

Isolation of VNC bacteria by the medium-dilution method

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The concept of 'viable but nonculturable (VNC)' has been developed for the two decades and recognized among many bacteria including environmental microb and human pathogens. Therefore, culture-independent techniques, such as fluorescence *n situ* hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE), are required for the detection of bacteria in the environment and foods. In this study, for detection of VNC bacteria, we tried to establish the medium-dilution method, as follows;

- 1) The confirmation of the existence of the bacteria is carried out from the turbidity of the medium in the culture,
- 2) The isolation of the bacteria is carried out using the micro-fraction that the diluted sample is divided with a micro-plate.

The viable count in the sample was estimated both by the medium-dilution method and by colony-counting method. In a seawater sample, the viable count estimated by the medium-dilution method was 10 times as large as that of colony-counting method. Therefore, the seawater sample seemed to contain VNC bacteria. In Taina pickles sample, the viable counts estimated by both methods were almost equal.

From these results, the medium-dilution method was found to be useful for the isolation of VNC bacteria from the environment and foods.