A Dual Antibiotic-Metabolite-based Approach to Improve Bacterial Vaginosis Treatment

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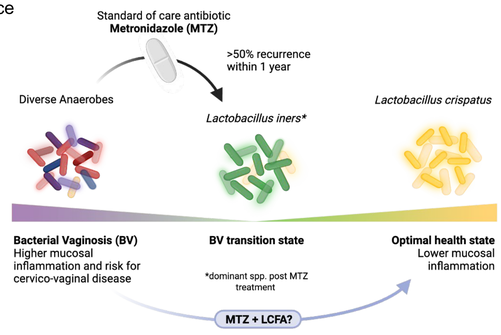
Motivation

- Bacterial vaginosis (BV) affects 20-30% of cisgender women worldwide[1]
- BV is associated with spontaneous preterm birth[2], cervical dysplasia[3], infertility[4], and STI acquisition risk,[5] including HIV[6]
- Microbiologically, BV is characterized by a paucity of health-associated lactobacilli and high abundance of diverse obligate anaerobes[7]
- Standard-of-care treatment with the antibiotic metronidazole (MTZ) results in recurrence of ≥50% of cases within 3-6 months[8] due to its propensity to promote dominance by recurrence-associated *Lactobacillus iners* rather than optimal health-associated *Lactobacillus crispatus*[6,9]
- Rapid antibiotic resistance development by BV-associated bacteria (BVAB) may also contribute to BV recurrence[10]
- Unsaturated long chain fatty acids (uLCFAs) such as oleic acid (OA) have been demonstrated to inhibit *L. iners* and promote *L. crispatus*[11]



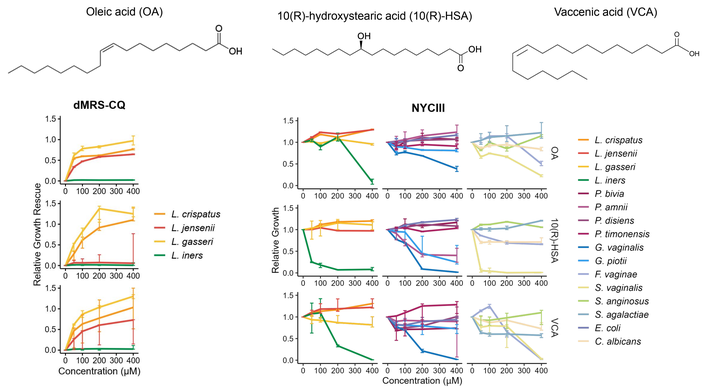
Questions & Objectives

1. Which uLCFAs exhibit differential growth effects between health-associated *Lactobacillus* versus *L. iners* and BVAB?
2. Can dual antibiotic-metabolite therapy improve BV treatment by providing greater selectivity and by hindering resistance development in BVAB?

- Promote the growth of *L. crispatus* while inhibiting *L. iners* and other BVAB
- Evaluate the efficacy of uLCFA and MTZ in driving desirable differential growth effects when co-administered and when covalently conjugated

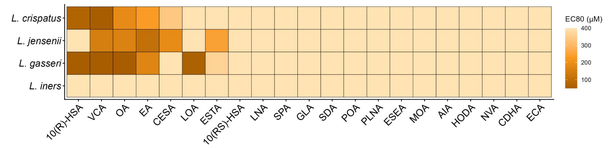
Results

Select uLCFAs promote the growth of *L. crispatus* while inhibiting the growth of BVAB

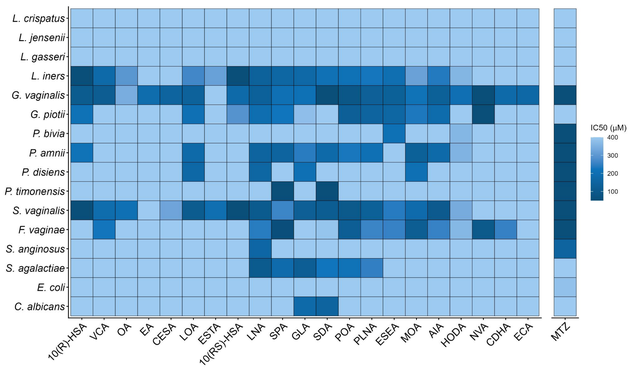


Results (cont.)

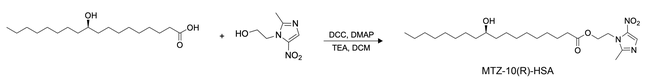
80% growth rescue concentration (EC80) in delipidated MRS-CQ media

