

Rotten Onions 101: Part I – What kind of rot you got?

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Empire State Producer's Expo – Onion Bulb Rot Session
Virtual: January 14, 2021

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Stop the Rot

*Combating onion bacterial
diseases with pathogenomic
tools and enhanced
management strategies*

<https://alliumnet.com/projects/stop-the-rot/>

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Department of
Agriculture

National Institute
of Food and
Agriculture

Video: <https://plantpath.wsu.edu/2020/12/15/combating-bacterial-diseases-onion-stop-rot/>

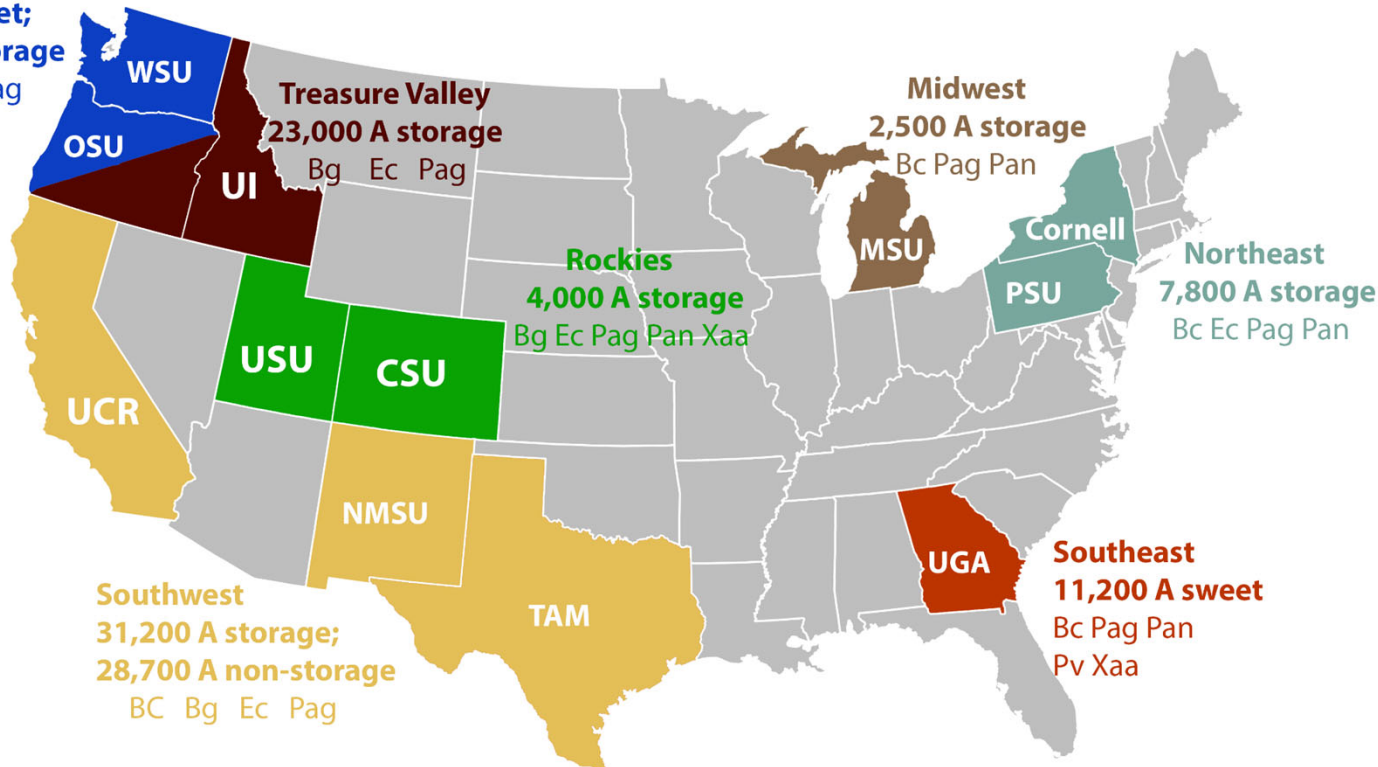
Combating Bacterial
Diseases of Onion: How Do
We Stop the Rot?



