Expression of *Chlamydia trachomatis* Topoisomerase I Can Compliment Conditional-lethal DNA Topoisomerase I Mutation in *Escherichia coli*

Noel S. Wolf, Caitlynn Diggs, Li Shen.

LSUHSC, Department of Microbiology

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

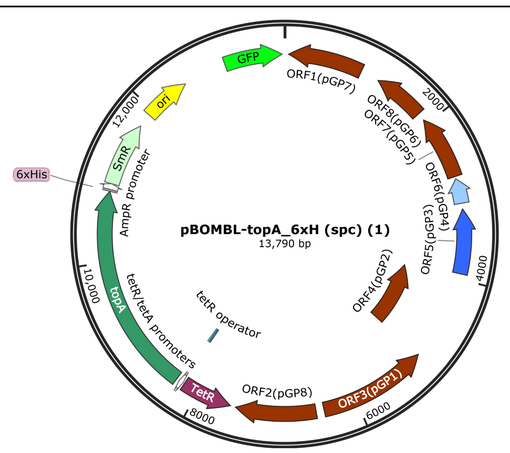

Figure 1. Map of pTopAH6 containing chlamydial *topA*-His6 under the control of aTC inducible P*tet* promoter

Growth Curve

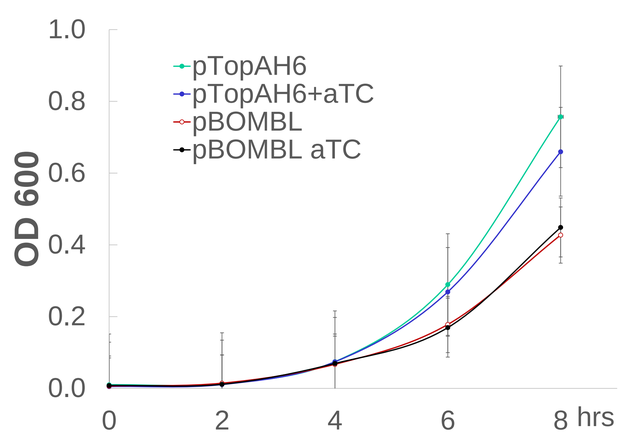

Figure 2. Growth of *E. coli* VS111-K2 -pTopAH6 vs -pBOMBL in the absence or presence of aTC after incubating 0-8 hrs.

Plate Assay

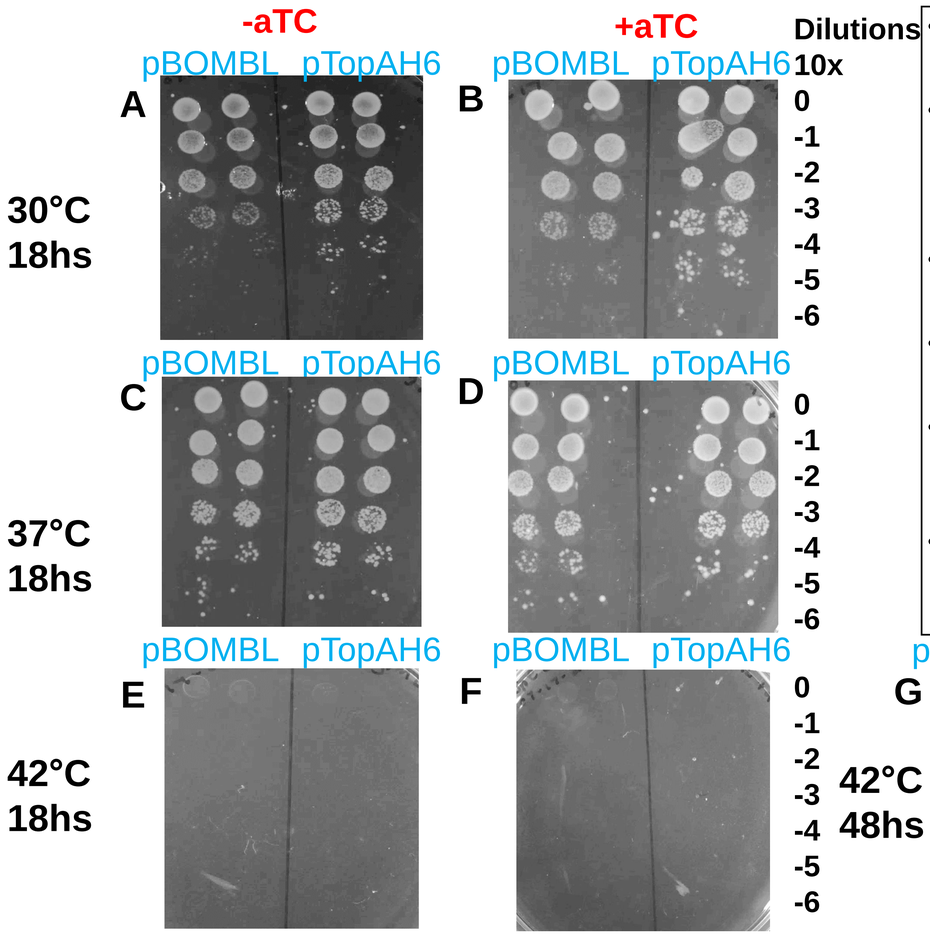

Figure 3. Growth of *E. coli* VS111-K2 pTopAH6 (right) vs pBOMBL (left) in the absence (A, C, E, G) or presence (B, D, F, H) of ATC after incubating at 30 °C, 37 °C, or 42 °C for 18 hours or 48 hours.

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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