Phage-mediated Shiga-toxin (Stx2d) gene transduction from O80:H2 Shiga toxigenic *Escherichia coli* (STEC) to non-STEC strains and *in vivo* virulence assessment

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The university of Liège provided financial support ('Fonds spéciaux de la Recherche Project HYBRID_COLI_O80 ')

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) are major foodborne pathogens causing human diseases ranging from diarrhea to life-threatening complications such as hemolytic–uremic syndrome. Virulence of STEC strains and their ability to cause severe diseases are linked to the activity of prophage-encoded Shiga toxins (Stxs). Stx phage acquisition and stability studies are crucial in terms of public health. The first objective of this work was to isolate and characterize the Stx2d phage isolated from STEC O80:H2, an emerging serotype in humans and calves, and then study the transduction of the *stx2d* gene phage-mediated in non-STEC strains. The second objective was to assess the survival of *Galleria mellonella* larvae inoculated with these convertant strains.

Results

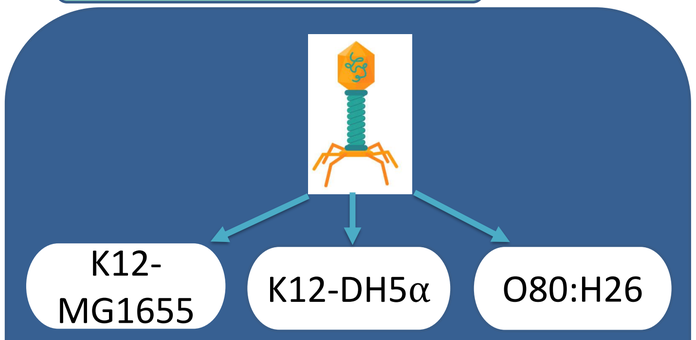

Fig1. *Stx2d* gene transduction
Three non-STEC strains were successfully converted with the *stx2d* gene through the Stx2d phage and confirmed by PCR

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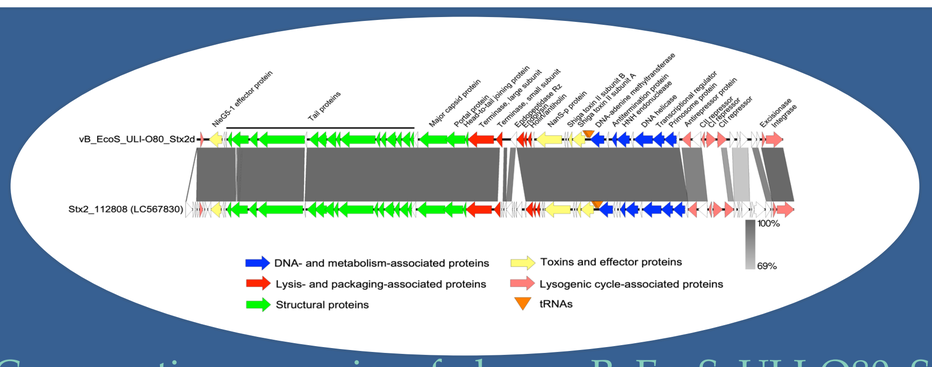

Fig2. Comparative genomics of phage vB_EcoS_ULI-O80_Stx2d and Stx2_112808
The two phages encode different lysogenic cycle associated proteins such as integrase, excisionase and different repressor such as repressor CI, CII and the antirepressor. Stx region is represented by the orange box.

2

Material and methods

In vitro: Three temperate phages were induced and isolated from a bovine STEC *E. coli* O80:H2 under UV radiation. The most stable phage vB_EcoS_ULI-O80_Stx2d was used to infect 5 non-STEC strains and its genome was analyzed.

In vivo: For each 3 transduced strains, 270 larvae were divided into 9 groups (8 ≠ concentrations and one control PBS). The larvae were incubated at 37°C and mortality was evaluated every 24h during 4 days.

