Assessing the mutational rates of bacterial foodborne pathogens in different agricultural environments during long-term colonization or environmental cycling

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Mutations can be a major driving force of bacterial adaptation. Therefore, it is important to quantitatively analyze mutational rates of bacterial foodborne pathogens that occur in different agricultural environments over a prolonged period or during cycling between environments. The purpose of this study was to determine the mutational rates of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria monocytogenes* in agricultural soil and irrigation water during long-term colonization or cycling between different environments. Microcosms of either 10 g of soil, 100 ml irrigation water, or 100 ml buffered peptone water (control) were inoculated with ~10^9 CFU of one of the three pathogens and then cultured/sampled every 2 week for 42 weeks. Long-term colonization microcosms were inoculated only once at the beginning of the 42 weeks, whereas environmental cycling consisted of inoculating a new microcosm with the pathogen isolated from the previous time point microcosm across 42 weeks. Shotgun metagenomics sequencing (Illumina NovaSeq S4) was performed on all samples (n=572) resulting in an average of 1.2 gigabases of sequencing data/sample. Reads were quality trimmed based on Phred score (Q33) using Trim-galore, assembled using MetaSPades, and finally contigs and filtered reads used to identify mutations or single nucleotide polymorphisms (SNPs) using the Bowtie2 plugin in Geneious Prime (v2022.1) against the corresponding assembled inoculum genome for each time point of the study. No mutations were observed in any long-term microcosms. However, soil cycling resulted in mutational rates of up to 2.48 mutations/yr for *E. coli* O157:H7, up to 2.48 mutations/yr for *S.* Typhimurium, and up to 6.19 mutations/yr for *L. monocytogenes*, whereas water cycling had up to 1.24 mutations/yr for *E. coli* O157:H7, up to 2.48 mutations/yr for *S.* Typhimurium, and up to 3.71 mutations/yr for *L. monocytogenes*. Our results provide critical insights into how these pathogens function and adapt to different agricultural environments.