An *In Vitro* Model to Test the effects of Tryptophan Metabolites on *Chlamydia trachomatis* development

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Introduction

Chlamydia trachomatis (Ct) is an obligate intracellular bacterium that is a common cause of genitourinary tract infections. Women with bacterial vaginosis (BV), a dysbiosis of the vaginal microbiota, characterized by an overgrowth of gram-negative anaerobes and a decrease in lactobacilli, have an elevated rate of Ct infection. Ct is a tryptophan auxotroph, but BV associated bacteria utilize and significantly lower vaginal tryptophan, producing an array of tryptophan catabolites in addition to those made by the host, and induce proinflammatory cytokines that can induce the tryptophan-degrading enzyme IDO1. In vitro assays have demonstrated that genital Ct strains have mechanisms to counteract this stress, but if, and how Ct development is modulated under specific BV-mediated environmental conditions remains unknown.

This study aimed to develop a new *in vitro* model that would enable us to determine if, and at which time points, tryptophan metabolites could modulate the Ct developmental cycle. We created a dual promoter reporter Ct strain, in which an early promoter was linked to the mCherry fluorescence protein and a late promoter to the green fluorescence protein GFP (Fig 1). This is used to visualize Ct development progression.

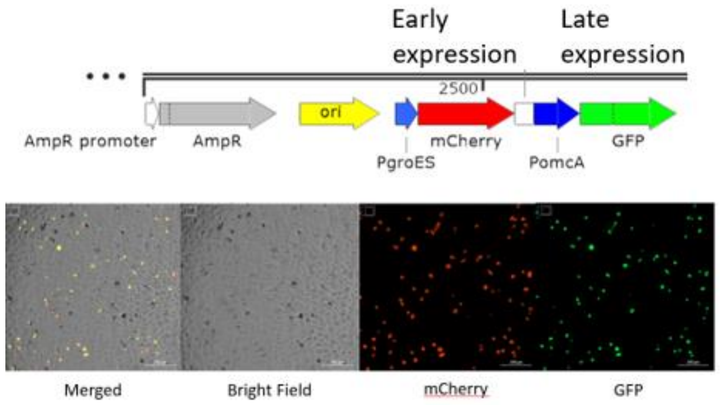

Figure 1. *C. trachomatis reporter strain used in this study. C. trachomatis strain was transformed with a shuttle plasmid, in which the early-promoter from the groES (PgroES) was linked to mCherry fluorescence protein. The late-promoter from omcA (PomcA) was inked to green fluorescence Protein (GFP).*

Tryptophan metabolites from multiple pathways were tested for their affects on Ct development in this study.

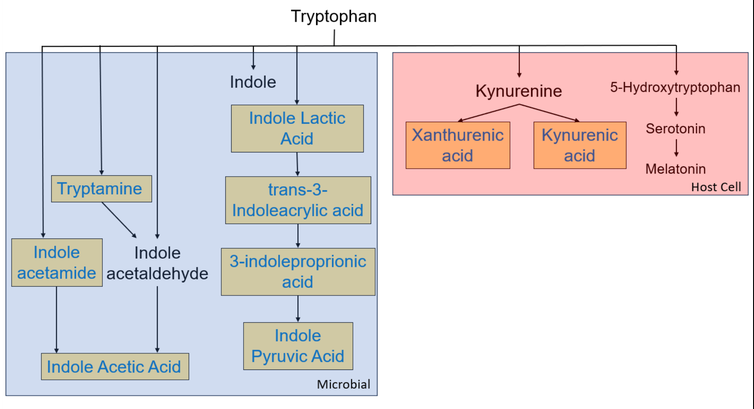

Figure 2. *This simplified diagram of tryptophan metabolism highlights the compounds tested in this study. There are multiple pathways of tryptophan metabolism that occur both in bacteria of the vaginal microbiome and in host cells as outlined.*

Methods

- Compounds were tested at supra-physiologic and physiologic concentrations for cell toxicity using CCK-8 assay
  - All concentrations used were within the physiologic range of BV(+) patients
- HeLa 229 cells were seeded on 96 well plates and cultured in RPMI 1640 medium containing 5% FBS.
- Plates were incubated for 24 hours prior to infection with *Chlamydia*.
- Medium containing the different compounds was added to the culture immediately after infection.
- Live cells were imaged at 24 hours & 42 hours post infection (h pi).
- The morphology of chlamydial inclusions was analyzed using Gen5.
- The data were compared using two-way ANOVA analysis in GraphPad Prism.

