

EVALUATION OF THE EXPRESSION OF VIRULENCE GENES IN *Aeromonas* spp. SUBJECTED TO DIFFERENT ENVIRONMENTAL CONDITIONS

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Abstract

Aeromonas spp is an opportunistic pathogen with multifactorial virulence, and the presence of virulence genes regulates the formation of intrinsic and extrinsic factors which increase the pathogenic potential of this bacteria. The objectives of this study were to evaluate the expression of virulence genes in *Aeromonas* spp. under different environmental conditions. In the research were used six isolates of *Aeromonas* spp., subjected to pH 7.0 and 10 temperatures of 28°C and 31°C and ammonia concentration of 0.1 and 0.9 mg/L in independent experiments. These environmental conditions were obtained in Trypticase Soy Broth medium modified. After 24 hours of incubation, the RNA was extracted from the isolates (Rneasy Qiagen® e Dnase Qiagen®), followed by cDNA synthesis (QuantiTect Reverse Transcription (Qiagen®)). It performed a quantitative analysis of aerolysin, lipase and *fla* genes by RT-qPCR. The sigma-70 from RNA polymerase (*rpoB*), 16S rRNA and DNA gyrase (*GyrA*) genes were tested as housekeeping. The 16S rRNA gene was the most stable and therefore was chosen as housekeeping. The qPCR analysis of the *fla* gene reveals that different tested ammonia concentrations (0.9 and 0.1 mg/L) influenced the expression of this gene ($p \leq 0.05$). The *fla* gene demonstrated to be more expressed in the group subjected to condition of 0.9 mg/L of ammonia in relation to the group of 0.1 mg/L and this gene is associated with biofilm formation by bacteria. The expression of aerolysin gene was not influenced by environmental factors and the lipase gene was shown to be weakly expressed *in vitro*. Increased expression of *fla* gene depending of the ammonia concentration in the culture medium demonstrates that bacteria may be protected in the biofilm structure, able to attack the host.

Keywords: Bacteria, biofilm, *fla*, housekeeping, RT-qPCR

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