Combining standard bacterial vaginosis treatment with cystine uptake inhibitors to block growth of *Lactobacillus iners* is a potential target for shifting the cervicovaginal microbiota towards health-associated *Lactobacillus crispatus*-dominant communities

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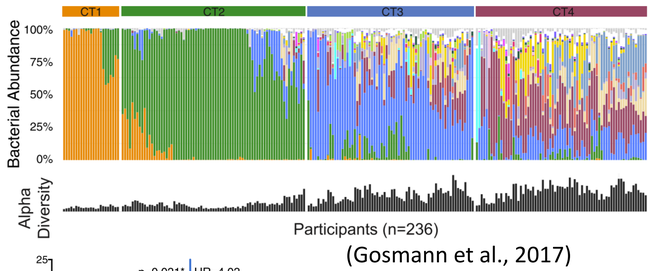
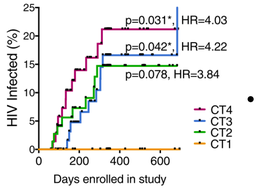
Introduction:

- Cervicovaginal microbiome domination by *Lactobacillus crispatus* is associated with protection from HIV and with other beneficial outcomes.
- By contrast, *L. iners*-dominance is associated with adverse outcomes and higher risk of bacterial vaginosis (BV) and HIV (Fig).



(Gosmann et al., 2017)

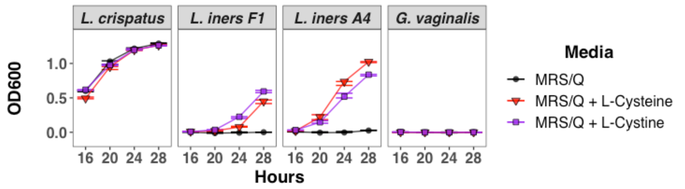


- BV treatment with metronidazole (MTZ), to which lactobacilli are resistant, typically produces *L. iners*-dominant (not *L. crispatus*-dominant) communities, and post-treatment relapse rates are high.
- Selectively inhibiting *L. iners* during BV treatment could mitigate HIV risk by favoring shifts to *L. crispatus*-dominant communities, but *L. iners* has been difficult to phenotypically characterize *in vitro* because it fails to grow in standard *Lactobacillus* MRS media unlike other cervicovaginal lactobacilli.
- **We investigated *L. iners* growth characteristics to identify the basis for its unique growth requirements and to develop strategies for inhibiting it in favor of *L. crispatus*.**

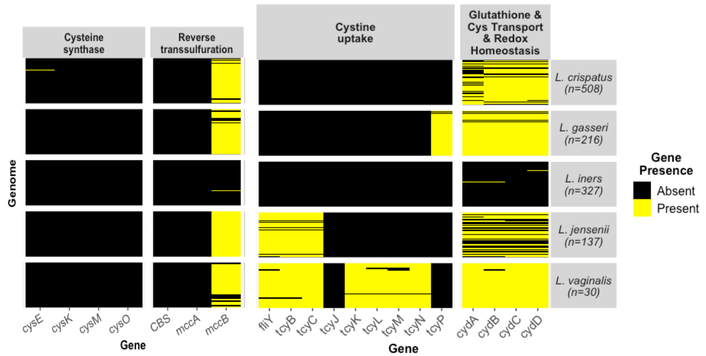
Methods:

- Bacteria were cultured anaerobically. Mono-culture growth in broth +/- supplements or inhibitors was quantified by optical density.
- Comprehensive *Lactobacillus* genome collections assembled from novel and published isolate genomes and from metagenomically assembled genomes were annotated using Prokka, Roary, and eggNOG.
- Competitive mixed cultures employing mock microbial communities +/- inhibitors were quantified via 16S rRNA gene sequencing.

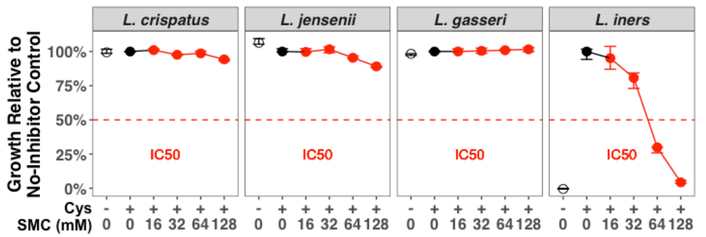
Cysteine supplementation supports *L. iners* growth in MRS media



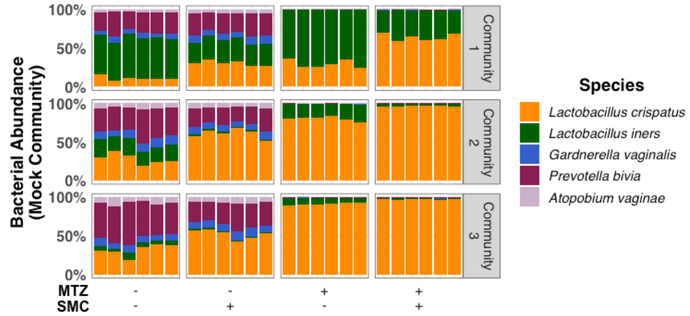
Major cervicovaginal *Lactobacilli* all lack canonical cysteine biosynthesis pathways, but *L. iners* uniquely lacks the cydABCD cysteine/glutathione transport operon

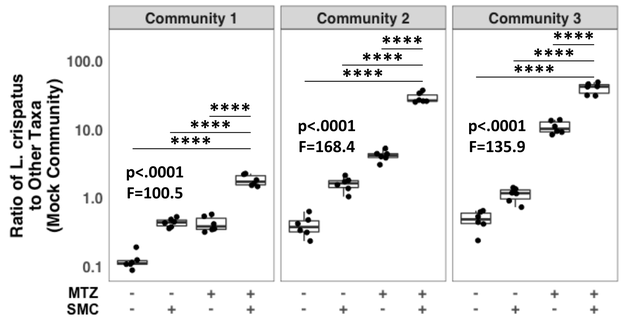


A cystine uptake inhibitor (SMC) selectively blocks growth of *L. iners*



MTZ combined with SMC shifts mock BV-like communities to *L. crispatus* dominance more effectively than MTZ alone





Conclusions:

- *L. iners* has unique nutritional requirements related to uptake of exogenous cysteine/cystine that can be selectively inhibited.
- Targeting cystine uptake to inhibit *L. iners* is a candidate strategy to shift the cervicovaginal microbiota toward *L. crispatus*-dominated communities associated with low HIV risk and other beneficial health outcomes.

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