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Ureolytic *Vibrio parahaemolyticus* Isolated from the Kamak Bay of Yeosu, in 2002 and 2003

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Five urease-positive *Vibrio parahaemolyticus* strains were isolated from Kamak Bay in Yeosu in 2002 and 2003. *V. parahaemolyticus* YKB4 and YKB14 were isolated from seawater, YFB20 from black rockfish (*Sebastes schlegeli*), and YFO21 and YFO22 from olive flounder (*Paralichthys olivaceus*). The five urease-positive strains (YKB4, YKB14, YFB20, YFO21, and YFO22) did not show hemolysin and protease activity, while they did alter in color (to red) as the bacteria grew in the urea broth medium. All samples showed identical biochemical characteristics as a reference strain, *V. parahaemolyticus* KCTC2471, except in urease production. The five urease-positive strains showed urease activities at a mid stationary phase, and their activity was maximal in the late stationary phase of their culture supernatant. The addition of urea to the Luria-Bertani (LB) broth medium significantly affected the initial production of urease of *V. parahaemolyticus* isolates. Mortality by urease-positive *V. parahaemolyticus* YKB4, YKB14, YFO21, and YFO22 was significantly high, being 60-80%, while YFB20 only reflected a rate of 20%. Protease-positive *V. parahaemolyticus* FM39 and FM50 showed a 40% and 60% mortality rate, respectively. However, hemolysin-positive *V. parahaemolyticus* had no mortality, like the non-pathogenic *V. parahaemolyticus* KCTC2471, while *V. vulnificus* resulted in a 40% mortality rate. Injection with urease-positive *V. parahaemolyticus* strains showed mortality within 12 hrs in mice, and the strains could be isolated from the dead mice.

Key words: *Vibrio parahaemolyticus*, Urease-positive, Protease-positive, Hemolysin-positive, Mortality

Introduction

Vibrio species produce various pathogenic factors (Edward et al., 1984; Larsen, 1984; Gray and Kreger, 1985; Kosary and Kreger, 1985; Ichinose et al., 1987; Honda et al., 1992; Kaysner et al., 1994; Suthienkul et al., 1995). Especially, *V. parahaemolyticus* and *V. vulnificus* have been known as very important strains because they were the major causal agents of seafood poisoning accidents during the 1970's and 1980's in Korea. They also produce proteolytic enzymes such as hemolysin and enterotoxin (Sakazaki et al., 1963; Sakazaki et al., 1968; Miyamoto et al., 1969; Dotevall et al., 1985; Honda et al., 1985; Chang and Shinoda, 1994; Kim et al., 1997). The bacterial hemolysin destroys red blood cells and helps the growth of pathogenic vibrios by supplying ferric ions, while the protease invades interstitial tissue space and damages hemorrhagic skin (Sakazaki et al., 1968; Finkelstein et al., 1983; Miyoshi et al., 1998; Shao and Hor, 2000). Many ureolytic bacteria are pathogenic to animals and human (MacLaren, 1969; Musher et al., 1975; Jones et al., 1990; Osterberg et al., 1990; Johnson et al., 1993; Mobley et al., 1995). Especially, it is well known that urease is essential for gastric colonization and plays a central role in the pathogenesis of *Helicobacter pylori* infection (Hawtin et al., 1990; Evans et al., 1991; Cover and Blaser, 1995). *Vibrio* species have usually been considered to be urease-negative: less than 10% of the strains are positive for urease activity (Twedt et al., 1969; Zen-Yoji et al., 1973; Fujino et al., 1974; Suthienkul et al., 1995). Suthienkul et al. (1995) reported that only 8% of 489 clinical *V. parahaemolyticus* strains were urease-positive. Recently, urease-positive strains

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among clinical *V. parahaemolyticus* isolates are gradually increasing worldwide (Huq et al., 1979; Oberhofer and Podgore, 1982; Nolan et al., 1984; Abbott et al., 1989; Kelly and Stroh, 1989; Honda et al., 1992; Magalhaes et al., 1992; Cai and Ni, 1996). However, reports on urease-positive *V. parahaemolyticus* in Korea are rare except for Kim (1999), where an environmental urease-positive *V. parahaemolyticus* KH410 strain was reported. The author isolated ureolytic *V. parahaemolyticus* from seawater, rockfish [black rockfish (*Sebastes schlegeli*) and olive flounder (*Paralichthys olivaceus*)] from Kamak Bay in Korea in 2002 and 2003, and examined the virulence of the ureolytic strains in mice.

