

A Chemical Approach To Understand Resistance To Soft Rot

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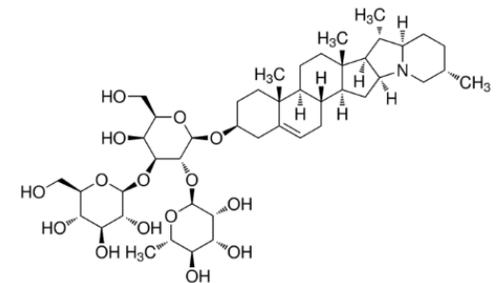
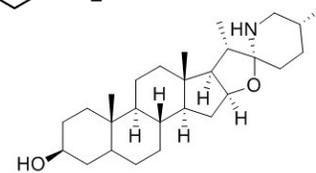
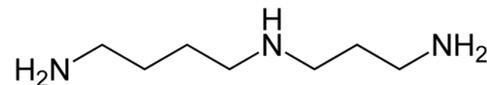
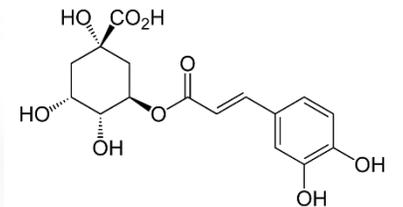
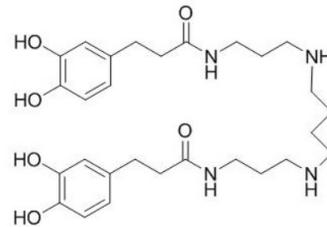
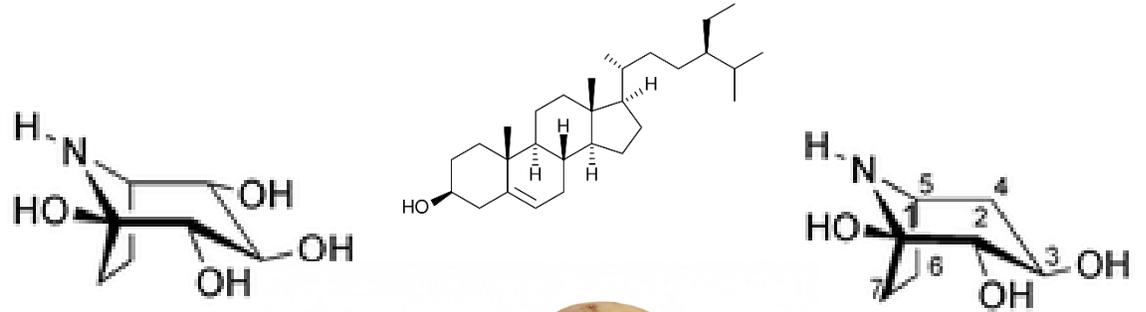
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We Are Working To Characterize Resistance in *Solanum chacoense* variety M6

S. chacoense M6 resistance may be due to
(i) toxins or (ii) avirulence factors

Key questions:

