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Standardization of A Polymerase Chain Reaction for the Identification of Salmonella Species

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Resumo

Introduction: Salmonella spp. is often associated with food borne outbreaks in several countries around the world, causing serious public health problems. The diagnosis of this species is usually performed by classical bacteriological cultures and serological testing, which are laborious and time-consuming methods. Aiming at an effective and rapid diagnosis, molecular techniques, such as PCR, allow for the rapid and specific detection of the pathogen. In this context, the present study aimed to perform a PCR standardization for the rapid and specific identification of Salmonella spp.. Methods: Primers designed for hilA gene, previously described in the literature, hilA2-F (5'-CTGCCGCAGTGTTAAGGATA-3') and hilA2-R (5'-CTGTCGCCTTAATCGCATGT-3') were selected and used for the internal standardization of optimal Salmonella spp.-specific PCR conditions. Thermal lysis was used to extract DNA from several Salmonella strains, used as positive reference controls, from Escherichia coli, Staphylococcus aureus, Enterobacter aerogenes, Klebsiella pneumoniae, used as negative controls, and from 16 environmental Salmonella spp. strains. The amplification conditions were adjusted in order to obtain the specific amplification of the fragment (496 bp) from the smallest possible amount of DNA. The resulting PCR products were

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analyzed by electrophoresis on ultrapure 1.5% agarose gels stained with ethidium bromide (10 mg mL-1). Results and Discussion: The optimum conditions determined in this study were: initial denaturation at 94°C for 5 min followed by 30 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1 min and a final extension at 72°C for 10 min, using a mixture of 5 μL reaction buffer (Invitrogen), 0.2 mM dNTP (Fermentas), 1.5 U recombinant Taq polymerase (Taq Platinum® - Invitrogen), 5 mM MgCl2 (Invitrogen) and 25 pmol of each primer (Invitrogen). The PCR amplified the expected fragment (496 bp) in these conditions only for the positive control and the previously identified environmental Salmonella spp. strains, showing 100% sensitivity and specificity and a limit of detection of 50 ng target DNA. Conclusion: It was possible to identify isolated Salmonella spp. colonies with 100% sensitivity and specificity using PCR with the optimum conditions standardized in the present study, reducing pathogen detection time to 5 hours.

Palavras-Chave: Salmonella , IDENTIFICATION , STANDARDIZATION , PCR

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