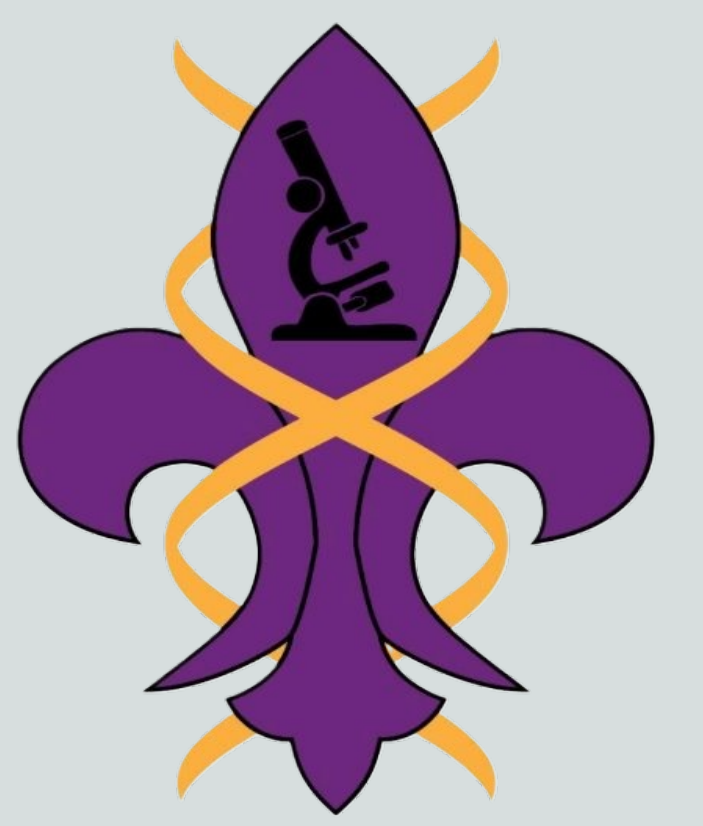


Could members of the maternal vaginal microbiome aid in the optimal maturation of their newborn infant's gastrointestinal microbiome?



Lillianne P. Ziegler¹, John W. Lammons BS², Li Shen MD PhD² and Alison J. Quayle PhD²

¹Benjamin Franklin High School, New Orleans and ²LSUHSC Department of Microbiology, Immunology, and Parasitology

Introduction

The bacterial metabolite indole-3-lactic acid (ILA) has an important role in maturing, and preventing inflammation in, a newborn infant's gastrointestinal (GI) tract^{1,2}.

ILA is a predominant metabolite produced by *Bifidobacter longum* subsp. *infantis* (*B.infantis*), a major 'pioneer' bacterium in the healthy infant GI microbiome. These *Bifidobacter* make ILA by metabolizing tryptophan, an essential amino acid found in high concentrations in breastmilk^{1,2}.

My lab recently observed a significant concentration of ILA in the vaginal secretions of some, but not all, young reproductive age women. We hypothesized that ILA-producing vaginal bacteria, transferred to newborns at birth, could aid in the optimal early development of their infant's GI tract.

The objectives of my study were to determine: (**Aim 1**) the association of ILA with specific vaginal communities and bacterial species and (**Aim 2**) if ILA concentrations found in the vaginal secretions can ligate the aryl hydrocarbon receptor (AhR), which is reported to play a key role in maintaining homeostasis and preventing inflammation at mucosal sites.

