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Biocontrol of verocytoxigenic *Escherichia coli* at key stages of the beef chain

Key external stakeholders:
Beef industry

Practical implications for stakeholders:
Verocytotoxigenic *Escherichia coli* (VTEC), particularly *E. coli* O157:H7 are a major food safety concern worldwide. Healthy ruminants can harbour VTEC in their gastrointestinal tract and can shed the pathogen in their faeces, leading to contamination of the hide, carcass and/or meat products posing a potential public health risk and commercial damage to the beef sector. There is a need for targeted controls against *E. coli* O157 at key points of the beef chain coupled with a demand for natural biological controls, due to increased consumer resistance to use of chemicals. The key finding from this study was that biocontrol agents (particularly phage and carvacrol) show great potential as novel controls against *E. coli* O157:H7 at key stages of the beef chain and further research on their development and application is being pursued.

Main results:
- Carvacrol and thymol were shown to inhibit and kill *E. coli* O157:H7 and other VTEC in a model broth system and retained their antimicrobial activities across a wide range of environmental conditions tested (e.g. temperature, pH, water activities etc).
- Carvacrol (3%) reduced *E. coli* O157:H7 numbers by 10 fold on beef hide and carcass
- Bacteriophages e11/2 and e4/1c inhibited and killed *E. coli* O157:H7 in a model broth system and retained activity under a range of environmental conditions.
- Bacteriophage significantly reduced *E. coli* O157:H7 in a model rumen system without affecting the natural microflora or fermentation.
- Bacteriophage sprayed on to hide could reduce *E. coli* O157:H7 by 100 fold.

Opportunity / Benefit:
Advice, consultancy work and/or technical services can be provided by Teagasc in the area of pathogen biocontrols.

Collaborating Institutions:
N/A

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1. Project background:

Healthy ruminants, particularly cattle can harbour *Escherichia coli* O157:H7 and other verocytotoxigenic *Escherichia coli* (VTEC) in their gastrointestinal tract and can shed the organism in their faeces. As a result pathogen carriage in food animals can lead to both direct and indirect contamination of raw and processed meats. Risk assessments have highlighted that contamination from the hide and/or faeces of the animal onto the carcass during processing are important areas for the control of *E. coli* O157:H7. Eradication of *E. coli* O157:H7 as well as other VTEC from farm livestock and the environment is not yet an achievable goal but risk reduction measures can be implemented on the farm and during processing to minimise the risk of infection. Current methods to ensure food safety and preservation may include a range of chemical preservative agents and/or physical processing intervention strategies. However increased consumer demand for healthier and minimally processed food with lower amounts of additives, as well as concerns regarding antibiotic resistance in foodborne bacteria has led to a greater interest and demand for natural, biological (biocontrol) methods of food preservation and safety. This project evaluated the use of selected biocontrol agents, namely antimicrobial peptides (AMPs; caseicin A and B and bovine beta defensive 3b), essential oil components carvacrol and thymol and bacteriophages (e11/2 and e4/1c) and their antimicrobial activities against VTEC *in vitro* laboratory systems and/or *in vivo* animal trials. The overall aim of the study was to determine whether these selected biocontrol agents could potentially be developed into a novel control strategy for *E. coli* O157:H7 and other VTEC at key stages of the beef chain.

2. Questions addressed by the project:

- Do the selected biocontrol agents (AMPs, carvacrol and thymol essential oils and bacteriophages e11/2 and e41/1c) inhibit *E. coli* O157:H7 and/or other VTEC in model broth and rumen conditions?
- Can the selected biocontrol agents be applied within the beef chain to reduce *E. coli* O157:H7 numbers in either the animal or meat product?
- Do the previously identified bacteriophages e11/2 and e4/1c harbour any undesirable genes (eg. virulence genes) that may hinder food industry applications?

3. The experimental studies:

Biocontrol agents.

