

Supplementary Materials: The Trx system in *Edwardsiella piscicida* contributes to oxidative stress tolerance, motility, and virulence

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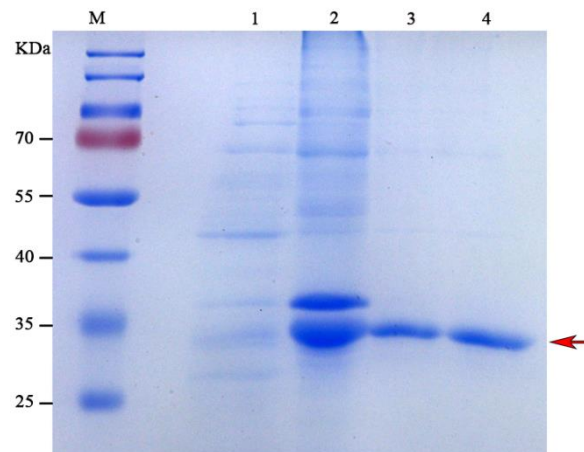


Figure S1. Expression and purification of rTrxB_{Ep}. The rTrxB_{Ep} carrying His-Tag is highly purified by affinity chromatography with Ni-NTA. The band indicated by the red arrow is rTrxB_{Ep} protein, which mainly appears in the SDS-PAGE gel stained with Coomassie blue. Lane M: PageRuler Prestained Protein Ladder (Thermo Scientific, US); lane 1: non-induced sample; lane 2: IPTG-induced sample; lane 3: purified sample; lane 4: renatured sample.

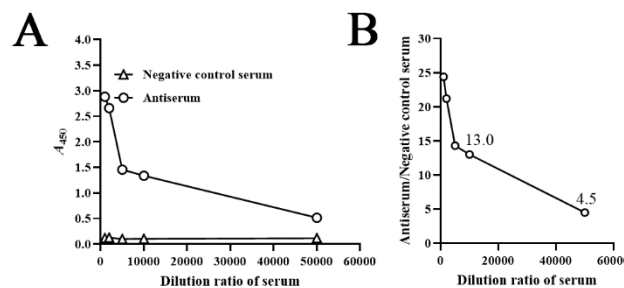


Figure S2. Determination of antiserum titer by ELISA. **A**, the antibody reacted with purified rTrxB_{Ep} protein by continuous dilution (from 1000-fold, 2000-fold, 5000-fold, 10000-fold to 50000-fold). The unimmunized mice serum was used as a negative control. **B**, the ratio of absorbance between anti-rTrxB_{Ep} serum and negative control serum. When the dilution ratio was 1:50000, the absorbance ratio of anti-rTrxB_{Ep} serum to negative control serum was 4.5.

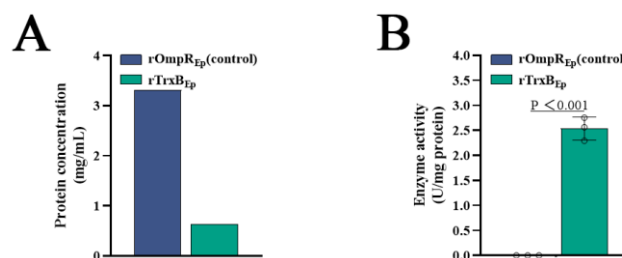


Figure S3. The thioredoxin reductase activity of the rTrxB_{Ep}. **A**, the content of rOmpR_{Ep} (control) and rTrxB_{Ep} was measured using the BCA Protein Assay. **B**, based on OD₄₁₂ analysis the activity of thioredoxin reductase compared with the control rOmpR_{Ep}.

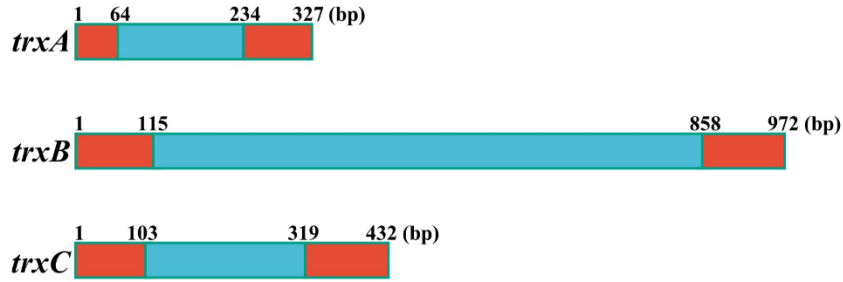


Figure. S4. The sequences knockout location map of *trxA*, *trxB*, *trxC*. Retained sequences are indicated by red shapes and knocked-out regions are indicated by blue shapes.

Table S1 Strains, plasmids used in this study

Strains or plasmids	References or sources
<i>Edwardsiella piscicida</i>	
TX01	Isolated from diseased Japanese flounder
$\Delta trxB$	This study
$\Delta trxA$	This study
$\Delta trxC$	This study
<i>Escherichia coli</i>	
DH5 α	TransGen Biotech (Beijing, China)
BL21 (DE3)	TransGen Biotech (Beijing, China)
D1000	Biomedal (Sevilla, Spain)
S17-1 λ pir	Biomedal (Sevilla, Spain)
BL21/pETTrxB	This study
Plasmids	
pEASY®-T1 Simple Cloning Kit	TransGen Biotech (Beijing, China)

Table S2. Oligonucleotide primers used in this study

Primer name	References or sources Sequence (5'-3')
TrxBKOF1	<u>GGATCCC</u> ATCAGATAAGAGCAAAC (<i>Bam</i> H I)
TrxBKOR1	TCCATGACCATGCCGGTGATCAGCAC
TrxBKOF2	CCGGCATGGTCATGGACCATATCTATCGT
TrxBKOR2	<u>GGATCCG</u> ACTGCGCTGTTTCAGC (<i>Bam</i> H I)
TrxBKOF3	TCGAGTCCAAGACGTACCAC
TrxBKOR3	ATATTACAACGCTGCTCAAGG
TrxBF	<u>GGATCC</u> ATGGTCTATGCGGCGCGT (<i>Bam</i> H I)
TrxBR	<u>AAGCTT</u> GTGGCGAGCGGCATC (<i>Hind</i> III)
TrxAkof1	<u>GGATCCC</u> GATCAGGATATCGACACC (<i>Bam</i> H I)
TrxAkor1	AGCAGCAGTTCAGCCTTCAGCACGTC
TrxAkof2	AGGCTGAACTGCTGCTGTTCAAGAAC
TrxAkor2	<u>GGATCC</u> ATCCTGCAGGATCTCCAG (<i>Bam</i> H I)
TrxAkof3	GTCGGCAAACCTTCTGTTTCAGTC