Genetic Characterization of DNA Region Containing the trh and ure Genes of Vibrio parahaemolyticus

KWON-SAM PARK, TETSUYA IDA,* YOSHIHARU YAMAICHI,† TOMOHITO OYAGI, KOICHIRO YAMAMOTO;‡ AND TAKESHI HONDA

Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565-0871, Japan

Received 25 February 2000/Returned for modification 31 May 2000/Accepted 29 June 2000

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 and that the location of the two genes are in close proximity on the chromosome. Here, 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). 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, ureDABCDEFG 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.

Vibrio parahaemolyticus is a gram-negative, halophilic marine bacterium which causes gastroenteritis in humans who are infected through consumption of raw or inadequately cooked seafood (15). V. parahaemolyticus strains that are isolated from diarrheal patients produce either the thermostable direct hemolysin (TDH) or the TDH-related hemolysin (TRH), or both, while hardly any isolates from the environment have these properties (15, 41). TDH and TRH, encoded by tdh and trh, respectively, are each composed of 165-amino-acid residues and show approximately 67% identity in their amino acid sequences. TDH and TRH show several common biological activities, such as hemolytic activity, enterotoxicity, cytotoxicity, and cardiotoxicity, and are considered to be the major virulence factors in the pathogenesis of V. parahaemolyticus (15, 35, 41, 47).

Urease is an enzyme that catalyzes the hydrolysis of urea to ammonia and carbon dioxide. It is found in a number of bacteria, plants, fungi, and algae (29). Several bacterial urease genes have been isolated and characterized. The types of organization of gene clusters for urease in most bacteria are basically similar, comprising structural genes, accessory genes involved in the incorporation of the nickel ion into the apoenzyme and, in some cases, genes involved in a nickel uptake or regulation of urease expression (30). In common with other Vibrio species, generally only a small population (several percent) of clinical V. parahaemolyticus strains produce urease (7, 13, 16, 46). The relatively rare urease-positive phenotype of V. parahaemolyticus is always associated with the possession of the trh gene (26, 36, 37, 46), making urease production a reasonably good clinical diagnostic marker for virulent (trh-positive) V. parahaemolyticus (46). Our previous research has shown that the association is due to a genetic linkage between the structural gene for urease (ureC) and trh on the chromosome of virulent V. parahaemolyticus strains (17). In one such strain, using long and accurate PCR (LA-PCR), the distance between trh and ureC was shown to be less than 8.5 kb (18). We also recently reported that V. parahaemolyticus and other Vibrio species possess two chromosomes and that, when present, the trh and ureC genes are localized on the smaller replicon (52).

In this study, to precisely understand the nature of the genetic linkage between the trh and ure genes in V. parahaemolyticus, we cloned and sequenced a DNA region (ca. 16 kb) containing the genes from a clinical V. parahaemolyticus strain. Furthermore, we constructed mutant strains with disruption in the genes on this DNA region to examine the role of the genes to urease production and hemolytic activity of V. parahaemolyticus.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Table 1 shows the bacterial strains and plasmids used in this study. Clinical V. parahaemolyticus strains, including
PCR conditions were as follows: after initial denaturation at 94°C for 3 min, a cycle of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min was repeated 30 times.

Construction of mutant strains. Mutant strains, derived from *V. parahaemolyticus* TH3996, were constructed as previously described (51), except that the homologous recombination was done by a single-crossover event. Briefly, to construct the mutants, using the chromosomal DNA of *V. parahaemolyticus* TH3996 as a template, we amplified partial sequences of *ureR*, *nikD*, and *ureC* by PCR. Each amplified fragment was cloned into a pT7-Blue vector and then digested with BamHI and PstI. These fragments were cloned into a R6K-ori suicide vector, pKY719 (33), digested with BamHI and PstI. These plasmids were introduced into *E. coli* SM10pir and then mated with *V. parahaemolyticus* TH3996. Conjugation was performed on nitrocellulose membrane laid over LB agar plates with incubation for 4 h at 37°C. The membrane was suspended in LB broth and inoculated onto TCSB agar plates containing 5 μg of chloramphenicol per ml and incubated overnight at 37°C. Chloramphenicol-resistant colonies were isolated and screened by Southern blot analysis for single-crossover mutation of *ureR*, *nikD*, or *ureC*.

