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Survival and transcriptional response of Salmonella enterica serovar Infantis isolated from food, veterinary and human sources in Brazil under stress conditions related to food safety and human health

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Salmonella enterica subsp. *enterica* serovar Infantis (*S*. Infantis) is a zoonotic, foodborne, and non-typhoid serovar widely distributed worldwide. Despite the clinical burden and the importance of its presence in the food-production chain, there is a lack of studies aiming to elucidate the survival capacity of *S*. Infantis under challenging survival conditions and its gene expression background. Therefore, this study aimed to analyze the survival and transcriptional response of *S*. Infantis isolated in Brazil against stress conditions. A total of 25 *S*. Infantis strains isolated between 2013 and 2018 from farm and industry settings, animals, animal feed (n=10), food items (n=9) and humans (n=6) were analyzed. An inoculum of approximately $1x10^8$ CFU/ml of each sample was exposed for 10 minutes and 1 hour to acid stress (HCl pH 2.6), oxidative stress (15mM H_2O_2), increased osmolarity (NaCl 9%) and minimum cooking temperatures (63 and 74°C), and for 1 and 24 hours to refrigerating (4°C) and freezing (-20°C) temperatures. Strain 1143/14, isolated from chicken feces, was selected for the transcriptome sequencing and identification of differentially expressed genes (DEGs) under acid, oxidative and increased osmolarity stresses. Under the acid stress, the strains analyzed showed an average survival of $2.8x10^7$ CFU/ml after 10 minutes and $3.7x10^4$ CFU/ml after 1 hour. Under the oxidative stress, the strains showed an average survival of $3.3x10^7$ CFU/ml after 10 minutes and $2.6x10^8$ CFU/ml after 1 hour. Under the increased osmolarity, the strains showed an average survival of $2.0x10^9$ CFU/ml after 10 minutes and 1 hour. At 4°C, the strains showed an average survival of $1.3x10^8$ CFU/ml after 1 or 24 hours. At -20°C, the strains showed an average survival of $3.7x10^7$ CFU/ml after 1 hour and $5.0x10^3$ CFU/ml after 24 hours. No strains survived to the minimum cooking temperatures tested. While no DEGs were found for the increased osmolarity, acid stress transcriptomes showed a total of 14 up-regulated and 38 down-regulated DEGs, and oxidative stress transcriptomes showed 407 up-regulated and 143 down-regulated DEGs. Identified DEGs were associated to biological processes of regulation, localization, locomotion, response to stimulus, and cellular, metabolic and homeostatic processes. In conclusion, *S*. Infantis from Brazil are able to successfully survive against most stress conditions, representing a challenge for its control in a food safety context, and the prevention of diseases in humans. The successful efficacy of minimum cooking temperatures observed for all strains reinforce the importance of heating to control *Salmonella* in food items. Moreover, the survival to acid and oxidative stresses are mediated by the expression of genes promoting the adaptation to these hazardous conditions. These preliminary results will be important for the future evaluation of the survival of these *S*. Infantis strains from Brazil in food, such as meat.

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