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NOTES

Identification of the *Vibrio vulnificus wbpP* Gene and Evaluation of Its Role in Virulence

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A *wbpP* gene encoding a putative UDP-*N*-acetyl-D-glucosamine C_4 epimerase was identified and cloned from *Vibrio vulnificus*. The functions of the *wbpP* gene, assessed by the construction of an isogenic mutant and by evaluating its phenotype changes, demonstrated that WbpP is essential in both the pathogenesis and the capsular polysaccharide biosynthesis of *V. vulnificus*.

The pathogenic marine bacterium *Vibrio vulnificus* is a causative agent of food-borne diseases, such as life-threatening septicemia and possibly gastroenteritis, in individuals with underlying predisposing conditions (8, 17, 31). Surface polysaccharides, such as capsular polysaccharides (CPS) and lipopolysaccharides, play crucial roles in the pathogenicity of gram-negative bacteria by assisting the bacteria to evade host defenses. CPS production is believed to be a major virulence factor of *V. vulnificus* that is essential for pathogenicity (8, 17, 31). Encapsulated strains of *V. vulnificus* that are virulent in mice have opaque colony morphologies on an agar surface, whereas acapsular transposon mutants are no longer virulent and appear translucent (34). Meanwhile, partially encapsulated *V. vulnificus* translucent-phase variants fall between the fully encapsulated wild type and acapsular transposon mutants in terms of their virulence and serum resistance (33, 34), thereby indicating that the amount of CPS on the cell surface correlates positively with the virulence of *V. vulnificus*.

However, very little is known about the biosynthetic pathway for the capsular polysaccharide of *V. vulnificus*, and the genes encoding the enzymes involved in the production of the capsular polysaccharide have not yet been identified. Nonetheless, it is generally believed that a similar biosynthetic pathway operates in gram-negative bacteria. Thus, the biosynthetic pathways and molecular genetics of surface polysaccharide production have been widely studied in *Pseudomonas aeruginosa* (1). The common glycolytic metabolite glucose 1-phosphate is first converted to UDP-*N*-acetyl-D-glucosamine (UDP-GlcNAc), the main activated precursor of surface-associated carbohydrate synthesis (1, 5). UDP-*N*-acetyl-D-galactosamine (UDP-GalNAc) is then formed by the C_4 epimerization of UDP-GlcNAc (5). UDP-*N*-acetyl-D-galactosaminuronic acid (UDP-GalNAcA), the product of the further dehydrogenation of UDP-GalNAc, is an important intermediate used for the biosynthesis of different uronic acid sugars of surface polysaccharides that contain GalNAcA or its derivatives, not only in *P. aeruginosa*, but also in other organisms (36). Also, it has been recently reported that the epimerization is performed by the gene products of *wbpP* (1).

So far, a great diversity of capsular types have been presented among different isolates of *V. vulnificus*, and more than 13 CPS chemotypes were identified by chromatographic analysis and nuclear magnetic resonance spectroscopy (9). Yet, compared with the substantial body of literature concerned with the structural determination of the CPS from *V. vulnificus* (4, 9, 24, 25), only a few studies have reported on the identification of the genes involved in *V. vulnificus* capsule expression (29, 32, 37). Accordingly, the present study screened a mutant exhibiting decreased opaque colony morphology from a library of *V. vulnificus* mutants constructed by random transposon mutagenesis, and a homologue of *P. aeruginosa wbpP* was identified and cloned by a transposon-tagging method. The functions of the *wbpP* gene in CPS production and in virulence were assessed by the construction of an isogenic mutant of *V. vulnificus* in which the *wbpP* gene was inactivated by allelic exchanges, and by evaluating its phenotype changes in vitro and in mice.

The bacterial strains and plasmids used in this study are listed in Table 1. Unless otherwise indicated, the *V. vulnificus* strains were grown in Luria-Bertani medium supplemented with 2.0% (wt/vol) NaCl (LBS). Cultures of the *V. vulnificus* strains were grown at 30°C with aeration; 5-ml samples were removed at log phase for determination of cell densities, WbpP activities, and cellular protein concentrations. The WbpP activities were determined according to the method of Creuzenet et al. (5). A unit of enzyme activity was defined as the conversion of 1 μmol of UDP-GlcNAc into UDP-GalNAc per 10 min as previously described (5). The protein concentrations were

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TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Relevant characteristics[a]	Reference or source
Bacterial strains		
V. vulnificus		
ATCC29307	Clinical isolate; virulent	Laboratory collection
NY018	ATCC 29307 with *wbpP*::*nptI*; Km^r	This study
E. coli		
SM10 λ *pir*	*thi thr leu tonA lacY supE recA*::RP4-2-Tc::Mu λ*pir* Km^r; host for π-requiring plasmids; conjugal donor	20
Plasmids		
Mini-Tn*5 lac*Z1	R6K γ *ori*; suicide vector; *oriT* of RP4; Ap^r	6
pDM4	R6K γ *ori; sacB*; suicide vector; *oriT* of RP4; Cm^r	21
pLAFR3	IncP *ori*; cosmid vector; Tc^r	30
pRK415	IncP *ori*; broad-host-range vector; *oriT* of RP4; Tc^r	13
pNY0400	Cosmid library containing *wbpP*; Tc^r	This study
pNY0413	pRK415 with *wbpP*; Tc^r	This study
pNY0421	pDM4 with *wbpP*::*nptI*; Cm^r Km^r	This study

[a] Ap^r, ampicillin resistant; Cm^r, chloramphenicol resistant; Km^r, kanamycin resistant; Tc^r, tetracycline resistant.

determined by the method of Bradford (3), with bovine serum albumin as the standard. The averages and standard errors of the mean (SEM) were calculated from at least three independent trials.