Stop the Rot: Combating onion bacterial diseases with pathogenomic tools and enhanced management strategies

Bacterial Bulb Rot Pathogens

Columbia Basin
1,000 A sweet;
24,000 A storage
Bg Ec Pag



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Center rot

Pantoea ananatis,
Pantoea agglomerans,
Pantoea allii

bugwood

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Bacterial streak & bulb rot

*Pseudomonas
viridiflava*

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Bacterial soft rots

Pantoea
agglomerans,
Pectobacterium
spp.

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UGA5076069 bugwood

Sour skin

Burkholderia cepacia

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Slippery skin - *Burkholderia gladioli* pv. *alliicola*



5362301 bugwood



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Enterobacter bulb rot - *Enterobacter cloacae*



bugwood

5359976

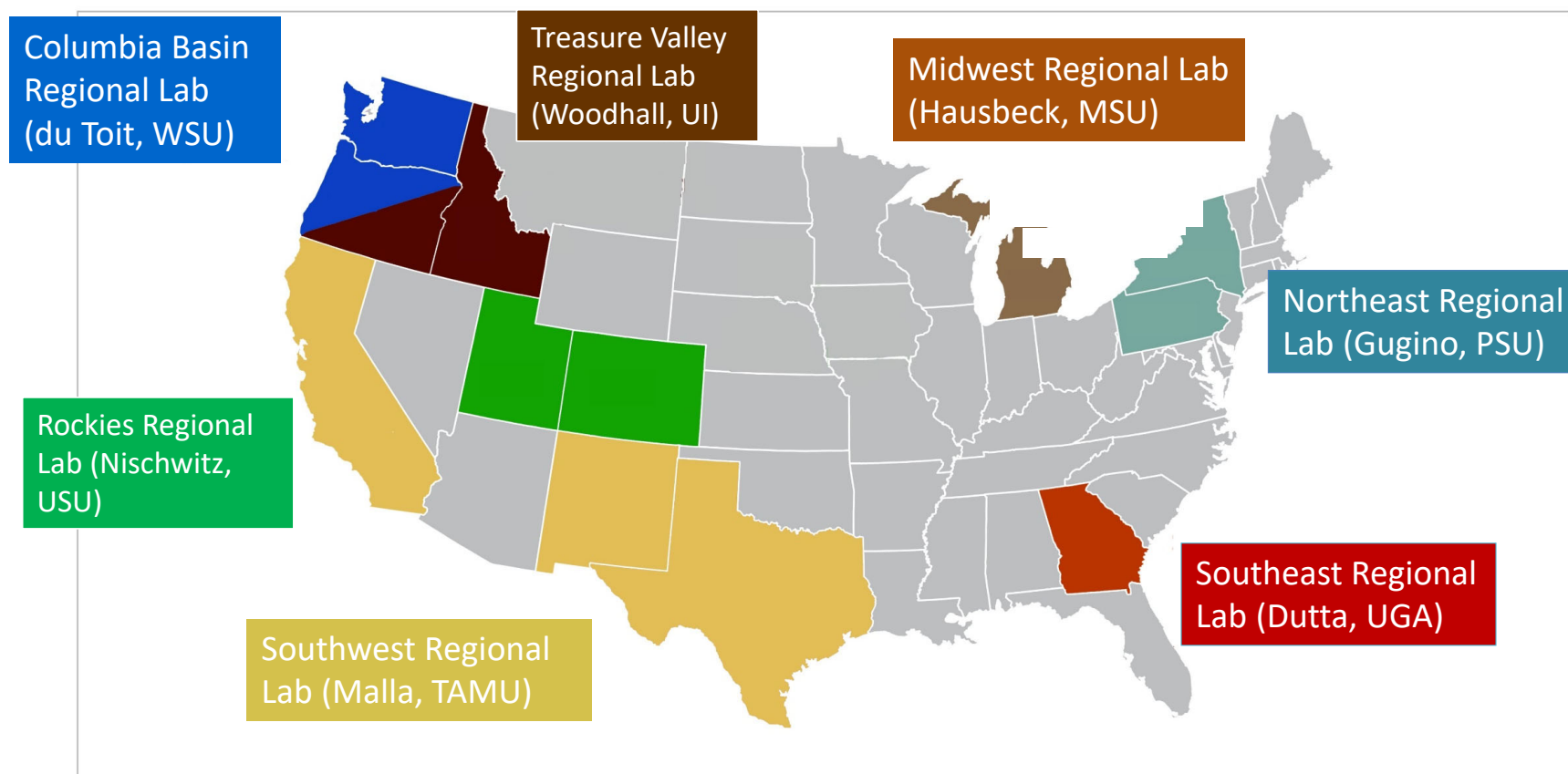
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Complexity of Accurate Bulb Rot Identifications

