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Pathogenesis and Genome of *Vibrio parahaemolyticus*

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It has been 50 years since *Vibrio parahaemolyticus* was discovered by Dr. Fujino in 1950 on the occasion of the "Shirasu food-poisoning" case occurred in Osaka. Since the discovery, this organism has been a major cause of food-poisoning in Japan, mainly associated with seafood consumption, and accounts for approximately 30~50% of the cases of bacterial food poisoning. Not only in Japan, now it is clear that this organism is present widely around the world as a causative agent of acute gastroenteritis. Recent report from the United States demonstrated that, besides gastroenteritis, *V. parahaemolyticus* can be a cause of wound infections and septicemia (1). Strains with invasive phenotype (2) could be responsible for such severe infections. In this symposium, I will talk on our recent study on the pathogenesis and genome of *V. parahaemolyticus*.

Presence of two circular chromosomes in *Vibrio* species. We have reported a physical map of the genomic DNA (5.1 Mb) for a clinical *Vibrio parahaemolyticus* strain AQ4673 by combining 17 adjacent *Not*I fragments (3). Surprisingly, the map showed two circular replicons of 3.2 and 1.9 Mb. Pulsed-field gel electrophoresis (PFGE) of undigested genomic DNA revealed two bands of corresponding sizes. Analysis both by *Not*I digestion and by Southern blot of the two isolated bands confirmed the existence of two replicons. The presence of genes for 16S rRNA on both the replicons indicates that the replicons are chromosomes rather than megaplasmids. The two bands were also seen after PFGE of undigested genomic DNA of *V. parahaemolyticus* strains other than AQ4673, and of strains belonging to other *Vibrio* species, such as *V. vulnificus*, *V. fluvialis* and various serovars and biovars of *V. cholerae* (3). These results suggest that a two-chromosome structure is common throughout *Vibrio* species.

Genetic linkage between *trh* and *ure*. We have demonstrated that possession of the gene for thermostable direct hemolysin-related hemolysin (*trh*) coincides with the presence of the urease gene among clinical *Vibrio parahaemolyticus* strains (4) and that the location of the two genes are in close proximity on the chromosome (5). Very recently, we cloned and sequenced the 15,754-bp DNA region containing the *trh* gene and the gene cluster for urease production from the chromosome of clinical *V. parahaemolyticus* (TH3996) (6). We found 16 open reading frames (ORFs) and a lower G+C content (41%) compared with the total genome of this bacterium (46 to 47%). The *ure* cluster consisted of eight genes, namely, *ureDABCEFG* and *ureR*. *ureR* was located 5.2 kb upstream of the other seven genes in the opposite direction. The genetic organization and sequences of the *ure* genes resembled those found in *Proteus mirabilis*. Between *ureR* and the other *ure* genes, there were five ORFs, which are homologous with the nickel transport operon (*nik*) of *Escherichia coli*. We disrupted each of the *ureR*, *ureC*, and

nikD genes in TH3996 by homologous recombination and analyzed the phenotype of the mutants. In the presence of urea these mutant strains had dramatically less urease activity than the strain they were derived from. Disruption of *ureR*, *nikD*, or *ureC*, however, had no effect on TRH production. The DNA region containing the *trh*, *nik*, and *ure* genes was found in only *trh*-positive strains and not in Kanagawa phenomenon-positive and environmental *V. parahaemolyticus* strains. At the end of the region, an insertion sequence-like element existed. These results suggest that the DNA region was introduced into *V. parahaemolyticus* in the past through a mechanism mediated by insertion sequences. This is the first reported case that the genes for an ATP-binding cassette-type nickel transport system, which may play a role in nickel transport through bacterial cytoplasmic membrane, are located adjacent to the *ure* cluster on the genome of an organism.

