Title : Development of a Multiplex PCR for Detection of Pathogenic Flavobacteria Causing Columnaris Disease in Tilapia and Asian Seabass

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ABSTRACT

Tilapia and Asian seabass have been considered as two important fish species for the economy of Thailand. However, the aquaculture of these species is frequently threatened by an infection of *Flavobacterium* spp. which are primary causative pathogens of columnaris disease. The disease outbreak can reduce production yields and cause significant economic losses. Therefore, accurate and rapid diagnostic techniques for early identification of bacterial infection is of great importance. This study aimed to develop a multiplex PCR technique for simultaneous identification of four bacterial species causing columnaris disease, including *Flavobacterium covae*, *F. davisii*, *F. oreochromis*, and *F. columnare*, in tilapia and Asian seabass. Four primer pairs were used in PCR experiments. The FC-aroB, FD-hemW, and FO-E1 primer pairs were specifically designed for *F. covae*, *F. davisii*, and *F. oreochromis*, respectively, whereas the Fcol-cslA primers were obtained from the previous work and used to identify all four species that had previously been classified into *F. columnare* group. Using the singleplex PCR, the optimal PCR condition included the annealing temperature at 54 °C, and the concentrations of FC-aroB, FD-hemW, FO-E1, and Fcol-cslA primers at 0.3, 0.2, 0.2, and 0.2 μM, respectively. However, the annealing temperature for the multiplex PCR assay was optimized at 55 °C with the same primer concentrations as used in the singleplex PCR. The developed multiplex PCR exhibited high specificity for the target DNA of all four flavobacteria, without non-specific amplification in other bacterial species tested. The multiplex PCR was highly sensitive with a detection limit of 2

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ng/µL for *F. covae*, 400 pg/µL for *F. oreochromis*, 80 pg/µL for *F. davisii*, and 16 pg/µL for *F. columnare*. After challenged with 25 bacterial DNA samples collected from local farms with the history of the disease outbreak, the multiplex PCR revealed 12 positive samples for *F. oreochromis*, 7 positive samples for *F. columnare*, and 6 positive samples for *F. covae*. However, no samples tested positive for *F. davisii*. These obtained data aligned well with the results from *16S rRNA* gene sequencing analysis. We anticipate that this multiplex PCR technique could be a useful and effective tool for controlling the outbreak of columnaris disease in tilapia and Asian seabass farming, which perfectly supports aquaculture industry of Thailand.

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