The Role of TonB Gene in Edwardsiella ictaluri Virulence

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Edwardsiella ictaluri is a Gram-negative facultative intracellular pathogen that causes enteric septicemia in catfish (ESC). Stress factors including poor water quality, poor diet, rough handling, overcrowding, and water temperature fluctuations increase fish susceptibility to ESC. The TonB energy transducing system (TonB-ExbB-ExbD) and TonB-dependent transporters of Gram-negative bacteria support active transport of scarce resources including iron, an essential micronutrient for bacterial virulence. Deletion of the tonB gene attenuates virulence in several pathogenic bacteria. In the current study, the role of TonB (NT01EI_RS07425) in iron acquisition and E. ictaluri virulence were investigated. To accomplish this, the E. ictaluri tonB gene was in-frame deleted. Growth kinetics, iron utilization, and virulence of the Ei/Delta1 tonB mutant were determined.

Loss of TonB caused a significant reduction in bacterial growth in iron-depleted medium (p > 0.05). The Ei/Delta1 tonB mutant grew similarly to wild-type E. ictaluri when ferric iron was added to the iron-depleted medium. The Ei/Delta1 tonB mutant was significantly attenuated in catfish compared with the parent strain (21.69 vs. 46.91% mortality). Catfish surviving infection with Ei/Delta1 tonB had significant protection against ESC compared with naïve fish (100 vs. 40.47% survival). These findings indicate that TonB participates in pathogenesis of ESC and is an important E. ictaluri virulence factor.

Keywords: tonB, iron, virulence, ESC, channel catfish

INTRODUCTION

Enteric septicemia of catfish (ESC) was first detected in the southern United States in 1976, and the disease was described in 1979 (Hawke, 1979). The etiologic agent of ESC is Edwardsiella ictaluri, which is in the family Enterobacteriaceae (Hawke et al., 1981). It is a facultative anaerobe that is motile with peritrichous flagella (Plumb and Sanchez, 1983). In its acute form, ESC causes gastroenteric septicemia, and its chronic form causes meningoencephalitis (Shotts et al., 1986; Newton et al., 1989) in cultured channel catfish (Ictalurus punctatus). Outbreaks of ESC occur during early summer and autumn, and fish are more at risk when water temperatures range from 22 to 28°C (Francis-Floyd et al., 1987). Stress and poor management practices increase susceptibility to ESC through alteration of host-defense mechanisms (Hawke and Khoo, 2004; Small and Bilodeau, 2005; Cunningham et al., 2014; Eissa and Wang, 2016).

Being the most prevalent bacterial pathogen of catfish (Wagner et al., 2006), E. ictaluri poses a significant economic threat to the commercial catfish industry (Shoemaker et al., 2009), the most
significant cultured finfish in the United States. Antimicrobials applied as a feed additive are the most common means to control ESC. However, anorexia is one of the first clinical signs associated with ESC, limiting the effectiveness of antimicrobial-mediated feed. Also, because E. ictaluri can survive in pond mud for an extended period (Plumb and Quinlan, 1986), recurrence of infection is common. Furthermore, antimicrobial treatment may result in emergence of resistant strains (Starlipe et al., 1993; Dung et al., 2008).

In Gram-negative bacteria, active transport of nutrients and substrates, including iron, hemin, vitamin B12, carbohydrates, and some transition metal elements are achieved by the TonB complex (TonB-ExbB-ExbD) and TonB-dependent transporters (Schauer et al., 2008; Lim, 2010). The TonB system consists of plasma membrane proteins ExbB-ExbD and periplasmic protein TonB, which provides energy to TonB-dependent receptors to transport substrates across the outer membrane (Liao et al., 2015). The tonB gene is located next to exbB and exbD in the order exbB, exbD, and tonB in some bacterial species, such as Neisseria meningitidis (Stojiljkovic and Srinivasan, 1997), Neisseria gonorrhoeae (Biswas et al., 1997), Xanthomonas campestris (Wiggerich et al., 1997), Pasteurella haemolytica (Graham and Lo, 1997), and Helicobacter pylori (Tomb et al., 1997). In contrast, the tonB gene of Enterobacteriaceae is not linked to the exbB and exbD genes (Hannavy et al., 1990; Bruske and Heller, 1993; Bruske et al., 1993).