Materials and Methods

Media and kit for rapid detection

All media was purchased from the Difco Co. (Sparks, MD, USA) except the tryptic soy agar plate (TSA, 5% sheep erythrocyte), which was from the Micromedia Co. (Daejeon, Korea). The API 20E kit was obtained from the bioMérieux Co. (Marcy I'Etoile, France), and reagents from Sigma-Aldrich (St. Louis, MO, USA).

Isolation of *V. parahaemolyticus*

The seawater and blackfish (black rockfish and olive flounder) were collected from Kamak Bay in Yeosu, Korea in 2002 and 2003. The isolation of *Vibrio* spp. from the seawater was conducted based on the methods of Park et al. (2002; 2003). The seawater was filtered with a millipore membrane filter (0.45 μm pore size, Millipore Co., Bedford, MA, USA) within a vacuum. Each 30 g of the muscle and skin from the black rockfish (*S. schlegeli*) and the olive flounders (*P. olivaceus*) was homogenated with 270 mL of phosphate buffered saline (PBS) for 2 min with Stomacher (Seward, London, England). The filter and fish hemogenate were inoculated and enriched in a peptone media (1% peptone, 0.5% NaCl). The enriched culture was spread on a thiosulfate citrate bile salt sucrose (TCBS) agar plate and incubated for 24 hrs at 37℃. The green colonies on the TCBS agar plate were selected and examined for their biochemical characteristics with an API 20E kit.

Urease activity assay

The isolates were inoculated in Luria-Bertani (LB) broth and cultured while shaking at 37℃. The culture supernatant (7,000×g for 20 min) was assayed for urease activity, employing *Proteus vulgaris* ATCC 6380 as a urease-positive reference strain. Each culture supernatant (50 μL) was mixed with 200 μL of a UHEP buffer (20 mM HEPES buffer, pH 7.5, 30 mM urea, 1 mM EDTA, 1 mM 2-mercaptoethanol) for 30 min at 37℃. Both phenol nitroprusside (1 mL) and alkaline hypochlorite (2 mL) were added to the reaction mixture, and the quantity of ammonia liberated in the reaction mixture was measured by absorbance at 625 nm (Weatherburn, 1967).

Pathogenicity test in mice

The pathogenicity of ureolytic *V. parahaemolyticus* was investigated based on the methods of Starks et al. (2000) and Park et al. (2004). The bacterial cells were suspended to 10^7-10^8 cfu/mL with PBS. The cell suspension (0.5 mL) was intraperitoneally injected into groups of 10 ICR mice (7- to 10-week-old males). The mice were observed for 48 hrs following infection. Each test was done duplicate. Mortality was shown the ratio of dead to total 10 mice inoculated.

Results and Discussion

Isolation of ureolytic *V. parahaemolyticus*

V. parahaemolyticus was isolated from seawater and fish from Kamak Bay during the summer of 2002 and 2003. Green colonies on a TCBS agar plate were examined for their biochemical characteristics with the API 20E kit. A total of 92 *V. parahaemolyticus* strains were isolated: 58 strains from seawater and 34 strains from fish. Among 92 of the *V. parahaemolyticus* strains, only 5 strains were urease-positive in a test using the API 20E kit. *V. parahaemolyticus* YKB4 and YKB14 were isolated from seawater, YFB20 from black rockfish (*S. schlegeli*), and YFO21 and YFO22 from olive flounder (*P. olivaceus*). However, the five urease- positive strains of *V. parahaemolyticus* (YKB4, YKB14, YFB20, YFO21, and YFO22) did not show hemolysin and protease activity (Table 1). They showed the same biochemical characteristics as the reference strain, *V. parahaemolyticus* KCTC2471, except in urease production (data not shown). Park et al. (2002; 2003) reported no urease-positive *V. parahaemolyticus* during 2001-2002 from the seawater of Gwangan Beach of Busan, but they isolated one strain in 2003 from the same area (Park et al., 2004). In present study, however, 5 urease-positive *V. parahaemolyticus* strains were isolated from the Kamak Bay of Yeosu. The urease activities of *V. parahaemolyticus*,

