Antimicrobial Tolerance in Listeria monocytogenes: Persister cells and the survival of the slowest

There are two ways in which bacteria survive killing by antibiotics. The most well-known, is antibiotic resistance, which results from the acquisition of a resistance gene or mutation that allows bacteria to grow and divide in the presence of antibiotic concentrations that would normally kill other bacteria. Antibiotic tolerance on the other hand, is the ability of bacteria to survive (but not grow) prolonged exposure to concentrations that should normally kill them. The predominant mechanism underlying tolerance is the so-called persister cell, a small subpopulation of dormant like cells that are completely refractory to antibiotics due to the inactivity of cellular processes. Persister cells have been linked to treatment failures in several bacterial infections including Mycobacterium tuberculosis, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.

Preceding the start of this PhD project, Listeria monocytogenes was observed to form these antibiotic tolerant persister cells. L. monocytogenes is a Gram-positive, foodborne pathogen that causes listeriosis, a rare, but often lethal disease, even with antibiotic treatment. It typically affects pregnant women, neonates, the elderly and the immunocompromised, and can cause mortality rates of up to 34%. In addition to the human host, this resilient organism has evolved to persevere in several environments, such as the soil and food processing environments, where it can cope with and even grow at temperatures between -0.4 and 45 °C or a pH range of between 4.6 and 9.6, and survive NaCl concentrations as high as 40%.

Given its resilience, as well as the industrial and clinical implications, we felt that L. monocytogenes would make a good model to study antibiotic tolerance and sought to further investigate persister cells and their underlying mechanisms in this organism. The body of work over the course of this PhD study has been organized into three manuscripts, which are summarized below.

The first manuscript sought to link the Small Colony Variant (SCV) to persister cells in L. monocytogenes. SCV cells of bacteria are a slow growing phenotype that can result from specific defects in the electron transport chain. We speculated that a stable L. monocytogenes heme deficient SCV would show a similar tolerance towards a broad range of antibiotics that persister cells do and found that apart from the macrolide erythromycin, the SCV exhibited increased tolerance towards the reactive oxygen species produced in the macrophage significantly better than the wildtype, both of which suggest that SCV of L. monocytogenes may be clinically relevant.

The two remaining manuscripts investigated the role of chromosomally encoded Toxin/Antitoxin (TA) systems in the ability of L. monocytogenes to tolerate and respond to different stress factors, including antibiotics. TA systems typically consist of a stable toxic protein that acts on some bacterial process, resulting in stasis, and a less stable antitoxin that binds and neutralizes the toxin. In the past few decades they have emerged as one of the primary mechanisms underlying the formation of persister cells.

In L. monocytogenes, the alternative sigma factor sigma B (σB) is responsible for the transcription of many stress and virulence genes, and transcriptomic studies indicate that the mazEF TA system is also part of the σB operon. In the second manuscript, we found that while the mazEF TA locus does not affect the level of persister formation during treatment with antibiotics in lethal doses, it does exert an effect on the expression of σB dependent genes, as well as the growth of this organism under stress.

Lastly, we looked deeper into the genome of L. monocytogenes for additional TA systems and, in addition to the two previously predicted TA systems, detected four loci with type II TA genomic features. However, due to unforeseen challenges we were unable to further characterize them and will employ fluorescence activated cell sorting in the future to both experimentally verify these putative TA systems and elucidate the conditions under which they are induced.

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