TonB-mediated active transport of nutrients is critical for survival of pathogenic bacteria during infection (Braun, 2001). Mutation of the tonB gene causes attenuation of virulence in several pathogenic bacteria (Jarosik et al., 1994; Seliger et al., 2001; Torres et al., 2001; Bosch et al., 2002; Hsieh et al., 2008). However, there is no information available on the importance of TonB in virulence of E. ictaluri. Therefore, the purpose of the current research was to delete the tonB gene of E. ictaluri and characterize virulence of the resulting mutant (EiΔtonB) in catfish. This study also elucidates the importance of TonB in iron acquisition, which has not been described previously.

**MATERIALS AND METHODS**

**Ethics Statement**
Catfish were used according to a protocol approved by the Institutional Animal Care and Use Committee at Mississippi State University.

**Bacterial Strains and Growth Conditions**

*Escherichia coli* C118 *λ* pir (Herrero et al., 1990) was used to clone the in-frame deleted tonB gene (ΔtonB) into pMEG-375 suicide plasmid (sacRB mobBR4 R6K ori Cm Amp) (Dozois et al., 2003). *E. coli* SM10 *λ* pir (Simon et al., 1982) was used as the donor strain in conjugation for transfer of the suicide plasmid into wild-type E. ictaluri stain 93–146 (Lawrence et al., 1997). Luria-Bertani (LB) and brain heart infusion (BHI) broth and agar (Difco, Sparks, MD) were used to culture *E. coli* at 37°C and *E. ictaluri* at 30°C, respectively. When needed, the following antibiotics and sugars (Sigma-Aldrich, Saint Louis, MN) were added to the culture medium; ampicillin (100 µg/ml), colistin (12.5 µg/ml), sucrose (5%), and mannitol (0.35%).

**In-Frame Deletion of the *E. ictaluri* tonB Gene**

The complete open reading frame of the tonB gene (locus tag = NT01E1_RS07425) was obtained from the *E. ictaluri* 93–146 genome (GenBank accession: CP001600) (Williams et al., 2012). To delete the tonB gene from *E. ictaluri*, gene splicing by overlap extension method was used as previously described (Horton et al., 1989). Briefly, the 1,114-bp upstream and 1,130-bp downstream fragments of the *E. ictaluri* tonB gene were amplified using EitonBF01-EitonBR42 and EitonBF807-EitonBR01 primer sets (Table 1), respectively. Fusion of upstream and downstream fragments was accomplished by a second PCR step using EitonBF01-EitonBR01 primers. The purified ΔtonB deletion fragment was cloned into pMEG-375 at the SacI and BamHI restriction sites using T4 DNA ligase (Promega, Madison, WI). Then the resulting plasmid (pEiΔtonB) was transferred into SM10 *λ* pir donor strain and mobilized into *E. ictaluri* by conjugation (Karsi and Lawrence, 2007) to obtain a single crossover strain on BHI agar plates containing ampicillin and colistin. The single crossover strain was streaked on LB agar with 5% sucrose and 0.35% mannitol to allow a second crossover to occur. Mutant verification was performed by ampicillin sensitivity to ensure loss of the plasmid and by PCR using the EitonBF01 and EitonBR01 primers to confirm ΔtonB. Final confirmation was conducted by sequencing the amplified ΔtonB fragment using the EitonBF01S primer. DNA sequencing was performed by Eurofins (Kentucky, USA).

**Growth of EiΔtonB under Iron-Depleted Conditions**

Growth of *E. ictaluri* ΔtonB and 93–146 were determined in iron-rich medium (BHI broth) and iron-depleted medium as previously described (Holden et al., 2012). Iron depletion in BHI broth was achieved using 100 µM 2’2’-dipyridyl (DPD), a ferrous iron chelator (Santander et al., 2012). Growth assays were performed in 24-well plates using a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek, Vermont, USA) at 30°C, with O.D. readings at λ = 600 nm taken every hour for 24 h. All growth

| Table 1 | List of primers with restriction enzyme used to construct EiΔtonB. |
|---|---|
| Primer | Sequence 5’-3’ | RE |
| EitonBF01 | AAAGAGCTGCTTTACAAAGTACCCACGCTGA | SacI |
| EitonBR42 | GGGCAGAAATTCATGTCAG | |
| EitonBF807 | CTGAAACGAAATTTCCTGCGTGTGACTGTCAT | TTTCGAGTC |
| EitonBR01 | AGGATCCATGGAACGCGCTGATGAAACAA | BamHI |
| EitonBF01S | CCTCTGACGATTCGAGTGA | |

* Bold sequences indicate the restriction enzymes (RE) added to the 5’ end primers. Two adenine nucleotides were added to the 5’ to increase the efficiency of restricting cut.

