Introduction

- Sexually transmitted infections caused by *Chlamydia trachomatis* represent the most commonly reported bacterial infections worldwide.
- New cases reported in the US have risen from ~0.7 million in 2000 to ~1.8 million in 2019.
- Over 70% of women with *C. trachomatis* genital tract infections are asymptomatic leading to pelvic inflammatory disease and infertility.
- The way in which the Chlamydia disease is caused by *C. trachomatis* is unclear, but it is likely due to its ability to adapt to, survive in, and replicate within the intracellular niche.

Topoisomerase A

- Topoisomerase I (TopA) is an essential enzyme that has a key role in removing excess negative supercoils in DNA by relaxing the supercoils.
- TopA can affect bacterial viability and is widely characterized in *Escherichia coli* (*E. coli*).
- *C. trachomatis* topA is distinct in that it consists of regions homologous to bacterial TopA, as well as the eukaryotic SWIB domain.

Methods

- The shuttle plasmid pTopAH6 expressing chlamydial topA (Fig. 1) and the vector control pBOMBL were constructed.
- *E. coli* strain VS111-K2 defective in topA gene was transformed with the plasmids, pTopAH6 and pBOMBL, respectively.
- A growth curve was taken to compare the growth of strains carrying pTopAH6 or pBOMBL at 37 °C during 8 hrs period in the presence or absence of aTC.
- Plate assay on LB agar was conducted to assess whether or not the *E. coli* cells grew at 30 °C, 37 °C, and 40 °C in the presence or absence of aTC.

Results

- **Figure 1. Map of pTopAH6 containing chlamydial topA-His6 under the control of aTC inducible Ptet promoter**

Plate Assay

- **Figure 2. Growth of *E. coli* VS111-K2-pTopAH6 vs -pBOMBL in the absence or presence of aTC after incubating 0-8 hrs.**

- **Figure 3. Growth of *E. coli* VS111-K2-pTopAH6 (right) vs pBOMBL (left) in the absence (A, C, E, G) or presence (B, D, F, H) of aTC after incubating at 30 °C, 37 °C, or 42 °C for 18 hours or 48 hours.**

Conclusion and future study

- The growth curve: adding aTC increased the growth of *E. coli* VS111-K2-pTopAH6 compared to VS111-K2-pBOMBL.
- Plate assay: *E. coli* VS111-K2-pTopAH6 grew better than that VS111-K2-pBOMBL at 30 °C; At 37 °C, both strains grew at the similar pattern; At 42 °C, both strains grew poorly at 18 h pi. Incubation for 48 h, VS111-K2-pTopAH6 grew better.
- The growth curve revealed that the *E. coli* strain VS111-K2-pTopAH6 grew faster than VS111-K2-pBOMBL.
- Expression of chlamydial topA in *E. coli* strain VS111-K2 was able to restore growth in *E. coli* strain VS111-K2.
- Future studies are required to clarify whether the DNA relaxation activity of the chlamydial topA is responsible for its role in restoring the viability of *E. coli* VS111-K2 cell.
- This system may be useful to continue this study in order to identify a functional domain and screen drugs acting on chlamydial topA.

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