Outer membrane protein OmpW is the receptor of typing phage VP5 of Vibrio cholerae O1 El Tor biotype

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Abstract

Phage-typing is used for the subtyping of epidemic clones of bacteria. In this study we identified the outer membrane protein OmpW as the receptor of phage VP5, one of the typing phages for *Vibrio cholerae* O1 El Tor biotype. The characteristic 11-bp deletion in *ompW* was observed in all epidemic strains resistant to VP5, suggesting that this mutation event can be used as a tracing marker in cholera surveillance.

**Key words:** *Vibrio cholerae*, phage typing, receptor, outer membrane protein
Seven cholera pandemics were recorded historically. The first six pandemics were putatively caused by the classical biotype of serogroup O1 *Vibrio cholerae*, whereas the ongoing seventh pandemic is caused by O1 El Tor biotype (1). Subtyping of *V. cholerae* strains is useful in epidemiological and microbiological studies. A phage-biotyping scheme, in which five typing phages were included, was established for the El Tor strain subtyping in the 1970s in China (2). During our surveillance, almost all El Tor strains from epidemics were found to be sensitive to all these five typing phages, which is designated as phage type 1 (PT1). However, during the 1998 cholera epidemic in Sichuan Province, some patient strains were resistant to phage VP5. These strains belong to PT6 and co-existed with PT1 strains. PT6 became the predominant phage type in 1999 and 2000, but it disappeared after 2001 (3), which showed a unique type of the epidemic strains.

Phages infect susceptible bacterial strains beginning with binding to receptors on the surfaces of bacteria. OmpW is an outer membrane protein that serves as a receptor of *E. coli* colicin S4 (6). Gene *ompW* has served as a species-specific gene of *V. cholerae* in many detection studies (4, 5). This gene was found to have an 11-bp deletion in the PT6 strains, but not detected in any PT1 strain (3). Therefore the role of OmpW in VP5 infection was suspected.

In this study, *ompW* genes from 44 strains isolated from 1998 to 2001, including 22 PT6 and 11 PT1 strains from Sichuan and 11 PT1 strains from other provinces were sequenced. The same 11-bp deletion was in all PT6 strains (Fig. 1). The deletion corresponds to 298–308 nt of *ompW*, and causes a shift in the reading frame. A new stop codon is generated 350 nt ahead of the original stop codon (Fig. 1). All the PT1 strains had...
the intact and identical \textit{ompW} sequences.

To assess the possible role of gene \textit{ompW} to VP5 infection, an \textit{ompW} deletion mutant, N16961-dompW, was constructed with suicide plasmid pWM91 (7) from the VP5 sensitive strain N16961. N16961-dompW lost sensitivity to VP5 (Fig. 2A). This sensitivity was restored when it was complemented with plasmid pBR322-ompW (designed strain N16961-dompW-R) containing the intact \textit{ompW} gene cloned from N16961, but not when it obtained pBR322-ompW(del) containing \textit{ompW} with an 11-bp deletion cloned from the PT6 strain VC631. VC631 became sensitive to VP5 when it obtained plasmid pBR322-ompW, but remained resistant when it obtained pBR322-ompW(del) (Fig. 2A). This indicated that the intact \textit{ompW} is needed to the sensitivity of \textit{V. cholerae} El Tor to VP5.

The role of OmpW in the infection of VP5 was further validated by strain adsorption tests. Phage VP5 (10\(^8\) PFU/ml) was mixed equally with strains N16961, N16961-dompW and N16961-dompW-R (10\(^8\) CFU/ml of each) respectively. The mixture was centrifuged to remove the cells and the adsorbed phage particles, the supernatants (containing the un-adsorbed phages) were diluted ten times serially, and dropped onto the double-layer agar plate containing VP5-sensitive strain 2477c. The plaques of 10\(^3\) dilutions of the supernatants were counted. When compared to N16961-dompW, the adsorption abilities of strains N16961 and N16961-dompW-R containing intact \textit{ompW} were much stronger, because there were less phage particles left in both supernatants and the differences were statistically significant (Fig. 2B). Therefore we identified OmpW as the receptor of VP5.