* Underlined sequences are the reverse-complement of the EitonBR42 primer.

The EitonBF01S primer was used in sequencing of the tonB gene amplified from EiΔtonB.
experiments were repeated twice. Each experiment was run with six replicates.

Iron Utilization of $Ei\Delta tonB$ under Iron-Depleted Condition

Effects of ferric chloride (FeCl₃), ferric nitrate Fe(NO₃)₃, and ferrous sulfate (FeSO₄) (Sigma) on the growth of $E. ictaluri \Delta tonB$ and 93–146 were determined under iron-depleted conditions as previously described (Khuon et al., 1998). To accomplish this, all iron sources were prepared fresh, sterilized through a 0.45 μ filter, and added to BHI broth at a final concentration of 10 μM. For each iron source, overnight cultures in BHI were adjusted to OD₆₀₀ = 1 before being subcultured at 1:100 into 5 ml BHI media containing 100 μM 2’-dipyridyl. Absorbance at OD₆₀₀ was measured after 18 h. All growth experiments were performed twice independently with four replicates.

Assessment of $E. ictaluri \Delta tonB$ Virulence

Assessment of virulence was conducted as described (Karsi et al., 2009). Briefly, 240 specific pathogen free (SPF) channel catfish (13.88 ± 0.27 cm and 27.77 ± 1.04 g) were transferred from the SPF fish hatchery at the College of Veterinary Medicine, Mississippi State University to 12 40 L flow-through tanks with aeration (20 fish per tank). Throughout the experiment, fish were kept at 25–28°C and fed to satiety using floating catfish feed. Experimental groups included wild-type strain 93–146, $Ei\Delta tonB$, and a sham control. Each group was assigned to four tanks randomly. After 1 week acclimation, the water level in tanks was lowered to 10 L. Bacterial cultures grown for 18 h were added to the tanks to provide an infection dose of ~3.32 × 10⁷ CFU per ml of water. CFUs were determined by plating serial dilutions on BHI agar. Fish challenge lasted 1 h, and the sham group was exposed to an equal volume of sterile BHI broth. Fish mortalities were recorded daily. The challenge agent was confirmed as cause of death by culturing anterior kidney swabs on BHI agar. After 21 days post-infection, all fish were re-infected with strain 93–146 (3.83 × 10⁷ CFU/ml water) as described above to evaluate protective immunity. Mortalities were recorded daily, and the mean percent survival for each treatment was calculated.

Statistical Analyses

In iron source utilization experiments, independent variables were time and iron source, while bacterial density (OD₆₀₀) was the dependent variable. Q-Q Plots and the Shapiro-Wilk normality test were used for checking normality of data. Homogeneity of variances was checked using Levene’s-Test. One-way ANOVA or Robust-Test of Equality of Means tables were used to determine the presence of significant differences among means ($p < 0.01$). The arcsine transformed percent mortality data were subjected to ANOVA using PROC GLM in SAS for Windows v9.4 (SAS Institute, Inc., Cary, NC) to assess significance. Dunnett’s post-hoc-test was applied to resolve differences between the means of groups. An alpha level of 0.05 was used in all analyses.

RESULTS

In-Frame Deletion of the $E. ictaluri \Delta tonB$ Gene

Using a double-selection strategy, we deleted 255 amino acids (including Arg-15 to Gln-269) from the 283 amino acid TonB protein, leaving 14 amino acids at both N- and C-terminals. $Ei\Delta tonB$ construction was confirmed by sequencing the amplified deletion site (Figures 1, 2).

Growth of $E. ictaluri \Delta tonB$ under Iron-Depleted Conditions

To assess the role of TonB in iron acquisition, we compared the ability of $Ei\Delta tonB$ and wild-type strain 93–146 to grow in standard and iron-depleted BHI broth. Loss of tonB caused a significant reduction in growth in standard BHI and when iron was limited by the addition 2,2’-dipyridyl (Figure 3).