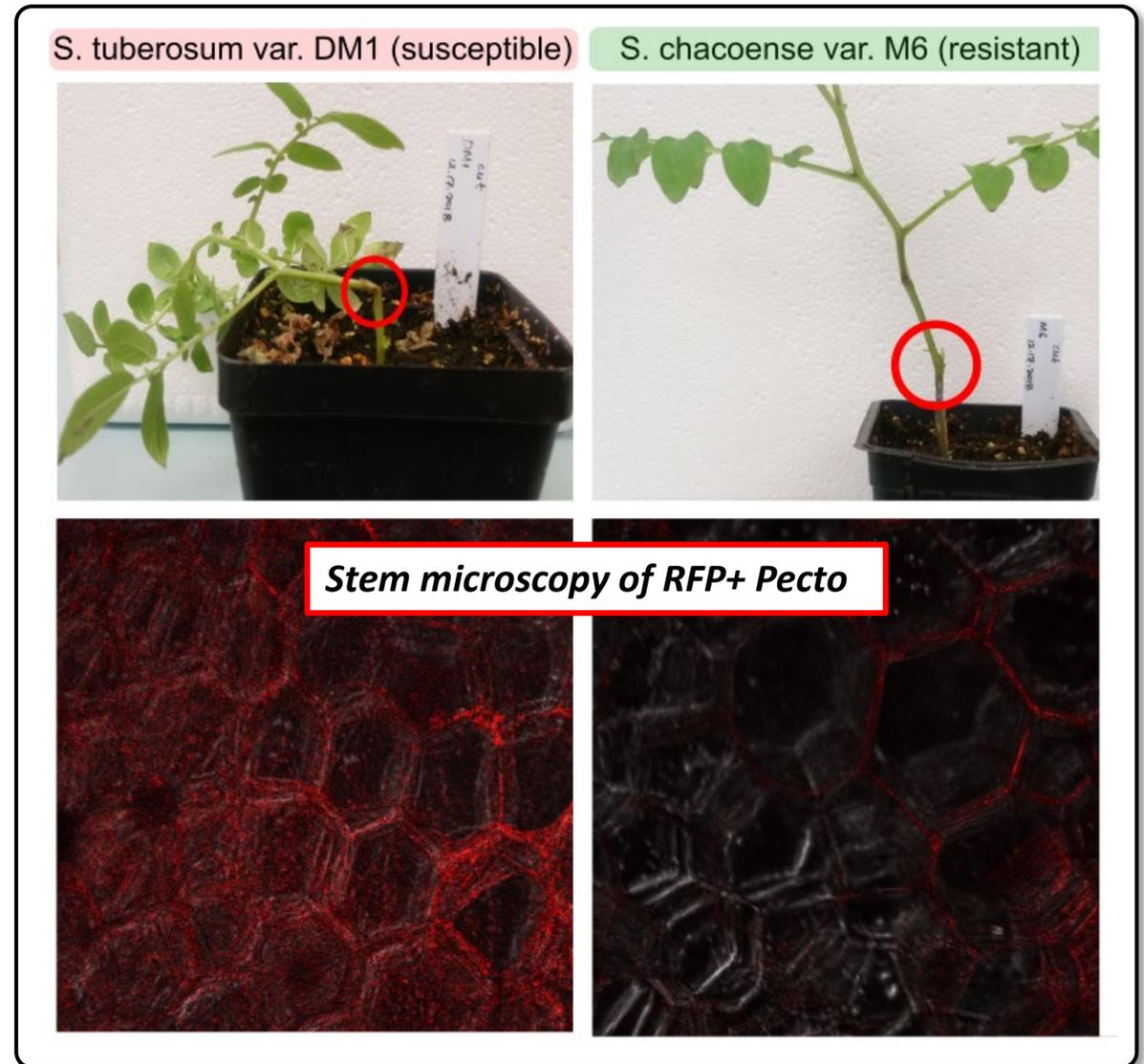
Why is M6 resistant?

How is M6 resistant?

When answered, can lead to:

Genetic targets to deploy resistance

Chemical targets to track resistance



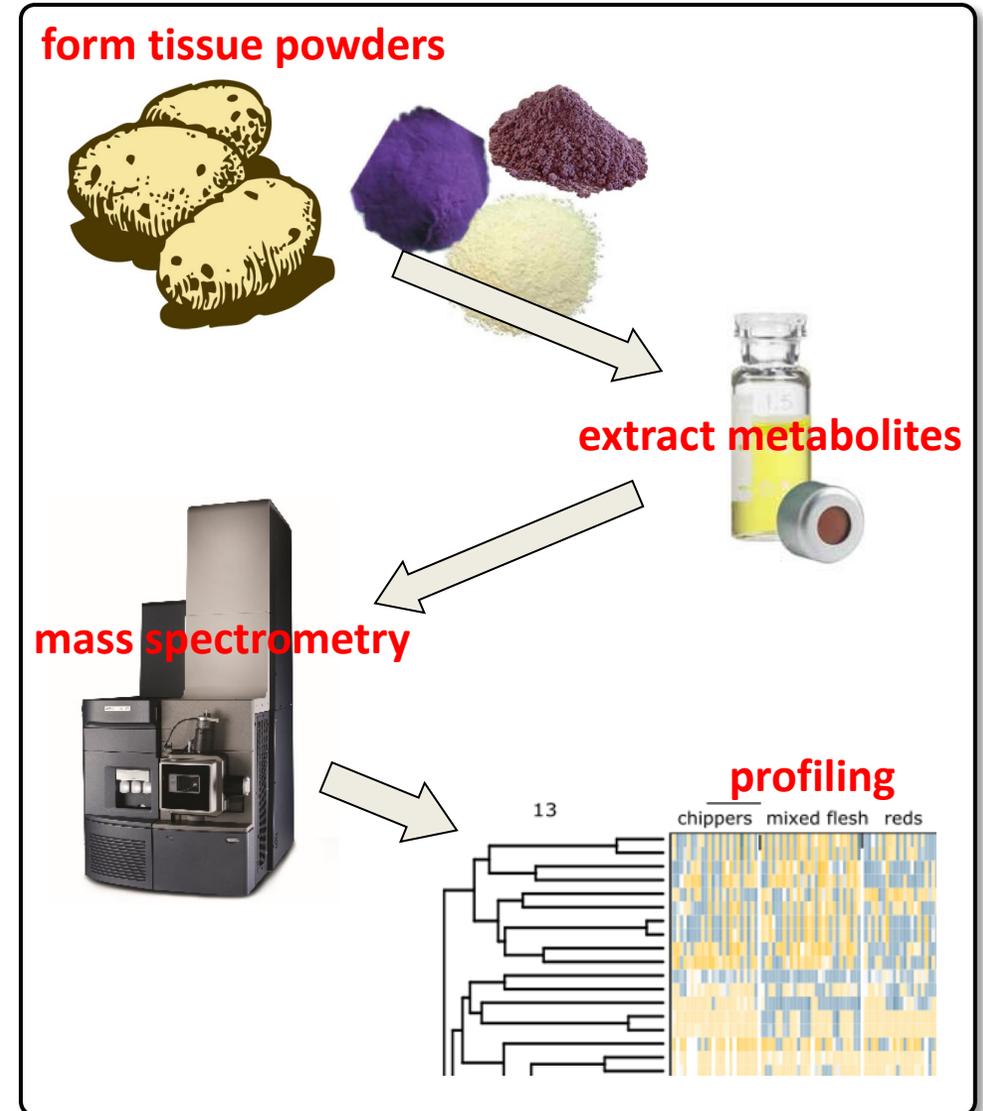
Potato Chemistry For Pathogen Resistance