- Many bacteria associated with onion bulbs
 - >13 genera
 - Several species within a genus
 - Different strains within a species
 - Some are virulent (cause bulb rots), some are not
- Multi-step process to diagnose bacteria causing rot in onion bulbs
 - Which sections of a rotten bulb to sample?
 - Whether to use surface-sterilization or not (concentration, duration)?
 - What types of agar media to use?
 - What physiological tests are relevant, and what DNA to sequence?
- With deviations at any step, five labs working on the same sample could, conceivably, get five different diagnoses



'Stop the Rot' regional labs in primary onion production regions

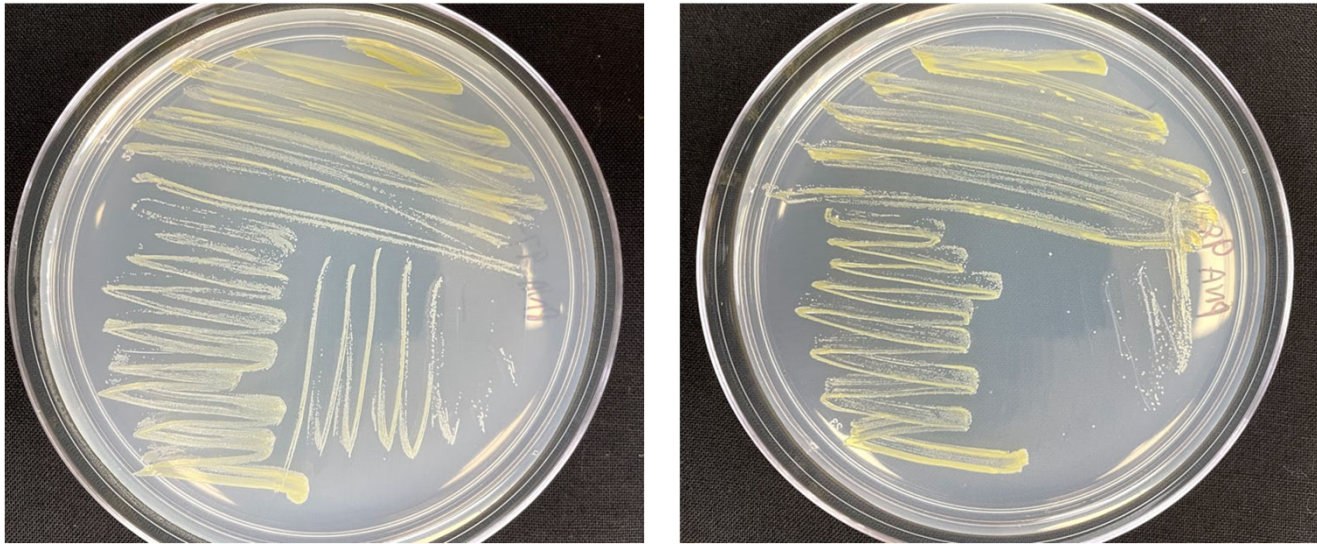


Stop the Rot: Combating onion bacterial diseases with pathogenomic tools and enhanced management strategies

Pathogenomic: Using genomics to spot problem pathogens

Can you tell these two bacterial strains apart?

