Salads and Salmonellas

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Introduction and Aims

***Salmonella* and salads:** Salad leaves are an important part of a healthy diet, but in recent years there have been reports of food borne illness associated with *Salmonella* and *E. coli* contamination of fresh salad produce. In particular, the consumer-popular ready-washed bagged mixed salads have been recent sources of *Salmonella* infection. The FSA reported that annually there are more than 500,000 cases of food poisoning in the UK, of which >48,000 were from fresh produce: vegetables, fruit, nuts and sprouting seeds, indicating enteric pathogen contamination of fresh produce is a growing consumer safety problem.

Aims: Salad leaves often become damaged during processing, and although much research has been made into improving the hygiene of salad cultivation, little is known about what happens to pathogenic bacteria when in the salad bag. The aim of this project is therefore to investigate the effects of fluid released from salad tissue that occurs during damage routine to commercial processing, on the growth and biofilm formation of *Salmonella enterica.*

Materials and Methods

Salads were sorted from bagged mixes, and extracted by grinding with a pestle and mortar (10g salad), then centrifuged, and filter-sterilised, now termed 'salad juice'.

For modelling behaviour of *Salmonella* within the salad bag, investigations of the salad leaf juice exposure on growth, biofilm formation and leaf colonisation were carried out. *Salmonella* were incubated in sterile water +/- 2% salad juices or salad bag fluids at 37ºC & 4ºC. **Motility assays** were performed in Dulbecco's Modified Eagle's Medium (DMEM). **Biofilm formation** on salad bag plastic was analysed microscopically using Green Fluorescent Protein-labelled *Salmonella*. **Salad leaf colonisation** investigated using Scanning electron micrographs (SEM).

Results

1. Salad extracts increase *Salmonella* growth in the fridge

Fig.1A shows that at 37ºC growth in water was supported in a dose dependent manner, while Fig. 1B shows that even traces of juice within salad bag fluids stimulated *Salmonella* growth by >1000-fold over the controls during a 5-day storage in a fridge set at 4ºC.

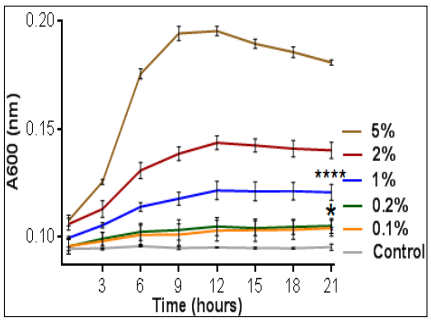


Fig 1A Timecourse of *Salmonella* growth at 37ºC in water supplemented +/-spinach juice

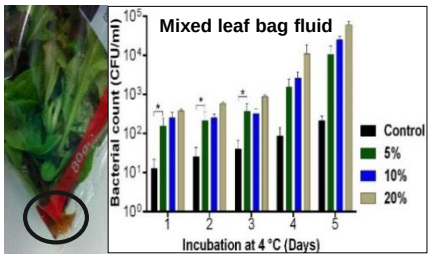
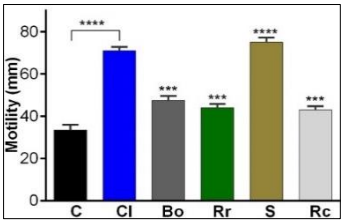


Fig 1B Histograms show growth of *Salmonella* in sterile water supplemented with 5-20 % (v/v) mixed leaf salad bag fluid

2. *Salmonella* motility and biofilm formation is enhanced by salad juice exposure

As motility is important in pathogen salad leaf colonisation we examined the motility of *Salmonella* in the presence of the salad leaf juices. Fig 2A shows that a 2% addition of any of the juices significantly increased motility relative to un-supplemented controls



Typical image of motility enhancement

Fig 2A 2% salad juice increase *Salmonella* motility in DMEM relative to controls
Key: C, control (no additions); Cl, cos lettuce; Bo, baby green oak lettuce; Rr, red romaine lettuce; S, spinach; Rc, red chard

Fig. 2B shows blue light microscopy images (x60 magnification) of GFP-*Salmonella* incubated in water and attaching to the salad bag plastic +/- 2 % spinach juice or 20% spinach bag fluid.

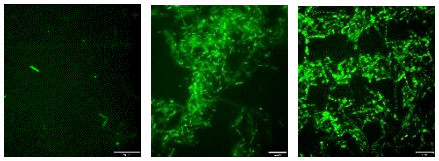


Fig 2B Microscopy images of untreated (left), spinach juice (middle) and spinach bag fluid.

3. Effect of salad extracts on *Salmonella* gene expression

To investigate salad extract effects on gene expression, analysis was made of the effect of extract exposure on protein expression. The results showed significant differences in expression profiles for all salad extract-treatments. Increased expression of infection related proteins was found: e.g SipA, SipB, SipC (which are essential for host cell invasion)

4. Salad juice enhances *Salmonella* salad leaf colonisation

In Fig 4 it can be seen that in comparison with non-treated controls (left) the association of *Salmonella* with the plant surface was greatly enhanced in the presence of the salad juice (right).

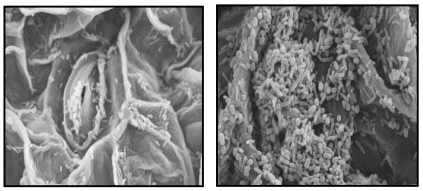


Fig 4 Spinach juice increases *Salmonella* colonisation of salad leaves SEMs of untreated (left) and spinach juice-exposed (right) *Salmonella* seeded onto spinach leaves.

5. Conclusion & Future work

It is clear that exposure to even very small volumes of salad leaf juices enhances *Salmonella* growth and biofilm formation, both of which are important in colonisation and persistence of the pathogen in bagged salads. It is therefore important to develop ways to prevent this interaction, and so we plan to use the many microbes that naturally live on the salad leaves or salad bag to prevent the *Salmonella* from attaching (Fig. 5)

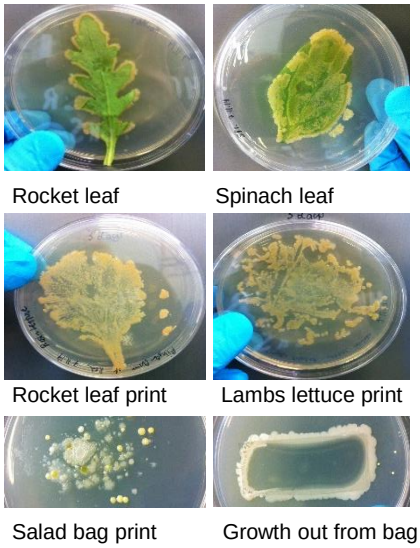


Fig 5 The natural bacteria and fungi living on salad leaves and attached to the salad bag they are contained in.

6. Acknowledgements

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