Iron Utilization of $E. ictaluri \Delta tonB$ under Iron-Depleted Condition

$Ei\Delta tonB$ was tested for its ability to utilize ferric iron sources in iron-depleted media. There was no significant difference in growth between $Ei\Delta tonB$ and 93–146 in medium containing ferric chloride, ferric nitrate, and ferrous sulfate as a sole iron source (Figure 4).
Virulence of *E. ictaluri* ΔtonB

Fish infected with *EiΔtonB* had significantly (*p* ≤ 0.05) lower percent mortalities than fish infected with 93–146 (21.69 vs. 46.91% mortalities) (Figure 5A). At 21 days post-infection, fish surviving *EiΔtonB* infection had no mortalities when challenged with wild-type strain 93–146, whereas naïve fish had 40.47% mean survival (Figure 5B).

DISCUSSION

TonB mediates transport of iron and vitamin B12, as well as nickel, carbohydrates, and other substrates (Noinaj et al., 2010). In almost all sequenced Gram-negative bacteria, one or more TonB complexes have been identified (Zimbler et al., 2013). The number of TonB proteins is highly variable among bacterial genomes.

The *E. ictaluri* 93–146 genome harbors four open reading frames (ORFs) annotated as TonB-dependent receptors (NT01EI_RS03180, NT01EI_RS07425, NT01EI_RS08370, and NT01EI_RS16830), which are typically involved in transduction of energy for transport of nutrients across the outer membrane. *E. ictaluri* TonB has the highest sequence similarity with *Edwardsiella piscicida* C07-087 TonB (82% identity), *E. tarda*
EIB202 TonB (81% identity), which is in agreement with species phylogeny. The goal of the current research was to elucidate the role of *E. ictaluri* TonB in iron acquisition and virulence.

Iron acquisition and utilization play a central role in bacterial growth. The results of *in vitro* growth assays demonstrated significant decrease in the growth rates in *EiΔtonB* compared to
parent strain 93–146 under both iron-replete and iron-depleted conditions. This suggests that TonB contributes to E. ictaluri growth and iron uptake. Interestingly, E. ictaluri encodes multiple iron acquisition systems in its genome, indicating the importance of iron uptake and suggesting it is needed during infection. Similar to our findings, mutation of the TonB protein in the fish pathogen Pseudomonas fluorescens resulted in decreased growth in LB medium with or without iron supplementation (Hu et al., 2012).

Our results also showed that addition of ferric iron improves growth of both Ei\textDelta{tonB} and wild-type E. ictaluri. In a previous study, E. ictaluri ferric hydroxamate uptake mutant (Ei\Delta{fluC}) was able to grow using various iron sources (Abdelhamed et al., 2013). Multiple TonB systems have been identified in several pathogenic bacteria such as Vibrio cholera, Vibrio anguillarum, Actinobacillus pleuropneumoniae, and P. aeruginosa (Stork et al., 2004). However, not all TonB systems are essential for virulence. For example, in V. anguillarum, only tonB2 is essential for the transport of ferric anguibactin and virulence; a tonB1 mutant is fully virulent (Occhino et al., 1998).

Lack of iron leads to significant stress for bacterial pathogens and is considered a signal that leads to changes in virulence gene expression (Massé and Arguin, 2005). In the gastric environment of catfish, E. ictaluri encounters iron starvation stress during the initial phase of infection. Our group identified E. ictaluri proteins that have increased abundance in iron-restricted conditions (Dumplaga et al., 2015). In the present study, catfish experiments demonstrated a 2.16-fold reduction in Ei\Delta{tonB} virulence compared with wild-type E. ictaluri. Similarly, P. fluorescens mutants defective in the TonB-dependent outer membrane receptor (TDRs) tdr1, tdr2, and tdr3, which had 26.7, 22.3, and 24.5% mean percent mortalities, respectively, compared with 70% mortality caused by the parent strain in a turbot (Seophilthalmus maximus) model fish (Zhang et al., 2014).

