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Isolation and characterization of VNC bacteria from food samples under high salt conditions

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Summary

Significant populations of viable but nonculturable (VNC) bacteria have reportedly existed in a number of natural environments, as well as foods. On the aspect of sanitary control in food industry, urgent attention should be paid on the fraction of VNC bacteria, which can grow under certain environmental conditions. We have previously constructed the medium-dilution method for enumeration and isolation of the in-liquid culturable (ILC) fraction of VNC bacteria using micro-plates. In the present study, we applied this method on a number of food samples as well as seawater samples to enumerate the population of ILC bacteria. By comparison with the estimated number by conventional colony-counting methods, we attempted to estimate the population of the ILC fraction of VNC bacteria in the food and seawater samples.

Thirty-five marine products, 26 salt-processed foods, 6 vegetables and 7 seawater samples were chosen for the samples. In most marine products, salt-processed foods and seawater samples, the cell numbers estimated by agar-plate cultivation using 1/5 strength ZoBell 2216E medium were approximately ten fold larger than those estimated by cultivation using Nissui TC medium. In contrast, approximately same cell numbers were estimated in vegetable samples by these two cultivation assay. These results suggest that ZoBell medium, designated for marine bacteria, was suitable for cultivation of bacteria predominant in marine products, salt-processed foods and seawater samples. The estimated cell numbers in liquid cultivation by medium-dilution method were almost same value with the cell numbers estimated by agar-plate cultivation in most food samples. In surface sea water samples, however, the cell numbers estimated by the medium-dilution method were approximately ten fold larger than those estimated by agar-plate cultivation. This result indicated that significant number of the ILC fraction of VNC bacteria in the sea water samples could be detected and cultivated using the medium-dilution method with ZoBell medium.

Total 10 bacterial isolates were obtained from a surface sea water sample by the medium-dilution method. One isolate, No. 5, showed growth ability in liquid medium, as well as agar plate. However, the other 9 isolates showed poor growth ability on agar plate, although they showed growth ability in liquid medium. These results suggested that these 9 isolates were ILC fraction of VNC bacteria. Phylogenetic analysis based on partial nucleotide sequence of 16S rRNA genes revealed that these 10 isolates were affiliated with the genus *Delftia* and were closely related to *Delftia tsuruhatensis* with 98 to 99% sequence identities. This finding suggested that even strains phylogenetically identical could show different phenotypes in the culturability on agar plate. Isolates No. 5, 8 and 21 were cultivated on agar plate after liquid cultivation with a high concentration of NaCl or at a low temperature. Isolate No. 5 could grow on agar plate after cultivation under these stress conditions. However, isolate No. 21 showed significantly limited growth on agar plate after liquid cultivation under a high concentration of NaCl and at a low temperature conditions. These results suggested that liquid cultivation with a high NaCl and/or a low temperature could promote the bacterial VNC state, at least for isolate No. 21.