Cloning and sequence analysis of the *wbpP* gene. Previously, we generated a library of *V. vulnificus* mutants by random transposon mutagenesis using a mini-Tn*5 lacZ1* (6, 27) (Table 1). Then, a mutant exhibiting decreased opaque colony morphology was screened from this mutant library, and a DNA segment flanking the transposon insertion was amplified from the genomic DNA of the mutant by PCR, as described previously (27). Since the nucleotide sequence of the resulting PCR product, a 310-bp DNA fragment, revealed 71% identity with that of *P. aeruginosa wbpP*, the DNA was labeled with [α-^{32}P]dCTP and named WbpPP. To clone the full *V. vulnificus wbpP* gene, a cosmid library of *V. vulnificus* ATCC 29307 constructed using pLAFR3 (27, 30) (Table 1) was screened using WbpPP as a probe. A colony exhibiting a positive signal was isolated, and the cosmid DNA was purified and named pNY0400 (Fig. 1A and Table 1).

The nucleotide sequence revealed a coding region consisting of 1,023 nucleotides, and the coding region was named *wbpP* of *V. vulnificus* (Fig. 1A). The amino acid sequence deduced from the *wbpP* nucleotide sequence revealed a protein, WbpP, composed of 340 amino acids with a theoretical molecular mass of 37,962 Da and a pI of 5.50. A database search for amino acid sequences similar to those deduced from the *wbpP* coding region discovered three other proteins involved in surface carbohydrate (polysaccharide) biosynthesis from *Shigella sonnei*, *Escherichia coli*, and *Salmonella enterica* serovar Typhi with high levels of identity (Fig. 1B) (http://www.ncbi.nml.nih.gov). The amino acid sequence of *V. vulnificus* WbpP was 70.7 to 63.8% identical to those of these proteins, and this identity appeared evenly throughout the proteins (data not shown).

Among these, the gene product of *P. aeruginosa wbpP* has been biochemically and genetically well characterized (1, 36). Also, the enzymatic characteristics of WbpP have recently been confirmed (5). WbpP is an NAD(H)-dependent UDP-GlcNAc C_4 epimerase that produces UDP-GalNAc, which is an important intermediate for surface carbohydrate biosynthesis in *P. aeruginosa* (1). The functions of WbgU of *S. sonnei*, WbqB of *E. coli*, and WcdB of *S. enterica* serovar Typhi are less well characterized, and hence, the proteins have different names. Nonetheless, recent genetic studies support the notion that they are all homologs with similar, if not identical, functions and that they all contribute to surface carbohydrate biosynthesis (28, 35). Therefore, all of this information suggests

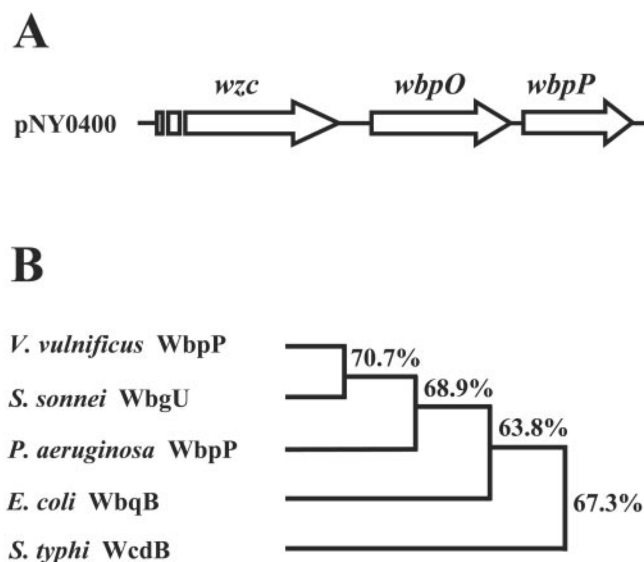

FIG. 1. *V. vulnificus wbpP* gene locus and relatedness of WbpP and other proteins involved in surface carbohydrate biosynthesis in Enterobacteriaceae. (A) Plasmid pNY0400 was used to determine the nucleotide sequence of *wbpP*. The open arrows represent the locations of a complete ORF (*wbpP*) and two less characterized ORFs (*wzc* and *wbpO*) and the directions of their transcription. (B) The dendrogram showing the amino acid sequence relatedness of *V. vulnificus* WbpP and gene products of putative polysaccharide biosynthesis genes was derived using the CLUSTALW alignment program (http://www.ebi.ac.uk/clustalw/) and is based on the amino acid sequences in the GenBank databases (NCBI).