However, it is possible that the function of TonB in E. ictaluri virulence may be distinct from its role in iron acquisition. There is substantial evidence that TonB function is not restricted to iron uptake. E. ictaluri TonB could be involved in transport of other substrates or the expression of yet-unidentified virulence factors in the host. The E. ictaluri genome does not have exbB and exbD genes, suggesting that E. ictaluri does not utilize the ExbB and ExbD proteins from the TonB-ExbB-ExbD complex. Moreover, deletion of tolQ and tolR genes, which are exbB and exbD homologs, does not affect E. ictaluri iron utilization (Abdelhamed et al., 2016). In Shigella dysenteriae, TonB is required for virulence and growth in the intracellular environment, but it is not required for intracellular iron acquisition (Reeves et al., 2000). Therefore, it is possible that E. ictaluri TonB may be required in vivo for something other than iron transport.

Catfish surviving infection by immersion with Ei\Delta{tonB} were completely protected against subsequent infection by the virulent parent strain, indicating that Ei\Delta{tonB} stimulated a protective immune response. Ei\Delta{tonB} is not safe to be considered a live attenuated vaccine candidate, but our results demonstrate that deletion of TonB causes attenuation without affecting protective immunogenicity. Therefore, it could be a viable gene to use in combination with other gene deletion(s) to develop a live attenuated vaccine.

In conclusion, our experiments showed that TonB participates in virulence of E. ictaluri and contributes to optimal host infection. To our knowledge, this study is the first to describe the contribution of TonB to E. ictaluri virulence. Further work is required to determine which iron transport system or combinations of systems are used to acquire iron during E. ictaluri infection.

**AUTHOR CONTRIBUTIONS**

HA, ML, and AK planned the experiments. HA and AK performed the experiments and analyzed the data. HA, ML, and AK wrote the manuscript.

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**REFERENCES**

Abdelhamed, H., Lu, J., Lawrence, M. L., and Karsi, A. (2016). Involvement of tolQ and tolR genes in Edwardsiella ictaluri virulence. Microb. Pathogen. 100, 90–94. doi: 10.1016/j.micpath.2016.09.011

Abdelhamed, H., Lu, J., Shaheen, A., Abbass, A., Lawrence, M. L., and Karsi. A. (2013). Construction and evaluation of an Edwardsiella ictaluri fhuC mutant. Vet. Microbiol. 162, 858–865. doi: 10.1016/j.vetmic.2012.11.006

Biswas, G. D., Anderson, J. E., and Sparling, P. F. (1997). Cloning and functional characterization of Neisseria gonorrhoeae gene gene. Mol. Microbiol. 24, 169–179. doi: 10.1046/j.1365-2958.1997.3421692.x

Bosch, M., Garrido, E., Lagostera, M., Pérez de Rozas, A. M., Badiola, I., and Barbé, J. (2002). Pasteurella multocida exbB, exbD and tonB genes are physically linked but independently transcribed. FEMS Microbiol. Lett. 210, 201–208. doi: 10.1111/j.1574-6968.2002.tb11181.x

Bruske, A. K., Anton, M., and Heller, K. J. (1993). Cloning and sequencing of the Klebsiella pneumoniae tonB gene and characterization of Escherichia coli-K. pneumoniae TonB hybrid proteins. Gene 131, 9–16. doi: 10.1016/0378-1119(93)90663-N

Braun, V. (2001). Iron uptake mechanisms and their regulation in pathogenic bacteria. Int. J. Med. Microbiol. 291, 67–79. doi: 10.1016/S0378-1119(01)00103

Cunningham, F. L., Jack, S. W., Hardin, D., and Wills, R. W. (2014). Risk factors associated with enteric septicemia of catfish on Mississippi commercial catfish farms. J. Aquat. Anim. Health 26, 84–90. doi: 10.1080/08997659.2014.886635

Dozois, C. M., Daigle, F., and Curtiss, R. III (2003). Identification of pathogen-specific and conserved genes expressed in vivo by an avian...
pathogenic Escherichia coli strain. Proc. Natl. Acad. Sci. U.S.A. 100, 247–252. doi: 10.1073/pnas.232687997

Dumplia, P. R., Peterson, B. C., Lawrence, M. L., and Karsi, A. (2015). Identification of differentially abundant proteins of Edwardsiella tarda during iron restriction. PLoS ONE 10:e0132504. doi: 10.1371/journal.pone.0132504

Dung, T. T., Hasebrouck, F., Tuan, N. A., Sorgeloos, P., Baele, M., and Decostere, A. (2008). Antimicrobial susceptibility pattern of Edwardsiella tarda Isolates from natural outbreaks of bacillary necrosis of Pangasianodon hypophthalmus in Vietnam. Microb. Drug Resist. 14, 311–316. doi: 10.1089/mdr.2008.0484