Our team has performed metabolomics and proteomics on tubers for many genotypes

We find variation in most metabolites:

- organic acids (malic acid, shikimic acid, etc.)
- amines/amino acids, saccharides
- lipids (terpenes/sterols, membrane lipids)
- alkaloids (glycoalkaloids, kukoamines, calystegines)
- phenolics
- peptides

We hypothesize that variation in stem/stolon/tuber chemistry is associated with resistance to soft rot pathogens



Experimental Design

S. chacoense var. M6 (res) vs.
S. tuberosum DM1 (susc)

Pathogen

Host

(A) Effects on Pathogen



Why is M6 resistant?

Can we define what “resistance” is?

Evaluate effects of chemical extracts on:

- Analysis 1: growth/viability
- Analysis 2: virulence/pathogenicity

(B) Non-targeted Metabolomics



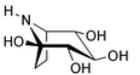
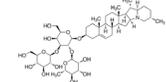
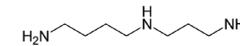
What does M6 ‘have’ vs. DM1?

Does M6 contain antimicrobials?

Evaluate chemistry using mass spectrometry for:

- Analysis: differences between DM1 vs. M6, stems and tubers

(C) Isolate Bioactive Chemicals



Can we purify a molecule or sets of molecules that inhibit soft rot?

Can we identify targets of pathogenicity?

- Analysis: fractionate extracts and test for bioactivity

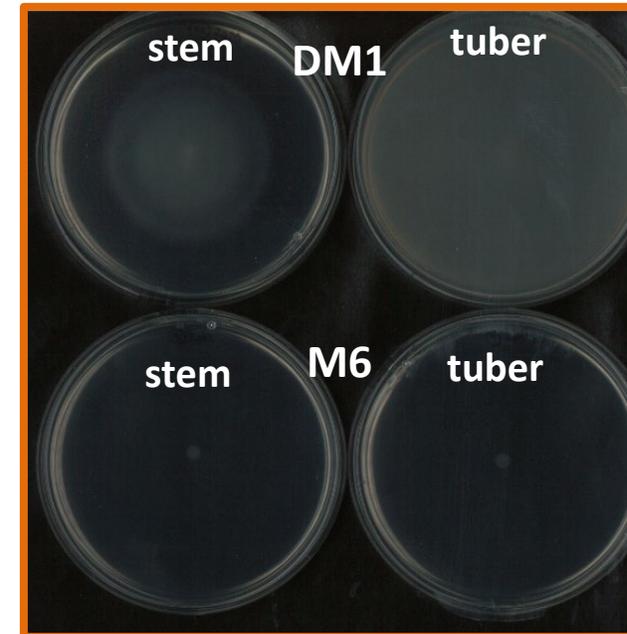
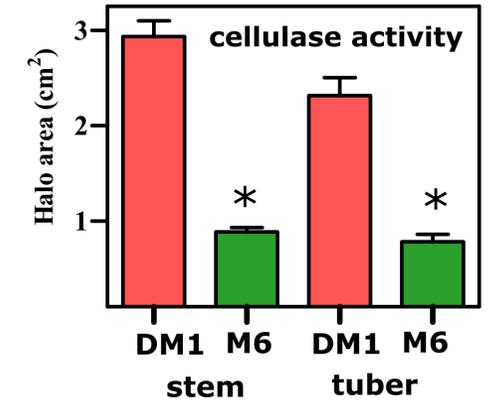
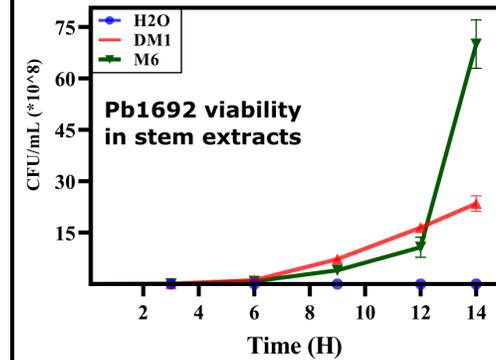
Part A: Effects on Host