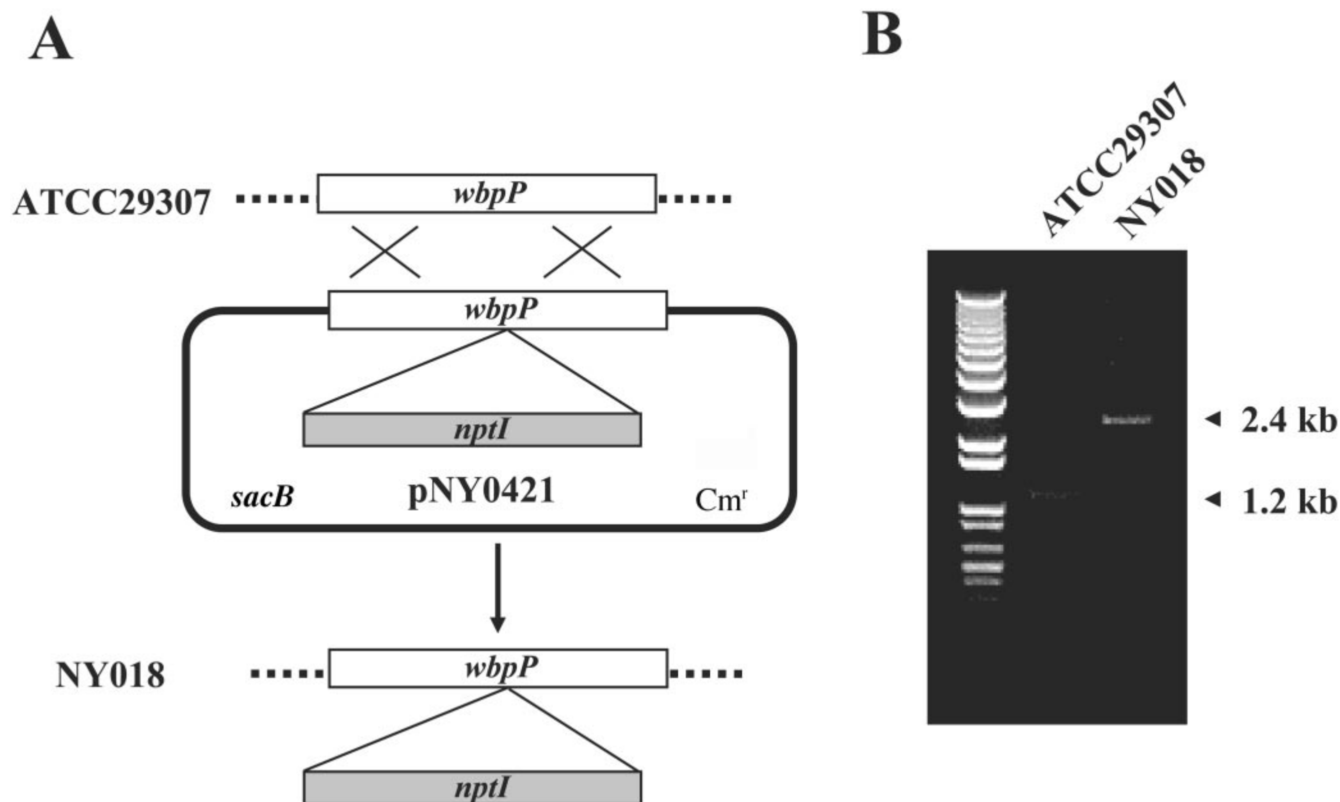

FIG. 2. Allelic-exchange procedure and construction of *wbpP*::*nptI* isogenic mutant. (A) Double homologous recombinations between the wild type (ATCC 29307) and plasmid pNY0421 led to an interruption of the *wbpP* gene and resulted in the construction of the *wbpP* mutant NY018. The dashed lines represent the bacterial chromosome; the full line, the plasmid DNA; the open box, the target *wbpP* gene; the shaded box, the *nptI* gene; and the large Xs, genetic crossing over. *sacB*, levansucrase gene. (B) PCR analysis of the wild type and NY018 generated by allelic exchanges. Molecular size markers (1-kb-plus DNA ladder; Invitrogen, Carlsbad, CA) and PCR products are indicated.

that the *wbpP* gene also encodes the protein required for surface carbohydrate biosynthesis by *V. vulnificus*.

