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APPLICATION OF RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS AND PLASMID PROFILES TO THE DIFFERENTIATION OF *VIBRIO PARAHAEMOLYTICUS* ISOLATED FROM COASTAL WATERS

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Abstrak: Kaedah profil plasmid dan amplifikasi DNA polimorfik secara rawak (RAPD) digunakan untuk menganalisis perbezaan genetik 57 pencilan *Vibrio parahaemolyticus* yang dipencilkan dari air pinggir pantai. Di kalangan pencilan, 16 corak plasmid diperhatikan, dengan plasmid bersaiz dari 1.5 ke 7.6 megadalton. Dua pencetus (Gen1-50-01, 5'-GTGCAATGAG-3' dan Gen1-50-02, 5'-CAATGCGTCT-3') menghasilkan profil fingerprint DNA genomik yang tetap dengan jalur-jalur dari 0.25 ke 5.0 kb. Profil RAPD menunjukkan tahap diversiti jujukan DNA yang tinggi di kalangan pencilan *Vibrio parahaemolyticus* yang diuji, kerana 48 jenis RAPD diperhatikan untuk setiap satu pencetus masing-masing. Jadi, profil plasmid dan analisis RAPD-PCR terbukti berkesan dalam membezakan pencilan-pencilan. Kaedah RAPD-PCR ternyata lebih sensitif. Data ini menunjukkan pencilan-pencilan *Vibrio parahaemolyticus* boleh dibahagikan kepada sekurang-kurangnya 56 subkumpulan epidemiologikal berasaskan keputusan-keputusan profil plasmid dan RAPD-PCR.

Abstract: Plasmid profiles and random amplified polymorphic DNA (RAPD) techniques were used to analyse the genetic differentiation of 57 isolates of *Vibrio parahaemolyticus* isolated from coastal water. Among the isolates, 16 plasmid patterns were observed, with plasmid sizes ranging from 1.5 to 7.6 megadalton. The two primers (Gen1-50-01, 5'-GTGCAATGAG-3' and Gen1-50-02, 5'-CAATGCGTCT-3') generated reproducible profiles of genomic DNA fingerprints producing bands ranging from 0.25 to 5.0 kb. The RAPD types profiles revealed a high level of DNA sequence diversity within the *Vibrio parahaemolyticus* isolates tested, as 48 RAPD types were observed for each primer respectively. Hence, plasmid profiles and RAPD-PCR analysis proved useful in discriminating the isolates. The later method proved to be more sensitive. Our data show that *Vibrio parahaemolyticus* isolates can be divided into at least 56 epidemiological subgroups on the basis of the plasmid profiles and RAPD-PCR results.

INTRODUCTION

Vibrio parahaemolyticus, a natural inhabitant of estuarine and marine environments is capable of causing acute gastroenteritis in humans. *V. parahaemolyticus* is an enteric pathogen transmitted to humans primarily through consumption of raw or mishandled seafoods, or through a wound; and this pathogen have been a source of disease outbreaks in Taiwan, Japan and other coastal regions (Joseph *et al.* 1982, Johnson *et al.* 1984, Janda *et al.* 1988, Chiou *et al.* 1991). Though the exact mechanism of its pathogenic effect is still not clearly understood, epidemiological studies have associated it with a lethal toxin (Sarkar *et al.* 1987), a vascular permeability factor (Honda *et al.* 1976), and thermostable direct haemolysin (TDH) and related haemolysins (Takeda 1983, Honda *et al.* 1991, Nishibuchi *et al.* 1989, Taniguchi *et al.* 1990). Production of TDH or Kanagawa phenomenon (KP) manifested as beta-haemolysis on a special agar called Wagatsuma agar (Miyamoto *et al.* 1969) has been used as a marker for virulent strains. It has now been demonstrated that not only all Kanagawa phenomenon-positive strains but also some Kanagawa phenomenon-negative strains have biologically active TDHs, indicating that all strains having the gene are potentially virulent (Nishibuchi *et*