Why is M6 resistant?

Can we define what "resistance" is?

In comparative assays (DM1 vs. M6, stem and tuber extracts), we find that:

- **Key Result 1:** Neither stem or tuber extracts of M6 or DM1 inhibit growth *in vitro* (i.e. bacteria can grow in these extracts just fine)
- **Key Result 2:** M6 extracts inhibit virulence factors of bacteria -- pectate lyase, cellulase, and protease activity, and inhibition of swimming/swarming (motility) phenotypes



Pb1692 swimming assay for motility

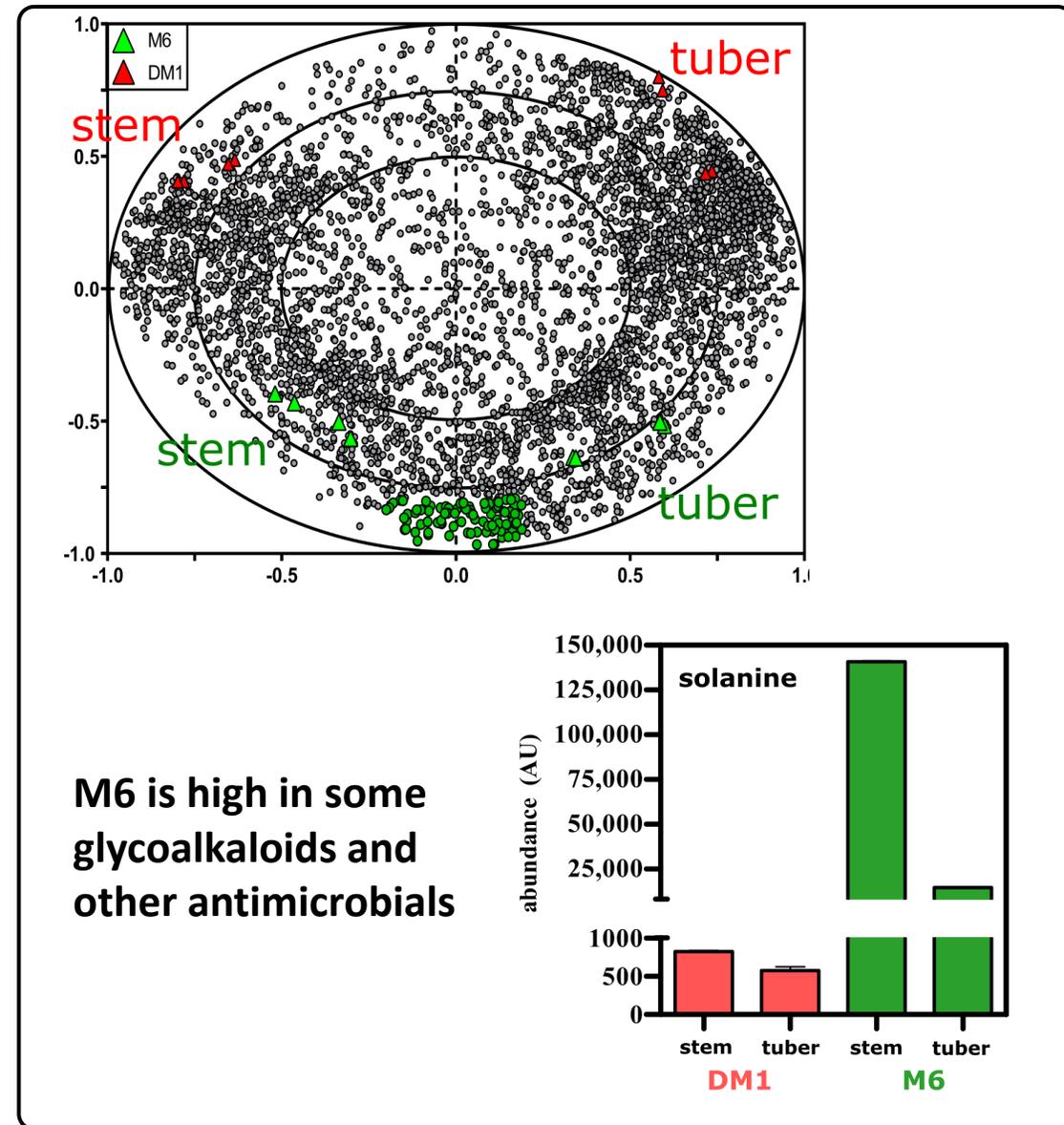
Part B: Is M6 Chemistry Different Than DM1?

