

Randomly amplified polymorphic DNA (RAPD) method for differentiating clinical and environmental isolates of *Vibrio parahaemolyticus*

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Abstract

Vibrio parahaemolyticus is a major human pathogen associated with consumption of fish and shellfish causing acute diarrhoea and vomiting within a few hours. However, it is likely that most *V. parahaemolyticus* isolates from the marine environment are harmless. Clinical isolates are difficult to differentiate from the majority of avirulent environmental isolates. The aim of this study is to develop a quick test to distinguish virulent isolates from avirulent isolates. Randomly amplified polymorphic DNA (RAPD) PCR has been performed on a collection of 24 clinical and 31 environmental *V. parahaemolyticus* strains isolated worldwide. Two clinical isolates C15 and C8 and two environmental isolates E22 and E2 were screened with 9 single primers OPK1-7, OPK12 and OPK18. The use of single primers failed to differentiate clinical and avirulent environmental isolates of *V. parahaemolyticus*. However, the use of two-primer RAPD is more promising. Out of the 29 different primer combinations, primers OPK13 (5'-d[GGTTGTACCC]-3'+OPK15 (5'-d[CTCCTGCCAA]-3' was most likely to produce a unique 300 base pairs band in 17 out of 24 (75%) clinical and 6 out of 31 (19.4%) environmental isolates of *V. parahaemolyticus*. DNA fingerprints of isolates were compared by visual inspection of band patterns. This work describes the use of combined RAPD primers that has been shown to produce unique bands and more complex banding patterns. The results of this study suggest that RAPD may be able to differentiate between most clinical and environmental isolates of *V. parahaemolyticus* which in turn could be used to distinguish virulent from avirulent isolates and developed into a quick test.