

Title Recovery and detection of *Vibrio vulnificus* during cold storage
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Abstract

Different cultural techniques and molecular methods for the detection of *Vibrio vulnificus* during cold storage in a model broth system were compared. Two strains of *V. vulnificus* were grown to stationary phase and inoculated (10^6 CFU/mL) into tryptic soy broth with 2% sodium chloride (TSBN₂) or artificial seawater (ASW), both pre-chilled to 5 °C. These were stored for 10 days, with sub-sampling conducted at time 0 and every 2 days thereafter. Each subsample was plated, by both pour and spread plate techniques, onto tryptic soy agar 2% sodium chloride (TSAN₂) with or without catalase (400 or 600 U) or sodium pyruvate (80 or 160 mg) supplementation. Nucleic acids were extracted from subsamples and subjected to PCR and RT-PCR with hemolysin as the target. Higher recoveries of *V. vulnificus* were obtained with spread plating compared to pour plating ($P<0.05$). The addition of sodium pyruvate (80 mg) or catalase (400 U) significantly increased cell recovery ($P<0.05$). PCR amplification signals were stronger than RT-PCR signals at each timepoint, and results were generally consistent between TSAN₂ and ASW for each strain. These results will aid in the design of optimum methods to recover and/or detect *V. vulnificus* cells subjected to sublethal stress that might be encountered in food processing and storage.