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Clonal relationship among the *Vibrio parahaemolyticus* isolates from coastal water in Saudi Arabia

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ABSTRACT

Vibrio parahaemolyticus is one of the microbiota of aquatic ecosystems. This halophilic bacterium is considered as one of the autochthonous microorganism in aquatic environments. During the last two decades, numerous clinical cases has been reported worldwide due to consumption of contaminated seafood products with *V. parahemolyticus*. Therefore, the ability to identify the source of a particular clone has important implications in epidemiological and ecological surveys. Only 28 (14%) out of 200 examined seawater samples were positive and yielded 36 isolates of *V. parahaemolyticus*. ERIC-PCR with two repetitive primers were applied to analyze all the positive isolates for thermolabile hemolysin (tlh) gene for clonal relationship. Both ERIC primers generated polymorphisms in all tested 36 isolates of *V. parahaemolyticus* and produced polymorphism bands ranging from 250 to 4000 base pairs. The genetic fingerprints patterns comprised by ERIC-PCR have provided evidence for strong genetic relationships within the analyzed isolates of *V. parahaemolyticus* isolated from this geographical area.

Introduction

The species of *Vibrio parahaemolyticus* is a halophilic, Gram-negative, facultative anaerobe organism and are inhabitants of marine and aquatic environments (West et al., 1986; Su and Liu, 2007; Ceccarelli et al., 2013). This organism is usually present as microbiota of marine animals and seafood for instance fish, shellfish, oysters, crabs, shrimp, and mussels. *V. parahaemolyticus* is responsible for causing food borne illnesses and is considered a leading pathogen among bacteria associated with ingestion of seafood and during illnesses. This bacterium binds to the fibronectin using the adhesion factors on the host cell; it then releases toxins into the cytoplasm causing infection (Gode-Potratz et al., 2011). However, in order for the toxigenic strains of this bacterium to cause the infection, they should usually harbor either the thermostable direct hemolysin (tdh) or thermostable direct hemolysin related (trh) gene, because these genes are considered as virulence factors (Nishibuchi and Kaper, 1995). In spite of the natural presence of these bacteria in marine environments and in seafood, there is great variability in its distribution and occurrence over different marine regions as this depends on the local seawater temperature and other environmental conditions (Martinez-Urtaza et al., 2012). Therefore, the recovered strains from environmental sources and seafood are reported in very low percentages to harbor virulence factor genes (DePaola et al., 2000; Haley et al., 2014). Thus, applying molecular typing to examine the genetic relatedness among the bacterial strains isolated from environmental sources, is very useful for correlating the abundance of *V. parahaemolyticus* in coastal environments with the reported cases of infection in humans and epidemiological investigations (Caburlotto et al., 2011).

PCR-based molecular methods are rapid, inexpensive, and requires less effort in comparison to pulsed filed gel electrophoresis (PFGE) as non-PCR established method. The method of repetitive-element PCR fingerprinting involves complementary primers to naturally resolve the repetitive DNA sequences that exist within the genome of bacteria (Lupski and Weinstock, 1992). The most widely used repetitive sequences are repetitive extra-genic palindromic (REP) sequence and enterobacterial repetitive intergenic consensus (ERIC) sequence for genotyping bacterial strains. Furthermore, the size of REP sequence ranges between 35 and 40 base pair and the ERIC size sequence usually ranges between 124 and 127 base pair. Both these repetitive sequences are capable of generating discriminative DNA fingerprinting polymorphisms for microbial genomes (Versalovic et al., 1991). This technique of using ERIC-PCR proved effective for tracking epi-

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demics, detecting prevalent clonal lineages, and uncovering genetic relationships among pathogenic bacterial strains. In this study, ERIC repetitive sequences were used to investigate the clonal relationships between the 36 *V. parahaemolyticus* isolates that had been recovered from different locations along the coastlines of Saudi Arabia using ERIC-PCR.

Materials and methods

Sample sites, isolation and identification

A total of 200 seawater samples were collected from 9 locations between February and December in 2015 as presented in Fig. 1 and Table 1. The sampled locations included Almorjan Island, Dammam corniche, Dammam marina front, Fanateer corniche, Half-moon Beach, Palm Beach Jubail, Qatif corniche, Sayhat corniche, and Tarout corniche (Fig. 1). The seawater samples were collected in sterilized 400-ml screw-capped glass bottles and analyzed to isolate *V. parahaemolyticus* (Elhadi et al., 2004; Tan et al., 2017). 25 ml from each seawater sample were enriched in 225 ml of alkaline peptone water (APW) and the salt content in APW was adjusted from 1% to 3% and incubated at 37 °C for 24 h. After incubation, a full wire loop from each enriched sample was streaked on CHROM *Vibrio* agar (CHROM, France). Presumptive mauve color colonies on CHROM *Vibrio* agar were purified and subjected to further confirmation using PCR amplification of gene to encode the thermolabile hemolysin (*tlh*) gene with species-specific primers (Brasher et al., 1998).

