Title Real-time reverse-transcriptase PCR for *Salmonella* Typhimurium detection from lettuce and tomatoes

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Abstract

Salmonella outbreaks linked to fresh produce stress the importance of rapid detection methods to help prevent disease. Real-time reverse-transcriptase-PCR (rt-RT-PCR) is based on the detection of mRNA (shorter half-life than DNA), showing promise of detecting viable pathogens while eliminating the need for gel electrophoresis. The research is aimed at applying rt-T-PCR to detect *Salmonella* from spiked lettuce and tomatoes within one day. Twenty-five grams of lettuce and 100 g of tomatoes were inoculated with 1–8 log CFU of an overnight culture of *Salmonella* Typhimurium. Bacteria were recovered with 0.05 mol/L glycine–0.14 mol/L saline buffer (containing 0.05 g/100 ml Tween-20, 3 g/100 ml beef extract). For low inocula (5 log to 1 log CFU), a short pre-enrichment of 6 h in peptone buffer was carried out to improve assay sensitivity. Serial dilutions were spread plated on Xylose Lactose Tergitol 4 (XLT4) agar and incubated at 37 °C for 48 h and portions used for RNA extraction using the Qiagen RNeasy® Mini Kit. SYBR Green one step RT-PCR kit with *invA* gene primers and an internal amplification control was used for detection. Reaction conditions were 50 °C/40 min, then 94 °C/45 s, 58 °C/45 s, 72 °C/45 s for 45 cycles followed by melt temperature analysis. This rt-RT-PCR procedure could detect 4 log CFU/25 g *Salmonella* from lettuce and tomatoes (100 g) after pre-enrichment. Without pre-enrichment, *Salmonella* detection from lettuce was 6 log CFU/25 g and from tomatoes was 6–7 log CFU/100 g. These results show that rt-RT-PCR can be used to detect *Salmonella* contamination in produce within 24 h.