Generation and confirmation of a *wbpP*::*nptI* mutant. The role of the gene product of *wbpP* in virulence was examined by constructing a *wbpP* mutant of *V. vulnificus*. To inactivate *wbpP* in vitro, 1.2-kb *nptI* DNA conferring resistance to kanamycin (23) was inserted into a unique BamHI site present within the *wbpP* open reading frame (ORF) to produce pNY0421 (Fig. 2A and Table 1). *E. coli* SM10 λ *pir* (containing pNY0421) was used as a conjugal donor to generate the *wbpP*::*nptI* mutant of *V. vulnificus* ATCC 29307 by homologous recombination (Fig. 2A). The *V. vulnificus wbpP* mutant chosen for further analysis was named NY018. The conjugation and isolation of the transconjugants were conducted using methods previously described (10), and a double crossover, in which the wild-type *wbpP* gene was replaced with the *wbpP*::*nptI* allele, was confirmed by a PCR as shown in Fig. 2B. The PCR analysis of the genomic DNA from ATCC 29307 using the primers WbpP005F 5′-ATTCTGCAGTGGGAGGATAGAGATAAA TCTTC-3′ and WbpP011R (5′-ATTGAATTCTATAATAGC TTCTTCATCATATGA-3′) produced a 1.2-kb fragment (Fig. 2B), whereas the genomic DNA from NY018 resulted in an amplified DNA fragment approximately 2.4 kb in length. This 2.4-kb fragment was in agreement with the projected size of the DNA fragment containing the wild-type *wbpP* (1.2 kb) and the *nptI* gene (1.2 kb).

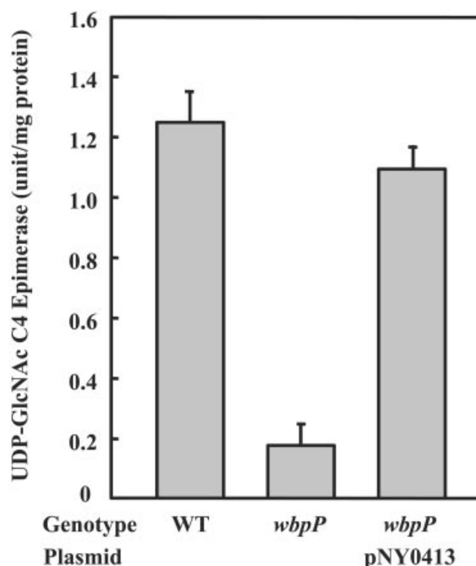

FIG. 3. Effect of *wbpP* gene mutation on UDP-*N*-acetyl-D-glucosamine C_4 epimerase. Cultures of the wild type (WT) and NY018 (*wbpP*) were grown in LBS, and the UDP-*N*-acetyl-D-glucosamine C_4 epimerase activities were determined from samples removed at an optical density at 600 nm of 0.8. Complementation of the mutant with a functional *wbpP* (pNY0413) is also presented as indicated. Relative activities of the UDP-*N*-acetyl-D-glucosamine C_4 epimerase were measured as described in the text. The error bars represent the SEM.

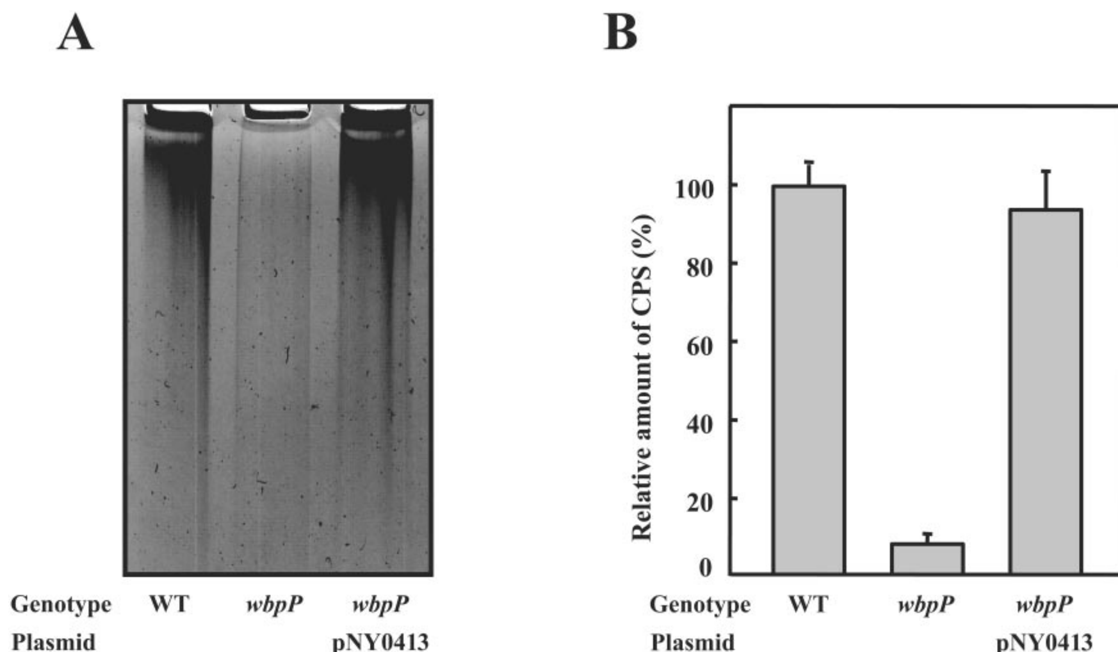

FIG. 4. Analyses of CPS. (A) The method described by Enos-Berlage and McCarter (7) was used to isolate the CPS from the wild type (WT), NY018 (*wbpP*), and complemented strain as indicated. After separation on 5% polyacrylamide gels, the CPS was visualized by silver staining as described by Kelley and Parker (14). (B) The relative amounts of CPS from each strain are presented based on the amount of the CPS of the wild type as 100%. The error bars represent the SEM.