Table 1. Ureolytic *Vibrio parahaemolyticus* isolated from the Kamak Bay in 2002 and 2003

Sample (total isolates)	Pathogenic factors			
	Strains	Hemolysin	Protease	Urease
Seawater in 2002 (22)	YKB4	-	-	+
	YKB14	-	-	+
Seawater in 2003 (36)		-	-	-
Fish in 2003 (34)	YFB20	-	-	+
	YFO21	-	-	+
	YFO22	-	-	+

Hemolysin, protease, and urease activity were confirmed with a TSA agar plate (5% sheep erythrocyte), 10% skim milk agar plate, and urea broth, respectively. YFB20, isolated from black rockfish (*Sebastes schlegeli*); YFO21 and YFO22, isolated from olive flounder (*Paralichthys olivaceus*); +, detected; -, not detected.

YKB4, YKB14, YFB20, YFO21, and YFO22, were measured with the culture super- natant during the bacterial growth in a LB broth medium. They showed urease activity from the mid stationary phase and activity was maximal at the late stationary phase. The maximum urease activity of *V. parahaemolyticus* isolates was twice as high as that of *P. vulgaris*, which is a urease-positive reference strain (Fig. 1). Kim (1999) reported that the urease activity of the environmental *V. parahaemolyticus* KH410 was the highest at the late exponential phase and decreased rapidly at the initial stationary phase. This result does not accord with those of the present study, where urease activity was recorded from the mid stationary phase and was maximal at the late stationary phase. Generally, urease is constitutively synthesized in some microbes, such as *Bacillus pasteuri*, *Sporosarcina ureae*, and *Morganella morganii*. It is also induced by urea in microbes such as *P. mirabilis* and those which bear plasmid-encoded ureases, including *Salmonella cubana*, *Providencia stuartii*, and some *E. coli* strains (Mobley et al., 1995). Kim (1999) reported that the urease of environmental *V. parahaemolyticus* KH410 was induced and that it was an intracellular enzyme. In order to investigate whether the ureases of *V. parahaemolyticus* YKB4, YKB14, YFB20, YFO21, and YFO22 are intracellular or extracellular enzymes, the cells were disrupted using a sonicator (30 minutes on ice at an amplitude of 10) (Sonics & Materials Inc., Danbury, U.S.A), and the urease activity from culture supernatant and disrupted cell fraction was compared. However, no urease activity from the disrupted cell fraction could be detected. The urease of *V. parahaemolytiuc* YFO22 was produced from normal LB broth medium, although urea (0.2%) promoted the initial production of urease (Fig. 2). These results indicate that the ureases of *V. parahaemolyticus* are constitutive and extracellular enzymes.

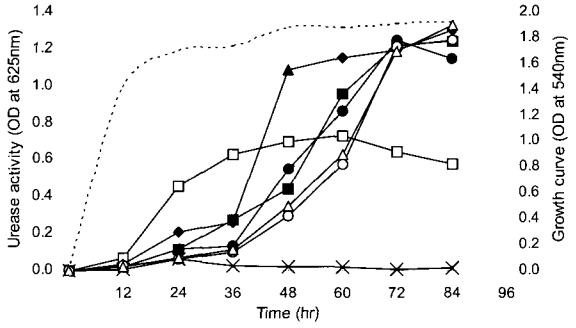

Fig. 1. Changes of urease activity during the growth of *Vibrio parahaemolyticus* isolates.
----, bacterial growth curve; X, *V. parahaemolyticus* KCTC2471 (a urease-negative reference strain); □, *P. vulgaris* ATCC6380 (a urease-positive reference strain); ○, *V. parahaemolyticus* YKB4; △, *V. parahaemolyticus* YKB14; ■, *V. parahaemolyticus* YFB20; ▲, *V. parahaemolyticus* YFO21; ●, *V. parahaemolyticus* YFO22.

