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Isolation and molecular characterization of toxigenic *Vibrio parahaemolyticus* from the Kii Channel, Japan

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Summary

Studies were conducted on the ecology of potentially pathogenic *Vibrio parahaemolyticus* in three coastal areas of Kii Channel, Tokushima, Japan. Seawater and seaweed samples were collected seasonally between June 2003 and May 2004. Total and toxigenic strains of *V. parahaemolyticus* were isolated using most probable number culture and colony blot hybridization. Toxigenic strains were serotyped and further characterized by random amplified polymorphic DNA (RAPD) and ribotyping. Six thousand strains of *V. parahaemolyticus* were isolated and 18 were found positive for *tdh*. *V. parahaemolyticus* were detected in all samples during summer and autumn, and from some samples during winter and spring. Among the toxigenic strains seven serotypes, five ribotypes and RAPD patterns were observed. Seven strains belonged to O3:K6 clone with identical ribotypes and RAPD patterns to that of a pandemic reference strain. The presence of toxigenic *V. parahaemolyticus* with pandemic potential might indicate a human health risk due to consumption of marine food sources.

Introduction

Vibrio parahaemolyticus, a halophilic bacterium, is the leading cause of seafood-borne gastroenteritis especially in countries where seafood consumption is high (Barker and Gangarosa, 1974; Trison and Kelly, 1984). The organism is ubiquitous in brackish and marine waters and can be found along the

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Asian, North American, African and Mediterranean coasts (Eko et al., 1994; Barbieri et al., 1999). *V. parahaemolyticus* was first identified as a causative agent of human disease after an outbreak of gastroenteritis in Japan in 1950 (Joseph et al., 1982). Food-borne outbreaks and isolated cases have occurred throughout the world and are commonly the result of consumption of contaminated seafood (Honda and Iida, 1993). Crabs, prawns, scallops, oysters and clams have been identified as sources of infection caused by *V. parahaemolyticus* (Joseph et al., 1982). Seaweed, another kind of seafood, which grows in marine environment where vibrios thrive (Sims et al., 1993; Vugia et al., 1997), was first implicated as source of gastroenteritis in 1997 (Vugia et al., 1997).

Serotyping can differentiate isolates of *V. parahaemolyticus* and 13 O types and 71 K types have been identified (Iguchi et al., 1995). Normally, a wide variety of serovars are involved in outbreaks. After 1996, an increasing incidence of gastroenteritis in many parts of the world has been linked to pandemic O3:K6 serotype (Okuda et al., 1997; Bag et al., 1999). The association of this new O3:K6 serotype with most of *V. parahaemolyticus* infections in India, Taiwan, Laos, Japan, Thailand, Korea and the United States in recent years suggests that this organism may have an unusual characteristic to be transmitted by food and has pandemic potential (Bag et al., 1999). Outbreaks with toxigenic strains of *V. parahaemolyticus*, especially with O3:K6 clone, have been epidemiologically linked to consumption of seafoods after 1997 in Japan (Infectious Diseases Surveillance Center, National Institute of Infectious Diseases, 1999). Association of such outbreaks due to consumption of seaweeds has not been intensively studied (Sims et al., 1993; Vugia et al., 1997).

Pathogenic *V. parahaemolyticus* has been known to produce either thermostable direct hemolysin (TDH), TDH-related hemolysin (TRH) or both (Honda and Iida, 1993; Nishibuchi and Kaper, 1995). TDH and TRH encoded by *tdh* and *trh* genes, respectively, are now recognized as major virulence factors for the pathogenesis of *V. parahaemolyticus* (Honda and Iida, 1993; Nishibuchi and Kaper, 1995). The frequency of *tdh* or *trh* detection in environmental samples and seafoods was reported to be very low in comparison to clinical isolates (Kiiyukia et al., 1989; Ogawa et al., 1989; Cook et al., 2002). A separate gene, thermolabile hemolysin (*tlh*) has also been characterized (Taniguchi et al., 1985, 1986). This gene was shown to be present in all of the *V. parahaemolyticus* strains tested previously (Taniguchi et al., 1985, 1986).

V. parahaemolyticus and many other vibrios become abundant during warmer months in seawaters of Japan and other parts of the world but they are rarely isolated in colder months (Kelly and Stroh, 1988; Chowdhury et al., 1990). However, the ecology of pathogenic *V. parahaemolyticus* present in environmental samples has not been fully understood. This study was, therefore, aimed to investigate the ecology of potentially pathogenic *V. parahaemolyticus* and to determine if pandemic serotypes prevailed in the seawater and seaweed samples collected from the coastal areas of the Kii Channel.

Materials and methods

Sample collection, its processing and the identification of *V. parahaemolyticus*

Seawater and seaweed (marine algae, attached to rocks or other solid surfaces) samples were collected seasonally from June 2003 to May 2004 from three coastal areas (Komatsushima, Tokushima and Naruto) of the Kii Channel, Tokushima, Japan (Fig. 1). Komatsushima coast is the south-eastern part of Tokushima that faces the Pacific Ocean. Tokushima and Naruto coasts, located in the central and northern part of Tokushima, respectively, are swimming beaches. Samples were collected at four different sites, 0.5 km far from each other within each coastal area following the method of American Public Health Association

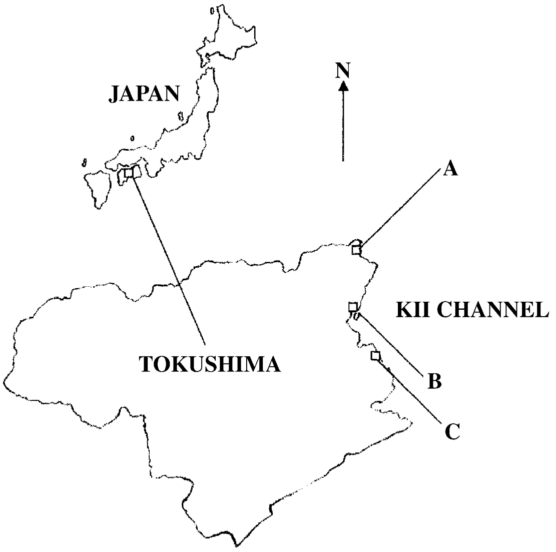

Figure 1. Map of the sampling area: (A) Naruto coast, (B) Tokushima coast, (C) Komatsushima coast.