Randomly amplified polymorphic DNA studies to evaluate discrimination between virulent and avirulent isolates of *Vibrio parahaemolyticus*

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Introduction

Vibrio parahaemolyticus is the most common cause of seafood related gastroenteritis in South East Asia but not all strains of this species can be considered truly pathogenic. Three serotypes, O3:K6, O4:K68 and O1:K untypeable are responsible for a pandemic *V. parahaemolyticus* infection. Strains belonging to the O3:K6 clone have been responsible for large oyster associated outbreaks in the United States but have not been identified from either seafood or clinical cases in Europe.

Most virulent isolates from clinical cases express *tdh* and *trh* genes, the genes responsible for thermostable direct haemolysin (TDH) and TDH-related haemolysin production. In addition, the presence of a type III secretion system has recently been implicated in the pathogenicity of *V. parahaemolyticus*[1]. A significant proportion of isolates from seafood do not exhibit or express these genes and consequently the significance of strains isolated from the environment is not clear. This has necessitated the development of rapid identification and detection methods for virulent isolates. The use of randomly amplified polymorphic DNA (RAPD) has been applied extensively as a relatively quick and easy method to subtype bacterial isolates of the same species[2].

We describe a method of using commercially available RAPD analysis beads (Amersham Biosciences) to characterize virulent and avirulent isolates from worldwide locations.

Methods

In our study we screened six 10-mer PCR primers, described elsewhere[3], in pairs, to evaluate their ability to obtain discriminatory banding profiles in a panel of virulent and avirulent isolates of *V. parahaemolyticus*. From this, we deduced that two pairs of primers were suitable to characterize 86 isolates of *V. parahaemolyticus* and closely related organisms of clinical and environmental origin from UK and worldwide locations

Results

Screening and primer selection

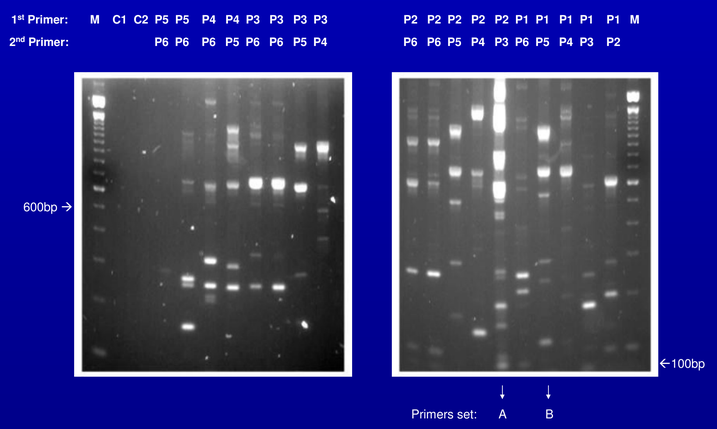

Fig . 1. RAPD primer screening of *V. parahaemolyticus* environmental isolate, VP350 (from *Crassostrea gigas*, UK). Primers P1 to P6 were used in pairs for RAPD PCR. Lanes M, 100bp Ladders (Gibco). Lane C1 and C2 are no DNA template and no primer controls respectively.

Acknowledgements

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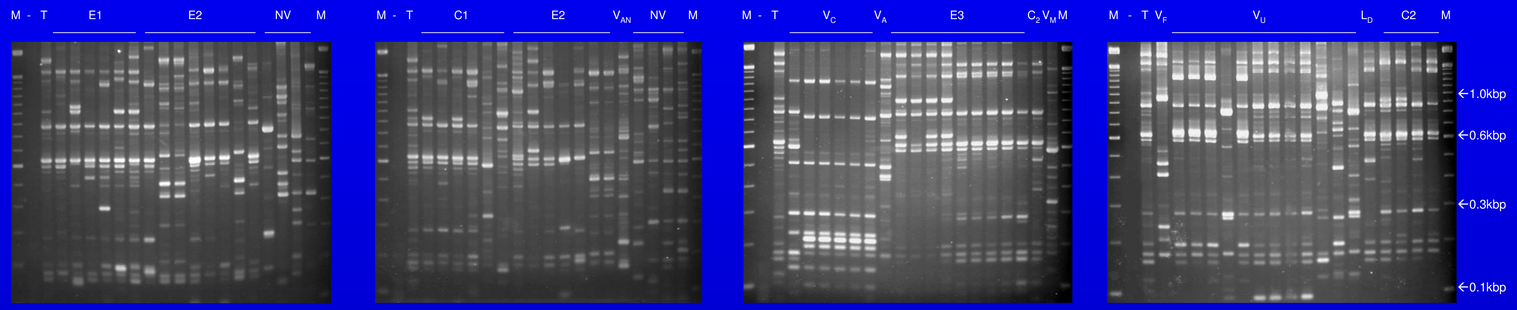