Results

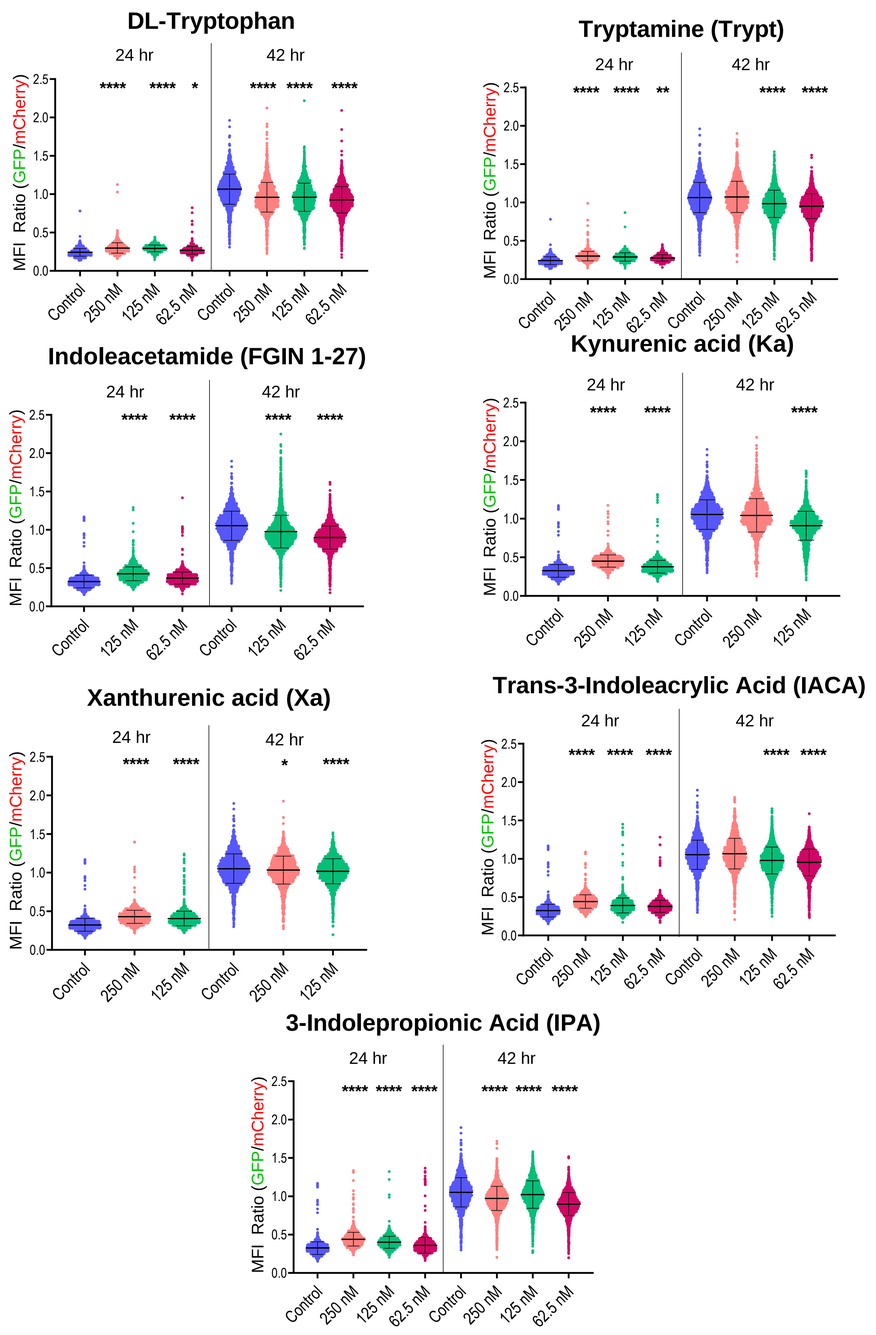

Figure 3. *Ratio of mean fluorescence intensity (MFI) of GFP versus mCherry for each inclusion from three replicates of each condition plotted with the mean ± one standard deviation (SD). Each concentration is plotted compared to the control for that time point tested. Asterisks (*) show the level of significant difference from the untreated control for each condition. This group of compounds saw significant increases in GFP/mCherry at the 24 h pi and significant decreases in GFP/mCherry at the 42 h pi.*

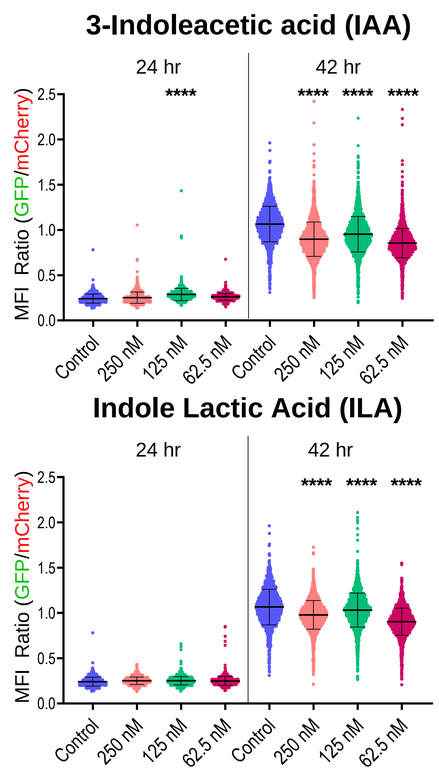

Figure 4. *. Ratio of mean fluorescence intensity (MFI) of GFP versus mCherry for each inclusion from three replicates of each condition plotted with the mean ± SD. Each concentration is plotted compared to the untreated control for that time point. Asterisks (*) show the level of significant difference from the control for each condition. IAA and ILA both saw little to no changes in GFP/mCherry ratio at the 24 h pi but showed significant decreases in the ratio at the 42 h pi*

Conclusions

Using HeLa cells infected with a *C. trachomatis* promoter reporter strain, we were able to assess the effect of different tryptophan metabolites on the developmental cycle of *C. trachomatis*. We observed that there were two subsets of compounds.

1.) ILA and IAA – showed no significant change from control at 24 h pi but did show significant decrease in expression of GFP/mCherry at the 42 h pi.

2.) DL-Trp, Trypt, FGIN 1-27, Ka, Xa, IACA, and IPA – showed significant increases in GFP expression at 24 h pi and significant decreases in GFP/mCherry at 42 h pi.

There are multiple potential explanations for this observed effect, one of which is outlined below.

Tryptophan metabolites stimulate faster growth

↓

Earlier expression of GFP late promoter

↓

Increased GFP/mCherry ratio at 24 hours

↓

Earlier start of secondary infection cycle

↓

Decreased GFP/mCherry ratio at 42 hours

Further investigation is required to determine the cause of the observed effects.