

Identification of a Novel TonB Family Member TonB4 Involved in Iron Adaptation in *Acinetobacter baumannii*

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BACKGROUND

Iron acquisition is critical for *Acinetobacter baumannii* to survive and establish infection in the iron-restricted host environment. Although TonB-dependent systems are central to outer membrane nutrient uptake, the contribution of distinct TonB-family members to *A. baumannii* pathogenic fitness remains poorly defined. By comparing respiratory infection- and colonization-derived isolates, we identified a previously uncharacterized TonB-family gene enriched in infection-associated strains. This gene was designated *tonB4* in the hypervirulent strain LAC-4, and its structural and functional roles were further investigated.

METHODS

■ Bioinformatic characterization:

Sequence, structural, phylogenetic, and genomic context analyses of *tonB4* were performed.

■ Strain construction:

$\Delta tonB4$ and the corresponding complemented strain were generated in the LAC-4 background.

■ Iron-dependent growth assessment:

Iron-dependent growth was assessed under iron-replete and iron-limited conditions supplemented with $FeCl_3$ or hemin.

RESULTS

1. Structural and genomic distinctiveness of TonB4

TonB4 retained the conserved C-terminal TonB domain but displayed an extended flexible region, indicating structural divergence from canonical TonB proteins (Figure 1 A-D). Genomic context analysis showed that, unlike *tonB1*–*3*, *tonB4* was not colocalized with *exbB/exbD*, but instead clustered with heme- and iron-associated genes (Figure 2).

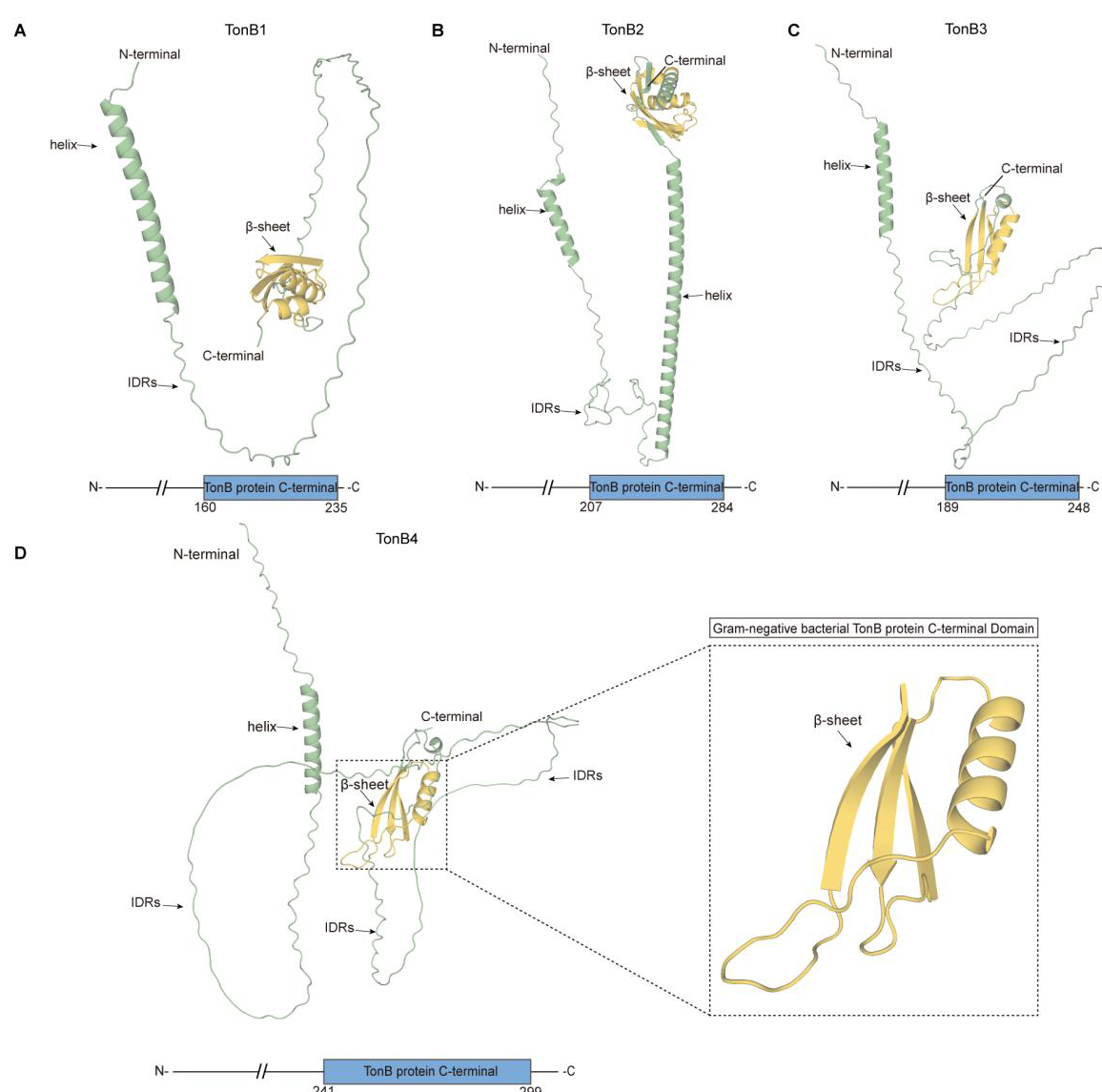


Figure 1 Structural features and conserved domain organization of four TonB proteins.

