Validation of a *Vibrio parahaemolyticus* predictive model in different species of Australian oysters

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Introduction

Recent data from the US Centers for Disease Control & Prevention indicate that humans infections caused by *Vibrio* spp. have increased in relation to other food borne pathogens (CDC, 2010). Climate change presents a compounding factor with the potential to affect exposure levels and alter geographical distribution (Marques et al., 2010; Martinez-Urtaza et al., 2010). Of all pathogenic *Vibrio* spp., *Vibrio parahaemolyticus* presents a significant hazard in shellfish when post-harvest temperatures are not properly controlled in supply chains. Predictive models can be effective risk management tools; however they have not been adequately developed and validated for *V. parahaemolyticus* in Pacific and Sydney Rock Oysters.

Materials and Methods

Storage trials

Sydney Rock (*Saccostrea glomerate*) and Pacific (*Crassostrea gigas*) Oysters were harvested from commercial growers in New South Wales, Australia in summer 2009 and 2010. Sydney Rock Oysters were stored at 15, 18, 22, 24 and 28°C; and Pacific Oysters at 15, 18, 23, 24 and 28°C.

V. parahaemolyticus enumeration

At selected time intervals, *V. parahaemolyticus* was enumerated using a 3-tube Most Probable Number (MPN) technique (FDA, 2004) with slight modification. Ten oysters were opened aseptically, meat and liquor placed in a filter stomacher bag with an equal weight of Peptone Salt Solution (PSS; 0.1% bacteriological peptone, 3% NaCl, pH 7.4), and stomached at high setting for 2 min. A 20-g sample of the homogenate was mixed with 80 g of modified Alkaline Peptone Water (mAPW; 1% bacteriological peptone, 3% NaCl, pH 8.4) and diluted in 10-fold increments with PSS. Ten and one milliliter from the initial dilution and 1ml of all other dilution were individually added to three tubes containing 10 ml mAPW (modified to 3%NaCl instead of 1%NaCl). Inoculated mAPW tubes were incubated at 37°C for 16-18 h. A 200 µl sample of each turbid broth was transferred to a sterile 96-well plate and stored at -20°C until PCR assay. *Vibrio parahaemolyticus* was identified using *tlh* primers (Nordstrom et al., 2007). The presence-absence of positive bands in the agarose gel were evaluated and total populations of *V. parahaemolyticus* determined using a MPN table.

Data analysis

For each storage experiment, a *V. parahaemolyticus* viability profile was obtained by plotting storage time (hours) versus $\log_{10}$ MPN/g. DMFit software was used to estimate growth rate ($\log_{10}$ MPN/h) using the Baranyi growth model (Baranyi and Roberts, 1994). Estimated growth rates were transformed to specific growth rate (SGR, ln MPN/h).

Results and Discussion

A model was previously produced for the viability of injected *V. parahaemolyticus* in Pacific Oysters (Fernandez-Piquer et al., 2009). To evaluate its use for commercial supply chains, it was necessary to validate predicted growth rates in oysters containing natural *V. parahaemolyticus*. In addition, the model was tested in both Sydney Rock and Pacific Oysters.

Five different viability profiles were obtained for each oyster species in storage experiments. When Pacific Oysters were tested, *V. parahaemolyticus* grew in oysters stored at 28 and 23°C, at growth rates of 0.205 and 0.036 log MPN/h, respectively. Populations at the last time point tested were found to be 4.5 and 4 $\log_{10}$ MPN/g at 23 and 28°C, respectively. A growth rate of 0.17 $\log_{10}$CFU/h was observed in American Oysters (*Crassostrea virginica*) at 26°C, with an associated higher maximum density of 5.8 $\log_{10}$ CFU/g (Gooch et al., 2002). Pacific Oysters stored at 15, 18 and 24°C did not show a significant increase in *V. parahaemolyticus* levels. At these temperatures, bacterial levels ranged from 2.5 to 4 $\log_{10}$ MPN/g during all storage experiments.

Interestingly, while counts were variable, there was no evidence of consistent growth of *V. parahaemolyticus* in Sydney Rock Oysters at any tested storage temperatures (15, 18, 22, 24 and 28°C). The highest bacterial level tested during storage trials was 4.5 $\log_{10}$ MPN/g. These results are in agreement with previous studies done for Sydney Rock Oysters, in which relatively small changes in *V. parahaemolyticus* levels at 15°C over 14 days, and at 30°C for 7 days; and counts no higher than 4 $\log_{10}$ MPN/g were observed (Eyles et al., 1985). This result indicates that different risk management practices might be considered for Sydney Rock and Pacific Oysters.

Validation data and the model predictions were plotted from 15-30°C (Figure 1). There was good agreement for *V. parahaemolyticus* growth rates in PO stored at 15 and 28°C. However, slower growth was observed at 18, 23 and 24°C than predicted by the model. This observation might result from different defense systems in these oyster species.

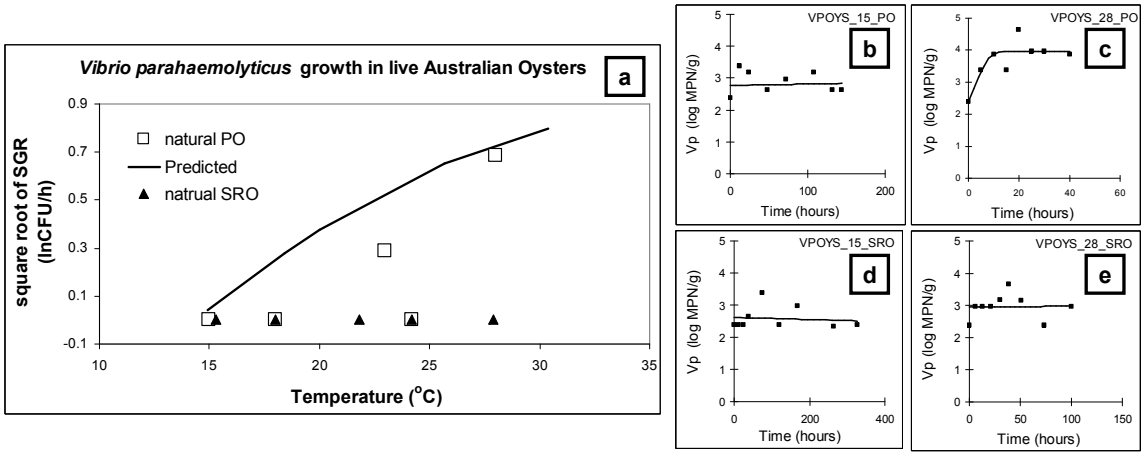

Figure 1. Model and validation data plot (a) and examples for *V. parahaemolyticus* viability profiles for Pacific (b, c) and Sydney Rock Oysters (d, e) at 15°C and 28°C.

Conclusions

The predictive model for *V. parahaemolyticus* in Pacific Oysters was 'fail-safe' for natural *V. parahaemolyticus* in Sydney Rock and Pacific Oysters, and can be used as a tool to design storage conditions in Oyster supply chains. Future research should consider potential variability among *V. parahaemolyticus* strains (pathogenic versus non-pathogenic).

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