Urease assay. Bacteria were grown at 37°C with shaking in 100 ml of LB broth (3% NaCl) supplemented with 0.1% sterile urea after autoclaving of the medium. The organisms were harvested by centrifugation at 10,000 × *g* for 10 min, washed three times with 20 mM sodium phosphate (pH 7.0–7.4) solution (100 μl), and disrupted by sonication. The supernatant was obtained after centrifugation at 4°C (12,000 × *g*, 20 min). The supernatant was used both for the urease assay and for the determination of protein quantity using the Bio-Rad DC protein assay kit (Bio-Rad). Urease activity was determined using an ammonia test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan) to measure the release of ammonia. The amount of ammonia produced was calculated by referring to a standard curve made for known concentrations of ammonium chloride. Urease activity was expressed as micromoles of urea hydrolyzed per minute per milligram of protein.

Hemolytic activity assay. Wild-type and mutant strains were cultured at 37°C for 16 h with shaking in SPP medium (14) (5 g of NaCl, 10 g of peptone, 2 g of glucose, and 5 g of dibasic sodium phosphate per liter in distilled water, pH 7.6). The supernatant was obtained by centrifugation (12,000 × *g*, 10 min) at 4°C. The hemolytic activity was determined by a modification of the previously described method (14). Briefly, sheep erythrocytes were washed in phosphate-buffered saline (PBS) three times and adjusted to a hematocrit of 4% with PBS. Equal volumes (100 μl each) of supernatant serially diluted with PBS and erythrocytes were mixed and incubated at 37°C in a water bath for 1 h and then centrifuged at 2,000 × *g* for 2 min. Supernatant samples (160 μl) of the reaction mixtures were taken for spectrophotometric measurement of released hemoglobin at 540 nm on a 96-well plate using a Multiskan Mcc340 (Labosystems, Tokyo, Japan).

**RESULTS**

Cloning and sequencing of the region containing the *thr* and *ure* cluster. For the cloning of the region containing the *thr* and *ure* cluster, total chromosomal DNA was isolated from *V. para-
$V.~parahaemolyticus$ TH3996 and completely digested with several restriction enzymes. The DNA fragments were Southern blotted with a digoxigenin-labeled $ure$ probe (17). The 11.8-kb $Xho\text{I}$ fragment and the 7.3-kb $Bgl\text{II}$ fragment which hybridized with the $ure$ probe were cloned to the pUC119 vector at the $Sal\text{I}$ and $Bam\text{HI}$ sites (designated pKS1 and pKS2, respectively). The adjacent region of the 11.8-kb $Xho\text{I}$ fragment, which as a 4.0-kb $Spe\text{I}$ fragment hybridized with probe A (Fig. 1), was cloned into the $Xba\text{I}$ site of the pUC19 vector (designated pKS3). In the end, the total region of approximately 16 kb that contains the $trh$ and $ure$ cluster was cloned (Fig. 1). After analysis of the restriction maps, each fragment was digested with appropriate restriction enzymes and subcloned into the corresponding sites of the pUC119 vector. The complete nucleotide sequence was determined in both directions.

**Nucleotide sequence analysis.** Nucleotide sequence analysis of the DNA region revealed that the region is 15,754 bp in size and possesses a total of 16 ORFs (Fig. 1 and Table 2). ORF1 as homologous with a transposase found in $Vibrio~anguillarum$ (88% identity; L40498) and was flanked by 18-bp inverted repeats (5'-GGCTTTGTTGCGTAATTC-3') in both sides. ORF2 was the $trh$ gene. ORF3 had some homology with two positive regulators for urease expression, $P.~mirabilis$ UreR (51% identity) and $E.~coli$ UreR (50% identity; P32326). In addition, the ORF contained a typical bacterial regulatory AraC family signature located at amino acid positions 215 to 257. Therefore, ORF3 was designated UreR.