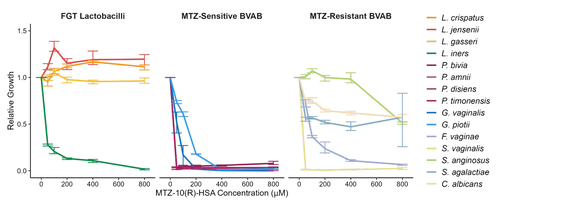


50% growth inhibitory concentration (IC50) in NYCIII media

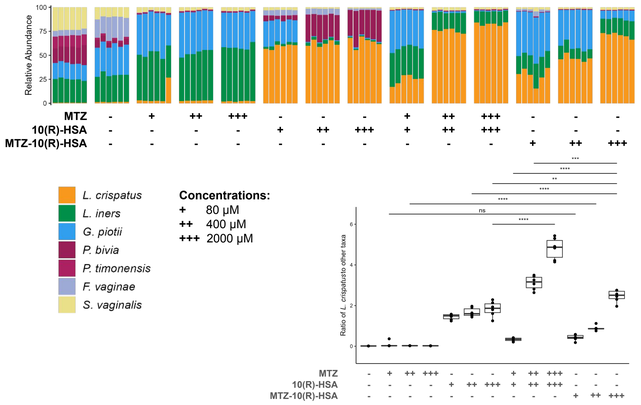


MTZ-10(R)-HSA conjugate inhibits the growth of *L. iners* and BVAB while promoting the growth of *L. crispatus*



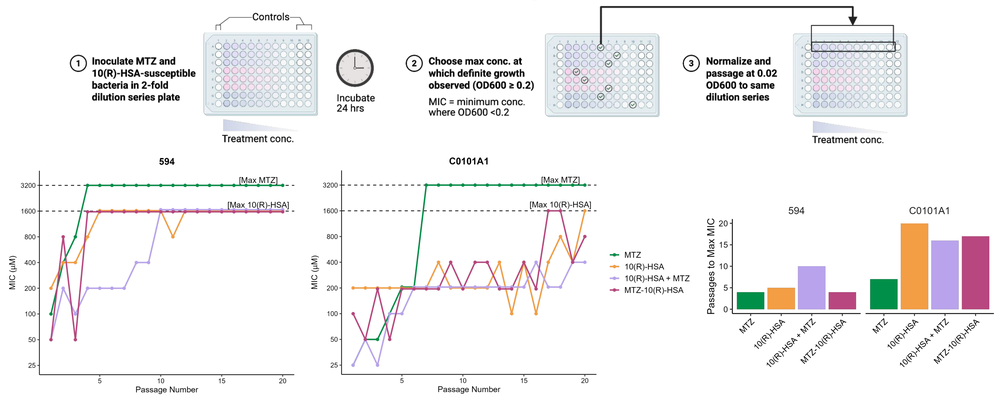


MTZ and 10(R)-HSA co-administration shifts mock BV communities toward *L. crispatus* dominance with a dose-dependent effect



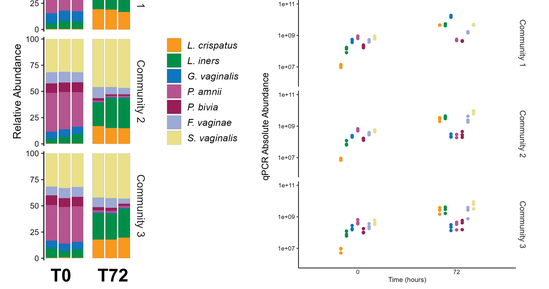
Results (cont.)

Resistance development against MTZ and 10(R)-HSA co-administration is hindered compared to MTZ alone in two strains of BVAB *Gardnerella vaginalis*



Isolate	Treatment	Gene Product	Mutation
G. vaginalis (594)	MTZ	Nitroreductase	Frameshift
	10(R)-HSA	Mbeg1-like protein	S281I
	10(R)-HSA + MTZ	Nitroreductase	Q26*
		Mbeg1-like protein	S281I
	MTZ-10(R)-HSA	Nitroreductase	Q26*
		Mbeg1-like protein	S281I
G. vaginalis (C0101A1)	MTZ	Nitroreductase	Q87*, D257N
	10(R)-HSA	Acyl-CoA synthetase	G277C, A353V
		plsC	M131V
	10(R)-HSA + MTZ	Nitroreductase	F247L, D257N
		Acyl-CoA synthetase	P406S
		plsC	K133N
	MTZ-10(R)-HSA	Nitroreductase	A214E, Q54*
		Acyl-CoA synthetase	T382A
		plsC	M197R, M131K

Communities:
1. WT *G. vaginalis* (P0)
2. 1:1 mixture of MTZ-resistant *G. vaginalis* (P4) and 10(R)-HSA *G. vaginalis* (P5)
3. 10(R)-HSA and MTZ resistant *G. vaginalis* (P10)



Conclusions & Future Directions

- Select uLCFAs simultaneously promote non-*iners Lactobacillus* while inhibiting *L. iners* and BVAB
- Co-administration of MTZ and top candidate 10(R)-HSA as well as the MTZ-10(R)-HSA conjugate maintains therapeutically relevant growth effects in single strain growth assays as well as mock communities
- MTZ and 10(R)-HSA co-administration hinders antibiotic resistance in the canonical BVAB *G. vaginalis*, while resistance against MTZ-10(R)-HSA displayed a similar profile to MTZ alone
- Mutations acquired in *G. vaginalis* resistance to 10(R)-HSA occur in broad lipid metabolism pathways, with said strains being outcompeted in a mock BV community

- Evaluate if MTZ and 10(R)-HSA co-administration can still achieve *L. crispatus* dominance in a mock BV community in the setting of resistant *G. vaginalis*

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