Abstract

Listeriosis is an important food-borne disease responsible for high rates of morbidity and mortality. *L. monocytogenes* has been the cause of several food-borne outbreaks and product recalls throughout the world. It can adapt and survive in a wide range of stress conditions which makes it difficult for food producers to eradicate. The goal of this study was to use phenotypic assays and whole genome sequencing to elucidate possible links between food-related stress resistance and virulence phenotypes in *L. monocytogenes* strains originating from different sources. Four *L. monocytogenes* isolates from sweetcorn and one isolate from a food processing environment (control) were sequenced and evaluated for the ability to survive in acid (pH 3.5, 15 min), in the presence of a commercial antimicrobial mixture (2% v/v, 90 min), heat (60°C, 5 min) and hydrogen peroxide (420 mM, 15 min). Results showed that the strains had different resistance levels to the above stressors with the environmental strain being more susceptible to heat and the commercial antimicrobial. Also, results showed that the four sweetcorn isolates were more virulent than the environmental isolate as they had significantly higher attachment and invasion capacity onto HCT-8 cell.

**Results and Discussion**

Figure 1. *L. monocytogenes* resistance to stressors and biofilm formation. (A) Heat resistance of *L. monocytogenes* strains in BH broth, (B) pH resistance, (C) H₂O₂ resistance, (D) biofilm formation, (E) motility in aerobiosis and (F) in anaerobiosis. Results are expressed as log reduction (N₀: control counts; N: counts after stress exposure). Error bars represent the standard deviation. Asterisks indicate significant differences (*p<0.05, **p<0.01, ***p<0.001; ****p<0.0001).

Our initial phenotypic assays (Fig. 1) allowed us to test a range of environmental associated stress conditions, with OT171-4 isolates displaying a higher level of adhesion and invasion, in vitro, compared to the reference isolate. Also, the in vivo experimental data (Fig. 2) showed that the four new isolates displayed significantly higher virulence properties when compared to the reference strain as the colonisation levels for the sweetcorn isolates were higher for both the liver and the spleen. It is important to note that the OT171-4 sweetcorn isolates all showed similar levels of colonisation in both organs. The in vivo findings reveal that the sweetcorn isolates have increased capacity at translocating from the gastrointestinal tract to other organs of mice or simply are more capable of surviving in the gastrointestinal tract.

**Conclusion**

We identified similar phenotypic properties such as in vitro adhesion and invasion, in vivo liver and spleen colonisation, and resistance to heat for all four *L. monocytogenes* outbreak-like strains (OT171-4) which have differed significantly from a reference strain (FMT 1750). SNP analysis explored what genetic changes may be causing these phenotypic observations. We highlight the importance of combining WGS strategies in conjunction with phenotypic methods as a key approach in the investigation of listeriosis.

**References**