ORF4 and ORF8 showed striking amino acid sequence similarities to components of the peptide-binding-protein-dependent transport systems and ATP-binding cassette (ABC) transporters of several gram-positive and gram-negative bacteria, such as the $dpp$ operon, membrane-associated complexes of five proteins belonging to the ABC transporter family of $Streptococcus~pyogenes$ (39), the $Salmonella~enterica$ serovar Typhimurium $opp$ operon (12), the periplasmic binding-protein-dependent transport system, and the $E.~coli$ $nik$ operon, which encode the specific transport system for nickel (34). To determine the possible contribution of these ORFs to urease production, ORF5 was disrupted by homologous recombination. The disruption of ORF5 resulted in the near abolishment of urease activity (see below) (Table 3). Thus, it seems likely that the five ORFs are components of an ABC transporter which mediates the transport of the nickel ion from the exterior to the cytosol and is designated $nikABCDE$.

The $nik$ operon was in the same direction of transcription as the ORF1 to ORF3 genes (Fig. 1). $NikA$, ORF8, comprised 546

**TABLE 2. ORFs identified in the region containing the $trh$ and $ure$ gene cluster**

<table>
<thead>
<tr>
<th>ORF</th>
<th>Gene</th>
<th>Position</th>
<th>No. of aa*</th>
<th>Accession no.</th>
<th>% Identity</th>
<th>Homologous ORFs in database</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Transposase</td>
<td>1042</td>
<td>125</td>
<td>306</td>
<td>10498</td>
<td>Transposase ($V.~anguillarum$)</td>
</tr>
<tr>
<td>2</td>
<td>$trh$</td>
<td>1936</td>
<td>1370</td>
<td>189</td>
<td>67850</td>
<td>TRH ($V.~parahaemolyticus$)</td>
</tr>
<tr>
<td>3</td>
<td>$ureR$</td>
<td>3220</td>
<td>2375</td>
<td>282</td>
<td>40644</td>
<td>UreR ($P.~mirabilis$)</td>
</tr>
<tr>
<td>4</td>
<td>$nikE$</td>
<td>3990</td>
<td>3370</td>
<td>207</td>
<td>67221</td>
<td>DppE ($S.~pyogenes$)</td>
</tr>
<tr>
<td>5</td>
<td>$nikD$</td>
<td>4682</td>
<td>3993</td>
<td>230</td>
<td>67220</td>
<td>DppD ($S.~pyogenes$)</td>
</tr>
<tr>
<td>6</td>
<td>$nikC$</td>
<td>5485</td>
<td>4673</td>
<td>271</td>
<td>67218</td>
<td>DppB ($S.~pyogenes$)</td>
</tr>
<tr>
<td>7</td>
<td>$nikB$</td>
<td>6449</td>
<td>5490</td>
<td>320</td>
<td>67218</td>
<td>Putative transmembrane protein ($S.~typhimurium$)</td>
</tr>
<tr>
<td>8</td>
<td>$nikA$</td>
<td>7983</td>
<td>6346</td>
<td>546</td>
<td>67218</td>
<td>Putative substrate-binding protein ($S.~typhimurium$)</td>
</tr>
<tr>
<td>9</td>
<td>$ureD$</td>
<td>8389</td>
<td>9228</td>
<td>280</td>
<td>7089</td>
<td>UreD ($P.~mirabilis$)</td>
</tr>
<tr>
<td>10</td>
<td>$ureA$</td>
<td>9249</td>
<td>9548</td>
<td>100</td>
<td>3282</td>
<td>UreA ($E.~coli$)</td>
</tr>
<tr>
<td>11</td>
<td>$ureB$</td>
<td>9562</td>
<td>9882</td>
<td>107</td>
<td>7087</td>
<td>UreB ($P.~mirabilis$)</td>
</tr>
<tr>
<td>12</td>
<td>$ureC$</td>
<td>9882</td>
<td>11582</td>
<td>567</td>
<td>7086</td>
<td>UreC ($P.~mirabilis$)</td>
</tr>
<tr>
<td>13</td>
<td>$ureE$</td>
<td>11640</td>
<td>12116</td>
<td>159</td>
<td>7090</td>
<td>UreE ($P.~mirabilis$)</td>
</tr>
<tr>
<td>14</td>
<td>$ureF$</td>
<td>12147</td>
<td>12809</td>
<td>221</td>
<td>7091</td>
<td>UreF ($P.~mirabilis$)</td>
</tr>
<tr>
<td>15</td>
<td>$ureG$</td>
<td>12821</td>
<td>13456</td>
<td>212</td>
<td>06206</td>
<td>UreG ($P.~mirabilis$)</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>14836</td>
<td>15660</td>
<td>275</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* aa, amino acid(s).
dine tail, which suggests a role in Ni2+
involved in the incorporation of nickel into the urease apoenzyme. UreF, and UreG, which encode the urease accessory proteins P. mirabilis UreE, UreF, and UreG was also found with aemolyticus (UreD) showed homology with transcribed in the direction opposite to ORF1 to ORF8. ORF9 identified at amino acid positions 317 to 323 of the V. para-
sequence for the urease active sites (MVCHHLD) (27) was K. aerogenes 136, and His-246 of the bacterial ureases, were conserved in relative positions in the which have been shown to have functional significance in other. Amino acid residues in the structural subunits, K. aerogenes and ters found in other bacterial species such as V. parahaemolyticus UreC. Furthermore, the consensus se-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean urease activitya (μmol of NH₃/min/mg of protein) ± SEM at:</th>
<th>Mean hemolytic activity (%b) ± SEM</th>
<th>Mean FAc 6 ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 h</td>
<td>16 h</td>
<td></td>
</tr>
<tr>
<td>TH3996</td>
<td>2.500 ± 0.200</td>
<td>2.460 ± 0.200</td>
<td>100</td>
</tr>
<tr>
<td>TH3996 (nikD)</td>
<td>0.020 ± 0.010</td>
<td>0.020 ± 0.010</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>TH3996 (ureR)</td>
<td>0.020 ± 0.010</td>
<td>0.020 ± 0.010</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>TH3996 (ureC)</td>
<td>0.010 ± 0.010</td>
<td>0.010 ± 0.010</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>TH3996 (trh)</td>
<td>2.490 ± 0.200</td>
<td>2.450 ± 0.020</td>
<td>0</td>
</tr>
</tbody>
</table>