Bacterial colonies isolated from onion bulb growing on nutrient agar



Neither can we!

- 99% of the DNA between these two strains is identical
- The differences in the remaining 1% of DNA is what determines whether the strain is pathogenic (= makes onion rot) or not.

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Pathogenomics: Using genomics to spot problem pathogens

An Analogy – *E. coli* food poisoning

- There are MILLIONS of *E. coli* that live inside of us that are harmless
- It is only the occasional specific strain (e.g. *E. coli* 0157:H7) that makes us sick (is pathogenic)
- We need to identify the strains that makes us sick in a sea of non-pathogenic strains



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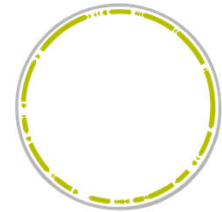
Pathogenomics: Using genomics to spot problem pathogens

What is Pathogenomics?

- Pathogenomics is **genomic** research on **pathogenic microorganisms**
- The **genome** is all the genetic material, the sequence of DNA, of an organism
- **Pathogenic microorganisms** in our case are **onion rot-causing bacteria**



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Allgenetics.eu

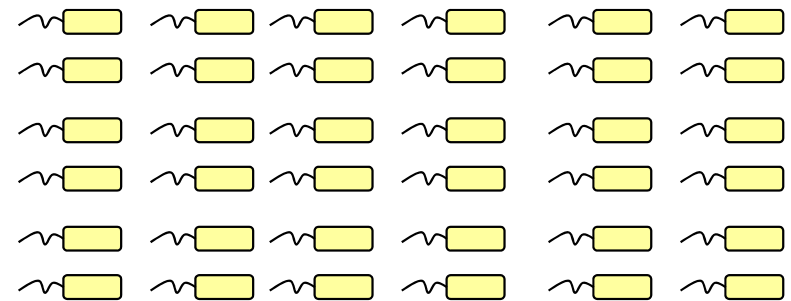
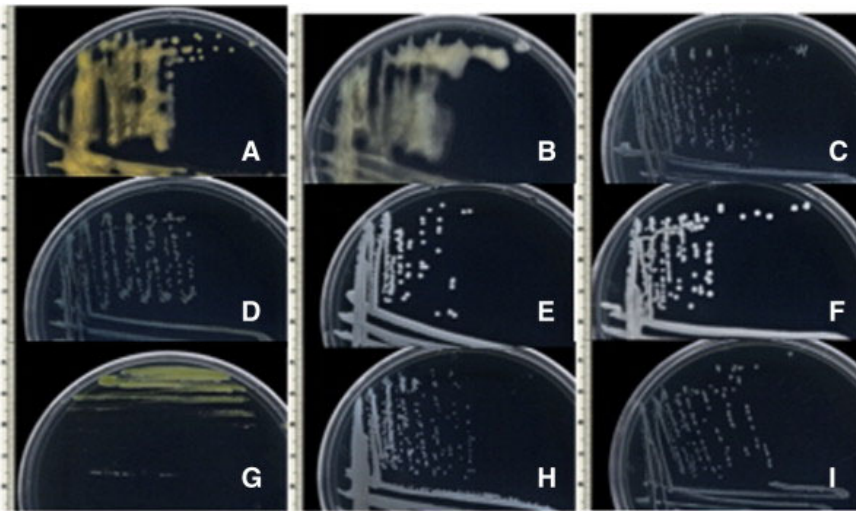
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Pathogenomics: Using genomics to spot problem pathogens

The Process

Step 1: Collect as many strains of a specific bacteria as possible to capture genetic diversity

- Plate out bacterial strains from 100s of onion bulb samples
- Pluck individual colonies off of plates = individual isolate