The virulence of environmental urease-positive *V. parahaemolyticus*

The virulence of urease-positive and hemolysin-negative *V. parahaemolyticus* isolated in the present study were investigated by injecting urease-positive strains (*V. parahaemolyticus* YKB4, YKB14, YFB20, YFO21, and YFO22), protease-positive strains (*V. parahaemolyticus* FM39 and FM50), hemolysin-positive (*V. parahaemolyticus* S34 and S72, *V. vulnificus* FM29, and *V. alginolyticus* FM13), *Vibiro* strains having both hemolysin and protease (*V. cholerae* non-O1 FM44 and *V. mimicus* FM4), and

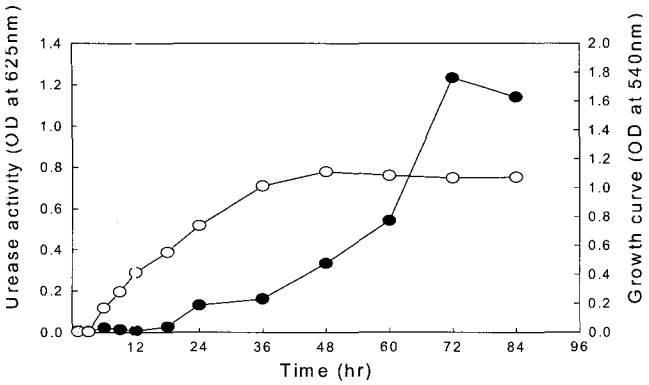

Fig. 2. Effect of urea on the urease productivity of *Vibrio parahaemolyticus* YFO22.
----, bacterial growth curve; ●, no urea in LB broth; ○, 0.2% urea in LB broth.

non-pathogenic *V. parahaemolyticus* (KCTC2471) into ICR mice. Each cell suspension (0.5 mL of 10^7-10^8 cfu/mL) was injected intraperitoneally into groups of 10 ICR mice (7- to 10-week-old male). The mortality from urease-positive *V. parahaemolyticus* YKB14, YKB4, YFO21, and YFO22 was significantly high, being 60-80%, while urease-positive *V. parahaemolyticus* YFB20 showed a mortality rate of 20%. Protease-positive *V. parahaemolyticus* FM39 and FM50 showed a 40 and 60% mortality rate, respectively. Hemolysin-positive *V. vulnificus* FM29 injected mice had a 40% mortality rate. With the exception of *V. vulnificus* FM29, however, hemolysin-positive *Vibrio* strains did not result in mortality, which is similar to non-pathogenic *V. parahaemolyticus* KCTC2471 (Table 2). The mortality rate of urease-positive *V. parahaemolyticus* strains in mice was measured for 12 hrs following infection, and the vibrio strains could be isolated from the viscera of the injected mice. The inoculated mice generally showed swollen maws and reddish viscera, compared to the non- inoculated mice (data not shown). From these results, the virulence of urease-positive *V. parahaemolyticus* was much stronger than those of hemolysin or protease-positive strains on ICR mice. Bacterial urease plays a central role in pathogenesis such as urolithiasis (stone formation), catheter encrustation, pyelonephritis, ammonia encephalopathy, hepatic encephalopathy, and inactivation of complement (Mobley et al., 1995). Urease of *P. mirabilis* could cause pyelonephritis in a rat or a mouse (Jones et al., 1990; Johnson et al., 1993). Urease-positive *Staphylococcus saprophyticus* is a frequent cause of UTI (urinary tract infection) in young, sexually active women (Osterberg et al., 1990). The active gastritis due to *H. pylori* is predominantly correlated to its urease (Hawtin et al., 1990; Evans et al., 1991; Cover and Blaser, 1995). Some recent studies indicated that the outbreak of gastroenteritis has been caused by hemolysin-negative and urease-positive *V. parahaemolyticus* (Honda et al., 1992; Suthienkul et al., 1995; Osawa et al., 1996; Okuda et al., 1997). In the present study, urease-positive *V. parahaemolyticus* strains showed much higher mortality than hemolysin

Table 2. The virulence of environmental *Vibrio* isolates producing various pathogenic factors