VP5 is one of the five typing phages in the phage-biotyping scheme, we have observed that almost all the toxigenic El Tor strains in the epidemics in China are VP5 sensitive, whereas some Sichuan strains from 1998 to 2000 are the exceptional with
ompW mutation and VP5 resistance. We searched the OmpW sequences in the GenBank Protein database. Within the obtained 142 V. cholerae strains, 97 have the same OmpW sequence with N16961, whereas some strains have some amino acid residue substitutions (Table 1). Twenty-five strains possess two sequence types of the truncated OmpW, one type includes 20 strains from Haiti in 2010 and a Brazil strain in 1978, and another type includes two strains which have the same sequence mutation with Sichuan PT6 strains. It showed the divergence of OmpW in V. cholerae, and most likely VP5 resistant strains may also appeared in other area.

Some biological effects of ompW inactivation were detected in V. cholerae. A quantitative biofilm formation assay was performed using 96-well cell plates and crystal violet staining as described (8). The OD_{570}/OD_{600} values of N16961 were 1.53±0.13, and 1.76±0.22 with N16961-dompW. Mild enhancement of biofilm formation was observed in the ompW mutant (P<0.05). This may be a compensatory response, promoting environmental survival. In our colonization competition test of the wild and mutant strains in mice, a slight decrease in the concentration of the ompW mutant (C6706-dompW, constructed from the VP5 sensitive El Tor strain C6707) was observed (Fig. 3). This was consistent with the results of a previous study that showed the intestinal colonization activities of ompW deficient mutants to be only marginally lower than those of wild-type O1 strains (9). In this way, ompW mutants are showed to have weaker adaptability to environment and human hosts than their wild-type counterparts have.

In conclusion, OmpW may act as the receptor of typing phage VP5. Cholera phages may play a role in the emergence of new V. cholerae pandemic serogroups or clones, and
phage-resistant strains may have a survival advantage (10). However, the PT6 strains disappeared while the PT1 strains continue to spread within the population. This suggests that the PT6 strain must have some deficiencies with respect to surviving in and outside of hosts. Nevertheless, when such strains contain the $ompW$ mutation, they can be used as a tracer, allowing their path through the environment and human population to be tracked.

**Acknowledgements**

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Table 1. The OmpW mutant *V. cholerae* strains used in the protein sequence comparison and their OmpW mutation.

<table>
<thead>
<tr>
<th>OmpW mutation*</th>
<th>Sequence characters</th>
<th>Strains and their sources</th>
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<tbody>
<tr>
<td>Residue substitution</td>
<td>T121A.</td>
<td>BJG-01 (NA).</td>
</tr>
<tr>
<td>Truncated</td>
<td>“KLHHL” from residue 131.</td>
<td>116063 (1978, Brazil); HC-02A1 (NA); HC-36A1 (NA); and the following 20 strains from Haiti, 2010: HC-02C1; HC-1A2; HC-41B1; HC-43B1; HC-44C1; HC-46B1;</td>
</tr>
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HC-50A1; HC-51A1; HC-52A1; HC-55A1; HC-55B2; HC-55C2; HC-56A1; HC-57A1; HC-59A1; HC-59B1; HC-60A1; HC-61A2; HC-78A1; HE-45.

“TFATYYGPILLW”
Truncated from residue 100, same as PT6 strains.

2740-80 (1980, US Gulf Coast); gi487821612 (NA).

*, Compared to strain N16961; NA, the epidemic data are not available.
Figure 1. Alignment of the *ompW* gene of the PT6 and PT1 *V. cholerae* strains, showing the same 11 bp deletion mutation in all PT6 strains. A new stop codon appeared 350 nt in the upstream of the original stop codon.

Figure 2. Experimental identification of the role of OmpW in VP5 infection.
A. Plaques formed by wide-type strains N16961 and VC631, deletion mutant N16961-dompW, and the strains complemented with different plasmids. B. VP5 adsorption with the wild-type strain N16961 and its ompW mutant type. The plaques were counted in the $10^5$ dilutions in a double-layer agar plate containing the VP5-sensitive strain 2477c. Strain N16961-dompW was used as the test and calculation control. Three independently prepared VP5 phages were used as the biological repeat tests. The plaque numbers of N16961 and N16961-dompW-R treated groups were divided by those of the N16961-dompW-treated group, respectively, producing the percentages of plaque formation compared to the control group, to show the adsorption ratios. Smaller values indicated more phage particles were adsorbed. Statistically significant comparisons between the groups were marked with * (ANOVA for randomized block design, $P<0.05$), no difference was found between N16961 and N16961-dompW-R groups.

Figure 3. Colonization competition test of the wild strain C6706 and its *ompW* mutant in mice. The short lines indicate the average CFU values of the tests.