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Optimizing a strategy for molecular identification of species from Mycobacterium avium-intracellulare complex

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The study was conducted to optimize a flow and techniques for rapid molecular identification of Mycobacterium avium-intracellulare complex (MAC) organisms. The PCR was optimized using two primer sets (MAChsp65 and Groel-2) which amplify 1621and 1564 base pair (bp) fragments of the heat shock protein 65 (hsp65) gene respectively. After conducting the gradient temperature for MAChsp65 primers, the first attempt of optimization at an initial denaturation temperature of 95°C was successful for M. tuberculosis but no amplification of 1621 bp was detected from Deoxyribonucleic acid (DNA) of MAC. Following this initial failure, Groel-2 primers were designed to amplify 1564 bp fragment of the hsp65. The gradient PCR which was conducted by Groel-2 primers for positive control resulted in very week DNA amplification. Buffer and MgCl2 alteration did not significantly improve the PCR product. In both primer sets increasing the initial denaturation temperature from 950C to 960C, adding acetamide to the PCR master mix and raising Taq DNA polymerase by 50% resulted in DNA amplification. The optimized annealing temperatures in this study were 54.2 and 58.5°C for MAChsp65 and Groel-2 primers respectively. 5µl of 50% acetamide showed better amplification than that without acetamide, but abundance of acetamide (10 µl) inhibited amplification. Sequencing of hsp65 by the two sets of primers showed concordant result between 16S and hsp65 analysis for 9 M. avium strains (with further subspecies identification into 8 M. avium subsp. avium and 1 M. avium subsp. hominisuis), 2 M. intracellualare strains and 2 discordant results identified as M. avium by one method and as M. intracellulare by the other. Generally four different hsp65 sequevars were identified among the 9 M. avium isolates tested by the MAChsp65 primers and six other hsp65 sequevars among the 7 MAC tested by Groel-2: two for M. intracellulare strains and four for M. avium strains. Therefore, hsp65 sequencing has a higher discriminatory power to resolve MAC strains to sub species levels.

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