Intermicrobial interactions and sustainable biological control of bacterial diseases of plants

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Features of bacterial diseases of plants that make them amenable to biological control

- Bacterial plant pathogens colonize surfaces of plants.

- Bacteria invade tissues through natural openings (e.g. stomates, hydrathodes, and nectarathodes) or wounds.

- Reducing surface populations of pathogens or protecting wounds and natural openings interrupts the disease process.

- Few chemical control methods are available or effective, so growers are interested in biological control.
Two classic bacterial diseases

Crown gall
*Agrobacterium tumefaciens*

Fire Blight
*Erwinia amylovora*
Crown gall
*Agrobacterium tumefaciens*

Historically a severe disease in plant nurseries. Infected plants must be destroyed, resulting in losses of ~80% of plants.

- Disease controlled by sanitation and biological control.

Disease cycle from Agrios, pTi representation from wikipedia
Agrobacterium radiobacter strain K84

- Discovered in Australia.
- Registered and commercialized in US in 1970s
- Used primarily in nurseries
- Applied by dipping plants in \( \sim 10^8 \) CFU/ml suspension prior to planting
- K84 colonizes wounds, occupies binding sites for the pathogen, and produces an antibiotic Agrocin 84
Efficacy of *Agrobacterium radiobacter* strain K84 on cherry rootstock

![Bar chart showing percent trees with crown gall across different locations (Corvallis, Aurora, Moses Lake, Ephrata). The chart compares the effects of Pathogen only, K84, then pathogen, and Water control treatments.]
Concern about sustainability of biocontrol of crown gall with K84

pAg84 carries Agrocin 84 production and resistance genes and is self-transmissible.

\textit{A. radiobacter} K84 \quad \textit{A. tumefaciens}

\begin{itemize}
  \item pAgK84
  \item Conjugation pilus
  \item Chromosome
\end{itemize}

\begin{itemize}
  \item Agrocin 84-sensitive pathogen
  \item Agrocin 84-resistant pathogen
\end{itemize}
Transfer of pAg84 to pathogenic agrobacteria in plants in fields

Strain K1026, a genetically engineered derivative of K84 – Deleted *tra* gene in pAg84

K1026 produces Agrocin 84 and controls crown gall as well as the wild type, but can no longer transfer pAg84 to the pathogen

Registered and commercialized in US in 1999
Fire blight: bacterial disease of pear and apple caused by *Erwinia amylovora*

Fire blight epidemics occur sporadically, resulting in losses of tens of millions of dollars to growers.
Erwinia amylovora grows as an epiphyte on floral stigmas to populations of $10^6$ to $10^7$ cells per flower then moves to infection sites on the nectary. Prevent disease by suppressing epiphytic growth of pathogen on stigmas, before migration to the nectary.

Micrographs by Mark Wilson and Larry Pusey
Biological control agents for fire blight

_Pseudomonas fluorescens_ A506 (BlightBan A506, NuFarm Americas)
- Isolated from pear in California by Steve Lindow.
- Primary mechanism: preemptive exclusion.
- Registered and commercialized for fire blight management (1996).
- Also used to reduce frost injury and russet of fruit.

_Pantoea vagans_ C9-1 (BlightBan C9-1, NuFarm Americas)
- Isolated from apple in Michigan by Carol Ishimaru.
- Mechanisms: peptide antibiotics and preemptive exclusion
- Formerly called _Erwinia herbicola_ C9-1, then _Pantoea agglomerans_ C9-1.
- Registered in 2006, but not yet commercialized, for control of fire blight.
Experimental orchard trials

Spray biological control agents at $10^8$ CFU/ml on flowers during early and near full bloom.

At full bloom, inoculate flowers by misting with low dose of the pathogen.

Monitor bacterial populations on flowers.