Fig. 2. RAPD fingerprinting profiles of *V. parahaemolyticus* and closely related organisms with primers P2 and P3 . E1, Environmental isolates (Spain); E2, Environmental isolates (Dorset, UK); E3, Environmental isolates (Southampton, UK). C1, Clinical isolates (Japan); C2, Clinical Isolates (NCTC). T, type strain (NCTC 1902); V_{AN}, *V. anguillarum*; V_C, *V. cholerae*, V_A, *V. alginolyticus*; V_M, *V. mimicus*; V_F, *V. fluvialis*; V_U, *Vibrio (unspeciated)*; L_D, *Listonela damesela*; NV, Non-*vibrios*; (-) No DNA template controls. Lanes M, 100bp Ladders with the sizes of selected markers indicated by the arrows.

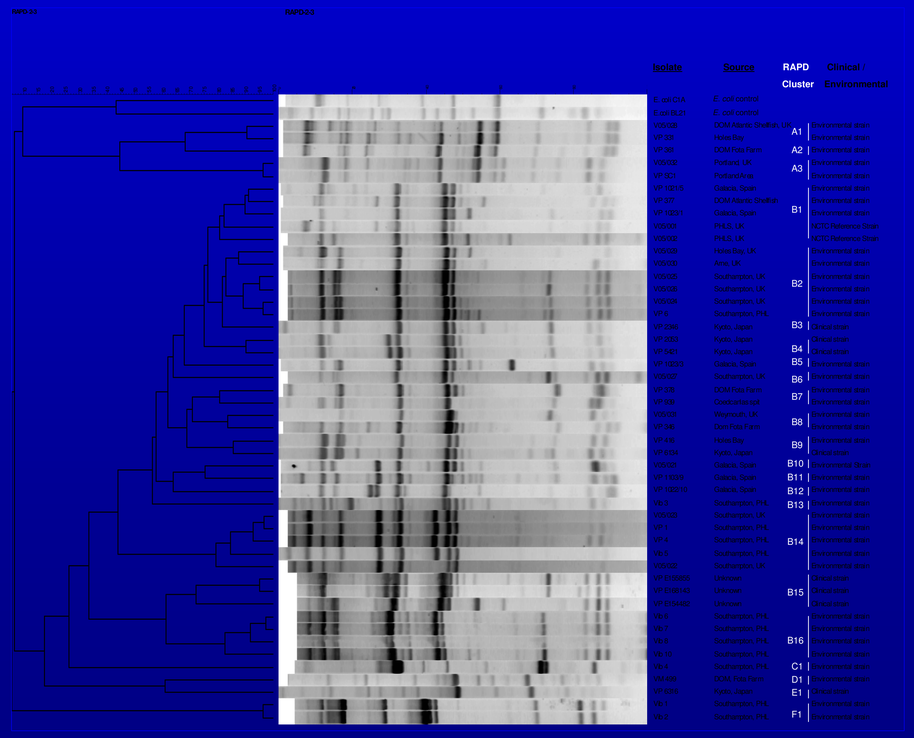

Fig . 3. UPMGA generated dendrogram showing clustering of RAPD patterns of *V. parahaemolyticus* and associated banding pattern. Intraspecies RAPD banding similarities are shown as percentage similarities. Last three columns indicate designation of name, geographical location of isolation, RAPD cluster and whether clinical or environmental respectively. Dendrogram was produced using BioNumerics software (Applied Maths, Belgium)

Discussion

RAPD Typing

We describe the first molecular characterisation of *V. parahaemolyticus* of UK isolates using RAPD. With these results, together with a further set of RAPD primers (Primer set B, results not shown), we were able to validate and support previous work suggesting that RAPD is a relatively quick, easy method to subtype bacterial organisms (Fig. 2.). Independent RAPD PCR results of type strain VP1902 (Fig.2., Lane T) show the reproducibility of this technique.

RAPD has been able to differentiate between different *Vibrio* species (Fig.2. and Fig.3., clusters C, D, E & F) as well as intra-species variation in *V. parahaemolyticus*.

Based on RAPD profiles we were able to categorise 9 unknown *Vibrio* species which previously had not been identified.

RAPD Clustering

V. parahaemolyticus fall into two distinct RAPD clusters (A and B). Clinical isolates fall under four distinct subclusters (B3, B4, B9 and B15). It is noteworthy that isolate VP416, an environmental isolate from the UK, that is also Kanagawa positive, is clustered with clinical isolate VP6134 from Japan. This is in agreement with previous reports that suggest clinical isolates, those of pandemic serotype 03:K6, may be clonally related.

Further Work

Ongoing work looks at pulse field gel electrophoresis (PFGE) profiles. Current work suggest that virulent isolates of *V. parahaemolyticus* are a more homogenous group despite variation in geographical sources of isolation and infection. Variation in the intergenic spacer regions (ITS) also suggest a similar trend.

References

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