a Values represent the means of three experiments, each conducted in duplicate.

b Hemolytic activity in culture supernatant after incubation of the strains in SPP medium at 37°C for 16 h with shaking. The percentage of hemolytic activity for the wild type was set at 100%. Values represent the means of six experiments.

c FA, amount of accumulated fluid in milliliters per length (in centimeters) of ligated rabbit small intestine. Values represent the means of six experiments.

during the assembly of urease or the insertion of Ni2+. We failed to find any significant homology of ORF16 protein with any sequences in GenBank.

The 16 ORFs appear to be grouped into two transcription directions. From ORF1 to ORF8 the genes are transcribed in the opposite direction to the remaining genes, ORFs 9 to 16. We found that the average G+C content of the DNA sequence, at 41.0%, was lower than the G+C content of the V. parahaemolyticus genome (46 to 47%) (1).

Construction of ureR, nikD, and ureC mutant strains. To determine the possible contribution of a putative nickel transport gene operon and urease gene cluster to urease production and hemolytic activity, each of the genes (ureR, nikD, and ureC) was disrupted by homologous recombination in V. parahaemolyticus TH3996.

In the wild-type strain, hybridization of a BglII and XhoI digests of chromosomal DNA using probes specific for ureR or nikD showed a common 5.3-kb fragment. This fragment was replaced with 9.4-kb hybridizing fragments in the ureR and nikD mutants. The BglII digest of chromosomal DNA in the wild-type strain showed a single 7.3-kb fragment using a specific ureC probe. This fragment was also replaced with an 11.4-kb hybridizing fragment, a finding consistent with the size expected from insertion of the suicide vector into each gene, in the ureC mutant (data not shown). When the same digest of chromosomal DNA was examined using a Cm (a gene of chloramphenicol resistance) probe, each of the mutant strains (ureR, nikD, and ureC) was hybridized with the probe but no signal was detected in the wild-type strain. Mutant strains grew at a similar rate to that of the wild type (data not shown). We used the mutant strains to examine urease activity and hemolytic activity.

