Integrated phenotypic and genomics analysis to elucidate differences in stress resistance and virulence of *L. monocytogenes* strains

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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.

Introduction

In 2018 the European Food Safety Authority (EFSA) released a report on an invasive *Listeria monocytogenes* outbreak which had caused lethal human infections, with the source initially attributed to Hungarian frozen sweetcorn and other frozen vegetables (1). Whole-genome sequencing (WGS) confirmed the link between the human isolates and frozen sweetcorn isolates and so in 2018 the Hungarian authorities banned the marketing of all frozen vegetables (1). The authorities in the United Kingdom and other countries in the European Union took a similar action. Currently WGS represents a powerful tool for molecular epidemiological studies; however, associating genotype with phenotype, is often lacking. Many foodborne pathogenic bacteria will behave differently upon colonization of the human host and by coupling genome sequencing with phenotypic experimental data, our understanding of bacterial pathogenesis and survival can vastly improve. A variety of genes involved in pathogenicity and survival in the host are likely to be activated only *in vivo*, which strengthens the importance of scrutinizing outbreak isolates in experimental infections. In order to understand their virulence, we have isolated *L. monocytogenes* from commercially available frozen processed sweetcorn from Hungary and performed genotypic and phenotypic characterization.

Aim

In order to understand the pathogenic potential of the sweetcorn *L. monocytogenes* isolates we have performed comparative genomic analysis in addition to phenotypic assays to correlate genotypic and phenotypic markers for a virulence profile of these *L. monocytogenes* outbreak-like isolates. Our study explores the resistance of these *L. monocytogenes* outbreak-like isolates to the major stresses encountered during host invasion and the establishment of a disease phenotype.

Results and Discussion

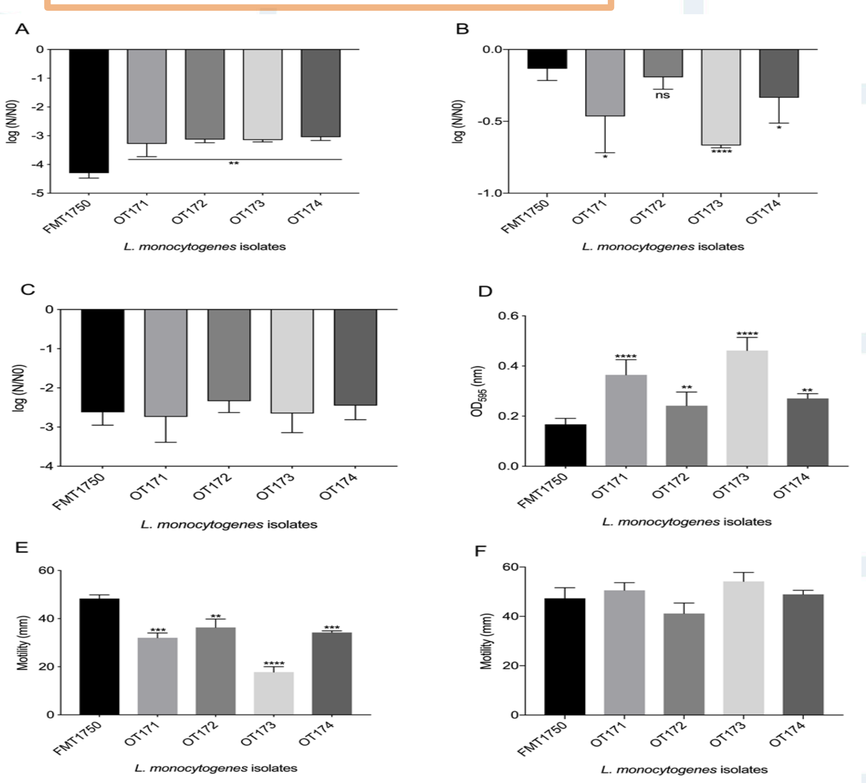

Figure 1. *L. monocytogenes* resistance to stressors and biofilm formation. (A) Heat resistance of *L. monocytogenes* strains in BHI broth, (B) pH resistance, (C) H_2O_2 resistance, (D) biofilm formation, (E) motility in aerobiosis and (F) in anaerobiosis. Results are expressed as log reduction (N0: 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.

