TITLE: DIFFERENTIAL PROTEIN EXPRESSION OF *SALMONELLA ENTERICA* SEROVAR TYPHIMURIUM BIOFILMS EXPOSED TO CARVACROL

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ABSTRACT:

Salmonella spp. is a one of the main causes of foodborne illness, causing thousands of cases of salmonellosis in the world. The ability this bacterium to form biofilms on different surfaces is an important factor to its resistance and persistence in different environments. Different strategies have been proposed to control biofilms based in their biological and physics characteristics. Many studies demonstrated the action of carvacrol against *Salmonella* spp biofilm. This study evaluated the differences in protein expression of biofilm of *Salmonella* Typhimurium ATCC 14028 on polypropylene (PP) with and without treatment with of carvacrol. The overnight culture of *S*. Typhimurium was diluted in Trypic Soy Broth (TSB) to yield 10^7 CFU mL^{-1} and placed in plates containing PP coupons, were incubated for 48 h at 35 ºC. After incubation, *S*. Typhimurium biofilm formed on PP was treated with 2 x CIM (624 µg/mL) of carvacrol for 1 hour. Thereafter, cells were recovered from coupons using a cell scrapper and ultrasonic bath (25 kHz for 10 min), centrifuged at 4500 x *g* for 5 min, washed with saline solution and pellet was used for protein extraction. Lysis buffer and sonication were used for protein extraction and total protein quantification was verified according to the Bradford method. Four Hundred µg/mL was separated in first dimension using Immobiline DryStrip gels (13cm and pH gradient 4-7), and rehydration and isoelectric focusing using isoeletric focusing system. Second dimensional separation was performed in SDS-PAGE gel (12.5% acrylamide/Bis-Acrylamide). After electrophoresis, proteins were stained overnight with Coomassie Blue G-250, gels were digitalized and analyzed using ImageMaster software. The proteins resulting of peptide digestion were separated by C18 (RP-nanoUPLC) coupled with a Q-Tof Premier mass spectrometer and peptide mass fingerprint data were searched using Mascot. The treatment with 2 x MIC of carvacrol changed the expression of 49 proteins, being that 40 were downregulated and 9 were upregulated after treatment with carvacrol. In general, the proteins were classified in to carbon metabolism, protein metabolism, transmembrane transport, oxidative stress, nitrogen metabolism, metabolism of nucleotides, amino acid metabolism and quorum sensing. Our results suggest that the treatment with carvacrol modify the global metabolism of *S.* Typhimrium biofilm, however more detailed analyzes should be performed to better study these changes.

Keywords: biofilm, carvacrol, proteomic, *Salmonella* Typhimurium,

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