Methods

Aim 1: We used n=109 vaginal samples from women (18-32 y.o) with ILA concentrations previously determined by ultra-performance liquid chromatography tandem mass spectrometry, and with associated 16S rRNA microbiome data. VALENCIA had been used to classify the vaginal community state type (CST) of each specimen resulting in the following assignments: **CST I**, the optimal microbiome type and dominated by *Lactobacillus crispatus*, **CST III**, dominated by *L. iners*, and **CST IV**, characterized by a diverse array of bacterial vaginosis (BV) associated anaerobes creating a non-optimal vaginal environment (**Fig.1**) CSTs were also subcategorized as **CST I-A** (highest relative abundance of *L. crispatus*), **CST I-B** (lower relative abundance of *L. crispatus*), **CST III-A** (higher relative abundance *L. iners*), and **CST III-B** (lower relative abundance of *L. iners*), **CST IV-A** (higher relative abundance BVAB1-*Candidatus Lachnocurva vaginae*) and **CST IV-B** (higher relative abundance of *Gardnerella vaginalis*).³

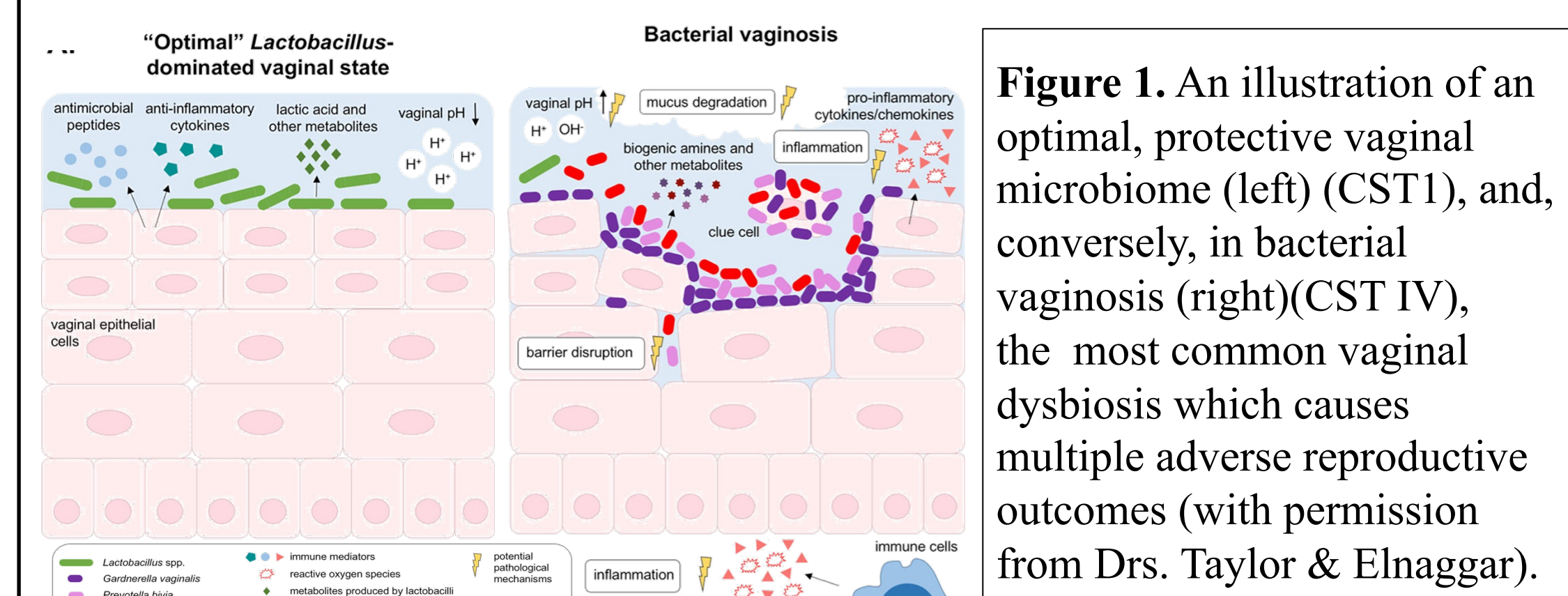


Figure 1. An illustration of an optimal, protective vaginal microbiome (left) (CST I), and, conversely, in bacterial vaginosis (right) (CST IV), the most common vaginal dysbiosis which causes multiple adverse reproductive outcomes (with permission from Drs. Taylor & Elnaggar).

