Comparison of Sample and Detection/Quantification Methods for *Salmonella enterica* from Produce

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Background

The purpose of this study was to identify and optimize fast and reliable sampling and detection methods for the identification of pathogens that may be present on produce grown in small vegetable production units on the International Space Station (ISS), thus a field setting. Microbiological testing is necessary before astronauts are allowed to consume produce grown on ISS where currently there are two vegetable production units deployed, Lada and Veggie. (Fig.1). Methods of analysis must be portable and microgravity compatible.

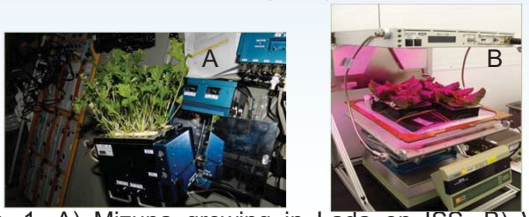


Fig. 1. A) Mizuna growing in Lada on ISS. B) Veggie ground experiment (Kennedy Space Center, FL).

Inoculation and Sample Methods

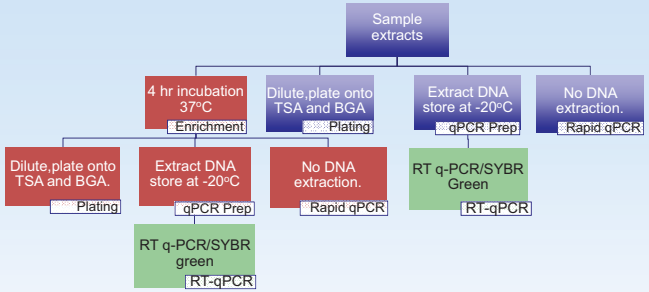
Plants/produce. Tomato (cv. Red robin), red romaine lettuce (cv. Outredgeous) and Radish (cv. Cherry Bomb II) where grown in pots in a controlled environment growth chamber at 50% RH, 25°C and 400ppm CO2. Produce was used immediately after harvest.

Inoculation. 18 hour broth cultures of *Salmonella enterica* Typhimurium were washed by centrifugation, re-suspended and inoculated by pipetting 10ul of approximately 10^7 bacteria /cm^2. Inoculum was allowed to dry before sampling.

Three sample methods.

1. Surface swabbed from marked and inoculated 1cm^2 and placed into sterile buffered peptone water (BPW) or water.
2. Adhesive tape (cellulose) pressed firmly onto inoculated measured surface and placed into buffer or sterile water with glass beads.
3. Inoculated produce pummeled in a bag blender after the addition of a measured volume of buffer.

Detection/Quantification



Culture based enumeration. Sample extracts (pre and post enrichment) were diluted and plated onto Brilliant Green Agar (BGA) and Trypticase Soy Agar (TSA). Incubated at 37°C for 24 hours before counting.

Quantitative Polymerase chain reaction (qPCR).

•DNA was extracted from samples using the Ultra Clean Microbial Kit (Mo bio, Carlsbad, CA) and stored at -20°C until use.

•PCR reaction was prepared with *inv*A gene primers and Light Cycler 480 SYBR Green I Master Mix (Roche, Indianapolis, IN).

•Serially diluted standard curves were prepared with DNA from known concentrations of *S. enterica*

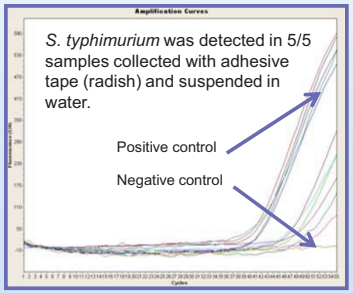
Rapid detection PCR using RAZOR®EX.

•Tape and swab sample suspended into sterile water.

•PCR reaction was prepared with 900 nM *inv*A gene primers and 100 nM TaqMan fluorescent probe then injected into a RAZOR(Biofire, Salt Lake, UT) pouch.

•Four minute enzyme activation, 15 s denaturing step, 60 s annealing. Fifty five cycles completed.

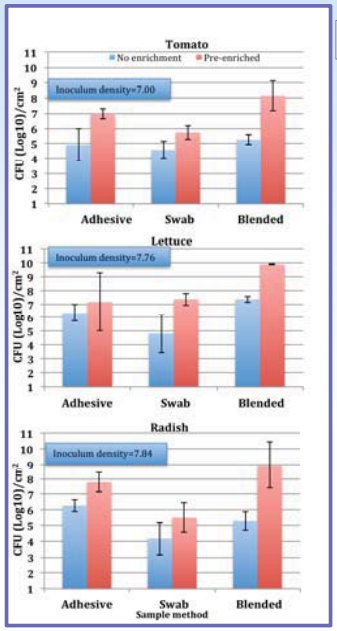
Results



Rapid qPCR

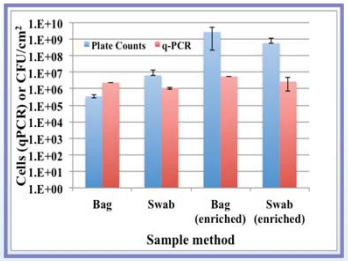
5/5 adhesive tape and swab samples from inoculated radish tested positive for the presence of *S. enterica*. BPW interfered with the reaction.

Results



Culture based enumeration

- No significant difference (P<0.05) between swab and adhesive tape sampling methods in the recovery of *S. enterica* from tomato and lettuce.
- Blended samples of the red leaf lettuce resulted in significantly higher (P<0.05) recovery of the bacterium before and after enrichment.
- Recovery was reduced from the inoculum density by 0.2 to 1.6 log CFU/cm^2 depending on the sample method and type of produce



RT-qPCR

Plated samples yielded greater CFU/cm^2 counts than the cell counts determined by q-PCR. There was no significant difference (P<0.05) between sampling methods (swab and tape shown)

Conclusion

These data suggest that swab or adhesive tape sampling methods are effective in the recovery of *S. enterica* from the surfaces of produce and may be appropriate methods for field analysis.

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