Getting outside the cell: characterization of holins encoded by two distinct phages to exit their *Bacillus cereus* host

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LYSIS PATHWAY

At the end of the phage replication cycle, the processes of bacterial lysis and release of progeny virions rely on the **holin-endolysin** dyad. In the **canonical model** (**Fig. 1**), the concerted action of these proteins provoke bacterial lysis through holin permeabilization of the inner membrane (IM), allowing cytoplasm-accumulated endolysins to reach the periplasm where it degrades the peptidoglycan (PG) meshwork.

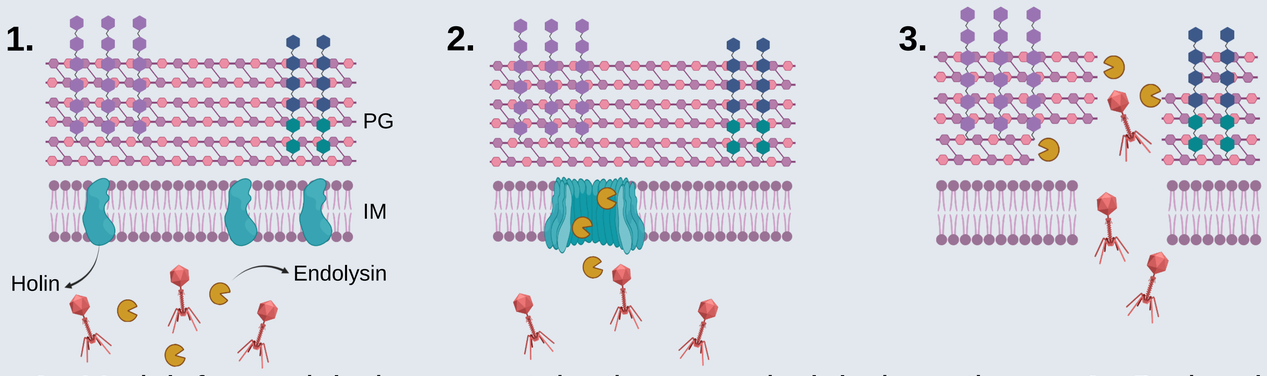
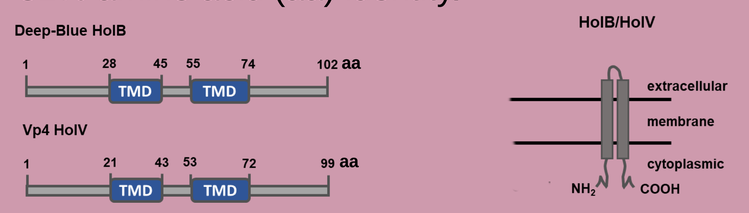


Figure 1. Model for endolysins export in the canonical lysis pathway. **1.** During late gene expression, holin and endolysin are produced and accumulated harmlessly in the IM and cytosol, respectively. **2.** Triggering event: holin redistribution and re-arrangements to form micron-scale holes, which allow endolysin to cross the IM. **3.** PG degradation and IM weakening leading to the release of progeny phages at the expense of the host cell.

HOLIN CANDIDATES

Deep-Blue and Vp4 phages belong to the *Herelleviridae* family (myoviruses) and infect strains within the *B. cereus* group [1]. This bacterial cluster includes closely related species. Among them, some strains are known to be pathogens involved in foodborne intoxications and infections.

Both phages encode a single candidate holin: HolB (gp133, 102 aa) and HolV (gp184, 99 aa), respectively, both displaying two transmembrane domains (TMD) and both termini presumably located in the cytoplasm. Despite similar arrangement, they share only 51% amino acid (aa) identity.



RESULTS

In *Escherichia coli*, HolB and HolV display holin activities

The growth of *E. coli* cells expressing HolB and HolV was optically monitored to evaluate the protein lytic activity (**Fig. 2**). Upon induction, holins presumably form non-specific holes allowing the passage of the T7 lysozyme that can mimic endolysin action by breaking down the PG.