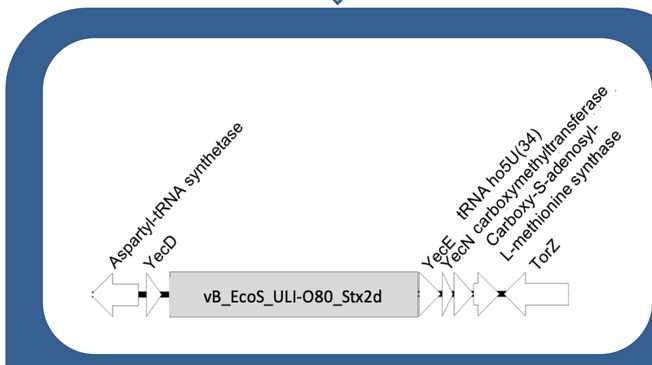

Fig3. Insertion site *yecE* of the three convertant strains

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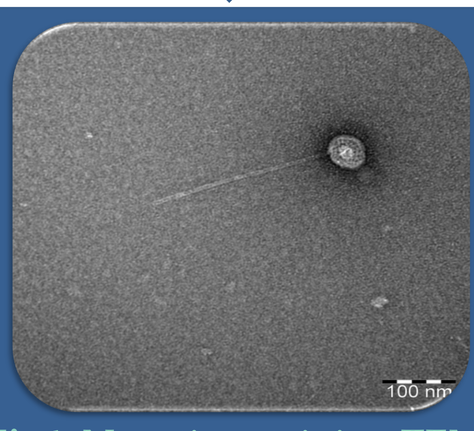

Fig4. Negative staining TEM showed that vB_EcoS_ULI-O80_Stx2d is attributed to the siphovirus morphology

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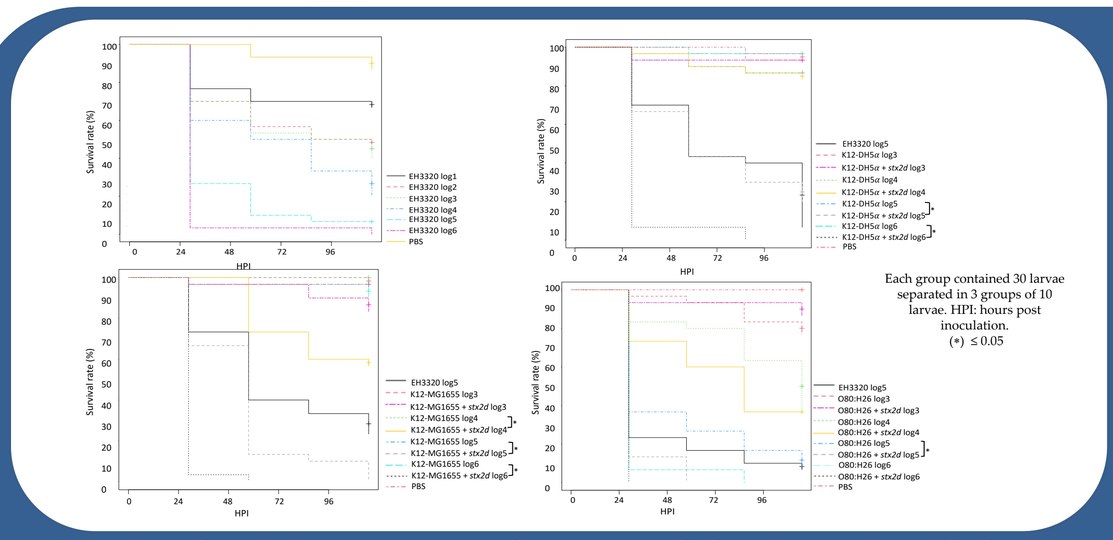

Fig5. Kaplan-Meier survival curves of the *G. mellonella* larvae experiments

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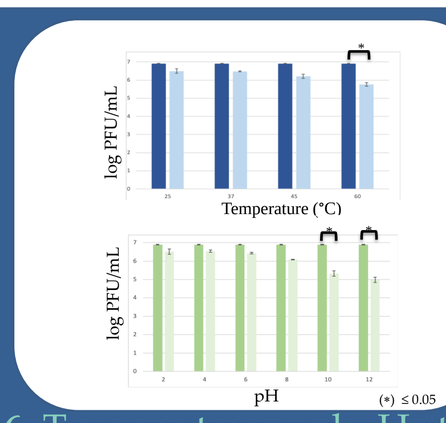

Fig6. Temperature and pH stability of vB_EcoS_ULI-O80_Stx2d

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Conclusion

The phage vB_EcoS_ULI-O80_Stx2d belongs to the *Caudoviricetes* class (currently unclassified genus and family) and to the siphovirus morphology, it is stable and can resist to moderate pH and temperature conditions. Induction of lambdoid prophages carrying *stx* genes can convert non-pathogenic *E. coli* into STECs. *In vivo* experiments showed that convertant strains caused significantly higher mortality rates than the corresponding non-STEC strains.