Effect of *wbpP* gene mutation on UDP-GlcNAc C_4 epimerase activity. For ATCC 29307, the UDP-GlcNAc C_4 epimerase was produced and reached a maximum 1.3 units (Fig. 3). The disruption of *wbpP* in the mutant NY018 reduced the UDP-GlcNAc C_4 epimerase activity ($P < 0.05$), which corresponded to only approximately one-sixth of that of the wild type, demonstrating that the *wbpP* gene encodes the UDP-GlcNAc C_4 epimerase of *V. vulnificus*. However, the UDP-GlcNAc C_4 epimerase activity was still evident in NY018 and significantly higher than the background level observed when the control assay was carried out in the absence of an enzyme source, indicating the production of at least one more UDP-GlcNAc C_4 epimerase (or its homolog) by *V. vulnificus* ATCC 29307. Consistent with this assumption, a putative nucleotide-sugar epimerase gene was also identified in the *V. vulnificus* 1003 strain (37), but its nucleotide sequence was not homologous to that of *wbpP* (data not shown).

We examined whether the reintroduction of pNY0413 carrying a recombinant *wbpP* could complement the decrease in UDP-GlcNAc C_4 epimerase activity of NY018 cells. For this purpose, pNY0413 was constructed by subcloning *wbpP* amplified by a PCR using the primers WbpP005F and WbpP010R (5′-ATTGAATTCGCTTCGAGTTTGATATTGCTCTA-3′) into the broad-host-range vector pRK415 (13). The UDP-GlcNAc C_4 epimerase activity of NY018(pNY0413) was restored to a level comparable to the wild-type level of ATCC 29307 (Fig. 3). Therefore, the decreased UDP-GlcNAc C_4 epimerase activity of NY018 was confirmed to result from the inactivation of functional *wbpP* rather than any polar effects on genes downstream of *wbpP*.

Effects of *wbpP* gene mutation on biosynthesis of capsule. To date, the WbpP protein of *P. aeruginosa* is the only UDP-GlcNAc C_4 epimerase that has been characterized at the molecular and biochemical levels. It has been suggested that the product of the epimerization of UDP-GlcNAc, UDP-GalNAc, is an important intermediate for surface carbohydrate biosynthesis in gram-negative bacteria (5). Thus, to examine whether WbpP is indeed involved in the surface carbohydrate biosynthesis of *V. vulnificus*, the capsules of the parental wild type and the *wbpP* mutant NY018 were compared.

For this purpose, the CPS was prepared from plate-grown cells and analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by Enos-Berlage and McCarter (7). The quantitative CPS measurement was assessed based on the intensities of each band using a UMAX digital imaging system (UTA-1100; UMAX Technologies, Inc., Fremont, CA) and Kodak 1D Image Analysis software (Eastman Kodak Co., Rochester, NY). Figure 4A shows the CPS from each strain after separation on the sodium dodecyl sulfate gel. The CPS synthesis was reduced in NY018, and the residual level of CPS corresponded to approximately one-sixth of that in the wild type (Fig. 4A and B). As such, it was apparent that the mutation in *wbpP* of *V. vulnificus* affected the amount of CPS. CPS production by NY018(pNY0413) was restored to the wild-type level (Fig. 4A and B).

WbpP is required for cytotoxicity toward epithelial cells in vitro. To examine the effects of the *wbpP* mutation on the ability of *V. vulnificus* to damage epithelial cells, two different assays were performed using INT-407 (ATCC CCL-6) human intestinal epithelial cells. The *V. vulnificus* strains were grown in an LBS broth, harvested by centrifugation, and suspended in a cell culture medium, minimum essential medium containing 1% (vol/vol) fetal bovine serum (GIBCO-BRL, Gaithersburg, MD), to appropriate concentrations. The preparation of the