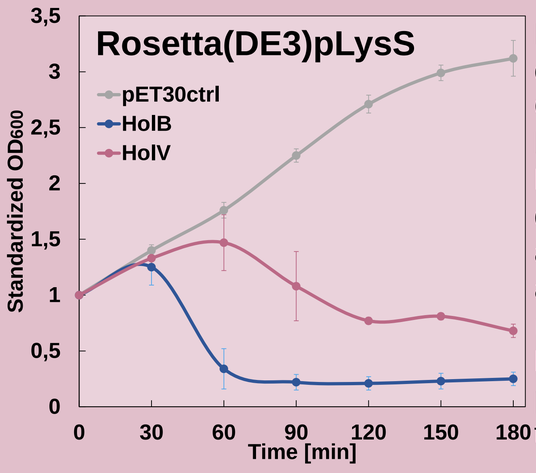


Figure 2. Expression of holins in *E. coli*. Cell growth assessed by OD_{600} monitoring. Holin induction was done at time 0 by adding 0.5 mM IPTG and measured by OD monitoring. Experiments were performed in triplicate.

HolB and HolV localization in *E. coli* was evaluated using a C-terminal GFP tag (**Fig. 3**). It showed that the proteins are localized at the cell periphery, presumably in the IM.

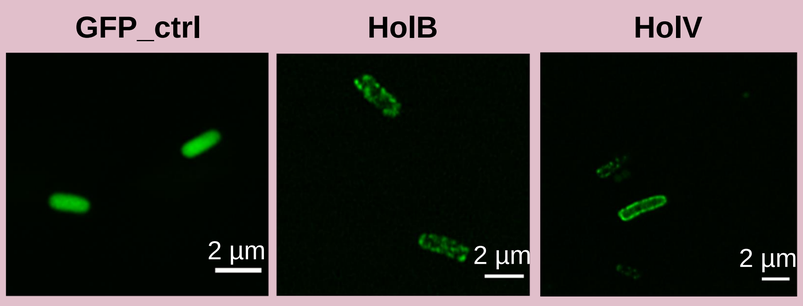


Figure 3. Confocal microscopic imaging of *E. coli* BL21(DE3) cells expressing GFP-tagged HolB and HolV.

In *B. thuringiensis*, the expression of holins alone is not sufficient to observe cell lysis

The effect of holins expression in *B. thuringiensis* was then evaluated by growth monitoring (**Fig. 4**). No bacterial lysis was observed upon HolB expression while cells expressing HolV only showed a moderate OD diminution after 24h. However, co-expression with their endolysin (PlyB and PlyV) did enhance toxicity. Confocal microscopy confirmed their localization at the cell periphery (**Fig. 5**)

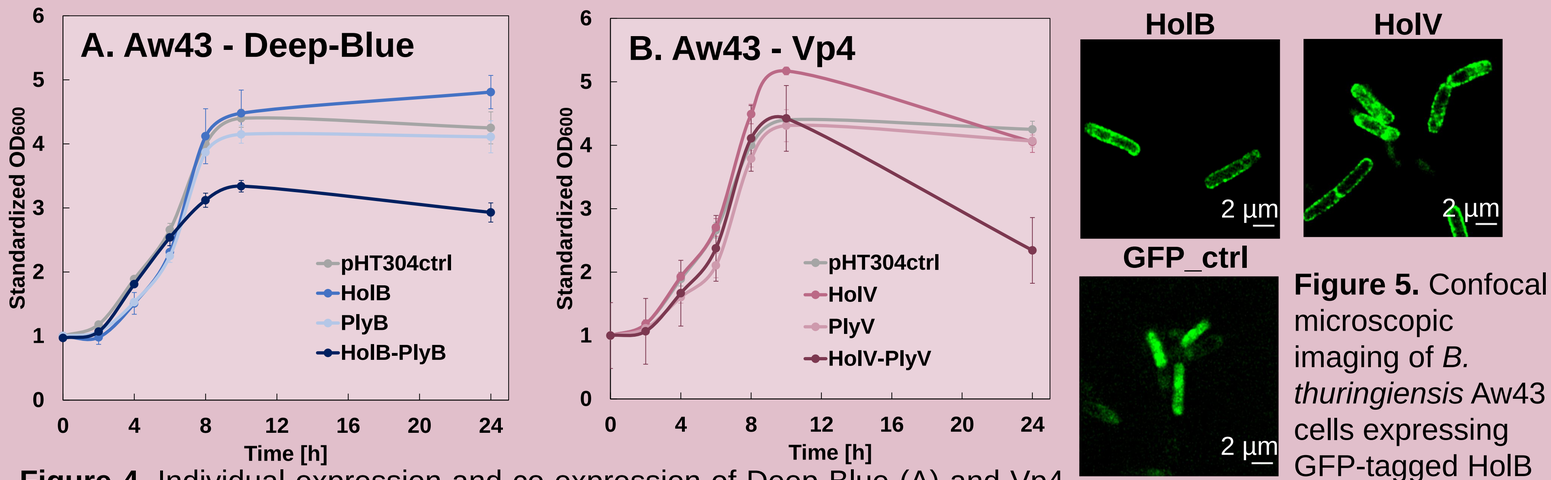


Figure 4. Individual expression and co-expression of Deep-Blue (A) and Vp4 (B) holins and endolysins in *B. thuringiensis* Aw43. Holin induction was done at time 0 by adding 20 mM xylose. Experiments were performed in triplicate.

