

Rapid Detection of *Flavobacterium psychrophilum* Using Fluorescent Magnetic Beads and Flow Cytometry

Kyoko Hibi, Yasutoshi Yoshiura¹, Hideki Ushio,
Huifeng Ren and Hideaki Endo*

Faculty of Marine Science, Tokyo University of Marine Science & Technology,
4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

¹Aquatic Animal Health Division, National Research Institute of Aquaculture,
Fisheries Research Agency, 224-1 Hiruta, Tamaki, Mie 519-0423, Japan

(Received July 25, 2011; accepted February 9, 2012)

Key words: fluorescent magnetic beads, immunomagnetic separation, fish disease, *Flavobacterium psychrophilum*, harmful bacteria, detection

Flavobacterium psychrophilum has emerged as one of the most significant bacterial pathogens in salmonid aquaculture worldwide. We have been studying the detection of harmful bacteria using immunomagnetic separation and flow cytometry (FCM). In this study, we used fluorescent magnetic beads and 5-cyano-2,3-ditolyl tetrazolium chloride (CTC). Bacteria were specifically collected using fluorescent magnetic beads with only one antigen-antibody reaction. CTC turns into a red fluorescent formazan that is detectable by FCM. *F. psychrophilum* cells were stained with CTC and labeled with fluorescent magnetic beads. Double-stained bacteria (red fluorescence by CTC and green fluorescence from fluorescent magnetic beads) were detected by FCM. Bacterial cell numbers were determined by FCM and compared with those measured by a traditional colony counting method in the range of 10^2 – 10^8 cells/ml. The FCM assay could provide a bacterial cell count within 1 min and the total assay time, including sample preparation, was less than 3 h.

*Corresponding author: e-mail: endo@kaiyodai.ac.jp