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Potential for transfer of *Escherichia coli* O157:H7, *Listeria monocytogenes* and *Salmonella* Senftenberg from contaminated food waste derived compost and anaerobic digestate liquid to lettuce plants

Suzannah Murphy [a, b], Michael T. Gaffney [a, c], Seamus Fanning [b, d], Catherine M. Burgess [a, *]

[a] Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland
[b] UCD-Centre for Food Safety, School of Public Health, Physiotherapy & Sports Science, University College Dublin, Belfield, Dublin 4, Ireland
[c] Horticulture Development Department, Teagasc Food Research Centre, Ashtown, Dublin 15, Ireland
[d] Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Stranmillis Road, Belfast BT9 5AG, United Kingdom

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ABSTRACT

The diversion of food wastes from landfill to sustainable disposal methods, such as composting and anaerobic digestion, has led to an increase in the soil amendment products that are now commercially available and which are derived from both of these processes. The use of such products as soil amendments during the production of ready-to-eat (RTE) crops is increasing worldwide.

The aim of this study was to investigate the potential of three well-recognised bacterial pathogens of importance to public health, namely *Escherichia coli* O157:H7, *Salmonella* Senftenberg and *Listeria monocytogenes*, to become internalised in lettuce plants from peat growing media amended with contaminated food waste derived compost and anaerobic digestion liquid. The results demonstrated both S. Senftenberg and *E. coli* O157:H7 are capable of internalisation at lower inoculation levels, compared to previous studies. The internalisation was visualised through confocal microscopy. Internalisation of *L. monocytogenes* did not occur, however significant levels of *L. monocytogenes* contamination occurred on the non-sterilised plant surface.

Assessing the internalisation potential for each of these pathogens, through the compost and anaerobic digestate matrices, allows for better risk assessment of the use of these products in a horticultural setting.

1. Introduction

The occurrence of foodborne illness attributed to ready-to-eat (RTE) vegetables is on the increase worldwide. In Europe it is thought that this is due to increased surveillance of RTE vegetables as potential vehicles for transmission of foodborne illness, and the increase in both the production and supply of RTE vegetables. An increase in consumer demand has led to the globalisation of the RTE vegetable production (Jacxsens et al., 2012). RTE sprouting seeds are increasingly being associated with outbreaks due to the ideal incubation environment for pathogens provided during germination of the seeds. Leafy greens have also been repeatedly implicated in foodborne outbreaks. In Europe fourteen outbreaks specifically linked to leafy greens were documented between 2004 and 2012 (Callejón et al., 2015). In the same period in the United States there were 37 outbreaks specific to leafy greens.

Contamination of leafy greens can occur at any stage of the farm-to-fork chain. This study however is concerned with the pre-harvest environment. In particular, the use of organic fertilisers such as composted food wastes may increase the risk of exposure of leafy greens to bacterial pathogens. Up to now there have been no outbreaks directly linked to contaminated organic fertilisers. However, these potential sources are not routinely tested after application to the land and are generally not available for testing once the outbreak occurs (EFSA, 2014). Although an efficient composting process should eliminate bacterial pathogens present in the waste, the product can become re-contaminated during storage or may contain discrete pockets within the matrix which do not reach required temperatures during composting (Avery et al., 2012). Both scenarios may lead to the survival and growth of

* Corresponding author.
E-mail address: kaye.burgess@teagasc.ie (C.M. Burgess).

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pathogens. The practice of burying the composted waste (CW) into the soil weeks before crops are planted may also not be sufficient to eliminate pathogens, as studies have shown enteric pathogens such as *Escherichia coli* O157:H7 and *Salmonella enterica* are capable of surviving for months in the soil environment (Jiang et al., 2002; You et al., 2006; Fremaux et al., 2008). As *Listeria monocytogenes* is an environmental pathogen it can easily survive for long periods in the soil environment (Kim and Jiang, 2010).

Anaerobic digestate liquid (AD) is an example of an organic fertiliser which has the potential to be used in horticultural settings. The use of anaerobic digestion to transform food waste to a fertiliser is increasing worldwide. Currently this product is mainly used on grasslands but its high nutrient content and sustainable production method means additional uses such as soil fertilisers for crops are being investigated (Alburquerque et al., 2012). As with composting the anaerobic digestion process should reduce or eliminate bacterial pathogens. Studies have reported on the identification of both *S. enterica* and *L. monocytogenes* in the final AD product (Bonetta et al., 2011). Other authors have also reported on the survival of bacterial pathogens in soil which has been amended with contaminated AD liquid (Goberna et al., 2011).