DNA extraction

Recovered isolates of *V. parahaemolyticus* on Vibrio chromogenic agar that were positive for the *tlh* gene were subcultured in Luria Bertani (LB) broth and kept in an incubator-shaker at speed of 240 rpm for 24 h. A volume of 1.5 ml from the incubated LB broth was transferred into a micro-centrifuge tube and the harvested pellets were mixed with 700 μl of nuclease free water (Promega, USA) and kept for 30 min in a water bath that had been adjusted to 100 °C to liberate the DNA using the boiling technique. The extracted DNA was immediately kept and preserved at −20 °C for further use.

PCR analysis of the tlh gene

PCR reaction targeting the detection gene encoding *tlh* gene was done in 25 μl volume per reaction test using Go Taq Flexi DNA polymerase (Promega, USA) and the forward and reverse primer sequences (F-tlh: 5′-AAAGCGGATGTATCAGAAGCACTG-3′, and R-tlh: 5′-GCTACTTTCTAGCATTTTCTCTGC-3′) (Bej et al., 1999). The program used for amplification reaction mixtures with a thermal cycler machine was performed as described by Taiwo et al. (2017). Ten μl from amplified products were separated on a 2% agarose gel, and positive isolates of *V. parahaemolticus* were compared to the 450-bp amplicon band produced by the positive control of the *V. parahaemolyticus* strain (ATCC 17802).

ERIC-PCR

The clonal relationship among the *Vibrio parahaemolyticus* isolates was carried out using ERIC-PCR fingerprinting method with two repetitive primer sequences ERIC1R (5′-3′ATGTAAGCTCCTGG GGATTCAC) and ERIC2 (5′-3′AAGTAAGTGACTGGGGTGAGCG) (Versalovic et al., 1991). The conducted amplification conditions with ERIC1R and ERIC2 primers were as described by Rivera et al. (1995). Ten μl from the PCR amplified products with both ERIC primers were separated with a 1.5% agarose gel electrophoresis for 120 min at 90 Volts. GelPiolt 1 kb Plus ladders (Qiagen, Germany) were used as molecular weight DNA markers.

ERIC-PCR fingerprinting analysis

All the gel images of electrophoresis agarose gels were processed using GelJ software for analyzing the DNA fingerprint (Heras et al., 2015). The dendrogram was constructed using the unweighted average pair group method (UPGMA) and a Dice coefficient to compare between the discriminatory power of ERICR1 and ERIC-2 primers.

Results

Isolation of V. Parahaemolyticus

Seawater samples in the present study were collected and analyzed from nine locations along the coastline of the Eastern Pro-

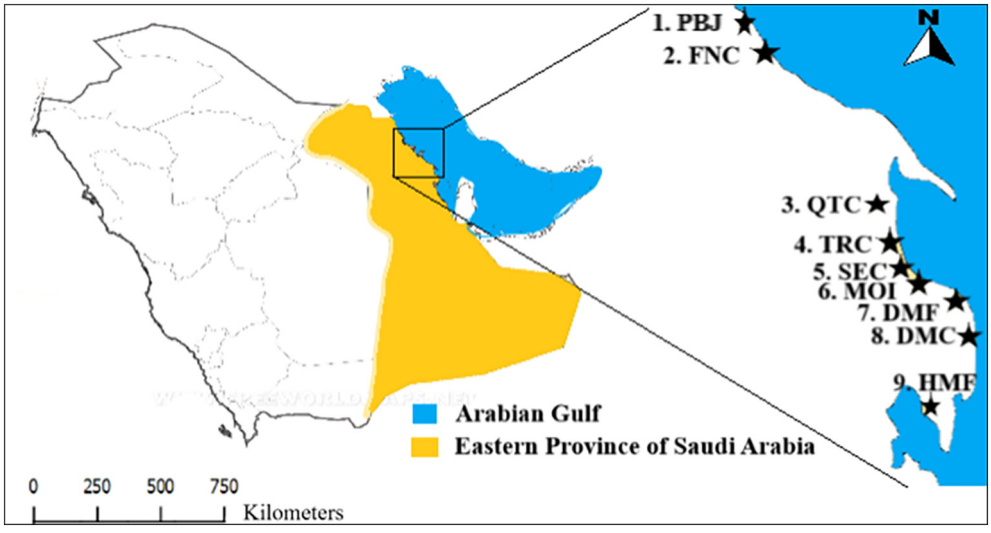

Fig. 1. Location map of seawater sampling sites in the Eastern Province of Saudi Arabia.

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