**Vibrio parahaemolyticus toxRS operon (VP0819-VP0820) regulates outer membrane proteins OmpU and OmpN that are important for survival in acid stress**

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**Introduction**

One of the main regulators of virulence in *Vibrio cholerae* is the two-component regulator ToxRS. In *V. cholerae*, ToxR binds to the promoter and activates the toxT gene, which in turn activates the expression of a number of virulence genes, such as ctxA and tcpA (DiRita et al. 1991). Additionally, ToxR has also been shown to regulate the two outer membrane porins, OmpU and OmpT in a manner that is independent of ToxT (Miller et al. 1988). It has also been demonstrated in *V. cholerae* that ToxRS is necessary for the survival of the organism when faced with certain environmental stresses such as low pH and bile (Merrel et al. 1999; Provenzano et al. 2000a; Provenzano et al. 2000b). Furthermore, it has been shown that the survival defect exhibited by toxRS knockout mutants is most likely due to the lack of OmpU in the outer membrane, though an exact mechanism has yet to be elucidated (Merrel et al. 2000).

*Vibrio parahaemolyticus* contains a homologue of the toxRS operon within its genome on chromosome 1 open reading frames (ORFs) VP0819 and VP0820 (Lin et al 1993). These ToxR and ToxS proteins of *V. parahaemolyticus* share a 53% and 67% amino acid identities with the *V. cholerae* ToxR and ToxS proteins respectively. Interestingly, a second ToxR homology, VPA1332 is present on chromosome 2 encoded with the VPaI-7 region (Kodama et al. 2010). No ToxT homology is present in *V. parahaemolyticus*. Recently, we demonstrated that a deletion mutant of the toxRS operon (VP0819-VP0820) from *V. parahaemolyticus* RIMD2210633 is hypersensitive to both inorganic and organic acid stress (Whitaker et al. 2010). In the present study, we investigated whether the *V. parahaemolyticus* toxRS operon is responsible for controlling the expression of ompU and to determine if the absence of this outer membrane protein is responsible for the acid sensitive phenotype in the toxRS deletion mutant.

**Methods and Materials**

*RNA extraction, cDNA synthesis, DNA microarray, and quantitative Real-Time PCR*

The *V. parahaemolyticus* strains used in this study were grown for four hours in LB broth containing 3% NaCl at 37°C and total RNA was extracted using the RNAprotect Bacteria reagent and RNeasy kit according to the manufacturer’s protocols. Samples were treated with DNase to ensure there was no contamination with genomic DNA and quantified using a Nanodrop spectrophotometer. Superscript II Reverse transcriptase was used to synthesize cDNA from the RNA samples as per the manufacturer’s protocols. For DNA microarray, 10 μg of RNA were used as a template for first and second strand cDNA synthesis. Double stranded cDNA was hybridized to a NimbleGen array. Samples were scanned and normalized by Roche NimbleGen and visualized using DNAstar Analysis software. For single stranded cDNA synthesis only, 500 ng of RNA was used as template and the reaction was primed with 200 ng of random hexamers. cDNA samples were diluted 1:250 and used as the template for quantitative PCR using Fast SYBR green master mix on an Applied Biosystems 7500 Fast Real Time PCR System.
Results and Discussion

*The toxRS operon regulates expression of ompU and a number of other genes*

As a first step to determine what genes fall under the regulation of ToxRS in *V. parahaemolyticus*, we performed DNA microarray analysis, comparing gene expression in the ΔtoxRS mutant with that of wild-type *V. parahaemolyticus*, when grown for 4 hours in LB containing 3% NaCl broth. The expression of a number of genes, belonging to many different functional classes, were found to be significantly altered in the ΔtoxRS mutant in comparison to the wild-type strain; a total of 57 genes showed significant increased expression and 39 showed significant decreased expression. Most notably, ORFs VP2467, which encodes an OmpU homologue (outer membrane protein U) and ORF VPA0527, which encodes an OmpN homologue, show decreased expression approximately 250-fold and 22-fold respectively, when compared with the wild-type strain (Table 1).

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Product</th>
<th>Functional Class</th>
<th>Relative Fold change</th>
</tr>
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<tbody>
<tr>
<td>VP2467</td>
<td>OmpU</td>
<td>Cell envelope</td>
<td>-251.181</td>
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<tr>
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<td>ToxS</td>
<td>Regulatory Functions</td>
<td>-42.256</td>
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<td>ToxR</td>
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<td>VPA0527</td>
<td>OmpN</td>
<td>Transport and binding proteins</td>
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<td>LeuO</td>
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To confirm our microarray data and to further investigate *ompU* expression in the ΔtoxRS background, we used quantitative real-time PCR. In these experiments, we examined the expression levels of *ompU* under different growth conditions in wild-type and mutant strains. For all of the *V. parahaemolyticus* strains tested, cultures were grown for 4 hours in either LB containing 1% NaCl or LB containing 3% NaCl adjusted to pH 5, conditions previously described to induce the expression of toxR (Whitaker et al. 2010). Firstly, expression of *ompU* in wild-type cells was increased significantly under acid stress conditions. Secondly, expression levels of *ompU* were significantly decreased in the ΔtoxRS mutant compared with levels in wild-type cells grown under the same conditions (Fig. 1).

Through the use of DNA microarray analysis, we have demonstrated that the toxRS operon in *V. parahaemolyticus* is a potential global regulator that may regulate directly or indirectly nearly 100 different genes on the genome. Of those genes, VP2467 (*ompU*) exhibited the greatest change in expression (~250-fold down). We used real-time PCR to confirm that expression of the *ompU* gene is greatly decreased in the ΔtoxRS mutant,
even under conditions that are favorable to toxR expression. Additionally, another outer membrane protein, OmpN shows decreased expression in the ∆toxRS mutant. Given the decrease in expression of ompN and ompU, in particular, we propose that the encoded proteins play an important role in protecting the bacterium from environmental stress and that a lack of these proteins results in the acid sensitive phenotype that is observed in the ∆toxRS mutant.

References


