Listeria spp IDENTIFICATION by PCR

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The genus *Listeria* spp. comprises fifteen species and six subspecies. Among these, *L. monocytogenes, L. ivanovii* and *L. seeligeri* can be associated with diseases in humans, and, specifically, *L. monocytogenes* can cause severe food-borne illness, where the manifestation can vary from a simple cold to meningitis, encephalitis, septicemia and abortion. The detection of Listeria *spp* is usually performed based on classical culture and biochemical identification, methods which are laborious and slow , requiring around ten days to complete, of which approximately two days are intended for selective enrichment only. Targeting a specific and quick test as a screening to replace this step, the Polymerase Chain Reaction (PCR) technique has proven efficient, reducing the assay time and cost of analysis. The present study aimed to perform a PCR standardization for the rapid and specific identification of *Listeria* spp. Primers designed for the *16S-23S* region, previously described in the literature, IGS 1 (5' -GGCCTATAGCTCAGCTGGTTA-3') and IGS 2 (5' -GCTGAGCTAAGGCCCCGTAAA-3'), were selected and used for the internal standardization of optimal *Listeria* spp.-specific PCR conditions. To optimize the amplification conditions, reference and environmental strains of *Listeria monocytogenes (*ATCC *19117)*, *Listeria inoccua*, *Escherichia coli (ATCC* 43895), *Staphylococcus aureus* and *Salmonella thyphi (*ATCC *10749)* after growth were subjected to DNA extraction with the aid of a commercial kit Blood and Tissue (Qiagen®) and quantification by fluorimetry using Qubit (Invitrogen). The conditions for the amplification of a 200 bp fragment were established and the analytical specificity and sensitivity were determined. The PCR was standardized using 66 ng target DNA, 100 mM dNTPs, 100 mM of each *primer*, 2 mM $MgCl_2$ and 1U Taq DNA polymerase. The amplification cycle adopted the following parameters: initial denaturation 95 ° C for 10 min, 30 cycles of 95 ° C for 1 min, 61 ° C for 1 min, 72 ° C for 1 min and final extension at 72 ° C for 7 min. The resulting PCR products were analyzed by gel electrophoresis on 1.5% ultrapure agarose gels stained red. The PCR reaction showed sensitivity and specificity of 100% for the tested conditions, detecting only members of the *Listeria* spp. genus. This PCR assay is efficient, therefore, in reducing the assay time and cost analysis, and could be used as a screening step to identify suspected *Listeria* spp. colonies.

Keywords: listeriosis, 16S-23S region, molecular diagnostics

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