Aim 2: The HT29-Lucia- AhR cell line (InvivoGen) is an epithelial cell line engineered to express endogenous AhR (**Fig.2**). I expanded and utilized the line according to the standard guidelines. This line permits the screening of potential AHR ligands by measuring secreted Lucia

luciferase reporter protein in the culture supernatant. ILA (Sigma-Aldrich) was utilized at the approximate concentrations we had observed across the spectrum of the patient vaginal secretions. An AHR agonist (FICZ) was used as a positive control at the recommended concentration.

Statistical analysis comparing group means was performed using a Wilcoxon rank sum test. An analysis testing the association between microbial abundance and ILA abundance was performed using linear modeling.

ILA association with vaginal CST

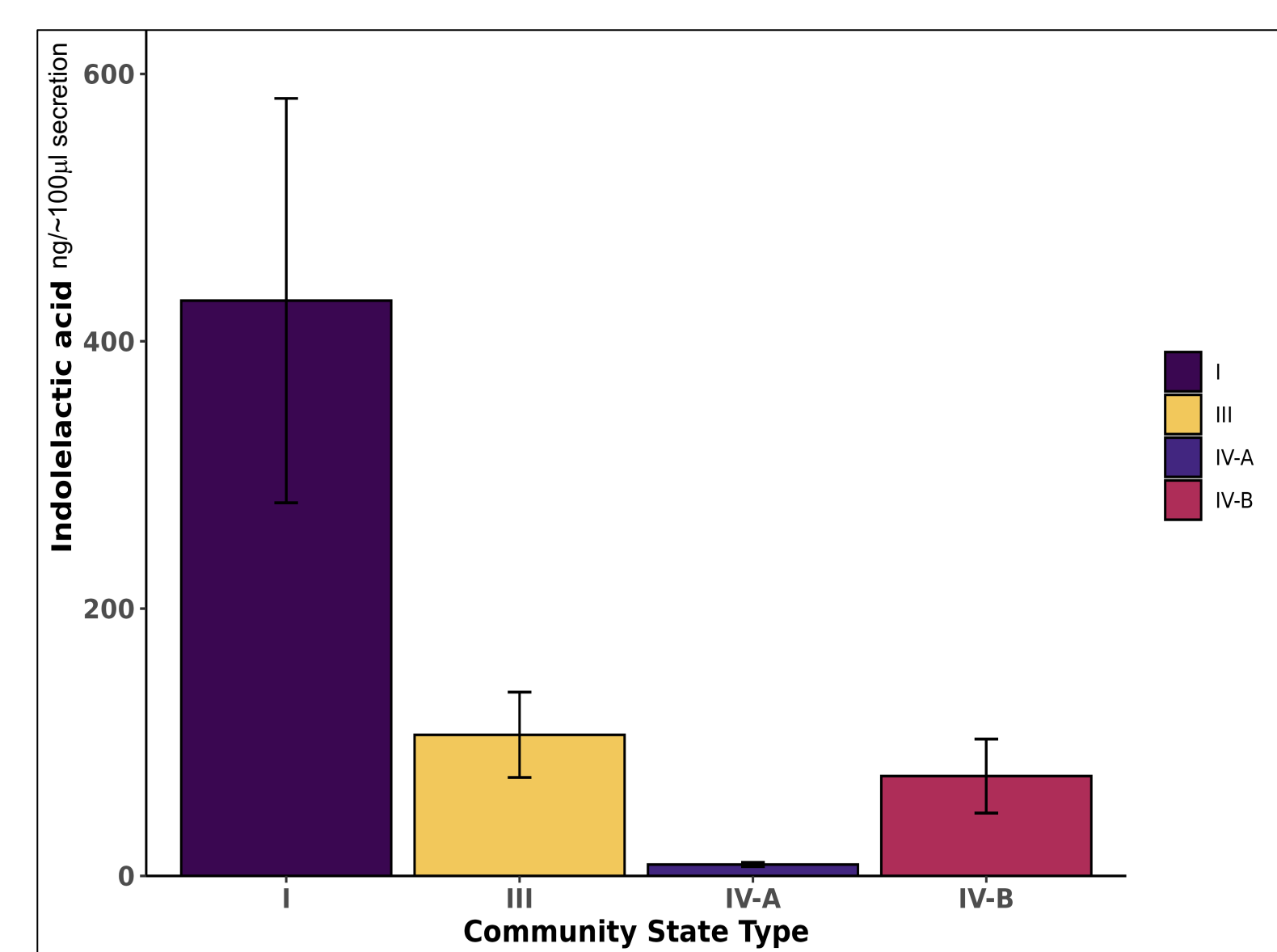


Figure 3. Abundance of ILA (ng/~100µl vaginal secretions) is significantly higher in women with CST I, the optimal vaginal microbiome. Participants were grouped based on CST and those in CST I, the optimal CST and dominated by *L.crispatus*, had a significantly greater abundance of ILA compared to the BV CST's IV-A & IV-B ($p < 0.001$).