Urease activity of wild and mutant strains. In P. mirabilis, which possesses the ureR gene, urease production is not regulated by the nitrogen regulatory system (ntr) or catabolite repression but is induced by urea and the induction is mediated by ureR (8). Because V. parahaemolyticus TH3996 has the ureR gene in common with P. mirabilis, we investigated whether or not the urease production of the TH3996 strain is affected by the presence of urea. In the LB broth with 3% NaCl without urea, there was little urease production. With 0.1% urea supplementing the medium, however, urease production was 100 times greater. These data suggest that urea is involved in the induction of V. parahaemolyticus urease. Therefore, the following experiments for examining urease production were conducted with media containing 0.1% urea. The urease activities of ureR, nikD, and ureC mutant strains were very low, even in the presence of 0.1% urea (Table 3). In contrast, under the same conditions the urease activity of the trh mutant strain (51) did not change compared with the wild-type strain. These results suggest that ureR, nikD, and ureC genes are essential for active urease production. From these results, we concluded that the ureR, nikD, and ureC genes are important for urease expression, whereas trh is not.

Hemolytic activity of mutant strains. Next, we investigated the possible contribution of the ureR, nikD, and ureC genes to hemolysin (TRH) production by the TH3996 strain by comparing the hemolytic activity of wild-type and mutant strains. As shown in Table 3, the nikD, ureR, and ureC mutant strains retained the same hemolytic activity.

Enterotoxicity of mutants as shown by the rabbit ileal loop test. To investigate the enterotoxicity of the mutant strains, we examined the enterotoxic activity of the wild-type and mutant strains in rabbit ileum. The wild-type or mutant strains (10⁶ CFU) were injected into the ligated ileal loops of rabbits which were sacrificed after 16 h. After removal of the small intestine
the fluid accumulation in the ligated ileal loops was measured (Table 3). Except for the thr mutant strain, no significant differences in fluid accumulation were observed in samples from wild-type and mutant strains. These results indicate that the presence or absence of urease production by the V. parahaemolyticus strain does not affect the enterotoxicity of the organism under these test conditions.

**Distribution of ureR and nik operon in clinical V. parahaemolyticus strains.** In previous studies, we demonstrated that the possession of thr completely coincided with the presence of the ureC (a structural gene of urease) by clinical V. parahaemolyticus strains and that, in strain AQ4673, which possesses the two genes, the distance between thr and ureC is within several kilobases (17, 18). Consistent with these previous results, nucleotide sequence analysis of TH3996 showed that the distance between the two genes is 7,945 bp. To investigate the distance between the two genes of the other thr-positive clinical strains of V. parahaemolyticus, we performed LA-PCR using oligonucleotide primer pairs targeting thr and ureC (18).

All of the strains for testing were amplified accurately to the same ampiclon size as the AQ4673 strain (data not shown). Next, we used colony hybridization with specific probes for ureR and nikD to test for the presence or absence of the ureR and nik operon in clinical and environmental strains of V. parahaemolyticus. The ureR and nikD genes were present in all of the thr-positive strains (n = 8) but not in Kanagawa phenomenon (KP)-positive strains (n = 12) and the environmental strains (n = 6) (data not shown).

These data suggest that the DNA region containing the thr and urease gene cluster is present in thr-positive and environmental V. parahaemolyticus.

**DISCUSSION**

Urease, which requires the nickel ion in the active site, catalyzes the hydrolysis of urea to ammonia and carbon dioxide, resulting in net increase in environmental pH (30). So far, urease gene clusters have been isolated in several bacteria, such as P. mirabilis (19, 20, 44), K. aerogenes (23, 31, 32), Helicobacter pylori (2, 4, 22), Bacillus sp. strain TB-90 (25), Streptococcus salivarius (42), and Yersinia enterocolitica (5, 43). The expression of bacterial urease genes is regulated by different mechanisms (3). For example, urease expression in K. aerogenes is activated only under nitrogen-limiting conditions (24).

P. mirabilis urease is induced by urea and is mediated by the positive transcriptional regulator ureR (8). S. salivarius urease is regulated by pH (42).

We previously reported that the urease production by clinical V. parahaemolyticus strains correlates completely with the possession of the thr gene, and research by others has shown similar results (26, 36, 37). We have also found that the distance between the two genes is within several kilobases (17, 18), suggesting the genetic linkage between the two genes. In the present study, we demonstrated this genetic linkage. To investigate the coexistence and proximity of the thr and ure genes, we cloned and sequenced the region containing the thr and ure gene cluster on the chromosome of TH3996, a clinical V. parahaemolyticus strain that has the thr gene and produces urease.

