Strand specific RNA-sequencing and membrane lipid profiling reveals growth phase-dependent cold stress response mechanisms in Listeria monocytogenes

The human pathogen Listeria monocytogenes continues to pose a challenge in the food industry, where it is known to contaminate ready-to-eat foods and grow during refrigerated storage. Increased knowledge of the cold-stress response of this pathogen will enhance the ability to control it in the food-supply-chain. This study utilized strand-specific RNA sequencing and whole cell fatty acid (FA) profiling to characterize the bacterium’s cold stress response. RNA and FAs were extracted from a cold-tolerant strain at five time points between early lag phase and late stationary-phase, both at 4°C and 20°C. Overall, more genes (1.3×) were suppressed than induced at 4°C. Late stationary-phase cells exhibited the greatest number (n = 1,431) and magnitude (>1,000-fold) of differentially expressed genes (>2-fold, p<0.05) in response to cold. A core set of 22 genes was upregulated at all growth phases, including nine genes required for branched-chain fatty acid (BCFA) synthesis, the osmolyte transporter genes opuCBCD, and the internalin A and D genes. Genes suppressed at 4°C were largely associated with cobalamin (B12) biosynthesis or the production/export of cell wall components. Antisense transcription accounted for up to 1.6% of total mapped reads with higher levels (2.5×) observed at 4°C than 20°C. The greatest number of upregulated antisense transcripts at 4°C occurred in early lag phase, however, at both temperatures, antisense expression levels were highest in late stationary-phase cells. Cold-induced FA membrane changes included a 15% increase in the proportion of BCFA and a 15% transient increase in unsaturated FAs between lag and exponential phase. These increases probably reduced the membrane phase transition temperature until optimal levels of BCFA could be produced. Collectively, this research provides new information regarding cold-induced membrane composition changes in L. monocytogenes, the growth-phase dependency of its cold-stress regulon, and the active roles of antisense transcripts in regulating its cold stress response.

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