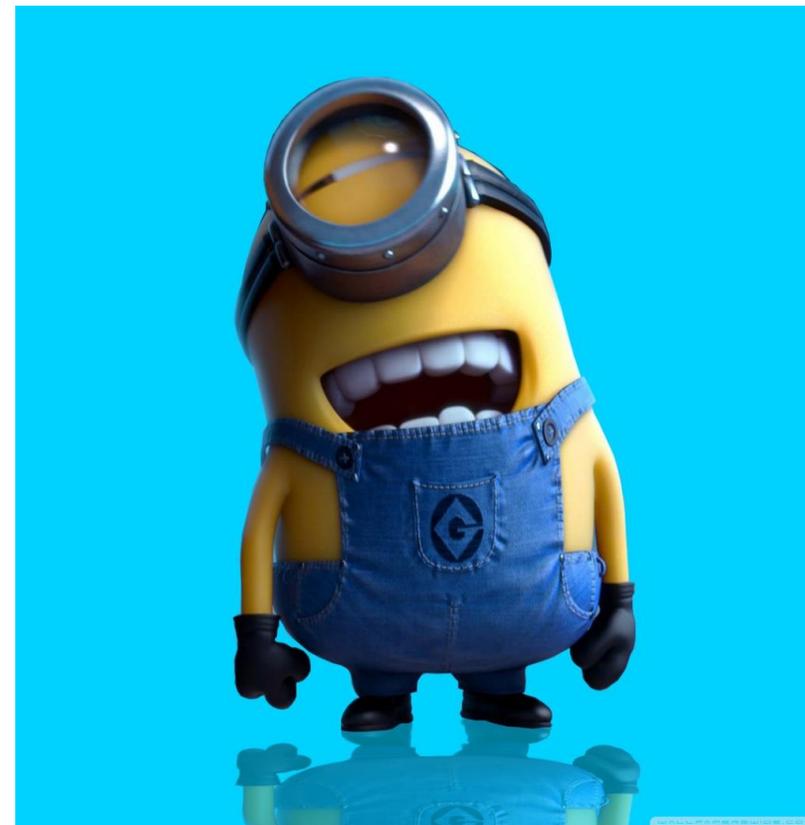
At American Crystal Sugar, we have identified many bacteria utilizing the MinION technology since 2021. Some of the locations/samples are:

- ❖ Soil
- ❖ Beets
- ❖ Brei (from quality lab)
- ❖ Flume
- ❖ Cossettes
- ❖ Raw Juice



Background, Details, Reasons

- ❖ The MinION technology is from Oxford Nanopore Technologies, UK, and is used for DNA sequencing.
- ❖ Papers have been presented at the last two ASSBT conferences about the MinION work being done at Crystal.





Background, Details, Reasons

- ❖ Many of the bacteria that have been identified are found in abundance, but are lesser known as far as the consequences of being present in the beets and the process
- ❖ For example, a well known bacteria such as *Leuconostoc* and other lactic acid bacteria (LAB) are well documented for the destruction of sugar, VFA and ethanol production, and process issues such as sliming and plugging filters, etc. in a sugar beet factory
- ❖ The effects can go beyond sugar loss and mechanical issues. The formation of these non-sugars can effect the sugar end as well with color rise, sugar boiling, more molasses generation, etc.

Storage Piles – Brei Samples – Crop 2020

11	Leuconostoc	Gluconobacter	Microbacterium	Cutibacterium	Sanguibacter
12	Leuconostoc	Pantoea	Microbacterium	Sanguibacter	Gluconobacter
13	Pseudomonas	Pantoea	Microbacterium	Gluconobacter	Sanguibacter
14	Leuconostoc	Pseudomonas	Gluconobacter	Microbacterium	Pantoea
21	Leuconostoc	Microbacterium	Pantoea	Gluconobacter	Cutibacterium
31	Leuconostoc	Marinilactibacillus	Microbacterium	Pseudomonas	Pantoea
32	Pseudomonas	Pantoea	Leuconostoc	Stenotrophomonas	Luteimonas
33	Pseudomonas	Pantoea	Luteimonas	Leuconostoc	Microbacterium
34	Leuconostoc	Pseudomonas	Pantoea	Marinilactibacillus	Microbacterium

