Listeria monocytogenes: illuminating adaptation with proteomics

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With increased consumption of minimally processed ready-to-eat foods the potential for exposure to Listeria monocytogenes has increased. Thus, there is a need to maintain a balance between food convenience and safety. L. monocytogenes is not a homogenous species; certain strains are more resilient to stressful conditions while others are potentially more virulent. To understand the basis of these differences we are applying proteomics to determine the molecular mechanism of adaptations of L. monocytogenes in food-relevant environments. The goal is to define how this species grows, behaves and survives thus allowing us to fine tune food safety risk management, especially when developing new minimal food processes or considering introduction of unpasteurised food such as raw milk cheeses.

Listeria monocytogenes causes listeriosis, a serious disease primarily acquired by food consumption, and that mainly impacts immunocompromised people, the elderly, and neonates. Listeriosis also occurs in livestock and was originally discovered in animals. Though the prevalence of listeriosis in Australia is relatively low it remains a major concern for the food industry. A case in point is the January 2013 brie and camembert cheese-associated outbreak that occurred in south-east Australia, associated with 5 deaths and one miscarriage. L. monocytogenes mainly occurs as an environmental contaminant and can enter food anywhere along the industrial food supply chain including within domestic settings. Despite being relatively nutritionally fastidious this tendency to be a frequent contaminant is due to the species inherent hardiness since it is low water activity and acid tolerant and able to grow at refrigeration temperatures. Fortunately it is readily eliminated by standard pasteurisation or by cooking so that the threat is limited to certain ready-to-eat foods, typically those with long refrigerated shelf-lives.

L. monocytogenes mainly causes disease by invading gastrointestinal epithelial cells by encouraging endocytosis. Once inside host cells other proteins aid intracellular survival, mobility, and cell-to-cell spread. L. monocytogenes is adept at evading and surviving within cellular and humoral immune systems. A fascinating aspect of L. monocytogenes is its ability to shift from an environmental saprophytic state where virulence genes are turned off to a parasitic state within animal or human hosts. At 37°C, if carbohydrate levels are low, many virulence genes are activated. This switch is mainly controlled by temperature sensitive small RNAs. The ability to respond to stress is also intertwined in this transitive process. The many sequenced genomes of L. monocytogenes are rich in transcriptional regulators controlled in overarching regulons by various “master” regulators, which functionally overlap in a complex network. This network allows L. monocytogenes to rapidly respond to changing environments, including switching on and off stress defence and virulence genes.

Using comprehensive proteomics we are attempting to understand more holistically the mechanistic basis of L. monocytogenes’ adaptation to different situations. State-of the art proteomics is now a very powerful tool and is becoming more cost-effective. Employing gel-free and label-free liquid chromatography (operated in either one or two dimensional modes) and sensitive, high resolution ion trap mass spectrometry, it is possible to take complex protein mixtures digested by a peptidase such as trypsin and identify and quantify peptides en-masse after bioinformatic comparison to proteome databases. This is possible due to better separation of individual proteomes.
peptides and highly accurate mass estimations to error levels of less than 1 part per 2 million. By counting each individual peptide spectrum (a spectral count) one can estimate the protein abundance of most proteins detectable within the proteome of a bacteria. This has major cost and labour advantages over gel-based proteomics. For *L. monocytogenes* which encodes some 2900–3000 proteins such an approach is very efficient with moderate depth peptide surveys able to detect >40% of its proteome. Though some limitations occur with this approach, such as accurately determining the abundance of inefficiently extracted proteins (e.g. proteins with several transmembrane helical domains) it is still readily possible to generate a large amount of data that can be used comparatively to

![Heat map showing comparative abundance ratios of proteins grouped in functional classes for two genome sequenced *L. monocytogenes* strains grown at 37°C and 25°C. The strains FW04/0025 (serotype 1/2a, food isolate) and ScottA (serotype 4b, clinical strain) were grown in brain heart infusion broth and proteins were extracted during exponential (log) and stationary growth phases with ~1200 proteins for each strain identified via LC/tandem MS/MS. Functional groups are based on gene ontology and calculated from accumulated spectral counts. Groups shown in grey did not have enough spectral counts for a calculated log ratio.](image-url)
“dissect” specific genomic functions and phenotypes. Details on the typical LC/MS methodology used has been reviewed\(^1\). A number of software- and statistical approaches for assessing protein abundance via spectral counting have been devised that have improved validation of sample comparisons\(^2,3\) and also have improved absolute protein quantitation in highly complex samples\(^4\).

We have used gel-free proteomics to investigate a variety of stresses and phenotypes of \textit{L. monocytogenes} and \textit{E. coli} O157:H7 strains, including water activity, cold, acid and alkaline stresses all relevant to either food or the food processing environment\(^5,6,7,8\). Essentially any prokaryote or eukaryote for which a proteome could be studied could be used in a similar fashion across a myriad of scenarios as long as the proteins can be obtained in sufficient quantities. As mentioned above \textit{L. monocytogenes} pathobiology is strongly controlled by temperature. As an example, we compared the proteomes of genetically different strains at two different temperature, 25°C and 37°C. Cells at these temperatures behave quite differently. At 25°C cells are actively swimming via peritrichous flagella while at 37°C cells lack flagella, become hydrophobic and better attach to surfaces. As can be seen in the accompanying heat map (Figure 1) when the proteins (covering 40–42% of the strain proteomes) are organized on the basis of their essential cellular function large differences between the temperatures occur, above and beyond growth phase- and strain-dependent changes. One of the obvious hallmarks of the temperature effect at 37°C is the suppression of motility and chemotaxis proteins with the most suppressed protein at 37°C being flagellin (reduced >50-fold). The most induced protein (40–60 fold) in both strains at 37°C is an OsmC family protein similar to the organic hydroperoxide resistance protein OhrA of \textit{Bacillus subtilis}. Organic peroxides are toxic metabolites that accumulate during metabolism\(^9\) thus it makes sense that at a more rapid rate of metabolism enhancement of peroxide detoxification is needed. Several other stress response proteins are also enhanced at 37°C including glutamate dehydrogenases required for survival against acid shocks, such as gastric passage, as well as superoxide dismutase, which protects against reactive oxygen radicals. Such protein changes likely also contribute to gastrointestinal, intracellular and external environmental survival. Hundreds of other protein changes also occur, including that of many involving uncharacterised proteins. Individual protein abundances within a given proteome differ at 4–5 orders of magnitude and, thus, defining and especially interpreting proteomic-level responses consistent at the species level as well as between strains represent substantial challenges to overcome.

In summary, proteomics is rapidly emerging as an accessible approach that can capture large amounts of functionally relevant proteomic, and by inference genomic information. The range of applications within microbiology itself is enormous in terms of understanding bacterial behaviour, physiology and pathogenesis.

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**References**


**Biographies**

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