What does M6 'have' vs. DM1?

Does M6 contain known antimicrobials?

In comparative assays (DM1 vs. M6, stem and tuber extracts), we find that:

- **Key Result 1:** Stem and tuber chemistry is very similar, but DM1 and M6 stem and tuber chemistry is very different
- **Key Result 2:** There are many chemicals that are known to facilitate resistance to pathogens that were higher in M6



Part C: Is M6 Chemistry Different Than DM1?

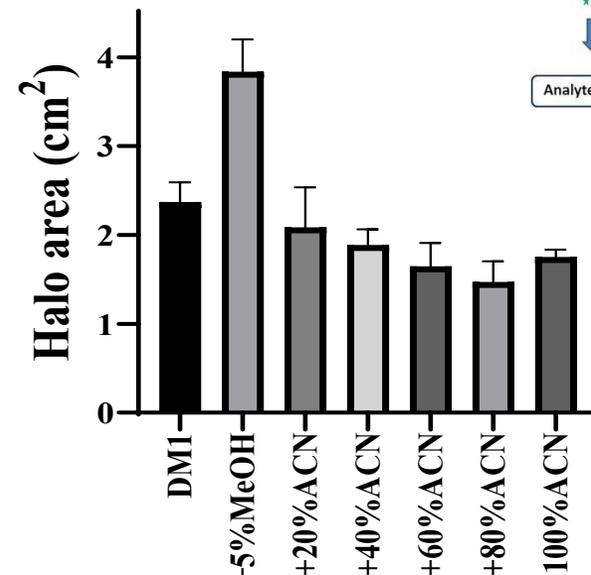
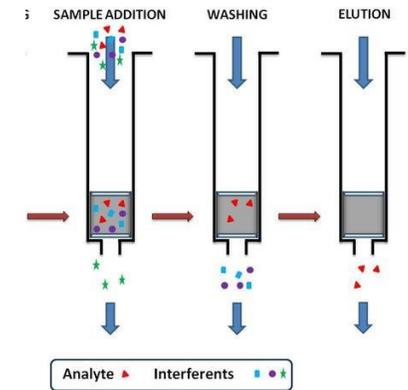
Can we purify a molecule or sets of molecules that inhibit virulence?

Can we identify targets of pathogenicity?

In fractionation assays, we find that:

- **Key Result 1:** M6 fractions vary in their effects on exoenzyme activity
- **In progress:** Omics analysis of bacteria treated with the different fractions

Solid Phase Extraction Bioassay-Guided Fractionation



Next Steps

(A) Effects on Pathogen



Why is M6 resistant?

Can we define what “resistance” is?

Next step is to validate individual potato molecules for their effects on inhibiting virulence

(B) Non-targeted Metabolomics

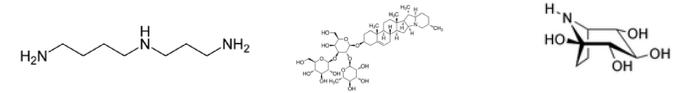


What does M6 ‘have’ vs. DM1?

Does M6 contain antimicrobials?

Publication in preparation describing M6 chemistry and how it differs from DM1

(C) Isolate Bioactive Chemicals



Perform different fractionations within ‘bioactive’ extracts

Perform genomics analysis for variation in pathways that produce these compounds