

Title Distribution of *Salmonella typhimurium* in romaine lettuce leaves
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Abstract

Leafy greens are occasionally involved in outbreaks of enteric pathogens. In order to control the plant contamination it is necessary to understand the factors that influence enteric pathogen–plant interactions. Attachment of *Salmonella enterica* serovar *typhimurium* to lettuce leaves has been demonstrated before; however, only limited information is available regarding the localization and distribution of immigrant *Salmonella* on the leaf surface. To extend our knowledge regarding initial pathogen–leaf interactions, the distribution of green-fluorescent protein-labeled *Salmonella typhimurium* on artificially contaminated romaine lettuce leaves was analyzed. We demonstrate that attachment of *Salmonella* to different leaf regions is highly variable; yet a higher attachment level was observed on leaf regions localized close to the petiole ($7.7 \log \text{CFU g}^{-1}$) compared to surfaces at the far-end region of the leaf blade ($6.2 \log \text{CFU g}^{-1}$). Attachment to surfaces located at a central leaf region demonstrated intermediate attachment level ($7.0 \log \text{CFU g}^{-1}$). *Salmonella* displayed higher affinity toward the abaxial side compared to the adaxial side of the same leaf region. Rarely, *Salmonella* cells were also visualized underneath stomata within the parenchymal tissue, supporting the notion that this pathogen can also internalize romaine lettuce leaves. Comparison of attachment to leaves of different ages showed that *Salmonella* displayed higher affinity to older compared to younger leaves (1.5 log). Scanning electron microscopy revealed a more complex topography on the surface of older leaves, as well as on the abaxial side of the examined leaf tissue supporting the notion that a higher attachment level might be correlated with a more composite leaf landscape. Our findings indicate that initial attachment of *Salmonella* to romaine lettuce leaf depends on multiple plant factors pertaining to the specific localization on the leaf tissue and to the developmental stage of the leaf.