ILA association with vaginal sub-CST

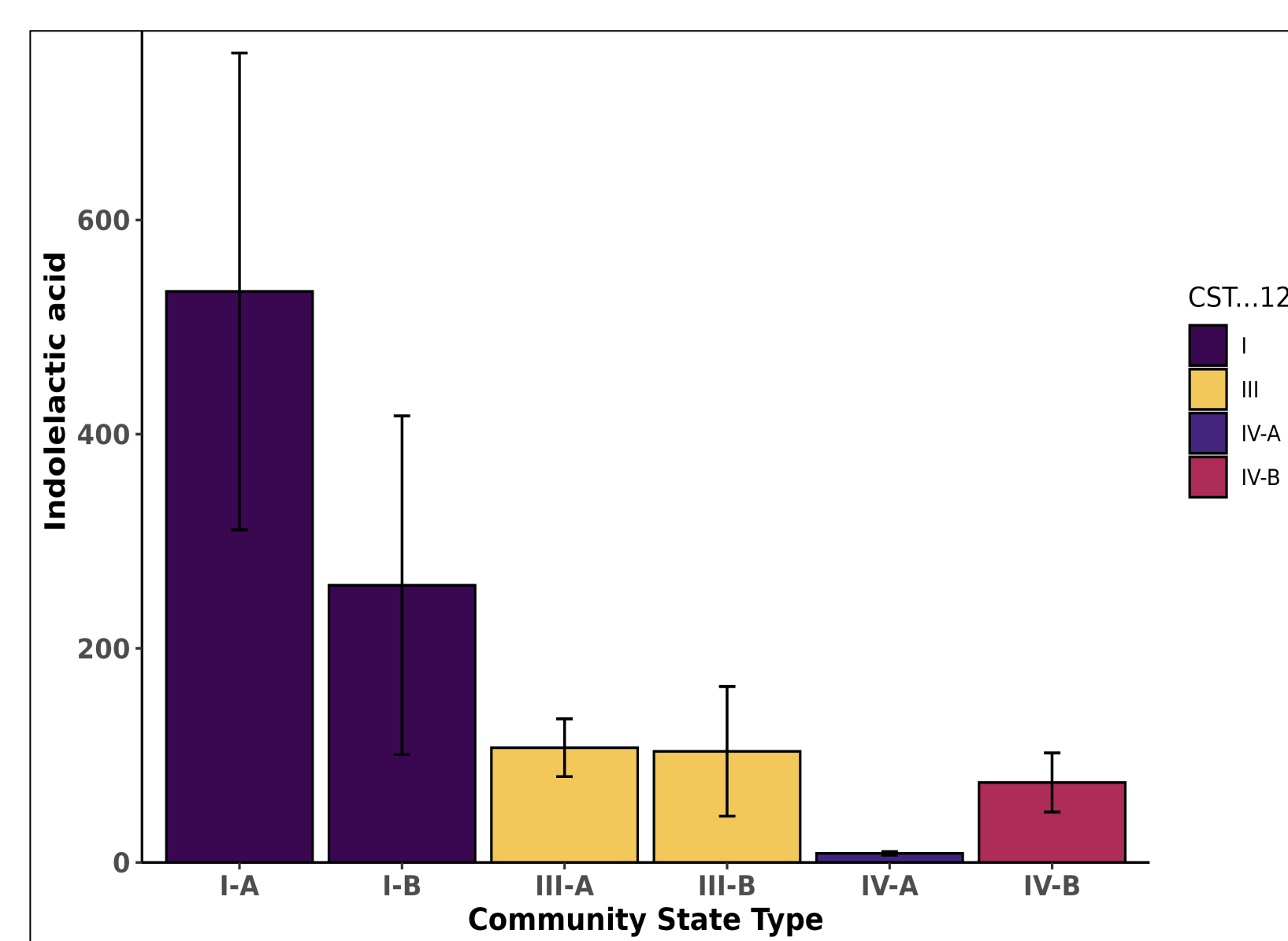


Figure 4. CST I-A was associated with the greatest ILA abundance. Participants were further divided into sub-CSTs and those grouped in CST I-A (the highest relative abundance of *L. crispatus*) had a significantly greater abundance of ILA than those in CST III-A ($p=0.02$), III-B ($p=0.01$), IV-A ($p<0.001$) and IV-B ($p<0.001$).

