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Luminescence-Based Acute Toxicity Bioassay for Heavy Metals Using Insoluble-Fibroin-Film-Immobilized *Vibrio fischeri*

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Luminescent organisms have been widely used to develop toxicity tests. However, bioluminescence-based methods are often nonspecific and unsuitable for storage. In this study, the marine luminescent bacterium *Vibrio fischeri* was grown in various media. After immobilization on insoluble fibroin film (IFF)-coated tubes, the luminescence intensity was measured. Late-log cultures grown in nutrient broth containing 3 or 5% NaCl were immobilized on IFF-coated tubes at high cell density and found to exhibit higher luminescence intensities. Seawater medium cultures in the stationary phase were also effectively immobilized on IFF-coated tubes and emitted high luminescence. Specifically, IFF-immobilized *V. fischeri* cultures in seawater medium maintained their high luminescence emission for 30 days when stored at –70°C. In addition, a bioluminescence toxicity assay with various heavy metal solutions was carried out. Almost all the heavy metals tested decreased the luminescence of IFF-immobilized *V. fischeri*. However, the concentrations required for the effective attenuation of the luminescence were relatively high. In contrast, mercury (Hg(II)) rapidly dampened the luminescence intensity within a very narrow concentration range. Our findings on the emission of high-luminescence signals by IFF-immobilized *V. fischeri* provide a specific and efficient tool to detect low concentrations of Hg(II) compounds.

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