Count and remove infected blossom clusters.
Disease control in inoculated orchard trials (antibiotic-sensitive isolate of *Erwinia amylovora*)

Oregon State Inoculated Fire Blight Trials 1991-2005

![Graph showing disease control effectiveness with various treatments. The graph compares different treatments like Biocontrol agents (BlightBan A506, P. vagans C9-1) and Antibiotics (Oxytetracycline, Streptomycin) along with Water control.](image-url)
Evaluation of intergeneric mixtures of antagonists for disease control
Populations of \( P_{vC9-1} \) and \( P_{fA506} \) on flowers when applied singly and as mixtures

- **Initial proportion of \( P_{vC9-1} \)**
- **1-Initial proportion of \( P_{fA506} \)**

**Total population**
Disease control with an intergeneric mixture of antagonists

Relative disease incidence

A506  C9-1  A506 & C9-1  Oxytetracycline  Streptomycin

Biologicals  Antibiotics

Water control = 100%

Stockwell et al 2010 Phytopathology
*Pantoea vagans* C9-1 and *Pseudomonas fluorescens* A506 co-colonize flowers when applied as a mixture.

Total antagonist population sizes were increased compared to single strain inoculants.

We anticipated enhanced control compared to single strains, but this did not occur.

We suspected that the interacting strains were interfering with a disease control mechanism.
Inhibition of *Erwinia amylovora* in culture by C9-1 and A506
Extracellular metalloprotease (AprX) of A506 inactivates a peptide antibiotic of *Pantoea* strains.

Antibiotic active against pathogen and A506 without protease is still resistant.

Disease control with *Pantoea agglomerans* Eh252 & *Pseudomonas fluorescens* A506 aprX::Tn5

![Graph showing disease control with different treatments](image-url)
Disease control with *Pantoea vagans* C9-1 & *Pseudomonas fluorescens* A506 aprX::Tn5

Stockwell et al 2010 Phytopathology
Why use mixtures of antagonists for control of fire blight?

• Greater population sizes with mixed inocula than single strain inoculants.

• Better disease control with compatible mixtures.

• Less variation in efficacy with compatible mixtures compared to single strains.

• Many pseudomonads produce extracellular metalloproteases.
  • We suspect that indigenous pseudomonads may reduce disease control by *Pantoea* spp., thereby contributing to variable efficacy.
Colonization of pear flowers by orchard bacteria

-Few bacteria are recovered from newly opened flowers.
-Applying bacterial antagonists reduced the incidence of detection of indigenous bacteria by petal fall
-Populations of indigenous bacteria were reduced 1000-fold on flowers colonized by biocontrol agents

Why use mixtures of antagonists for control of fire blight?

• Greater population sizes with mixed inocula.
• Better disease control with compatible mixtures.
• Less variation in efficacy with compatible mixtures compared to single strain inoculants.

• Many pseudomonads produce extracellular metalloproteases and mixtures of biocontrol agents reduce their populations.

• Conservation of antibiotic sensitivity of the pathogen. Mixtures of biocontrol agents also reduce populations of indigenous bacteria carrying antibiotic resistance genes, thereby reducing the probability of *Erwinia amylovora* acquiring genes for resistance to agricultural antibiotics.
Summary

Biological control of crown gall and fire blight represent two successes. Products are commercially available and are used by growers.

Inter-microbial interactions could have affected sustainable control with these antagonists:

1) Biological control of crown gall is an example where interactions between pathogens and the antagonist may lead to loss of efficacy. - Genetically modified derivative of K84 unable to transfer its plasmid to the pathogen mitigated this problem. Product is called “NOGALL.”

2) Biological control of fire blight is an example where interactions between antagonists (or antagonist and indigenous microbes) can reduce efficacy. - Extracellular protease deficient derivate of A506 reduced variation in disease control with mixtures of antagonists, future commercial use uncertain.

Use of molecular methods may reveal other impacts of intermicrobial interactions on sustainable, efficacious biological control. Reducing sources of variation in biocontrol effectiveness likely will lead to more adoption of microbial-based pest management strategies by growers.
Thank you for your attention...

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