Lactobacillus crispatus association with ILA abundance

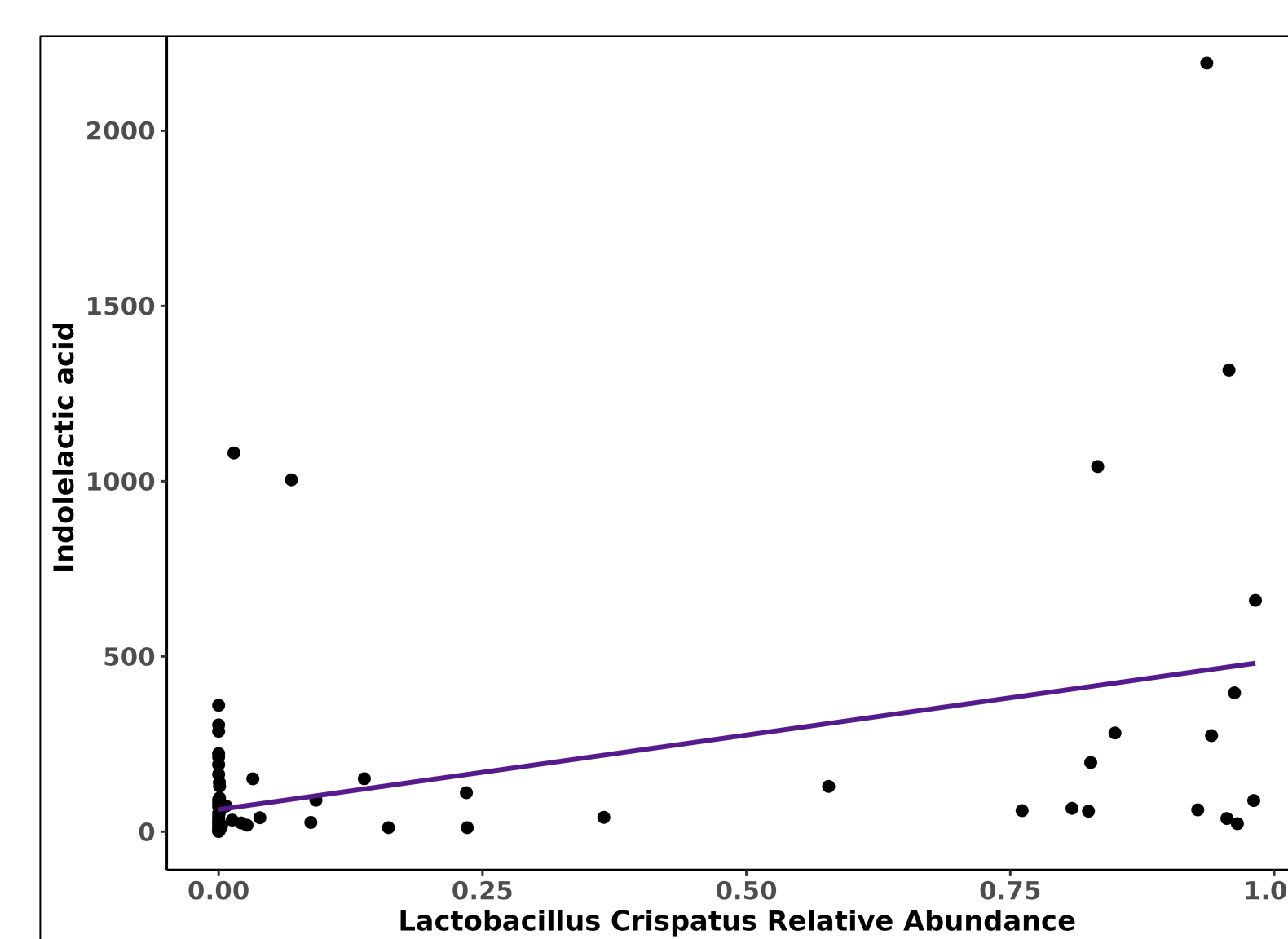


Figure 5. *Lactobacillus crispatus* abundance is associated with ILA abundance. The relative abundance of participants *L. crispatus* was assessed in comparison to vaginal ILA abundance. Linear modeling results indicate that *L. crispatus* relative abundance is positively associated with ILA abundance ($b = 425$, p value < 0.001).

