SALMONELLA ENTERICA INTERACTIONS WITH PLANTS AND THEIR ASSOCIATED MICROBIOTA

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Fresh fruit and vegetables have been associated recurrently with outbreaks of enteric illness. This microbial contamination of produce has become a significant problem for public health and the produce industry. Research on produce safety has improved our understanding of the ecology of enteric pathogens on plants. Interactions with the plant microbiota are among the critical factors that enable human pathogens to colonize produce. While human pathogens have to compete for resources with other inhabitants of the plant environment, their interactions with certain plant-associated microbes may also enhance their growth, survival, or dissemination.

Salmonella enterica is frequently isolated from leafy vegetables and herbs, and has caused outbreaks such as those linked to cilantro, cantaloupe and tomato in the USA (1). *Salmonella* contamination of retail produce has been correlated positively with the presence of post-harvest and soft rot disease (2, 3). We observed that population sizes of *S. enterica* Typhimurium increased 56-fold when inoculated alone onto cilantro leaves versus 2,884-fold when co-inoculated with *Dickeya dadantii* (*Erwinia chrysanthemi*), a prevalent pathogen that macerates plant tissue (4). A similar trend in *S. enterica* populations was observed in soft-rotted lettuce leaves. Transcriptome analysis in *S. enterica* cells that colonized *D. dadantii*-infected lettuce and cilantro leaves revealed a clear shift toward anaerobic metabolism and catabolism of substrates that are available due to degradation of plant cells by the pectinolytic pathogen. Twenty nine percent of the genes that were upregulated in cilantro macerates were previously observed to increase in expression also in the chicken intestine. Anaerobic conditions and the utilization of nutrients in the macerated plant tissue that are present also in the animal intestine due to dietary intake and digestion indicate a niche overlap that may explain the high adaptation of *S. enterica* to soft rot lesions. This human pathogen appears to have enhanced growth also in leaf tissue infected by *Bremia lactucae*, the causal agent of downy mildew disease of lettuce. Contamination of lettuce with *S. enterica* has led to recalls in the USA and to outbreaks in Europe. In experiments with Romaine lettuce in our laboratory, *S. enterica* population sizes increased 10^2-fold on healthy leaf tissue under conditions of warm temperature and free water on the leaves, but increased by 10^5-fold in necrotic lesions caused by *B. lactucae* (5). Others have shown that *Alternaria* *alternata* and *Cladosporium* spp. have a positive effect on *S. enterica* colonization of tomato fruit, one of the major produce sources of outbreaks of salmonellosis in the USA (6). Given that *S. enterica* has a high infectious dose, its association with plant pathogens on produce may be an important factor in its causation of human illness and the occurrence of outbreaks linked to this commodity.

We have observed also the rapid attachment and biofilm formation by *S. enterica* Typhimurium on *Aspergillus niger*, a common resident on agricultural crops and in soil (7). Several serovars of *S. enterica* associated similarly with *A. niger* whereas other bacterial species, such as *Pseudomonas* spp and *Xanthomonas* spp were unable to bind to the fungus, suggesting a certain level of specificity in this interaction. N-acetylglucosamine, a major component of chitin and therefore, of fungal cell walls, inhibited *S. enterica* attachment to chitin beads and to *A. niger* hyphae, indicating a role for chitin in the binding of the pathogen to the fungus. A cellulose-deficient mutant of *S*. Typhimurium did not bind to chitin beads nor to the fungus, and was unable to form a biofilm. Our results support the hypothesis that encounters with chitinaceous alternate hosts may contribute to the ecological success of human pathogens and likely to their dispersal.

Although few studies have investigated the role of protists in the microbial dynamics that take place on plants, protozoa are common members of the natural microflora of plant surfaces. Several species of amoebae have been found to be associated with fresh salad vegetables (8) and the commonly studied model ciliate, *Tetrahymena pyriformis* was isolated from spinach. In a collaborative study, we observed the presence of various types of protozoa on lettuce and spinach purchased at supermarket. We demonstrated the release of viable *E. coli* O157:H7 and *S. enterica* cells, but not of *Listeria monocytogenes* cells in the fecal pellets of diverse ciliated protozoa (9) and more specifically, the enhanced survival of *S. enterica* in fecal pellets released by a *Tetrahymena* sp. (10). Transcritome analysis of *S. enterica* in *Tetrahymena* phagosomes revealed the induction of numerous genes involved also in the survival and replication of this enteric pathogen in macrophages and human intestinal cells (11). This includes genes that play a role in the acid stress response and led to our observation that *S. enterica* cells gain enhanced acid resistance through their passage in *Tetrahymena*. Thus, the release of viable *S. enterica* as an undigested product by the protist may further increase its survival to the acidic stomach of the human host, thereby reducing the dose required to cause enteric disease.

Numerous studies have shown that human enteric pathogens join microbial consortia on plants, whether in aggregates or biofilms, in which they may gain protection from harsh environmental conditions and from the sanitizers used by the industry to decontaminate produce. In order to design effective crop management and sanitization strategies to improve the microbial safety of produce, it is imperative that the role of plant-associated microbes in the physical protection, multiplication and physiology of foodborne pathogens in the plant habitat be further investigated and included in models of foodborne disease risk assessment.

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