Pseudomonas

- ❖ First bacterial study is on *Pseudomonas punonensis*
 - ❖ Culture from Leibniz Institute DSMZ, Germany
- ❖ This genera was chosen as it has been found abundantly in brei (ACS-TSC quality lab), flume, cossettes, etc.
- ❖ There isn't much research on this species. It was first isolated from straw in Peru.
- ❖ *Pseudomonas* is a genera that grows at cooler temperatures
 - ❖ Optimal growth temp is 25-30°C
 - ❖ Different strains can range from 0-42°C

Study Details

- ❖ Media used was Tryptic Soy Broth (TSB) at half strength due to the media containing glucose
- ❖ Media was spiked with 20% sucrose
- ❖ *P. punonensis* was incubated at $<10^{\circ}\text{C}$ and at 28°C , aerobically and anaerobically
- ❖ Multiple trials (Trial 6 results used for this paper – aerobic at 28°C)
- ❖ Chemistry analysis for the sucrose, inverts and VFAs was done on HPLC

Results

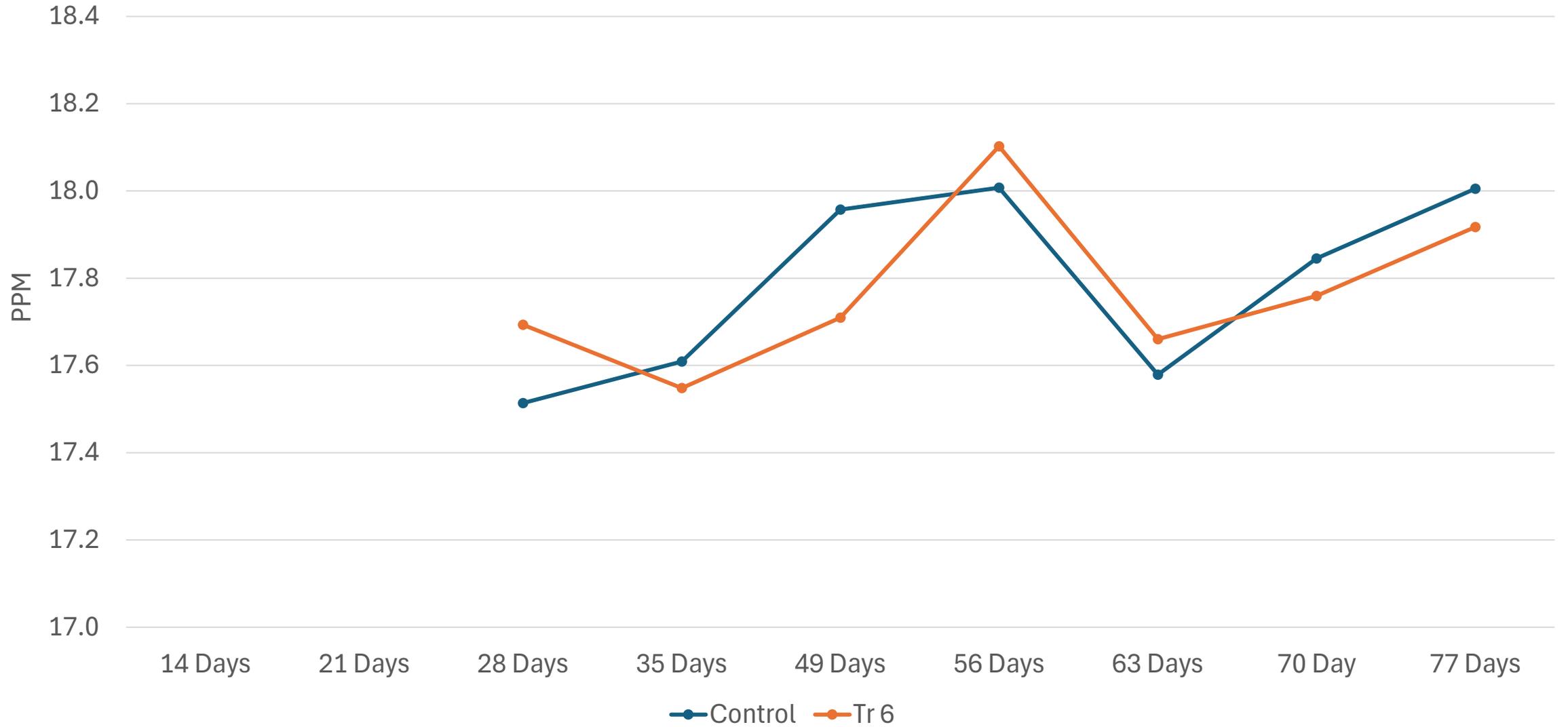
❖ The following slides will show results of:

❖ Sucrose

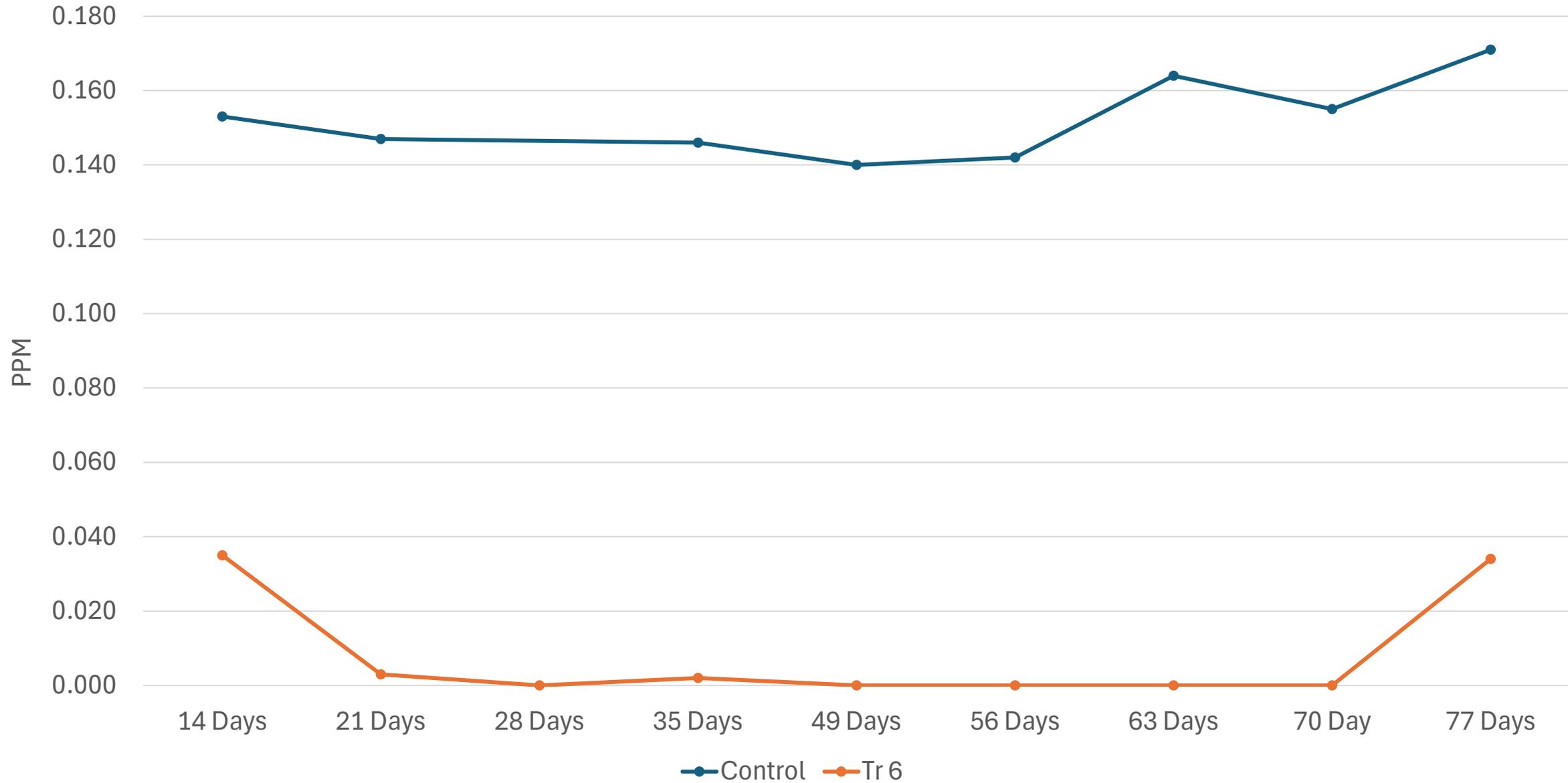
❖ Inverts

❖ VFAs

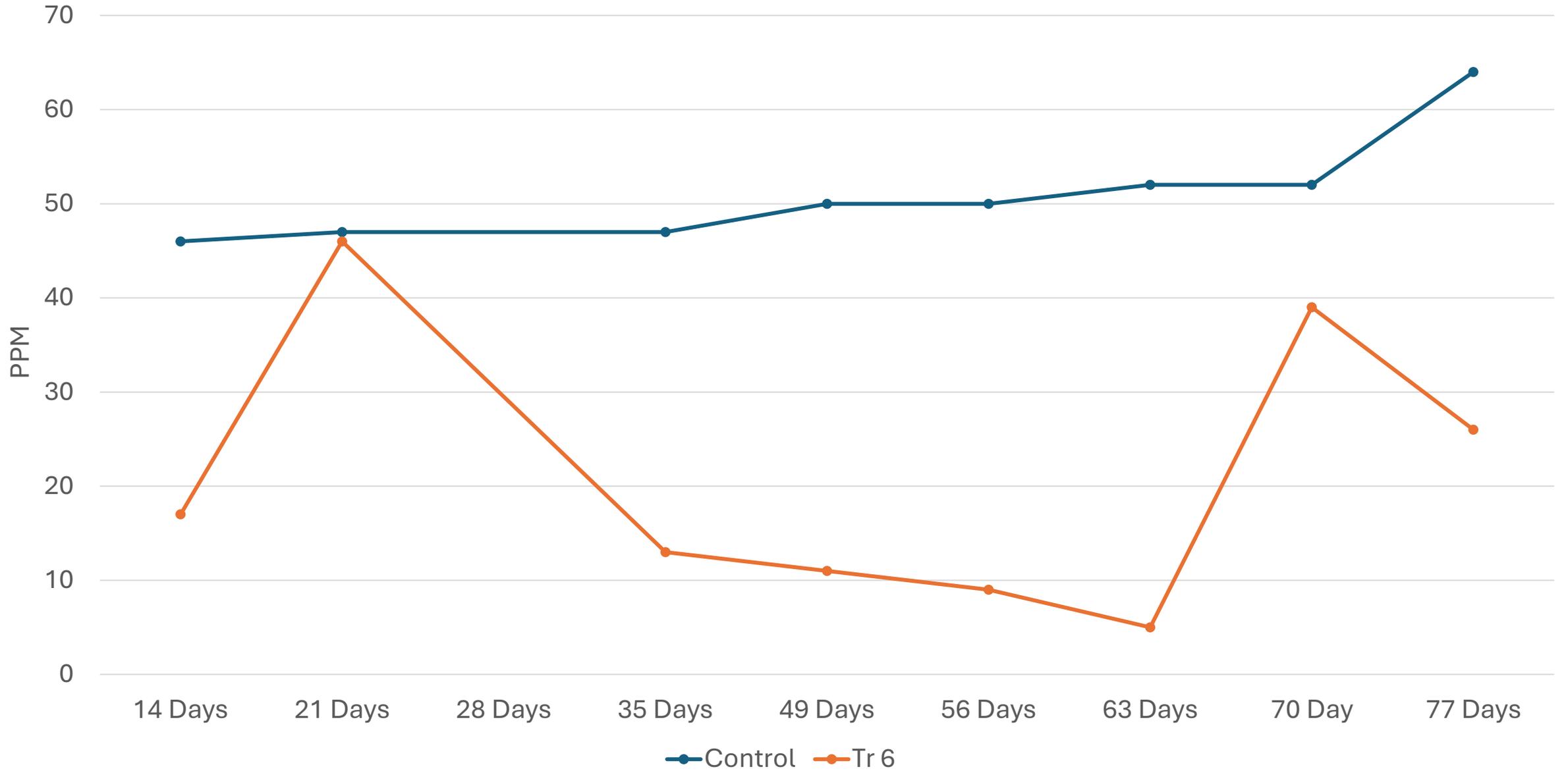
Sucrose



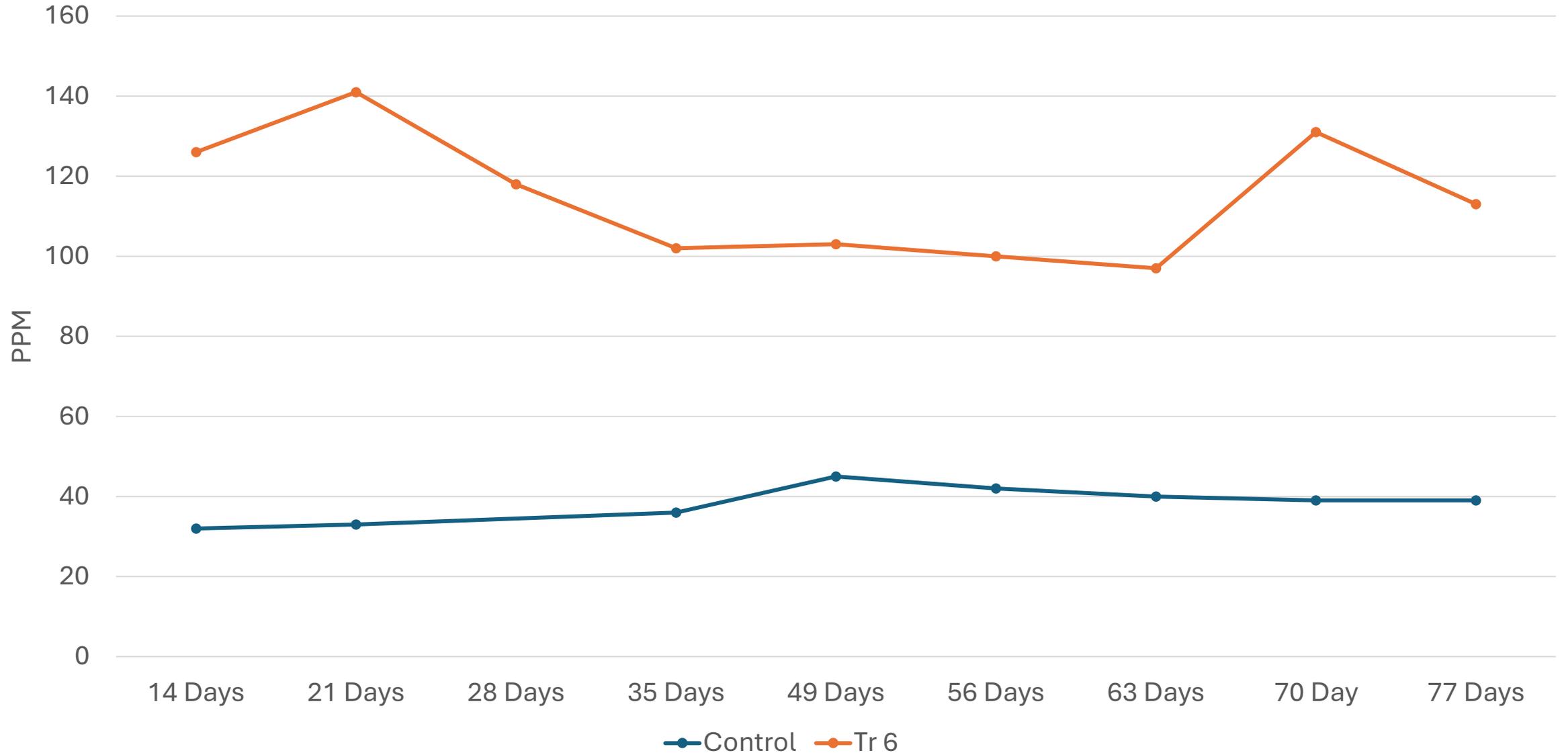
Invert



Acetic



Formic



More to Come

- Moving forward
 - Analyzed another *Pseudomonas* species – similar results
 - Currently working with a *Staphylococcus* species
- More bacterial strains on the agenda such as *Pantoea*, *Microbacterium*, *Cutibacterium*, *Arthrobacter*, *Bacillus*, etc
 - Have about a dozen cultures to analyze
 - Utilizing the MinION to identify unknowns found at Crystal

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Summary

- *Pseudomonas punonensis* study
- No invertase formed as sucrose was not broken down
- Inverts were utilized
- Acetic acid was utilized
- Formic acid was produced
- No ethanol or other VFAs were formed
- More studies will be done with additional bacterial strains

Questions???