Abstract

Listeria monocytogenes is a Gram-positive bacterium and a foodborne pathogen, known for sustaining a saprophytic lifestyle in a soil-plant environment as well as in food sources and food-processing environments. However, the ingestion of contaminated food allows the bacterium to infect host cells and become an opportunistic intracellular pathogen, capable of causing disease. Listerial infections are responsible for several cases of meningitis and septicemia in immunocompromised individuals, as well as abortions in pregnant women or birth of severely ill newborns. As a result, listeriosis has an average case-fatality rate of 20–30 % despite adequate treatment. Therefore, *L. monocytogenes* faces a variety of harsh conditions during its life cycle, which promoted the development of several mechanisms that support its growth, survival and dissemination between environments.

In the past decades, regulatory RNAs became the focus of several studies in *L. monocytogenes* and evidence suggests that they are central to environmental adaptation, contributing to the passage from saprophytism to virulence. The existence of multiple classes of regulatory RNAs is acknowledged but the prime focus in the field are the *trans*-encoding antisense RNAs, also known as small RNAs (sRNAs). sRNAs are usually encoded on the genome far from their target genes and act by basepairing with the target transcripts through limited and imperfect complementarity, making them potentially able to interact with multiple target genes. sRNA-mRNA basepairing can either affect positive or negatively the protein levels of the targets by respectively enhancing or preventing translation initiation and/or mRNA stability. A good example of sRNAs with roles in stress responses and virulence in *L. monocytogenes* is the LhrC family. In fact, the LhrCs can play a role in the transmission of this bacterium between environments, as some of the members were shown to be repressed in external environment conditions, whereas in host-relevant conditions, such as blood, intestinal lumen and inside macrophages, specific members of the LhrC family were highly expressed. In particular, LhrC1-5 were shown to contribute to replication of *L. monocytogenes* inside macrophages and to contribute to the adaptation of the pathogen to excess heme, suggesting that these sRNAs may play a role in the initial steps of infection as well as in the later stages when the bacterium reaches the bloodstream and blood-rich organs.

The contribution of LhrC1-5 in the prevention of heme toxicity and their massive induction upon heme stress, together with the fact that LhrC1-5 down-regulate genes encoding for proteins involved in heme uptake and utilization, encouraged the search for the unknown contributors that assist *L. monocytogenes* in dealing with the excess amount of heme it may encounter during the infection process. The results revealed that *L. monocytogenes* transcriptome undergoes severe rearrangements upon exposure to excess heme, which includes the shutting down of genes involved in iron/heme uptake and utilization, while a heme detoxification system (HrtAB) is activated. Another facet in the response of *L. monocytogenes* to heme toxicity is the induction of virulence factors, such as the *Listeria* adhesion protein LAP, suggesting that excess heme is seen by the bacterium as a host-derived signal that requires *Listeria*'s virulence potential to be at its fullest. Altogether, the work presented in this thesis contributed for a better understanding of the physiology of *L. monocytogenes* as well as the regulatory mechanisms and stress responses taking place during infection-relevant conditions. The full disclosure of those mechanisms will have a strong impact on food safety and on the farm-to-fork concept.