Phenotypic pathogenic factor	Strains	Lethal time (hr)	Mortality (%)
Buffered saline	Control	-	0
None	*V. parahaemolyticus* KCTC2471	-	0
Urease	*V. parahaemolyticus* YKB4	4-11	60
	V. parahaemolyticus YKB14	9-12	60
	V. parahaemolyticus YFO21	6-7	80
	V. parahaemolyticus YFO22	6-7	80
	V. parahaemolyticus YFB20	7.0-10	20
Protease	*V. parahaemolyticus* FM 39	4.0-13	40
	V. parahaemolyticus FM 50	5.0-8.0	60
Hemolysin	*V. parahaemolyticus* S34	-	0
	V. parahaemolyticus S72	-	0
	V. vulnificus FM29	8.0-16	40
	V. alginolyticus FM13	-	0
Both hemolysin and protease	*V. cholearae* non-O1 FM44	6.0-18	100
	V. mimicus FM4	6.0-7.0	60

FM and S strains were isolated in 2002 (Park et al., 2003) and in 2003 (Park et al., 2004), respectively, from Gwangan Beach seawater. Mortality indicates the ratio of death to the 10 mice inoculated.

or protease-positive strains. Therefore, the authors could suggest that the urease of *V. parahaemolyticus* was a very important phenotypic pathogenic factor.

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References

Abbott, S.L., C. Powers, C.A. Kaysner, Y. Takeda, M. Ishibashi, S.W. Joseph and J.M. Janda. 1989. Emergence of a restricted bioserovar of *Vibrio parahaemolyticus* as the predominant cause of Vibrio-associated gastroenteritis on the West Coast of the United States and Mexico. J. Clin. Microbiol., 27, 2891-2893.

Cai Y.L. and Y.X. Ni. 1996. Purification, characterization, and pathogenicity of urease produced by *Vibrio parahaemolyticus*. J. Clin. Lab. Anal., 10, 70-73.

Chang, D.S. and S. Shinoda. 1994. Toxin produced by pathogenic vibrios isolated from seafood. Bull. Kor. Fish. Soc., 27, 107-113.

Cover, T.L. and M.J. Blaser. 1995. *Helicobacter pylori*: a bacterial cause of gastritis, peptic ulcer disease, and gastric cancer. ASM News, 60, 21-26.

Dotevall, H., G. Jonson-Stromberg, S. Sanyal and J. Holmgren. 1985. Characterization of enterotoxin and soluble hemagglutinin from *Vibrio mimicus*: Identify with *V. cholerae* toxin and hemagglutinin. FEMS Microbiol. Lett. 27., 17-22.

Edward, P.D., J.M. Janda, I.A. Frederic and J.B. Edward. 1984. Comparative studies and laboratory diagnosis of *Vibrio vulnificus*, an invasive *Vibrio* sp. J. Clin. Microbiol., 19, 122-125.

Evans, D.J., D.G. Evans, S.S. Kirkpatrick and D.S. Graham. 1991. Characterization of the *Helicobacter pylori* urease and purification of its subunits. Microb. Pathog., 10, 15-26.

Finkelstein, R.A., M. Boesman-Finkelstein and P. Holt. 1983. *Vibrio cholerae* hemagglutinin/lectin/protease hydrolyzes fibronectin and ovomucin. Proc. Natl. Acad. Sci. USA, 80, 1092-1095.

Fujino, T., R. Sakazaki and K. Tamura. 1974. Designation of types strain of *Vibrio parahaemolyticus* and description of 200 strains of the species. Int. J. Syst. Bacteriol., 24, 447-449.

Gray, L.D. and A.S Kreger. 1985. Purification and characterization of an extracellular cytolysin produced by *Vibrio vulnificus*. Infect. Immun., 48, 62-72 .

Hawtin, P.R., A.R. Stacey and D.G. Newell. 1990. Investigation of the structure and localization of the urease of *Helicobacter pylori* using monoclonal antibodies. J. Gen. Micobiol., 136, 1995-2000.

Honda, S., S. Matsumoto, T. Miwatani and T. Honda. 1992. A survey of urease-positive *Vibrio parahaemolyticus* strains isolated from traveller's diarrhea, seawater and imported frozen seafoods. Eur. J. Epidemol., 8, 861-864.