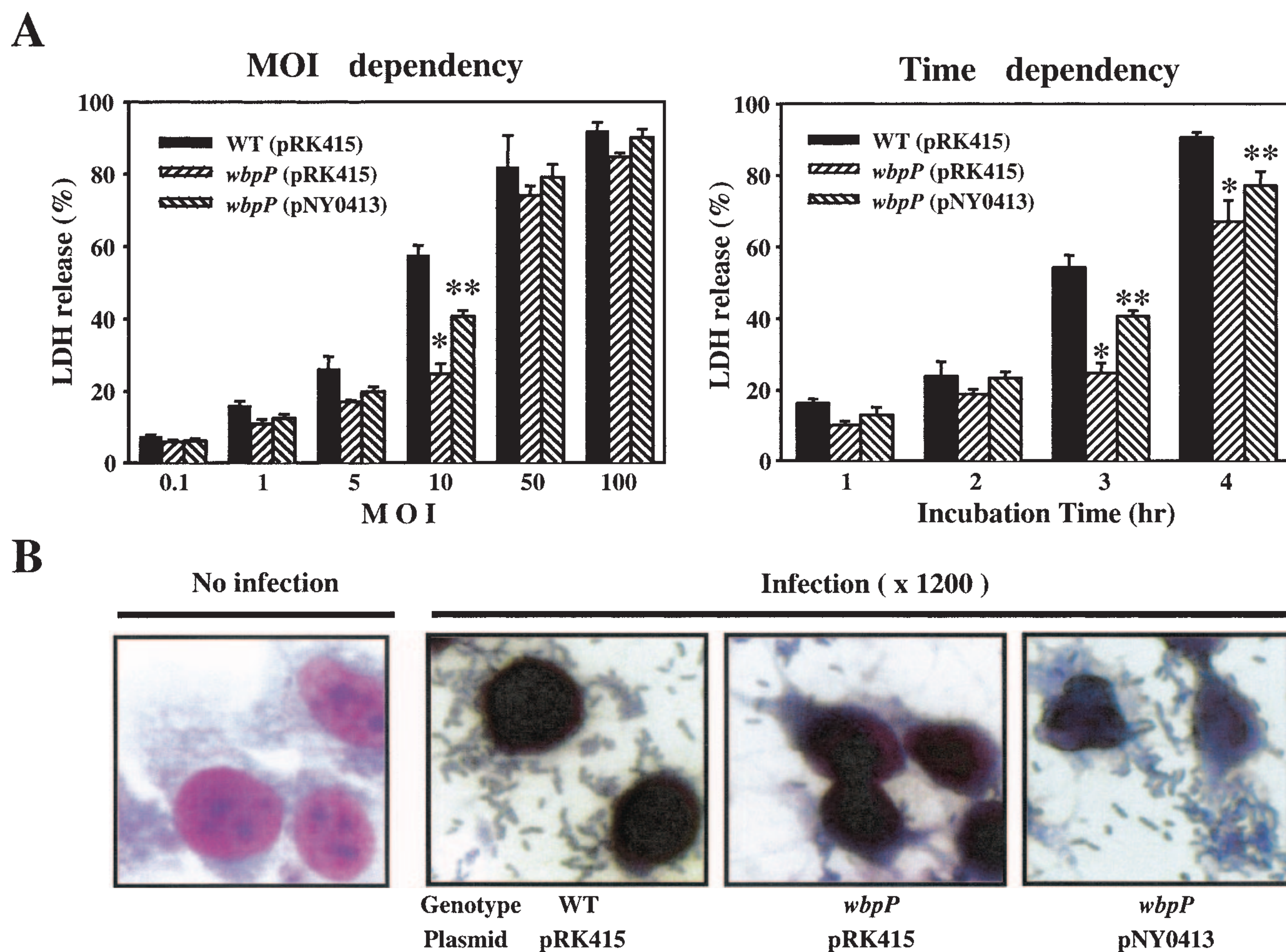

FIG. 5. Effect of *wbpP* mutation on virulence of *V. vulnificus* toward INT-407 cells. (A) INT-407 cells were infected with the wild-type, *wbpP* mutant, or complemented strain of *V. vulnificus* at various MOI for 3 h (left) or at an MOI of 10 for various incubation times (right). Thereafter, the cell cytotoxicity was determined by an LDH release assay. The data represent the means plus SEM from three independent experiments. *, $P < 0.01$; **, $P < 0.05$ relative to groups infected with the wild type of *V. vulnificus* at each MOI or each incubation time. (B) Microscopic observation of INT-407 infected with the *V. vulnificus* strains at an MOI of 10 for 3 h. From the left, uninfected (control) and infected with wild type (WT), NY018 (*wbpP*), or the complemented strain.

INT-407 cells and infection with the bacterial cultures were performed in a 96-well tissue culture plate (Nunc, Roskilde, Denmark) as described previously (11). The cytotoxicity was then determined by measuring the activity of lactate dehydrogenase (LDH) in the supernatant using a Cytotoxicity Detection Kit (Roche, Mannheim, Germany) and expressed using the total LDH activity of the cells completely lysed by 1% Triton X-100 as 100%.

The LDH activities from monolayers of INT-407 cells infected with 20 μl of a suspension of the wild type, NY018, and NY018(pNY0413) strains at different multiplicities of infection (MOI) and incubated for 3 h were determined (Fig. 5A). The *wbpP* mutant NY018 exhibited significantly less LDH activity when the MOI was up to 10. The level of LDH activity from the INT-407 cells infected with NY018 was almost twofold less than that from the cells infected with the wild type. The INT-407 cells were also infected at an MOI of 10, and the LDH activities from the cells were compared at different incubation times, as indicated in Fig. 5A. The cells infected with NY018 exhibited lower levels of LDH activity than the cells infected with the wild type when the cells were incubated with the bacterial suspension for as long as 4 h. The lower LDH activities were restored, although not to the level obtained from the cells infected with the wild type, when the cells were incubated with NY018(pNY0413).