Induction of AhR activity by ILA

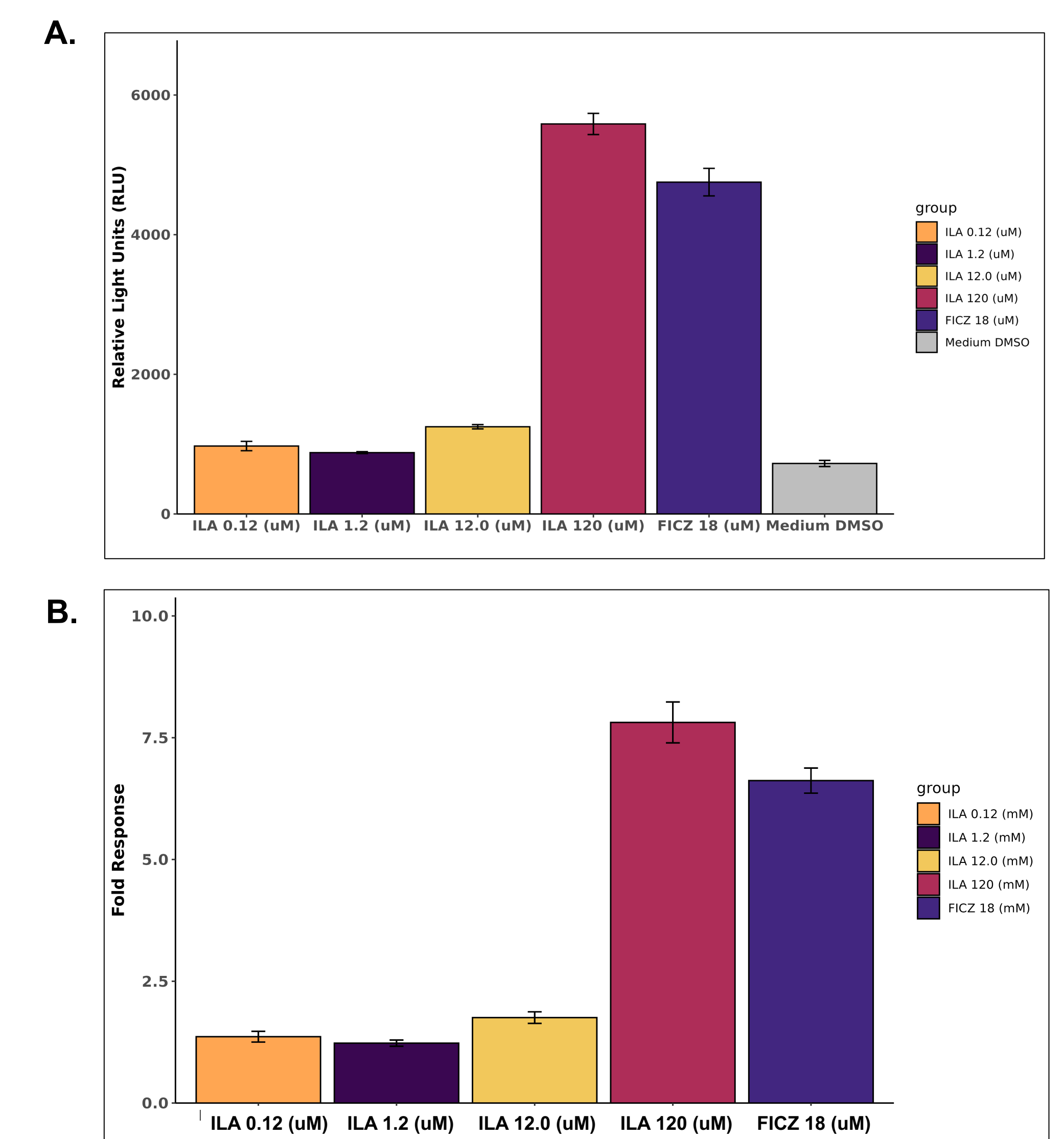


Figure 6. Induction of AhR activity by ILA in HT29-Lucia™ AhR cells. Cells were seeded at 15,000 cells/well in 96-well plates for 48 hours. Cells were then incubated with medium containing 1% DMSO, 18µM FICZ (a known AhR agonist used at the recommended optimal concentration) or 120, 12, 1.2 or 0.12µM ILA which is the approximate range of ILA in vaginal secretions. Each condition had 5 replicates. All conditions were significantly different compared to the medium/DMSO ($p < 0.01$ for all parameters except ILA 1.2µM, $p < 0.05$). (A) Results expressed in RLU and (B) fold change over medium/DMSO control.

Conclusions

- The highest ILA abundance in the vaginal tract of reproductive-age women is associated with an optimal vaginal microbiome dominated by *Lactobacillus crispatus*. While ILA has been reported to be made by several lactobacillus species (eg. *L.plantarum*, *L.reuteri*)⁴ this is the first time this level of association has been made with the optimal lactobacillus spp. found in the vaginal microbiome.
- I found that ILA was an AHR ligand, confirming several other reports¹.
- Recent studies indicate that members of mom's vaginal microbiome are transiently or persistently shared with their infant. Importantly, this raises the possibility that an optimal, lactobacillus dominant microbiome in mom could potentially contribute to early ILA production in the infant⁵. Further, studies suggest that an optimal early microbiome seeding decreases an infant's lifelong risk for some chronic diseases⁵.
- Time permitting, I could have taken this very preliminary study in several different directions, for example (I) growing and isolating lactobacillus cultures from CST I-dominant patients, after which I could sequence and study the growth and functional properties of these clinical isolates or (2) I could determine if ILA is a potential robust biomarker of a healthy vaginal microbiome.

References: (1) Ehrlich *et al*, BMC Microbiology 2020 (2) Meng *et al*, Pediatric Research 2019 (3) Ardizzone *et al*, Frontiers in Cellular and Infection Microbiology 2023 (4) Huang *et al*, Medicine in Microecology 2023 (5) Mortensen *et al*, eLife Computational and Systems Biology 2021.