Honda, T., M. Arita, T. Takeda, M. Yoh and T. Miwatani. 1985. Non O1 Vibrio cholerae produces two newly identified of toxins related to *Vibrio parahaemolyticus* hemolysin and *Escherichia coli* heat-stable enterotoxin. Lancet, II, 163-164.

Huq, M.I., D. Huber and G. Kibryia. 1979. Isolation of urease producing *Vibrio parahaemolyticus* strains from cases of gastroenteritis. Indian J. Med. Res., 70, 549-553.

Ichinose, Y., K. Yamamoto, N. Nakasone, M.J. Tanabe, T. Takeda, T. Miwatani and M. Iwanaga. 1987. Enterotoxicity of El Tor like hemolysin of *Vibrio cholerae* non-O1. Infect. Immun., 55, 1090-1093.

Johnson, D.E., R.G. Russell, C.V. Lockatell, J.W. Warren and H.L.T. Mobley. 1993. Contribution of *Proteus mirabilis* urease to persistence, urolithiasis, and acute pyelonephritis in a mouse model of ascending urinary tract infection. Infect. Immun., 61, 2748-2754.

Jones, B.D., C.V. Lockatell, D.E. Johnson, J.W. Warren and H.L.T. Mobley. 1990. Construction of a urease-negative mutant of *Proteus mirabilis*: analysis of virulence in a mouse model of ascending urinary tract infection. Infect. Immun., 58, 1120-1123.

Kaysner, C.A., C. Abeyta, P.A. Trost, W.E. Hill and M.M. Wekell. 1994. Urea hydrolysis can predict the potential pathogenicity of *Vibrio parahaemolyticus* strains isolated in the Pacific Northwest. Appl. Environ. Microbiol., 60, 3020-3022.

Kelly, M. and E.M.D. Stroh. 1989. Urease-positive, Kanagawa negative *Vibrio parahaemolyticus* from patients and the environment in the Pacific Northwest. J. Clin. Microbiol., 27, 2820-2822.

Kim, J.S. 1999. Characteristics of urease produced by *Vibrio parahaemolyticus*. Ph.D. Thesis, Dongeui University, Busan, Korea. pp. 99.

Kim, S.H., M.Y. Park, Y.E. Lee, M.H. Cho and D.S. Chang. 1997. Characteristics of hemolysin produced by *Vibrio cholerae* non-O1 FM-3 isolated from seawater. J. Kor. Fish. Soc., 30, 556-561.

Kosary, M.K. and A.S. Kreger. 1985. Production and partical characterization of an elastolytic protease of *Vibrio vulnificus*. Infect. Immun., 50, 534-540.

Larsen, J.L. 1984. *Vibrio angillarum*: Infection of temperature, pH, NaCl concentration and incubation time on growth. Appl. Bacteriol., 57, 237-246.

MacLaren, D.M. 1969. The significance of urease in *Proteus pyelonephritis*: a histological and biochemical study. J. Pathol. Bacteriol., 97, 43-49.

Magalhaes, M., Y. Takeda, V. Magalhaes and S. Tateno. 1992. Brazilian urease-positive strains of *Vibrio parahaemolyticus* carry genetic potential to produce the TDH-related hemolysin. Mem. Inst. Oswaldo Cruz., 87, 167-168.

Miyamoto, Y., T. Kato, Y. Obara, S. Akiyama, K. Takizawa

and S. Yamai. 1969. In vitro hemolytic characteristics of *Vibrio parahaemolyticus*: its close correlation with human pathogenicity. J. Bacteriol., 100, 1147-1149.

Miyoshi, S.I., H. Nakazawa, K. Kawata, K.I. Tomochika, K. Tobe and S. Shinoda. 1998. Characterization of the hemorrhagic reaction caused by *Vibrio vulnificus* metalloprotease, a member of the thermolysin family. Infect. Immun., 66, 4851-4855.

Mobley, H.L.T., M.D. Island and R.P. Hausinger. 1995. Molecular biology of mirobial ureases. Microbiol. Rev., 59, 451-480.

Musher, D.M., D.P. Griffith, D. Yawn and R.D. Rossen. 1975. Role of urease in pyelonephritis resulting from urinary tract infection with *Proteus*. J. Infect. Dis., 131, 177-181.