Zaid et al 2012

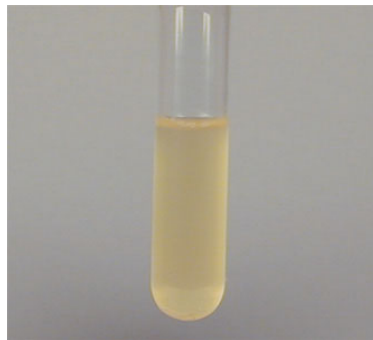
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The Process

Step 2: Screen to identify which isolates are aggressive pathogens

- Make a bacterial “broth” (high concentration of bacteria) from a single strain = inoculum
- Inoculate onion scales, bulb, leaf tissue, etc.
- If rot develops, the strain is virulent



Gary E. Kaiser, Ph.D.

Strain A

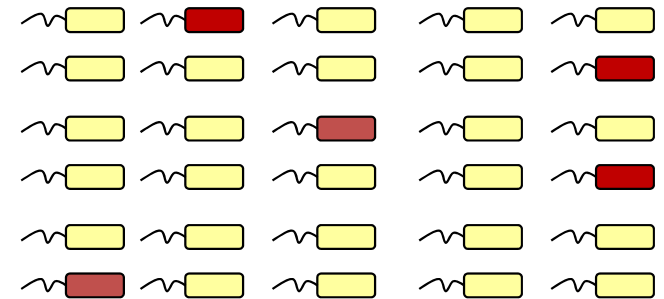


Virulent
= pathogenic

Strain B



Non-Virulent
= nonpathogenic

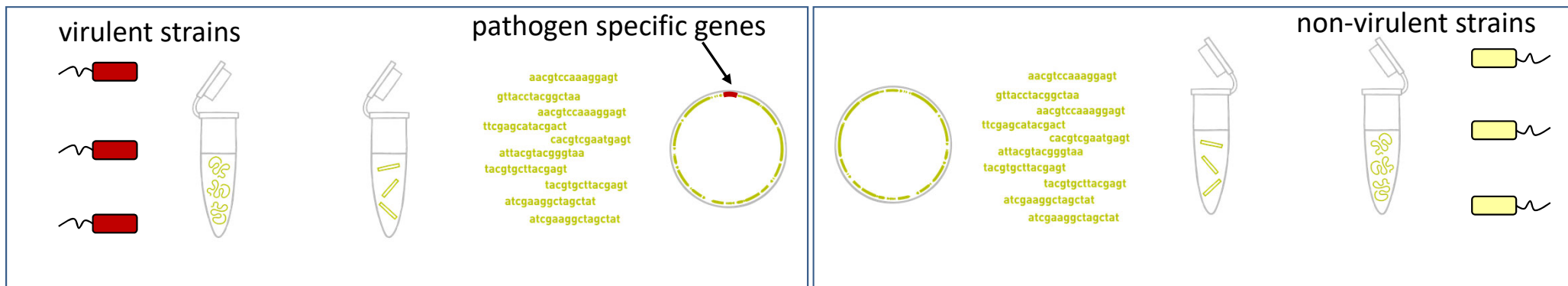


Pathogenomics: Using genomics to spot problem pathogens

The Process

Step 3: Use genome sequencing to find genes common to only the pathogen strains

- **Whole genome sequencing** is the process of determining the complete order of genes of the entire DNA of the organism.
- Collect DNA sample for each strain.
- Conduct genome sequencing.
- Compare genomes among different strains
 - Look for <1% of DNA that is different – this may confer whether strain does or does not rot onion



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The Process

Step 4: Develop new diagnostics based on the pathogen-specific genes

- Develop tool to that will identify pathogen-specific gene(s) within whole DNA
- Test for accuracy of diagnosis
- Once genetic diagnostic tools are developed, pathogenic bacterial strains can be identified very quickly in comparison to traditional techniques.
- Note, these genetic diagnostic tools are for specific of bacteria
 - Separate tools need to be developed for *Pantoea*, *Burkholderia* etc.

Acknowledgements

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More information about the project:

- <https://alliumnet.com/projects/stop-the-rot/>
- Lindsey du Toit dutoit@wsu.edu