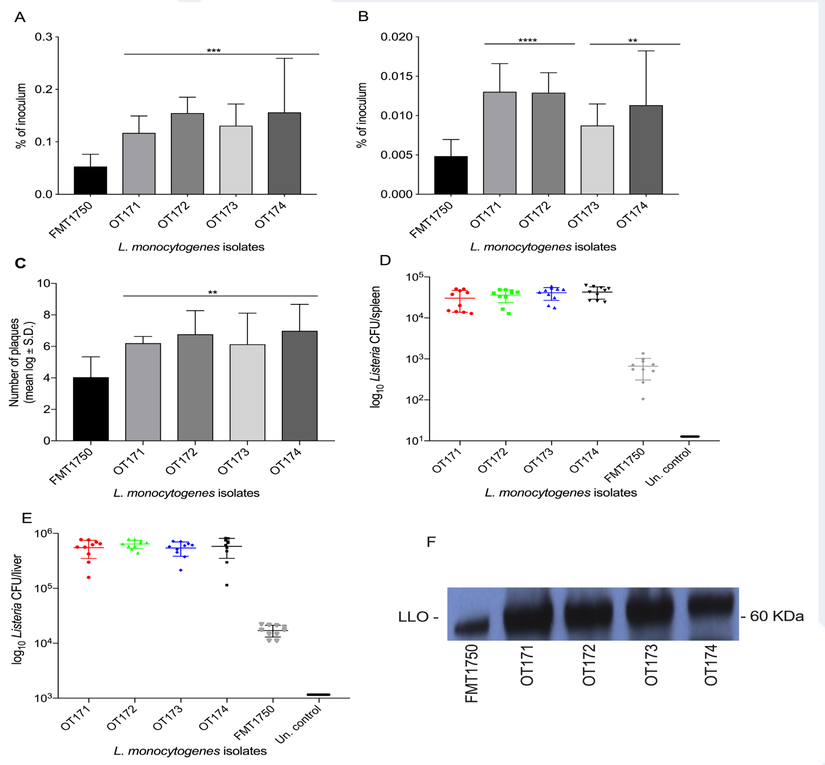

Figure 2. *In vitro* and *in vivo* infection abilities, LLO secretion and plaque formation of *L. monocytogenes* isolates. *In vitro* adherence (A), invasion (B) of HCT-8 cells of *L. monocytogenes* sweetcorn isolates compared to the FMT1750 reference strain. Panel C, represents the number of plaques. Panels D (spleen) and E (liver) show the levels of *in vivo* infection expressed as $\log_{10}$ CFU/ml. The levels of LLO production between strains is presented in panel F. Error bars represent the standard deviation. *Asterisks* indicate significant differences (*p<0.05, **p<0.01, ***p<0.001; ****p<0.0001).

The higher colonisation levels (Fig. 2) may imply that the sweetcorn isolates are better at tolerating the acidic conditions of the stomach. However, the *in vitro* resistance results showed that all isolates used in this study had a similar resistance to acidic conditions. In addition, the OT171-4 sweetcorn isolates also had the same susceptibility to oxidative stress, which could suggest that increased survivability in the gastrointestinal tract is not the determining factor that influences colonisation.

SNP analysis revealed:

- Flagellar hook protein FlgE as a potentially modified gene between OT171-4 sweetcorn isolates and FMT 1750.
- Transketolase (TKT) as affected significantly between the OT171-4 sweetcorn isolates and the reference strain. Salmonella spp., lacking transketolase have been described as avirulent in mice suggesting their possible polar effect in pathogenesis (2). Increased resistance to stresses in the OT171-4 isolates may also be related to these significant changes in the TKT enzymes (3).
- Precorrin-3B C(17)-methyltransferase and sirohydrochlorin cobaltochelatase genes were predicted as mutated, which are involved in nutrient and survival needs in anaerobic conditions (4,5).

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

1. European Food Safety Authority, 2015. EFSA Journal. 13(1):3991-n/a.
2. Shaw JA, et al. 2018. The Journal of biological chemistry. 293(29):11271-82.
3. Varghese S, et al. 2003. Journal of bacteriology. 185(1):221-30.
4. Camejo A, et al. 2009 . PLoS pathogens. 5(5):e1000449.
5. Moore SJ, et al. 2013. The Journal of biological chemistry, 288(1):297-305.