Eissa, N., and Wang, H.-P. (2016). Transcriptional stress response to environmental and husbandry stresses in aquaculture species. Rev. Aquacult. 8, 61–88. doi: 10.1111/raq.12081

Francis-Floyd, R., Beleau, M. H., Waterstrat, P. R., and Bowser, P. R. (1987). Effect of environmental and husbandry stressors in aquaculture species. Progr. Fish Cult. 48, 212–214. doi: 10.1577/1557-1548-8640(1986)48<212:SOIRIP&G:CO;2

Hawke, J. P. (1979). A bacterium associated with pond cultured channel catfish, *Ichthyobodo necator*. J. Fish. Res. Board Can. 36, 1508–1512. doi: 10.1139/f79-219

Holden, K. M., Browning, G. F., Noormohammadi, A. H., Markham, P. M., and Quinlan, E. E. (1986). Survival of *Edwardsiella tarda* in pond water and bottom mud. *Arch. Microbiol.* 141, 1–8. doi: 10.1577/1548-8667(1993)005<0001:PMRROE&gt ;2.3.CO;2

Horton, R. M., Hunt, H. D., Ho, S. N., Pullen, J. K., and Pease, L. R. (1989). Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension. *Science* 244, 68–71. doi: 10.1126/science.2832934

Hsieh, P. F., Lin, T. L., Lee, C. Z., Tsai, S. F., and Wang, J. T. (2008). *A* TonB-dependent outer membrane receptor. *Exp. Cell. Res.* 314, 396–400. doi: 10.1016/j.yexcr.2007.11.007

Hu, Y. H., Dang, W., and Sun, L. (2012). A TonB-dependent outer membrane receptor of *Pseudomonas fluorescens*: virulence and vaccine potential. *Trends Biochem. Sci.* 37, 129–138. doi: 10.1016/j.tibs.2011.12.004

Jarosik, G. P., Sanders, J. D., Cope, L. D., Muller-Eberhard, U., and Hansen, E. J. (1984). *Neisseria meningitidis* causing primary pyogenic liver abscess. *J. Infect. Dis.* 149, 439–443. doi: 10.1093/infdis/149.4.439

Karsi, A., Gülsoy, N., Corb, E., Dumpala, P. R., and Lawrence, M. L. (2012). Fur-regulated iron transport: haem transport genes are linked to one of two sets of tonB, exbB, exbD genes. *Microb. Biotechnol.* 5, 1493–1507. doi: 10.1111/mib.2010.00134

Karsi, A., Gülsoy, N., Corb, E., Dumpala, P. R., and Lawrence, M. L. (2008). Antimicrobial susceptibility pattern of *Edwardsiella tarda* Isolates from experimental enteric septicaemia in channel catfish, *Ictalurus punctatus* (Rafinesque), following immersion-exposure to *Edwardsiella tarda*. *J. Fish. Dis.* 31, 335–347. doi: 10.1111/j.1365-2761.1989.tb03322.x

Lim, B. L. (2010). TonB-dependent receptors in nitrogen-fixing nodulating bacteria. *Microbes Environ.* 25, 67–74. doi: 10.1264/jsme2.ME10102

Liu, M., and Cheng, A. (2015). [Structural features and functional mechanism of TonB in some Gram-negative bacteria-a review]. *Acta Microbiol. Sin.* 55, 529–536.

Maseé, E., and Arguin, M. (2005). Ironing out the problem: new mechanisms of iron homeostasis. Trends. Biochem. Sci. 30, 462–468. doi: 10.1016/j.tibs.2005.06.001

Ochoino, D. A., Wyckoff, E. E., Henderson, D. P., Whan, T. J., and Payne, S. M. (1998). *Vibrio cholerae* iron transport: haem transport genes are linked to one of two sets of tonB, exbB, exbD genes.* Mol. Microbiol.* 29, 1493–1507. doi: 10.1111/j.1365-2958.1998.02273.x

Plumb, J. A., and Quinlan, E. E. (1986). Survival of *Edwardsiella tarda* in pond water and bottom mud. *Microb. Drug Resist.* 14, 311–316. doi: 10.1089/mdr.2008.0484

Plumb, J. A., and Sanchez, D. J. (1983). *Edwardsiella icatuluri*. *Int. J. Syst. Bacteriol.* 33, 396–402. doi: 10.1099/0022-2836(99)90009-4