Morphological studies were also carried out using INT-407 cells, which were seeded onto glass coverslips placed at the bottom of the tissue culture plate and infected with the *V. vulnificus* strains at an MOI of 10 for 3 h (Fig. 5B). The cells were fixed in methanol, stained with 0.4% Giemsa, and examined under a light microscope (15). The stained cells were assessed for size, regularity of the cell margin, and the morphological characteristics of the nuclei. As shown in Fig. 5B, many Giemsa-stained INT-407 cells exhibited marked cellular damage after infection with the wild type and NY018(pNY0413). Cytoplasmic loss and nuclear-material condensation, typical phenotypes of cell death, were observed in the intestinal cells infected with the wild type and NY018(pNY0413). In contrast,

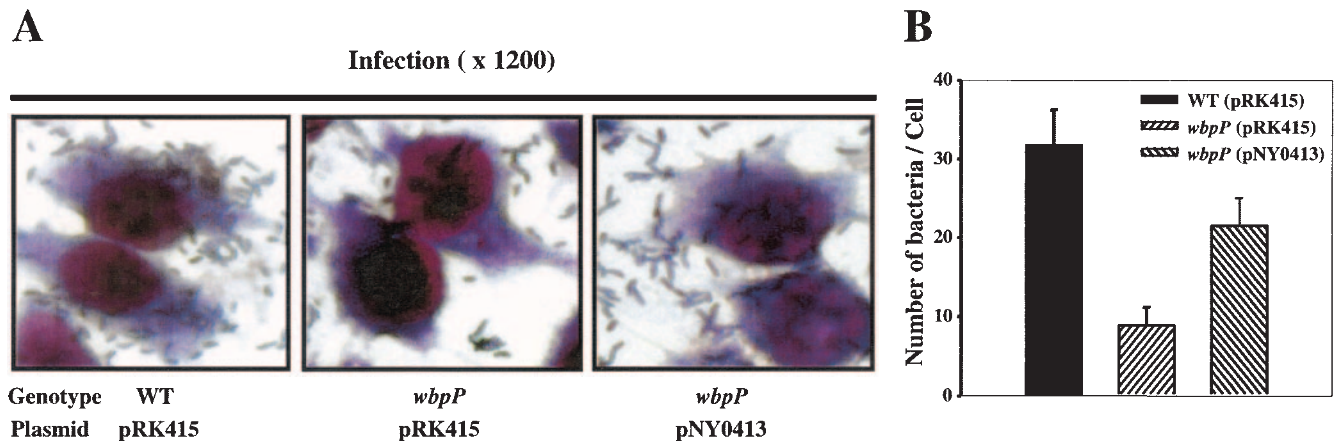

FIG. 6. Adhesion of *V. vulnificus* wild-type, *wbpP* mutant, and complemented strains to INT-407 cells. (A) INT-407 cells were cultured on glass coverslips and infected at an MOI of 10. After incubation with the bacteria for 2 h, the INT-407 monolayers were rinsed to remove any nonadhering bacteria. Light micrographs show the adhesion of the wild-type (WT), NY018 (*wbpP*), and complemented strains to the INT-407 cells. (B) The adherent bacteria were quantified and expressed as the number of bacteria per cell in the coverslip tissue culture. The error bars represent the SEM.

fewer dead cells were observed after incubation with NY018. The cells infected with NY018 exhibited a less damaged surface and less cytoplasmic loss. These results suggest that WbpP is important for the ability of *V. vulnificus* to infect and injure host cells.

WbpP is required for adhesion to epithelial cells in vitro. For the adhesion assay, INT-407 monolayers were prepared and infected as described above at an MOI of 10. The bacteria were allowed to adhere for different lengths of time. After thorough washing with phosphate-buffered saline (pH 7.4), the mean number of attached bacteria per cell calculated by examining 100 cells was used to represent the adhesion index for the strains.

The wild type and NY018(pNY0413) revealed the formation of small clusters of aggregated bacteria on the INT-407 cell surface (Fig. 6A). After 2 h of infection, the wild type and NY018(pNY0413) adhered to the INT-407 cells reached adhesion indexes of 32.0 and 22.5, respectively (Fig. 6B). In contrast, with NY018, a much smaller area of the intestinal-cell surface was covered with the bacteria, and no clusters of aggregated bacteria were observed (Fig. 6A and B). NY018 was consistently and significantly less adherent than the wild-type parent strain at all time points studied (data not shown). When infected for 2 h, the number of *wbpP* mutants per cell of the INT-407 monolayers was about threefold less than that for the wild type (Fig. 6B). Adhesion assays with an incubation period longer than 3 h were impossible, since most of the INT-407 cells were lysed. The results suggested that the *wbpP* mutant was significantly impaired in its ability to attach to the epithelial cells.

Virulence in mice is dependent on *wbpP*. The role of the *V. vulnificus wbpP* gene in virulence was also examined using a mouse model. The 50% lethal doses (LD_{50}s) of the wild type and the *wbpP* mutant were compared using ICR mice (specific pathogen free; Seoul National University), as described elsewhere (11, 16). The infected mice were observed for 24 h, and the LD_{50}s were calculated using the method of Reed and Muench (26). The mice were also injected intraperitoneally with 250 μg of iron dextran per g of body weight immediately before being injected with the bacterial cells.