Figure 5. Confocal microscopic imaging of *B. thuringiensis* Aw43 cells expressing GFP-tagged HolB and HolV.

Despite sharing similar organizations, HolB and HolV display distinct cell toxicity levels

In order to assess which parts of HolB and HolV were involved in cell toxicity, truncated versions were constructed. The effect of these *ad hoc* deletions on cell growth was assessed in *E. coli* (**Fig. 6**). Results indicated that despite similar predicted topologies, different behaviours are observed, most likely due to differences in amino acid composition and charge.

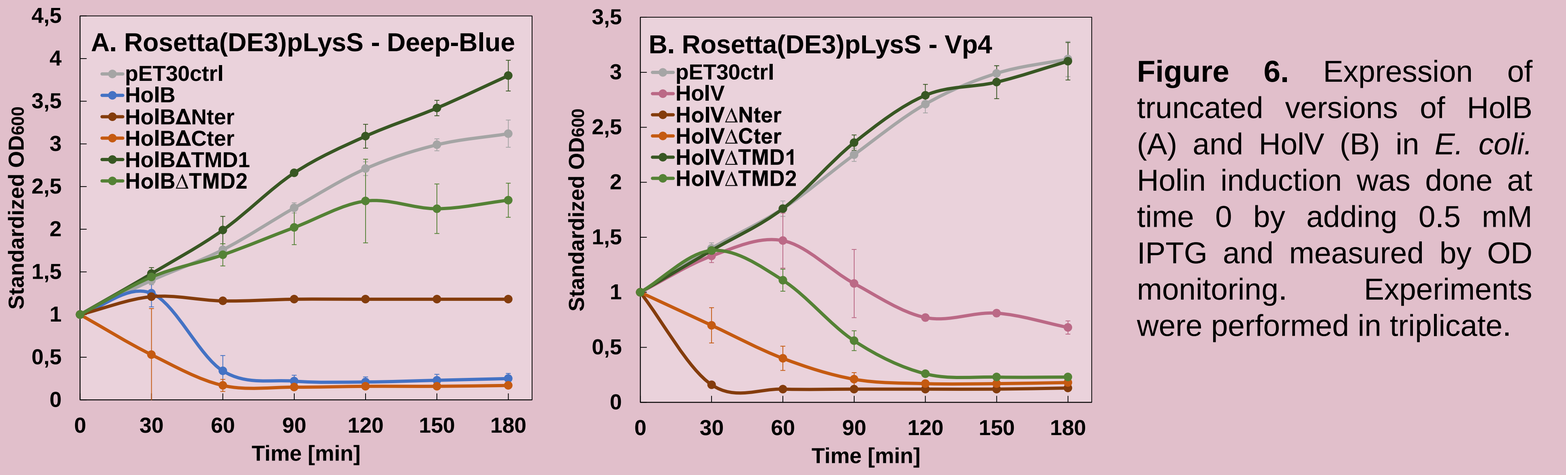


Figure 6. Expression of truncated versions of HolB (A) and HolV (B) in *E. coli.* Holin induction was done at time 0 by adding 0.5 mM IPTG and measured by OD monitoring. Experiments were performed in triplicate.

CONCLUSION

In contrast to holins encoded by phages targeting Gram-negative hosts, especially *E. coli*, those found in phages infecting Gram-positive bacteria have not been characterized in great detail.

In this work, HolB and HolV have been identified as the cognate holins of phages Deep-Blue and Vp4, respectively, and their functional characterization in *E. coli* and protein localization have been assessed. In *Bacillus*, enhanced lysis was observed when *holV* was expressed with its cognate endolysin within 24h while co-expression of *holB* and *plyB221* only led to a growth arrest. This slow or absent lysis could be due to a missing lysis partner that may be necessary to achieve an optimal and timely lysis.

All in one, this work provides novel insights into the holins involved in the phage-mediated lysis of Gram-positive hosts.

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References

[1] Hock, L., Gillis, A., & Mahillon, J. (2016). Complete genome sequence of bacteriophage Deep-Blue infecting emetic *Bacillus cereus*. *Genome announcements*, *4*(3), e00115-16.

Illustrations were created with BioRender.com