The following agents were evaluated in the project:

- Caseicin A and B are two casein-derived AMPs generated by a strain of *Lactobacillus acidophilus* which were identified at Teagasc Food Research Centre. Moorepark (Hayes *et al.* 2006, *Appl. and Environ. Micro*. 72: 2260-2264).
  Bovine beta defensin 3b is an AMP produced by the bovine immune response and has been previously been identified by Professor O'Farrelly and colleagues at the Trinity College Dublin.
- Carvacrol and thymol are two active components found in thyme/oregano essential oils.
- Bacteriophages are viruses that only infect specific bacteria. Bacteriophages e11/2 and e4/1c were previously identified by Teagasc Food research Centre, Moorepark (O'Flynn et al. 2004, *Appl. and Environ. Micro*. 70:3417-3424).

Model broth assays.

The abilities of all the agents to inhibit a panel of VTEC strains and other foodborne pathogens and spoilage bacteria were evaluated in a model broth assay. The abilities of selected agents to inhibit *E. coli* O157:H7 in various environmental conditions found in the food environment, including various temperatures, pHs, water activities and sodium chloride concentrations were also determined.

Model rumen assays.

Based on the broth results, only Caseicin A, carvacrol and the bacteriophages were carried through for

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assessment of activity in model rumen assays. This experiment was used as a model to determine whether the agents would be appropriate for animal application to control pathogen shedding. The assay involved inoculating a model rumen with *E. coli* O157:H7 and adding the agent at a range of concentrations and determining the number of surviving *E. coli* O157:H7 over 24 h. A control consisted of the assay inoculated with *E. coli* O157:H7 only.

Animal application experiments.
Following the results of the model broth and rumen experiments, the bacteriophages were chosen for *in vivo* application. The purpose of the experiment was to determine whether the administration of a bacteriophage cocktail could reduce the shedding of *E. coli* O157:H7 by cattle. Twenty cattle were inoculated with a cocktail of non-toxigenic marked (to aid experimental recovery) *E. coli* O157:H7 strains and then ten animals were then dosed daily for three days with a bacteriophage cocktail of e11/2 and e4/1c. The remaining ten animals were not dosed and remained as controls for the experiment. The experiment was repeated using two fistulated animals to determine the survival of *E. coli* O157:H7 and phage in the rumen as well as in the faeces following dosing. The numbers or presence of *E. coli* O157:H7 in the rumen/faecal samples were determined by plate counts and/or enrichment.

Hide/carcass application.
Carvacrol and the bacteriophages were examined as agents to control *E. coli* O157:H7 on hide and carcass. Hide and carcass pieces were inoculated with a cocktail of marked *E. coli* O157:H7 strains and a spray application of carvacrol (1-3%) or a bacteriophage cocktail was administered. Inoculated hide/carcass pieces treated with water only washes were used as a control.

Meat application experiments.
The ability of carvacrol to inhibit *E. coli* O157:H7 in meat products was also determined. Mince meat and salami slices were inoculated with *E. coli* O157:H7 and various concentrations of carvacrol (1-3%) were added to the product. *E. coli* O157:H7 inoculated but untreated meat samples were used as controls. The numbers of *E. coli* O157:H7 surviving over 10 days of storage at 4 and 10ºC was determined.

Genomic characterization of bacteriophages.
The genomes of bacteriophages e11/2 and e4/1c were determined using pyrosequencing and analysis of the genomes for undesirable genes that may hinder the use of the phages in food applications was performed.

4. Main results:

This study evaluated the use of selected biocontrol agents to inhibit *E. coli* O157:H7 and/or other VTEC within the beef chain.

- Concentrations of ~1 to >2 mg/ml of caseicin A and B AMPs inhibited *E. coli* O157:H7 and other VTEC, foodborne pathogens and food spoilage organisms in a model broth assay. However, the abilities of the AMPs to inhibit *E. coli* O157:H7 in different environmental conditions varied and were found to not affect *E. coli* O157:H7 in conditions when the organism was not growing (eg. low temperatures).
- Concentrations of 0.05-0.2% (v/v) of carvacrol and thymol significantly inhibited or killed *E. coli* O157:H7 and other VTEC, foodborne pathogens and food spoilage organisms in a model broth assay and remained active in a range of environmental conditions.
- Caseicin A at concentrations of >2mg/ml did not inhibit *E. coli* O157:H7 in a model rumen assay, whereas a concentration of 0.05% carvacrol significantly reduced *E. coli* O157:H7 numbers in the model rumen assay within 30 min. Unfortunately the same concentration of carvacrol was found to significantly inhibit the amount of *in vitro* gas produced within the assay indicating that carvacrol could reduce the natural rumen microflora which would be detrimental for rumen fermentation and animal production.
- The application of a bacteriophage cocktail to animals inoculated with *E. coli* O157:H7 did not affect the numbers of *E. coli* O157:H7 shed by the animals. The result may indicate that the dose may not be sufficient for the animals or that the phages were inhibited in the gastrointestinal tract. Indeed, phages were recovered in the rumen and/or faeces of the animals but numbers were very low. Future research to improve the activities and application procedures of the phage to animals is required.
- Concentrations of 3% carvacrol sprayed onto hides and carcass pieces inoculated with *E. coli* O157:H7 significantly reduced *E. coli* O157:H7 numbers (1.3 log cfu/cm^2) on the samples compared