Plumb, J. A., and Sanchez, D. J. (1983). Susceptibility of five species of fish to *Edwardsiella icatuluri*. *J. Fish. Dis.* 6, 261–266. doi: 10.1016/0143-0429(83)90075-0

R. D., et al. (1997). The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* 387, 61–68. doi: 10.1038/378111a0

Seliger, S. S., Mey, A. R., Wall, A. M., and Payne, S. M. (2001). The two TonB systems of *Vibrio cholerae* redundant and specific functions. *Mol. Microbiol.* 39, 801–812. doi: 10.1046/j.1365-2958.2001.02273.x

Shoemaker, C. A., Kleusis, P. H., Evans, J. J., and Arias, C. R. (2009). Use of modified live vaccines in aquaculture. *J. World Aquac. Soc.* 40, 573–585. doi: 10.1749/jwa.2009.00279.x

Small, B. C., and Bödeleau, A. L. (2005). Effects of cortisol and stress on channel catfish (*Ictalurus punctatus*) pathogen susceptibility and lysozyme activity following exposure to *Edwardsiella tarda*. *Gen. Comp. Endocrinol.* 142, 256–262. doi: 10.1016/j.ygcen.2004.12.004

starliper, C. E., Cooper, R. K., Shotts, E. B., and Taylor, P. W. (1993). A broad host range mobilization system for in vivo genetic engineering: transposon mutagenesis in gram-negative bacteria. *Biotechnology* 11, 784–791. doi: 10.1038/nbt1183-784

Stojilkovic, I., and Srinivasan, N. (1997). *Neisseria meningitidis* tonB, exbB, and exbD genes: Ton-dependent utilization of protein-bound iron in Neisseriae. *J. Bacteriol.* 179, 805–812. doi: 10.1128/jb.179.3.805-812.1997

Stork, M., Di Lorenzo, M., Mourão, S., Osorio, C. R., Lemos, M. L., and Crosa, J. H. (2004). Two TonB systems function in iron transport in *Vibrio anguillarum*, but only one is essential for virulence. *Infect. Immun.* 72, 7326–7329. doi: 10.1128/IAI.72.12.7326-7329.2004

Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., et al. (1997). The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* 388, 539–547. doi: 10.1038/31483

Torres, A. G., Bedford, P., Welch, R. A., and Payne, S. M. (2001). TonB-dependent systems of uropathogenic *Escherichia coli*: aerobactin and hemep transport and TonB are required for virulence in the mouse. *Infect. Immun.* 69, 6179–6185. doi: 10.1128/IAI.69.10.6179-6185.2001

Wagner, B. A., Wise, D. J., Kho, J. H., and Thibodeau, S. D. (2006). The epidemiology of bacterial diseases in food-size channel catfish. *J. Aquat. Anim. Health* 18, 263–272. doi: 10.1577/1554-8667(2002)014<0263:TOBEDI>2.0.CO;2
Wiggerich, H. G., Klauke, B., Köplin, R., Priefer, U. B., and Puhler, A. (1997). Unusual structure of the tonB-exb DNA region of Xanthomonas campestris pv. campestris: tonB, exbB, and exbD1 are essential for ferric iron uptake, but exbD2 is not. J. Bacteriol. 179, 7103–7110. doi: 10.1128/jb.179.22.7103-7110.1997

Williams, M. L., Gillaspy, A. F., Dyer, D. W., Thune, R. L., Waldierser, G. C., Schuster, S. C., et al. (2012). Genome Sequence of Edwardsiella ictaluri 93-146, a strain associated with a natural channel catfish outbreak of enteric septicemia of catfish. J. Bacteriol. 194, 740–741. doi: 10.1128/JB.06522-11

Zhang, S. R., Zhang, L., and Sun, L. (2014). Identification and analysis of three virulence-associated TonB-dependent outer membrane receptors of Pseudomonas fluorescens. Dis. Aquat. Organ. 110, 181–191. doi: 10.3354/dao02771

Zimbler, D. L., Arivett, B. A., Beckett, A. C., Menke, S. M., and Actis, L. A. (2013). Functional features of TonB energy transduction systems of Acinetobacter baumannii. Infect. Immun. 81, 3382–3394. doi: 10.1128/IAI.00540-13

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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