If horticultural growth substrates are contaminated with foodborne pathogens there is the potential for subsequent crop contamination to occur. However, previous studies in this area have reported conflicting results. *S. enterica* were shown to internalise into the root structure and to translocate to the edible part of RTE plants (Bernstein et al., 2007; Ge et al., 2012; Gorbatsevich et al., 2013). The results for *E. coli* O157:H7 vary, with some studies showing apparent internalisation (Solomon et al., 2002; Erickson et al., 2010), while others report an inability to internalise into the lettuce plant (Johannessen et al., 2005; Erickson et al., 2014a). By comparison limited research has been done on *L. monocytogenes*, but a recent study by Chitarra et al. (2014) demonstrated the internalisation of lettuce plants at 24 °C. Several factors determine the internalisation ability of these pathogens, such as temperature, moisture content of growing media, soil type and also the stage of growth of the plant and the plant type itself (Ge et al., 2012). The inoculum level has also been shown to affect internalisation ability (Erickson et al., 2014b). Internalisation has mainly been observed at high levels (Log_{10} 6–9 CFU g^{-1}) of pathogen inoculation. These bacterial numbers may not reflect actual pathogen numbers normally encountered under field conditions.

The variation in results highlights the need to further investigate the internalisation potential of each of these pathogens so as to assess the potential risk posed for contamination of RTE crops. Gu et al. (2013) reported that the internalisation of *S.* Typhimurium into tomato seedlings was reduced when organic fertilisers were used. The impact of the feedstocks for both composted waste and AD liquid on the internalisation ability may also play a role, but is rarely considered. To assess the risk posed by the use of these products as a fertiliser for RTE crop production, the potential for foodborne pathogens to internalise RTE crops from growth media amended with contaminated food waste derived compost and AD liquid should be investigated.

The aim of this study was to investigate the potential of three bacterial pathogens, *E. coli* O157:H7, *S. enterica* species and *L. monocytogenes,* to internalise lettuce plants from peat growing media amended with contaminated food waste derived compost and AD liquid. Plant surface sterilisation techniques were applied to ensure any positive samples were the result of internalisation and not the presence of the pathogens on the surface of the plants. Assessing the internalisation potential of each of these pathogens provides valuable information on the potential risk posed and enables the development of appropriate mitigation strategies.

2. Materials & methods

2.1. Bacterial strains and media

E. coli O157:H7 (ATCC 43888), *L. monocytogenes* and *S.* Senftenberg were used throughout this study. The *E. coli* O157:H7 is a non-toxigenic reference strain. *S.* Senftenberg is a compost isolate, and was kindly supplied by the Irish Department of Agriculture, Food and the Marine. Both *E. coli* O157:H7 and *S.* Senftenberg were transformed with the GFP containing plasmid pGFPuv (Clontech), by electrotransformation (Ma et al., 2011). The *L. monocytogenes* strain was kindly supplied by the National University of Ireland, Galway (Utratna et al., 2012). The latter contains an integrated GFP gene; the expression of which is dependent on the exposure of the *L. monocytogenes* to stressful environments. A second non-fluorescent strain of *Listeria, L. monocytogenes* 403T12B, was also used.

All bacterial strains were stored on Protect Beads, at −80 °C (Technical Service Consultants Ltd, UK). Throughout the study strains were maintained on tryptone soya agar (TSA) and over night cultures were grown in tryptone soya broth (TSB) at 37 °C and overnight cultures were washed twice in phosphate buffered saline (PBS), by centrifuging at 8000 rpm for 10 min, prior to use.

All broths and agars used in this study were purchased from Oxoid, (Hampshire, U.K.). Xylose lysine deoxycholate (XLD) agar was used for *S.* Senftenberg recovery. For *E. coli* O157:H7 recovery, sorbitol MacConkey agar with cefixime and tellurite (CT-SMAC) was used. *Listeria* chromogenic agar (CLA), with supplements was used to recover *L. monocytogenes*. Buffered *Listeria* enrichment broth (BLEB) was used for the detection of *L. monocytogenes* by enrichment.

AD samples were taken from a commercial facility and the feedstocks included food waste and animal manures. The pH and total solid content of the AD was 7.92 and 4.67%, respectively. CW samples were also taken from a commercial facility; feed-stocks here included food waste and green waste. The pH, electrical conductivity and organic matter content of the CW were 6.98, 4.12 and 42.85, respectively. The lettuce used throughout this trial was a commercial variety called *Lactuca sativa* var *capitata* (AMICA). The growing media mixed with both the CW and AD was a commercial peat based growing substrate, Jack's Magic peat based compost (Westlands, United Kingdom).

2.2. Inoculation of CW waste and AD

One ml of washed overnight cultures of either *E. coli* O157, *S.* Senftenberg and *L. monocytogenes* were inoculated into 60 g (w/w) CW or 10 ml AD. The inoculated CW/AD samples were the added to each pot containing peat growing media to give a final concentration in each 180 g pot of Log_{10} 5 CFU g $(CW/AD)^{-1}$.

2.3. Experimental setup

All lettuce plants were grown from seeds for three weeks before transplanting the plugs to individual 9 × 9 cm^2 pots. To prepare the pots for these plugs two approaches were used; one for compost and the other for AD. For the compost treatment, 120 g (w/w) peat growing media was weighed. This was mixed with 60 g (w/w) of inoculated CW and placed into a labelled pot. A divet was made in the centre of the pot and the lettuce plug planted. For the AD treatment the 10 ml volume of inoculated AD was mixed using a large tongue depressor with 180 g (w/w) of the growing media (GM). The lettuce plug was planted using the same method as for the CW treatment. Lettuce plants were grown at room temperature under artificial lights. The lighting was set up to reflect day light