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to the controls. The application of the bacteriophage as a spray onto inoculated hide pieces and allowed to stand for 1 h resulted in significant reduction (2.0 log cfu/cm^2) of *E. coli* O157:H7 numbers on the samples compared to the controls. Enumeration of *E. coli* O157:H7 immediately after phage application onto hide or carcass pieces did not affect *E. coli* O157:H7 numbers compared to the controls. This result suggests that the phage require time to penetrate and inhibit *E. coli* O157:H7 on the samples and may not be appropriate for use within the slaughter process where the line speed is typically very quick. The application of the phage could potentially occur in the lairage prior to slaughter whereby the animals have more time following transport to the factory.

- Concentrations of >2% carvacrol inhibited *E. coli* O157:H7 in minced meat and salami slices but these concentrations resulted in major sensory issues with the product.
- The genomes of both bacteriophages e11/2 and e4/1c were sequenced using pyrosequencing and analysis found no undesirable genes (e.g. toxigenic genes) that would hinder application of the phage in the food chain.

5. Opportunity/Benefit:

The technology developed in this project will benefit the beef industry, and public health of the consumer.

Industry

The biocontrol agents, investigated in this project show potential for application in the industry, however, more work is required to optimise their use and to gain regulatory approval in the EU. Internationally, successes has been reported with the use of phage against food pathogens in the US and Canada.

Consumers and regulatory authorities

Intervention strategies to decrease the numbers of VTEC in the food chain would decrease of risk of infection for the consumers. Such impacts would be of great benefit to both the consumers and the authorities in charge with ensuring compliance with EU food safety regulations.

6. Dissemination:

Information from the project has been disseminated via scientific publications, popular articles and stakeholder workshops

Main publications:

Rivas, L., McDonnell, M.J., Burgess, C.M., O'Brien, M., Navarro-Villa, A., Fanning, S. and Duffy, G. (2010): Inhibition of verocytotoxigenic *Escherichia coli* in model broth and rumen systems by carvacrol and thymol. *International Journal of Food Microbiology*.139: 70-78

Rivas, L, Coffey, B., McAuliffe, O., McDonnell, M.J., Burgess, C.M., Coffey, A., Ross, R.P. and Duffy, G. (2011): The *in vivo* and *ex vivo* evaluation of bacteriophage e11/2 and e4/1c for the control of *Escherichia coli* O157:H7. *Applied and Environmental Microbiology* 76(21):7210-7216

Coffey, B., Rivas, L., Duffy, G., Coffey. A., Ross, P. and McAuliffe, O. (2011): Assessment of *Escherichia coli* O157:H7-specific bacteriophages e11/2 and e4/1c in model broth and hide environments. *Int Journal of Food Microbiology* 147(3):188-94

Popular publications:

Coffey, B. (2010): Science Week 2009 Biocontrol of *Escherichia coli* O157:H7. T-Research. Volume 5, Number 1, Spring 2010 (ISSN 1649-8917). Pg 10.

Duffy, G. (2010): Control of Verocytotoxigenic *E. coli* in ruminant animals. *Irish Vet Journal*

McDonnell, M., Rivas, L., Burgess, C., Fanning, S. and Duffy, G. (2009): The use of carvacrol for the inhibition of *Escherichia coli O157:H7* in different environmental conditions using a model broth system. *ProSafeBeef* conference proceedings. *ProSafeBeef* conference. Dublin, Ireland. 25-26 March 2009.

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