Nolan, C.M., J. Ballard, C.A. Kaysner, J.L. Lilja, L.P. Williams and F.C. Tenover. 1984. *Vibrio parahaemolyticus* gastroenteritis: an outbreak associated with raw oysters in the Pacific Northwest. Diagn. Microbiol. Infect. Dis., 2, 119-128.

Oberhofer, T.R. and J.K. Podgore. 1982. Urea-hydrolyzing *Vibrio parahaemolyticus* associated with acute gastroenteritis. J. Clin. Microbiol., 16, 581-583.

Okuda, J., M. Ishibashi, S.L. Abbott, J.M. Janda and M. Nishibuchi. 1997. Analysis of the thermostable direct hemolysin (tdh) gene and the tdh-related hemolysin (trh) genes in urease-positive strains of *Vibrio parahaemolyticus* isolated on the west coast of the United States. J. Clin. Microbiol., 35, 1965-1971.

Osawa, R., T. Okistum, H. Morozumi and S. Yamai. 1996. Occurrence of urease-positive *Vibrio parahaemolyticus* in Kanagawa, Japan, with specific reference to presence of thermostable direct hemolysin (TDH) and the TDH-related hemolysin genes. Appl. Environ. Microbiol., 62, 725-727.

Osterberg, E., H.O. Hallander, A. Kallner, A. Lundin, S.B. Svensoon and H. Aberg. 1990. Female urinary tract infection in primary health care: bacteriological and clinical characteristics. Scand. J. Infect. Dis., 22, 477-484.

Park, M.Y., H.J. Kim, S.T. Choi, E.K. Oh and D.S. Chang. 2002. Pathogenic factors of *Vibrio* spp. isolated from seawater of Gwangan Beach in Busan. J. Fish. Sci. Tech., 5, 178-182.

Park, M.Y., H.J. Kim and D.S. Chang. 2003. Pathogenic *Vibrio* spp. isolated from the Gwangan Beach of Busan, 2002. J. Fish. Sci. Techn., 6, 105-109.

Park, M.Y., C.W. Park, C.S. Kwon and D.S Chang. 2004. Distribution of pathogenic *Vibiro* spp. in the Gwangan Beach of Busan, 2003. J. Fish. Sci. Tech., 7, 10-15.

Sakazaki, R., S. Iwanami and H. Fukumi. 1963. Studies on the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*, I. Morphological, cultural, and biochemical properties and its taxonomical position. Jap. J. Med. Sci. Biol., 16, 161-188.

Sakazaki, R., K. Tamura, T. Kato, Y. Obara, S. Yamai and K. Hobo. 1968. Studies of the enteropathogenic, facultatively halophilic bacteria, *Vibrio parahaemolyticus*. III. Enteropathogenicity. Jap. J. Med. Biol., 21, 325-331.

Shao, C.P. and A.I. Hor. 2000. Metalloprotease is not essential for *Vibrio vulnificus* virulence in mice. Infect. Immun., 68, 3569-3573.

Starks, A.M., T.R. Schoeb, M.L. Tamplin, S. Parveen, T.J. Doyle, P.E. Bomeisl, G.M. Escudero and P.A. Gulig. 2000. Pathogenesis of infection by clinical and environmental strains of *Vibrio vulnificus* in iron-dextran-treated mice. Infect. Immun., 68, 5785-5793.

Suthienkul, O., M. Ishibashi, T. Iida, N. Nettip, S. Supave, J.B. Eampokalap, M. Makino and T. Honda. 1995. Urease production correlates with possession of the trh gene in *Vibrio parahaemolyticus* strains isolated in Thailand. J. Infect. Dis., 172, 1405-1408.

Twedt, R.M., P.L. Spualding and H.E. Hall. 1969. Morphological, cultural, biochemical, and serological comparison of Japanese strains of *Vibrio parahaemolyticus* with related cultures isolated in the United States. J. Bacteriol., 98, 511-518.

Weatherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem., 39, 971-974.

Zen-Yoji, H., R.A. Clair, K. Ohta and T.S. Montague. 1973. Comparison of *Vibrio parahaemolyticus* cultures isolated in the United States with those isolated in Japan. J. Infect. Dis., 127, 237-241.

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