Nucleotide sequencing showed that the ure gene cluster of V. parahaemolyticus is comprised of eight genes. The structural genes, ureA, ureB, and ureC, which encode subunits of the enzyme, are flanked immediately upstream by ureD, encoding a chaperone-like protein, and downstream by the ureE, ureF, and ureG accessory genes that encode the proteins required for incorporating the nickel ion into the metallocenter within the active site (30). In addition, ureR, the positive regulatory gene, lies 5.2 kb upstream of ureD and is oriented opposite the other seven genes. The predicted polypeptide sequence from this gene contains a putative helix-turn-helix DNA-binding motif, suggesting that this protein belongs to the AraC family of positive regulators. Members of the AraC family are primarily involved in the positive regulation of a number of genes (10).

As our data show, in the absence of urea, expression of V. parahaemolyticus urease is very low, but when 0.1% urea is present in the medium, urease expression is about 100 times greater. This increase was not seen in samples of the mutant strain which had undergone disruption of ureR. From this we conclude that V. parahaemolyticus urease is induced by urea and that this induction is mediated by ureR. The organization and sequences of the ure gene cluster of V. parahaemolyticus were similar to that of P. mirabilis. Furthermore, the regulation of the expression of urease is similar in the two species (Table 3). The major difference between the V. parahaemolyticus and P. mirabilis urease gene clusters was the presence or absence of a nickel transporter operon between ureR and ureD.

Between ureR and ureD of V. parahaemolyticus there were five overlapping genes that had significant homology to the nik operon of E. coli (34). These genes appear to encode a typical ABC transporter system, including ATP-binding polypeptides and integral cytoplasmic membrane proteins (9). NikB and NikC were highly hydrophobic proteins with six potential transmembrane domains, which may form the channel for the substrate transport. NikD and NikE contain Walker A and B motifs responsible for coupling energy to the nickel transport system (49). To investigate the role of the ABC transport system, we constructed a nikD disrupted mutant by homologous recombination. The mutant strain showed almost complete absence of urease activity compared with the wild-type strain. Consequently, it is plausible to suggest that the ABC transport system that we found contributes to nickel transport through the bacterial cytoplasmic membrane. ABC-type transporters of nickel ions from the extracellular milieu into bacterial cells, such as an ABC transporter in H. pylori (11), have also been found; however, similar genes have not been reported in other bacteria that possess the urease gene cluster. In H. pylori, the ABC transporter genes are not located close to the urease gene cluster on the genome (48). Interestingly, the nik genes of V. parahaemolyticus are located between the ureR and the ure genes. Thus, this is the first report of finding genes for the ABC-type nickel transport system adjacent to the urease gene cluster on the genome of an organism.

LA-PCR analysis showed the distance between the thr and ureC of all the thr-positive V. parahaemolyticus samples we tested to be precisely 7.9 kb. ureR and nik operon were consistently present in thr-positive strains (regardless of tdh possession) but not in KP-positive strains, which possess two copies of tdh, or in environmental strains. The sequencing results showed ORF1 to be a transposase. These findings suggest the possibility that the thr, nik operon, and ure gene cluster were transmitted into V. parahaemolyticus strains through a mechanism mediated by insertion sequences in the past.

Although the rabbit ileal loop test showed that the enterotoxicity of the ureR, ureC, and nikD mutant strains did not change compared with the TH3996 strain that they were derived from, we cannot completely rule out the possibility that the V. parahaemolyticus urease plays a certain role in the pathogenesis of this bacterium. In the case of Y. enterocolitica, urease appears to act as a virulence factor by enhancing the survival of bacteria during their passage through the stomach, presumably by neutralizing hydrogen ions which penetrate
the bacterial cell wall (6). The contribution of urease to the pathogenesis of *V. parahaemolyticus* is currently being analyzed in our laboratory.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan; a Grant for International Health Cooperation Research from the Ministry of Health and Welfare of Japan; and the “Research for the Future” Program of the Japan Society for the Promotion of Sciences (JSPS-RFTF 97L00704).

REFERENCES


complete genome sequence of the gastric pathogen Helicobacter pylori. Nature 388:539–547.


