Evaluation of different procedures for the optimized detection of Vibrio parahaemolyticus in mussels and environmental samples

Detection and isolation of V. parahaemolyticus from natural sources is often problematical because of limitations in the analytical procedures. We evaluated a combination of conventional and molecular protocols previously described for the investigation of V. parahaemolyticus, with the aim of identifying the best procedures for improved detection of this organism in environmental matrices. A total of 259 samples of zooplankton (103), mussels (48) and seawater (108) were investigated by an Absence-Presence method (A/P), whereas 118 samples of zooplankton (70) and mussels (48) were analyzed by the Most Probable Number (MPN) method. All samples were processed by a two-step enrichment procedure, firstly with APW broth and then with SPB as selective secondary broth. Detection of V. parahaemolyticus was by direct-PCR and by plate culture on TCBS and CHROMagar Vibrio, after sample enrichment in APW and SPB. With the A/P method, V. parahaemolyticus was detected in 23.6% samples by direct-PCR, whereas only 11.2% samples were positive with the plate culture method. With the MPN method, V. parahaemolyticus was detected in 54.2% and 27.1% of the samples by direct-PCR and plate culture respectively; this indicated the existence of 31% false negative results with the A/P method. No significant differences between the use of a single (APW) or two-step enrichment (APW+SPB) were observed by direct-PCR with A/P or MPN, although a significant higher presence of V. parahaemolyticus was detected by plate culture in both protocols with the two-step enrichment procedure. In conclusion, direct-PCR after sample enrichment in APW broth was the most successful method for detection of V. parahaemolyticus with the A/P procedure and enumeration by MPN. Better detection was obtained with MPN than with the A/P protocol. Conversely, the plate culture procedure showed better results with the two-step enrichment protocol in which CHROMagar Vibrio was used as the selective agar.

Résumé / Abstract
Vibrio parahaemolyticus is a marine bacterium with a worldwide distribution and is frequently associated with human outbreaks of infection. Detection and isolation of V. parahaemolyticus from natural sources is often problematical because of limitations in the analytical procedures. We evaluated a combination of conventional and molecular protocols previously described for the investigation of V. parahaemolyticus, with the aim of identifying the best procedures for improved detection of this organism in environmental matrices. A total of 259 samples of zooplankton (103), mussels (48) and seawater (108) were investigated by an Absence-Presence method (A/P), whereas 118 samples of zooplankton (70) and mussels (48) were analyzed by the Most Probable Number (MPN) method. All samples were processed by a two-step enrichment procedure, firstly with APW broth and then with SPB as selective secondary broth. Detection of V. parahaemolyticus was by direct-PCR and by plate culture on TCBS and CHROMagar Vibrio, after sample enrichment in APW and SPB. With the A/P method, V. parahaemolyticus was detected in 23.6% samples by direct-PCR, whereas only 11.2% samples were positive with the plate culture method. With the MPN method, V. parahaemolyticus was detected in 54.2% and 27.1% of the samples by direct-PCR and plate culture respectively; this indicated the existence of 31% false negative results with the A/P method. No significant differences between the use of a single (APW) or two-step enrichment (APW+SPB) were observed by direct-PCR with A/P or MPN, although a significant higher presence of V. parahaemolyticus was detected by plate culture in both protocols with the two-step enrichment procedure. In conclusion, direct-PCR after sample enrichment in APW broth was the most successful method for detection of V. parahaemolyticus with the A/P procedure and enumeration by MPN. Better detection was obtained with MPN than with the A/P protocol. Conversely, the plate culture procedure showed better results with the two-step enrichment protocol in which CHROMagar Vibrio was used as the selective agar.

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