The LD_{50}s in the iron-overloaded mice after intraperitoneal infection with *V. vulnificus* strains are shown in Table 2. The LD_{50} for NY018 was greater than 10^7 CFU, compared with an LD_{50} of 10^2 CFU for the wild type. Therefore, for the mouse model of intraperitoneal infection, in which the *wbpP* mutant exhibited more than a 5-log-unit increase in LD_{50} over the wild type, the *wbpP* mutant appeared to be signifcantly less virulent than its parental wild type. This result indicates that WbpP of *V. vulnificus* is apparently important for the pathogenesis of the bacteria. Thus, when taken together, the results of the present study make it reasonable to conclude that the *wbpP* gene is essential for the virulence of *V. vulnificus* in mice, as well as in tissue cultures.

A variety of endotoxins and exotoxins have been implicated as putative virulence factors for *V. vulnificus* (8, 17, 31). However, to date, only a few virulence factors, such as CPS and the iron acquisition system (18, 34), have been confirmed as essential for the virulence of *V. vulnificus* by using the molecular version of Koch's postulates, where mutations are introduced into genes encoding putative virulence factors, followed by an evaluation of any attenuating virulence (8). In the present study, the *wbpP* mutant NY018 was a CPS mutant and was less adherent and less toxic to intestinal epithelial cells in vitro and also exhibited significantly diminished virulence in mice, as measured by its ability to cause death. However, when virulence was determined in mice (LD_{50}), the complemented

TABLE 2. Effects of *wbpP* mutation on lethality of *V. vulnificus* to mice

Strain[a]	Intraperitoneal LD_{50} (CFU)
ATCC 29307 (n = 6)	1.1×10^2
NY018 (n = 6)	9.3×10^7

[a] n, number of iron-treated mice for each inoculation group, ranging from 10^1 to 10^8 CFU in 10-fold increments.

strain NY018(pNY0413) was not able to recover the reduced virulence of NY018 even after several attempts (data not shown). As shown in Fig. 5, the cytotoxicity of NY018(pNY0413) was still less than that of the wild type, indicating that the *wbpP* mutation was not fully complemented by pNY0413. pNY0413 was not stably maintained in NY018 when the bacteria were present in the cell culture system and in mice, as determined by maintenance of tetracycline resistance (data not shown). Although other explanations are still possible, it is most likely that the lack of full complementation of the *wbpP* mutation by pNY0413 might be attributed to the instability of the plasmid.

Adhesion to intestinal epithelial cells is an important step in the disease process of pathogenic bacteria, yet the contribution of CPS molecules to bacterial attachment to epithelial cells is still not well understood. It has been reported that the presence or absence of CPS polymers influences the surface hydrophobicity and surface charge of the bacterial cell, and thus, altering the physiochemical characteristics of the cell surface has been postulated to modify the relative adhesive properties of the bacteria (2, 19, 34). When the autoagglutination activities of NY018 and the wild type were compared according to the procedures of Misawa and Blaser (22), the mutant was not as agglutinating as the wild type, indicating that the impaired adherence exhibited by the *wbpP* mutant might be related to its decreased autoagglutination activity (data not shown). However, it has recently been observed that the production of CPS and the ability of *V. vulnificus* to form a biofilm on abiotic surfaces are inversely related (12). This suggests that the correlation between CPS and *V. vulnificus* adhesion may vary with complex parameters that still remain to be determined.

Although the elucidation of the genomic sequences for two *V. vulnificus* strains revealed the presence of intact genes for group 1 CPS biosynthesis (http://www.ncbi.nml.nih.gov; accession numbers NC 004459, NC 004460, NC 005139, and NC 005140), insufficient information on the functional characteristics of the genes is still one of the greatest limitations in studies of the *V. vulnificus* CPS. Through transposon insertion mutagenesis, the functions of several genes required for CPS biosynthesis and transport have been identified. For example, an epimerase gene encoding a putative nucleotide-sugar epimerase that differs from *wbpP* in its nucleotide sequence has been identified in *V. vulnificus* 1003. A null mutation of this epimerase gene led to loss of the ability to produce CPS, and hence, loss of virulence of the organism (37). In addition to the epimerase gene, 16 other genes involved in CPS biosynthesis and the *wza* gene encoding a membrane transporter for a group 1-like CPS have also been identified (32). In the course of the current sequencing analysis, parts of *wzc* and *wbpO* homologs flanking *wbpP* were found (Fig. 1A). However, although it has been reported that these genes are clustered and are required for the biosynthesis of surface carbohydrates in Enterobacteriaceae (1, 28, 35), it is still unclear whether the genes are also involved in the synthesis of surface carbohydrates in *V. vulnificus*. Nonetheless, since the organizations of the genes are similar, along with the sequence homology, it is most likely that the roles of *wzc* and *wbpO* are analogous to those observed in other Enterobacteriaceae.

Nucleotide sequence accession number. The nucleotide sequence of the *wbpP* gene of *V. vulnificus* ATCC 29307 was deposited in GenBank under accession number AY350749.

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