Mem. Inst. Oswaldo Cruz, Rio de Janeiro,
Vol. *87* (1): 167-168, jan./mar. 1992

Research Note

BRAZILIAN UREASE-POSITIVE STRAINS OF *VIBRIO PARAHAEMOLYTICUS* CARRY GENETIC POTENTIAL TO PRODUCE THE TDH-RELATED HEMOLYSIN

MARCELO MAGALHÃES; YOSHIFUMI TAKEDA; VERA MAGALHÃES & SEIKI TATENO

Laboratório de Imunopatologia Keizo Asami, Universidade Federal de Pernambuco, Cidade Universitária, 50670-901 Recife, PE, Brasil

Long ago, it has been recognized that most clinical isolates of *Vibrio parahaemolyticus* induce a characteristic beta-type hemolysis when grown on a special high salt-mannitol blood agar medium, Kanagawa phenomenon (KP). In contrast, strains derived from marine environment are usually KP negative (R. Sakazaki et al., 1968, *Jpn. J. Med. Sci. Biol., 21:* 325-331; Y. Miyamoto et al., 1969, *J. Bacteriol., 100:* 1147-1149). Hemolysis was attributed to a thermostable direct hemolysin (TDH), whose encoding gene was cloned in *Escherichia coli* (J. B. Kaper et al., 1984, *Infect. Immun., 45:* 290-292) permitting the construction of a specific gentic probe (M. Nishibuchi et al., 1985, *Infect. Immun., 49:* 481-486).

Despite the narrow epidemiologic relationship between TDH production and human disease, papers reporting cases of gastroenteritis associated with KP-negative strains of *V. parahaemolyticus* have appeared (S. Honda et al., 1987, *Lancet, i:* 331-332; M. T. Kelly & E. M. D. Stroh, 1989 (*J. Clin. Microbiol., 27:* 2820-2822). Concerning this, over the last two years, we also have found in Recife, a tropical city situated in Northeast Brazil, some enteric isolates of *V. parahaemolyticus* producing low levels of TDH or do not producing that hemolysin at all. These strains belong to diverse serovars, and most are urease positive (M. Magalhães et al., 1991, *Rev. Inst. Med. trop. S. Paulo, 33:* 263-265).

In this vein, T. Honda et al. (1988, *Infect. Immun., 56:* 961-967) have shown that strains of *V. parahaemolyticus* isolated during an outbreak of gastroenteritis in the Maldives islands, despite the absence of TDH activity, produce another hemolysin related to it (TRH). It was presumed that TRH is an important virulence factor in KP-negative strains (T. Honda et al., 1989, *FEMS Microbiol. Lett., 57:* 241-246). Since immunological techniques for detecting that hemolysin are not yet standardized, we used DNA colony hybridization tests (M. Nishibuchi et al., 1985, *Infect. Immun., 49:* 481-486) to determine whether our urease-positive and KP-negative strains of *V. parahaemolyticus* also have genetic potentialities to produce TRH.

Methods used for recovering vibrios, serotyping, biochemical characterization, urease activity, and KP tests have been described elsewhere (M. Magalhães et al., 1991, *Rev. Microbiol. (S. Paulo), 22:* 83-88). For comparison, we also included in the study seven high TDH-producer clinical isolates and nine KP-negative strains of *V. parahaemolyticus* recovered from oysters.

As expected, none of the oyster strains harbored the hemolysin encoding genes. On the other hand, most human isolates carried the genes *tdh, trh,* or both (Table). It was noted a close association between urease activity and the presence of *trh.* Otherwise, the six urease-negative and KP-positive cultures just showed the *tdh* gene. This gene, however, although phenotypically not expressed, also was present in most of the *trh*-positive colonies. M. Nishibuchi & J. B. Kaper (1990, *Mol. Microbiol., 4:* 87-99) have demonstrated that the strains of *V. parahaemolyticus* showing a typical hemolysin-positive phenotype carry two chromosomal gene copies, *tdh*1 and *tdh*2. Thus, it appears that, when both *tdh* and *trh* hemolysin encoding genes are simultaneously present

Work supported by CNPq (grant 416241/89) and Japan International Cooperation Agency.

Received 10 September 1991.
Accepted 21 October 1991.

in our cultures, *tdh* gene is incorrectly expressed or not expressed at all. Whether this is a coincidence, requiring little genetic explanation, or such KP-negative and urease-positive strains of *V. parahaemolyticus* have only one copy of the *tdh* gene remains to be determined. Anyway, in the KP-negative strains of *V. parahaemolyticus* recovered from the Maldives islands and Brazil Northeast the *trh* gene would represent a potential trait for enteropathogenicity.

TABLE

Results of colony hybridization and urease tests in 18 clinical strains of *Vibrio parahaemolyticus*

Gene present		Urease test	
tdh	*thr*	positive[a]	negative
+	+	9	0
+	–	0	6
–	+	1	0
–	–	0	2

a: eight strains were KP-negative.

Despite hemolysins are believed to be a major factor for *V. parahaemolyticus* virulence, we could not demonstrate *tdh* or *trh* genes in two of our isolates. These isolates were recovered as the sole enteropathogen from adult patients with profuse watery diarrhea. Whether the virulence of these strains was linked to another hemolysin different from TDH or TRH, rests to be seen. In this connection, H. Toniguchi et al. (1990, *FEMS Microbiol. Lett., 55:* 339-345) cloned a new thermostable hemolysin gene, delta-VPH, from a KP-negative strain into *E. coli* K12, and H. Shirai et al. (1990, *Infect. Immun., 58:* 3568-3573) suggested that a family of *tdh*-related hemolysin genes not detectable by hybridization with the *tdh* or *trh* gene probe may be present in some *tdh* gene-negative clinical isolates. More recently, a third type of hemolysin (named Vp-TDH/I), which is produced by a KP-negative clinical strain of *V. parahaemolyticus* was described (T. Honda et al., 1991, *J. Gen. Microbiol., 137:* 253-259). These findings point out that many other studies are still needed before we clarify the actual diversity of *V. parahaemolyticus* hemolysins and their relationships with virulence.