Bacteriophage Management of *Salmonella* Contamination of Vine Fruits

Dr Alison Blackwell, APS Biocontrol Ltd.
The Problem
Contamination of drying fruit
The Problem

• The majority of vine fruits supplied to the UK market are grown and dried in Turkey

• Advantages:
  • excellent quality
  • Established relationship with a large group of producers

• Disadvantages
  • a known risk of microbial contamination of the drying fruit; e.g. through fouling by birds, reptiles & a variety of wild animals and subsequent distribution during fruit handling
The Problem

• *Salmonella* is the most important foodborne-pathogen and concern to the dried-fruit industry

• *Salmonella* may survive the stringent washing procedure post drying and continue undetected through the supply chain
The Problem

• Confirmed contamination issues are very rare but when they do occur they can cause significant financial losses, with potential impacts on a supplier’s share of a very competitive market

• Innovations put in place to **de-risk** the system would add confidence to the grower, packer, retailer and also the consumer
Bacteriophage
The prefect biocontrol agents
• Bacterial viruses
• The most abundant organisms in the world ($10^{13}$ on Earth)
• For almost every bacterial species, there exists at least one bacteriophage that can specifically infect & ultimately destroy that particular bacterial group
• Could bacteriophage act as effective biocontrol agents of microbial contaminants of dried fruit?
Bacteriophage as biocontrols
Bacteriophage as biocontrols

- Advantages over mainstream pesticides & biopesticides:
  - safe, naturally-occurring within the pathogen’s environment
  - highly specific, reducing non-target risks and potential environmental residue issues to zero
  - rapid mode of action
  - applicable to both mainstream & organic agriculture
  - continue to work long after chemicals have lost their potency
Commercialising Bacteriophage

APS Biocontrol Ltd.
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- Innovative biocontrol company, developing technologies which address an increasing need for effective and environmentally-sustainable replacements for synthetic chemicals
- The core technology is based on bacteriophage; naturally-occurring, safe antimicrobials
- The technology is being developed in a number of market sectors, with the lead product, Biolyse® being a world first; a bacteriophage-based solution for use post harvest and in particular, for washed and packed fresh produce
Reducing shelf-life losses: Biolyse®

- A specific bacteriophage mix targeting spoilage pathogens on potatoes
- Marketed as a processing aid*
- Provides significantly greater control than chemical products & addresses potential issues associated with chlorate residues on food
- UK customers: approx. 40% of the industry, supplying all of the major multiples

* "... any substance not consumed as a food by itself, intentionally used in the processing of raw materials, foods or their ingredients, to fulfil a certain technological purpose during treatment or processing, and which may result in the unintentional but technically unavoidable presence of residues of the substance or its derivatives in the final product, provided that these residues do not present any health risk and do not have any technological effect on the finished product*
Reducing shelf-life losses: Biolyse®

Estima: packing + 7d

Crombie Desiree: packing + 2d
Dried Fruit Contamination

Bacteriophage as a solution?
Tackling *Salmonella* contamination of dried fruit
Progress to date

- 22 distinct *Salmonella* isolates recovered from 35 batches of fruit
- Identified by PCR to two distinct serovars:
  - *S. enterica ssp diarizonae* – sheep/reptile isolate
  - *S. enterica ssp. salamae* Clifton – reptile isolate
Progress to date

• Bacteriophages can be recovered from many different environmental niches

• An environmental sample (fruit wash water, sheep or reptile faeces, sewage) is incubated in culture with the bacterial host

• Bacteriophage presence within the sample is indicated by clear plaques representing bacterial lysis

• A library of 42 bacteriophage active against the *Salmonella* isolates has been created
  • 4 bacteriophage with the broadest activity spectrum (50% of *Salmonella* isolates) selected for efficacy trials
Trial bacteriophage mix composition

[SIPHOVIRIDAE]

[MYOVIRIDAE]
Combining bacteriophage increases activity

**Single phage vs single host**

**4-phage mix vs mixed hosts**

[Graphs showing the comparison of single phage vs single host and 4-phage mix vs mixed hosts]
The Next Steps

*In situ* trials
**In situ trials**

A. Misted onto drying fruit

B. Applied as an emulsion in the oil coating post washing and processing in the factory
Conclusions
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• Bacteriophage have significant potential to act as effective biocontrol agents to de-risk the dried-fruit industry from potential microbial contamination issues, particularly by *Salmonella*

• Bacteriophage extreme sensitivity is an advantage re. environmental impact issues but presents technical challenges for developing effective mixes which will also cope with different pathogen serovars

• APS has a track record of commercialising bacteriophage for fresh produce and processes are in place to develop effective treatments for drying vine fruits

• The technology is also suitable for other fruit and nut products susceptible to bacterial contamination
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