

Title *Salmonella* Typhimurium internalization is variable in leafy vegetables and fresh herbs
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Abstract

Despite washing and decontamination, outbreaks linked to consumption of fresh or minimally-processed leafy greens have been increasingly reported in recent years. In order to assure the safety of produce it is necessary to gain knowledge regarding the exact routes of contamination. Leaf internalization through stomata was previously reported as a potential route of contamination, which renders food-borne pathogens protected from washing and disinfection by sanitizers. In the present study we have examined the incidence (percentage of microscopic fields harboring ≥ 1 GFP-tagged bacteria) of *Salmonella* Typhimurium on the surface and underneath the epidermis in detached leaves of seven vegetables and fresh herbs. The incidence of internalized *Salmonella* varied considerably among the different plants. The highest incidence was observed in iceberg lettuce ($81 \pm 16\%$) and arugula leaves ($88 \pm 16\%$), while romaine ($16 \pm 16\%$) and red-lettuce ($20 \pm 15\%$), showed significantly lower incidence ($P < 0.05$). Internalization incidence in fresh basil was $46 \pm 12\%$, while parsley and tomato leaves demonstrated only marginal internalization ($1.9 \pm 3.3\%$ and $0.56 \pm 1.36\%$, respectively). Internalization of *Salmonella* in iceberg lettuce largely varied (0–100%) through a 2 year survey, with a higher incidence occurring mainly in the summer. These results imply that *Salmonella* internalization occurs in several leafy vegetables and fresh herbs, other than iceberg lettuce, yet the level of internalization largely varies among plants and within the same crop. Since internalized bacteria may evade disinfection, it is of great interest to identify plants which are more susceptible to bacterial internalization, as well as plant and environmental factors that affect internalization.