A filamentous phage associated with recent pandemic *V. parahaemolyticus* O3:K6 strains. A specific serotype, O3:K6, of *V. parahaemolyticus* has recently been causing epidemics of gastroenteritis in Southeast Asia, Japan, and North America (1,7,8). To examine whether the new O3:K6 strains possess characteristics that may exacerbate outbreaks, we compared *V. parahaemolyticus* O3:K6 strains with non-O3:K6 strains using strains isolated from individuals with traveler's diarrhea at Kansai International Airport Quarantine Station, Osaka, Japan (9). All 24 O3:K6 strains possessed a common plasmid, pO3K6 (DNA size, 8,784 bp, with 10 open reading frames [ORFs]). The gene organization of pO3K6 was similar to that of CTXΦ, a filamentous phage previously described in *V. cholerae*, which encodes the genes for the cholera enterotoxin (10). We isolated a phage (phage f237) from the culture supernatant of *V. parahaemolyticus* O3:K6 strain KXV237, which formed a turbid plaque on an indicator strain. The genome of f237 was single-stranded DNA, and the double-stranded DNA obtained by treatment of the genome with DNA polymerase was identical to that of pO3K6 when analyzed by agarose gel electrophoresis after *Hin*dIII digestion. Furthermore, the N-terminal amino acid sequence of the f237 major coat protein was found in ORF4 of pO3K6. Our results showed that pO3K6 is a replicative form of f237. Among the ORFs found in the f237 genome, the sequence of ORF8 had no significant homology to those of any proteins in databases. ORF8 was located on a region corresponding to the distinctive region of CTXΦ, and its G+C content was apparently lower than that of the remaining DNA sequence of f237. By colony hybridization, ORF8 was detected only in O3:K6 strains isolated since 1996 and was not found in O3:K6 strains isolated before 1996 and clinical *V. parahaemolyticus* strains other than those of serotype O3:K6. Thus, this study shows that f237 is exclusively associated with recent *V. parahaemolyticus* O3:K6 strains. The ORF8 gene can be a useful genetic marker for the identification of the recently widespread O3:K6 strains of *V. parahaemolyticus*.

REFERENCES

1) Daniels NA, MacKinnon L, Bishop R, Altekruse S, Ray B, Hammond RM, Thompson S, Wilson S, Bean NH, Griffin PM, Slutsker L: *Vibrio parahaemolyticus* infections in the United States, 1973-1998. *J Infect Dis* **181:** 1661-1666, 2000.

2) Akeda Y, Nagayama K, Yamamoto K, Honda T: Invasive phenotype of *Vibrio parahaemolyticus*. *J Infect Dis* **176:** 822-824, 1997.

3) Yamaichi Y, Iida T, Park K-S, Yamamoto K, Honda T: Physical and genetic map of the genome of *Vibrio parahaemolyticus*: Presence

of two chromosomes in *Vibrio* species. *Mol Microbiol* **31:** 1513-1522, 1999.

4) Suthienkul O, Ishibashi M, Iida T, Nettip N, Supavej S, Eampokalap B, Makino M, Honda, T: Urease production correlates with possession of the *trh* gene in *Vibrio parahaemolyticus* strains isolated in Thailand. *J Infect Dis* **172:** 1405-1408, 1995.
5) Iida T, Park K-S, Suthienkul O, Kozawa J, Yamaichi Y, Yamamoto K, Honda T: Close proximity of the *tdh*, *trh* and *ure* genes on the chromosome of *Vibrio parahaemolyticus*. *Microbiology* **144:** 2517-2523, 1998.
6) Park K-S, Iida T, Yamaichi Y, Oyagi T, Yamamoto K, Honda T: Genetic characterization of DNA region containing the *trh* and *ure* genes of *Vibrio parahaemolyticus*. *Infect Immun* **68:** 5742-5748, 2000.
7) Okuda J, Ishibashi M, Hayakawa E, Nishino T, Takeda Y, Mukhopadhyay AK, Garg S, Bhattacharya SK, Nair GB, Nishibuchi M: Emergence of a unique O3:K6 clone of *Vibrio parahaemolyticus* in Calcutta, India, and isolation of strains from the same clonal group from Southeast Asian travelers arriving in Japan. *J Clin Microbiol* **35:** 3150-3155, 1997.
8) Matsumoto C, Okuda J, Ishibashi M, Iwanaga M, Garg P, Rammamurthy T, Wong H, DePaola A, Kim YB, Albert MJ, Nishibuchi M: Pandemic spread of an O3:K6 clone of *Vibrio parahaemolyticus* and emergence of related strains evidenced by arbitrarily primed PCR and *toxR* sequence analyses. *J Clin Microbiol* **38:** 578-585, 2000.
9) Nasu H, Iida T, Sugahara T, Yamaichi Y, Park K-S, Yokoyama K, Makino K, Shinagawa H, Honda T: A filamentous phage associated with recent pandemic *Vibrio parahaemolyticus* O3: K6 strains. *J Clin Microbiol* **38:** 2156-2161, 2000.
10) Waldor MK, Mekalanos JJ: Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* **272:** 1910-1914, 1996.