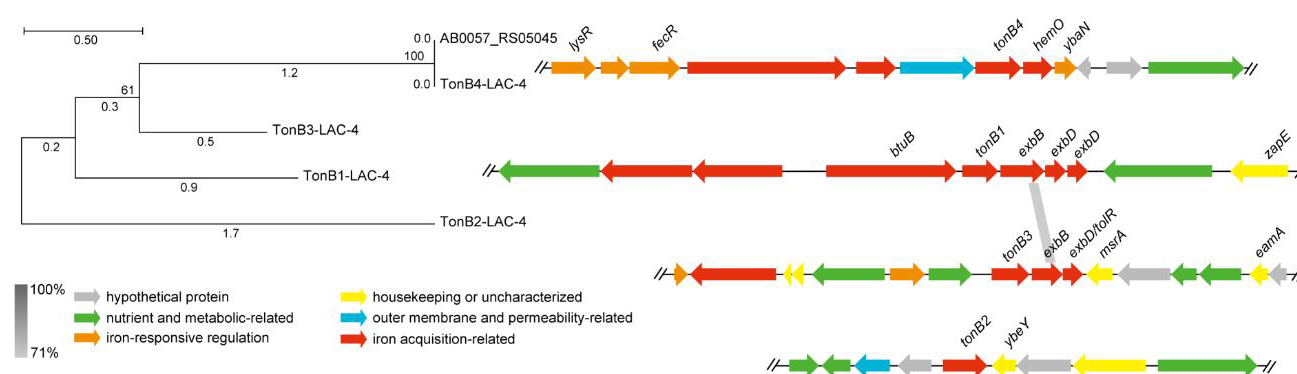


Figure 1 Genomic context comparison of the four *tonB* genes.

2. TonB4 contributes to iron acquisition under iron limitation

$\Delta tonB4$ grew normally under iron-replete conditions but showed impaired growth and increased siderophore production under iron limitation, indicating compromised iron acquisition. Growth was rescued by $FeCl_3$ and more efficiently by low concentrations of hemin (Figure 3).

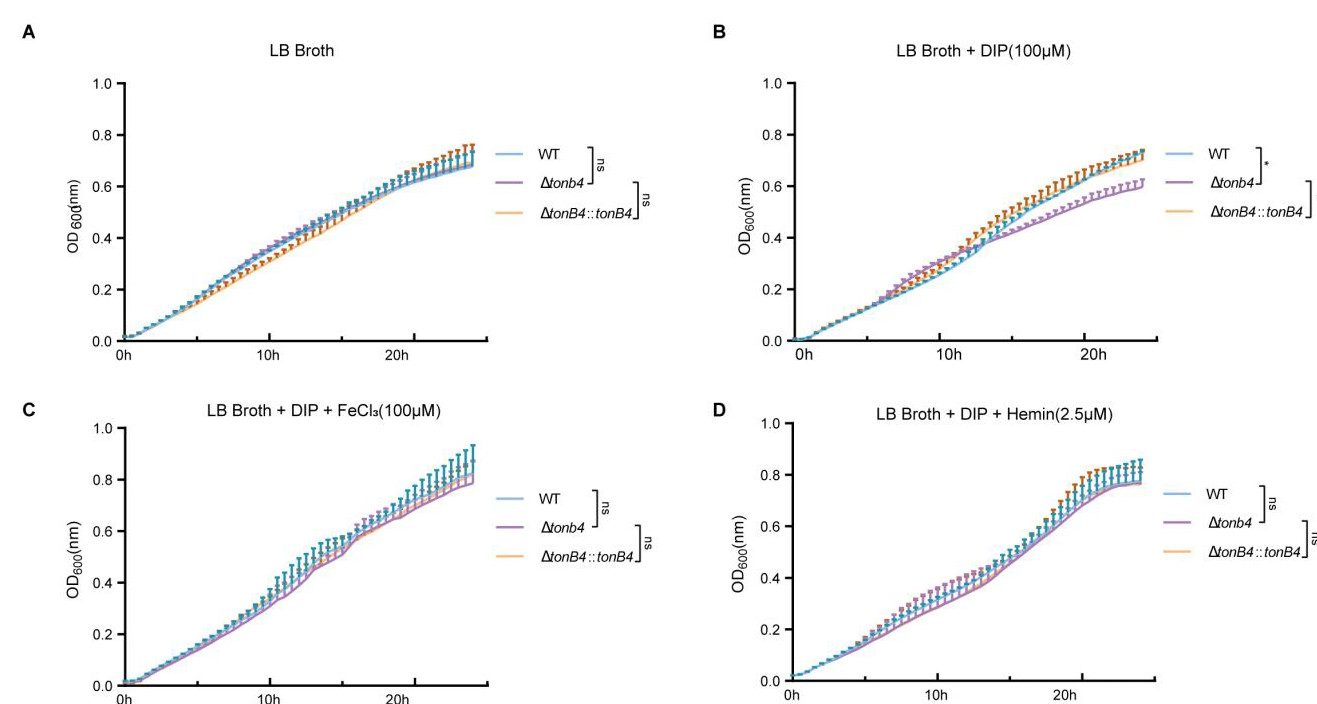


Figure 3 Effect of *tonB4* deletion on bacterial growth under iron limitation and iron-source rescue.

CONCLUSION

TonB4 represents a structurally and genomically distinct TonB-family member that contributes to adaptation under iron-restricted conditions, likely through a non-canonical iron acquisition pathway. These findings highlight functional diversification among TonB-family proteins in *A. baumannii*.

References:

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