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Development and validation of ready-to-use PCR kit for *Aeromonas hydrophila* strains*

Alper Ciftci[1], **Ertan Emek Onuk**[1], **Arzu Findik**[1], **Mehtap Unlu Sogut**[1], **Fikri Balta**[2] and **Gulay Ciftci**[1]
[1]Ondokuz Mayis University, Turkey
[2]Recep Tayyip Erdogan University, Turkey

Statement of the Problem: *Aeromonas hydrophila* is known to be the causative agent of bacterial hemorrhagic septicemia in fresh water fish. Zoonotic properties of *A.hydrophila* increase the importance of infection in terms of public health. The aim of this study was to develop the ready-to-use-PCR-kit and evaluate the stability of kit for *A. hydrophila*.

Methodology & Theoretical Orientation: The oligonucleotide primers which targeted *phospholipase* C gene were designated with using PUBMED-Blast. The optimization of PCR was conducted through combination of reactives. After optimization, the most suitable PCR conditions were performed for specifity. For this aim, related and unrelated bacterial strains were used. The sensitivity of PCR was investigated by the dilutions of DNAs of *A.hydrophila* strain. For the determination of stability of optimized PCR, 52 PCR mixtures without template DNA were lyophilized. These mixtures were diluted every week and *A.hydrophila* template DNA was added to the mixture. The mixtures were amplified with optimized PCR conditions.

Findings: The optimized PCR conditions with using designated primers were gave specific bands of 303 bp for *A.hydrophila*. When used, related and unrelated bacterial strains except for *A.hydrophila*, no band were shown in the PCR. These results showed us that the designated primers with optimized conditions were specific for *A.hydrophila*. The sensitivity of the PCR was determined as 70 pg of DNA. This concentration was used to determine the stability of lyophilized PCR mixtures. During 22 weeks period, the PCR gave positive band of 303 bp for *A.hydrophila*. After 23 weeks, no bands were shown.

Conclusion & Significance: For the development of ready-to-use-PCR-kit, the oligonucleotide primers were designated. After optimization, the kit gave results up to 22 months, that the primer used in this study was only *A.hydrophila* specific and was able to detect 70 pg of DNA.

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Biography

Alper Ciftci is an expert in Molecular microbiology and vaccine development. He finished his PhD at Ankara University, and now he works as associate professor at Ondokuz Mayis University, Samsun, Turkey. He focuses on working development and validation of commercial products such as vaccine and diagnostic kits.

aciftci@omu.edu.tr

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