Can the eel-nonvirulent biotype 1 *Vibrio vulnificus* strains be converted into eel-virulent ones by acquiring the virulence plasmid via conjugation between the two biotypes?

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Introduction

Vibrio vulnificus is pathogenic to both the human (Tacket *et al.*, 1984) and eel (Tison *et al.*, 1984), and may produce severe systemic infection with a high mortality rate in the susceptible hosts. The strains of this species are divided into three biotypes (BTs), BT1, BT2 and BT3, based on their differences in the phenotypic trait and host range (Bisharat et al., 1999), among which only BT2 strains are able to cause diseases, called vibriosis, in the eel (Biosca et al., 1996). We have previously demonstrated that a plasmid commonly and exclusively present in the BT2 strains is essential for the bacterial survival in eel serum and, consequently, virulence in the eel (Lee *et al.*, 2008). We have also shown that this BT2 virulence plasmid, aided by a conjugative plasmid found in most BT2 strains, can be transferred from a wild-type BT2 strain to a plasmid-cured derivative of this strain by conjugation at a frequency of 10^{-2} (Lee *et al.*, 2008). It is then intriguing why only a fraction of *V. vulnificus* strains possess the virulence plasmid and thus are virulent for the eel, despite that this plasmid can be readily transferred between the BT2 strains in the presence of a conjugative plasmid. In this study, we determined whether the BT2 virulence plasmid can be transferred to the BT1 strains by conjugation and, further, if it alone can render the transconjugants virulent for the eel. These data may provide insights of the emergence of eel-virulent BT2 strains.

Transfer of BT2 virulence plasmid from BT2 to BT1 *V. vulnificus* strains by conjugation.

The conjugation experiment was performed between a BT2 strain, which harbored both the virulence plasmid, pR99 (inserted with a chloramphenicol-resistance cassette), and conjugative plasmid, pC4602-1 (inserted with an ampicillin-resistance cassette), and the streptomycin-resistant mutants of various BT1 strains. As shown in Table 1, pR99 can be transferred into the tested clinical (YJ016, SW058 and SW059) and environmental (CG024, CG027 and CG028) BT1 isolates at a frequency ranging from 10^{-3} to 10^{-7}.

Table 1: Frequencies of transferring BT2 virulence and conjugative plasmids from a BT2 strain to various BT1 strains by conjugation.

Recipient	pC4602-1::Amp^r	pR99::Cm^r	pC4602-1::Amp^r and pR99::Cm^r
CT218	7.01×10^{-1}	2.08×10^{-2}	2.78×10^{-1}
CG024	7.65×10^{-5}	1.95×10^{-3}	6.12×10^{-5}
CG027	ND	1.30×10^{-6}	1.30×10^{-7}

CG028	4.92×10^{-5}	6.54×10^{-4}	2.42×10^{-5}
YJ016	5.57×10^{-7}	5.19×10^{-7}	ND
SW058	3.20×10^{-7}	7.36×10^{-6}	8.00×10^{-8}
SW059	3.49×10^{-4}	6.43×10^{-4}	7.14×10^{-4}

CT218: plasmid-cured BT2 strain; Cm^r: chloramphenicol-resistance cassette; Ap^r: ampicillin-resistance cassette; ND: not detectable.

Characterization of the BT1 transconjugants that possess the BT2 virulence plasmid.

As the BT2 virulence plasmid confers resistance to eel serum bactericidal effect, the resultant transconjugatns of BT1 strains were further examined for their survival in the eel serum. As shown in Figure 1, the survival of all transconjugants was significantly enhanced, compared to their parent BT1 strains, however, only to a level that was about 20% to 40% of that of a BT2 strain.

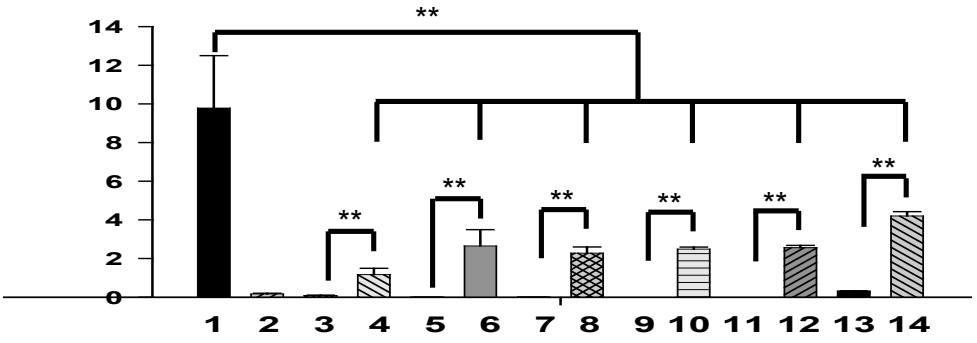

Figure 1: Survival of the BT1 transconjugants in eel serum. Lanes 1: CECT4999; 2: CT218; 3: YJ016; 4: HC131; 5: SW058; 6: HC137; 7: SW059; 8: HC145; 9: CG028; 10: HC141; 11: CG024; 12: HC147; 13: CG027; 14: HC168. CECT4999: a BT2 strain. CT218: CECT4999 cured of the virulence plasmid. HC131, HC137, HC145, HC141, HC147 and HC168 are the transconjugants (possessing pR99) of YJ016, SW058, SW059, CG028, CG024 and CG027, respectively. ** $P < 0.01$.

The difference in resistance to eel serum between the BT2 strain and BT1 transconjugants, both of which possess the BT2 virulence plasmid, may be due to their differences in the plasmid copy number or expression level of virulence genes. Therefore, we further checked the copy number of pR99 by quantitative PCR and the expression of *vep07*, a novel gene in pR99 that is required for bacterial survival in eel serum (Lee *et al.*, 2008), by reverse transcription-PCR and Western blotting. The copy numbers of pR99 (ranging from 1.5 to 2.0) in these BT1 transconjugants were comparable to that in the BT2 strain (about 3). The transcriptional levels of *vep07* in these tranconjugants were also similar to that in the BT2 strain. However, the amounts of Vep07 in the outer membrane of some of the BT1 transconjugants were much lower than that of the BT2 strain (Figure 2).

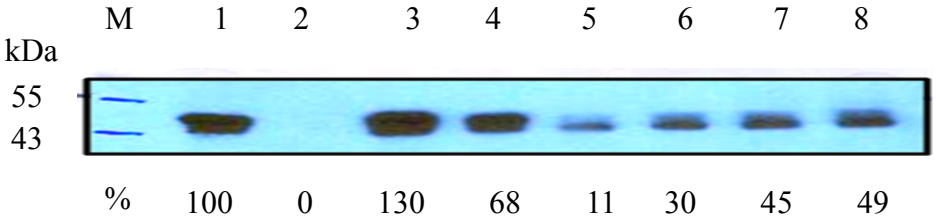

Figure 2: Expression of Vep07 in the outer membrane of BT1 transconjugants. The outer membrane fraction of bacteria incubated in 50% heat-inactivated human serum for 4 h were prepared and then analyzed for the amount of Vep07 by Western blotting. Lanes 1: HC134; 2: YJ016; 3: HC131; 4:HC137; 5: HC141; 6: HC145; 7: HC147; 8: HC168. HC134: CECT4999, a BT2 strain, with the conjugative plasmid. HC131, HC137, HC145, HC141, HC147 and HC168 are the pR99-possessing transconjugants of YJ016, SW058, SW059, CG028, CG024 and CG027, respectively. The % of intensity of each signal compared to that of CECT4999 is indicated.

Conclusions and discussion.

We demonstrate in this study that the BT2 virulence plasmid could be transferred to a BT1 strain by conjugation in the presence of a conjugative plasmid, albeit at a very low frequency (Table 1). Acquiring the virulence plasmid resulted in better survival of the BT1 transconjugants in eel serum, but not to the level of a BT2 strain (Fig. 1). Unlike the BT2 strains, the BT1 transconjugants did not require *vep07* for their survival in eel serum (data not shown). Besides, the amount of Vep07 in the outer membrane did not correlate well with the growth of the BT1 transconjugants in the eel serum (Fig. 2). Other genes in pR99 and the chromosome of a BT2 strain may be involved in bacterial survival in eel serum.

References

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