From Metabolomics to Mechanism: Characterizing Hippurate Metabolism in the Vaginal Microbiome

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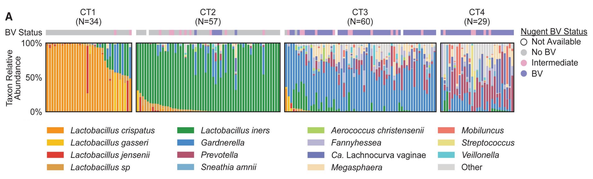
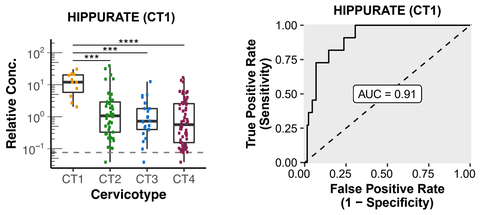
INTRODUCTION

- Demonstrating causality and functional significance of microbiome-metabolome associations is often difficult due to challenges in identifying underlying mechanisms.
- We investigated metabolomic associations within the human vaginal microbiota.
- Vaginal bacterial communities dominated by *Lactobacillus crispatus* are generally associated with optimal health.
- In contrast, communities dominated by *Lactobacillus iners* or diverse anaerobes are linked to adverse health conditions such as HIV, cervical cancer, and bacterial vaginosis (BV).

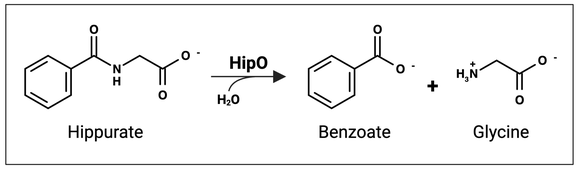
- Vaginal microbiota can be classified into “cervicotypes” (CTs) depending on abundance of specific bacteria[1,2].



- Vaginal bacterial communities dominated by *L. crispatus* (CT1) have uniquely high vaginal hippurate concentrations compared to communities dominated by *L. iners* (CT2) or various anaerobes (CT3 and CT4).



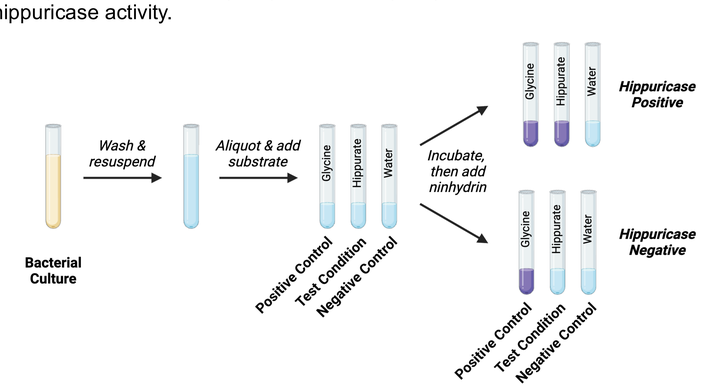
<u>Hypothesis:</u> High vaginal hippurate concentrations in CT1 are due to *L. crispatus*’s inability to metabolize hippurate, while *L. iners* and diverse anaerobes hydrolyze hippurate via hippuricase activity.



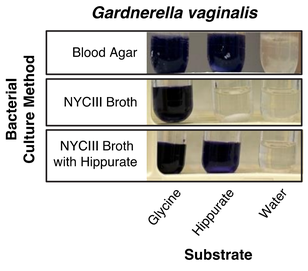
- Hippurate is a glycine conjugate of benzoic acid formed in the mammalian liver and kidneys [3,4].
- Some bacteria possess hippuricase enzymes that hydrolyze hippurate into benzoate and glycine, but the role of hippuricases in bacterial and microbiome physiology remains largely unknown[5].

METHODS AND RESULTS

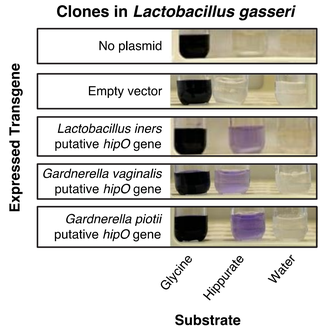
- Using a colorimetric assay, hippuricase (HipO) activity was detected in many diverse vaginal bacteria including *Lactobacillus iners* (CT2), *Gardnerella* (CT3), *Prevotella* (CT4) and other BV-associated bacteria, while *Lactobacillus crispatus* (CT1) lacks hippuricase activity.



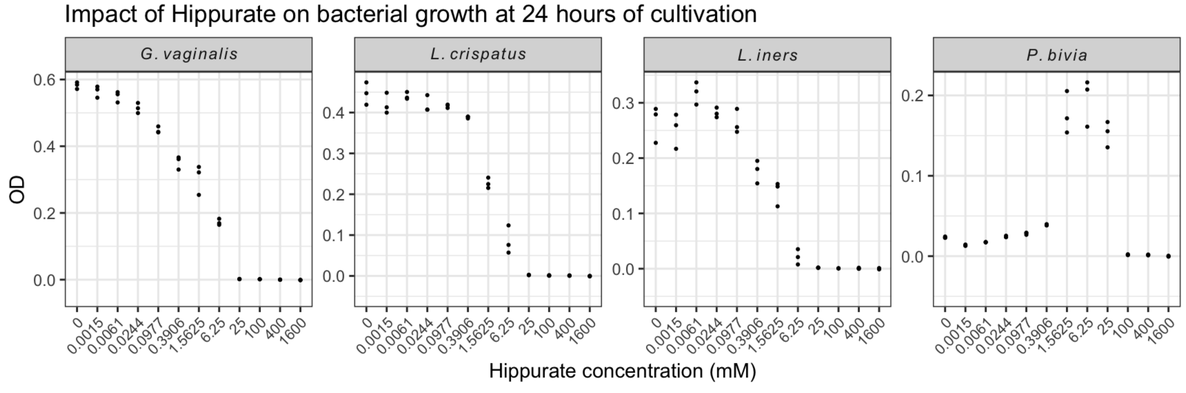
- In *Gardnerella vaginalis*, hippuricase activity appears to be inducible by specific growth stimuli.
- *G. vaginalis* grown on Columbia Blood Agar shows robust hippuricase activity, while *G. vaginalis* cultivated in liquid NYCIII did not show any hippuricase activity.
- Hippuricase activity also appears inducible in *L. iners* and *P. bivia* (not shown).



- Genomic analysis identified candidate hippuricase genes (*hipO*) in several vaginal bacteria, including *L. iners*, and multiple *Gardnerella* species.
- Cloning and expressing these candidate *hipO* genes in *Lactobacillus gasseri* (which is intrinsically hippuricase negative) confirmed they had *bona fide* hippuricase activity.



- High hippurate concentrations inhibited bacterial growth, but hippurate boosted P. bivia growth at lower concentrations.



CONCLUSION

- Diverse vaginal bacteria including *L. iners*, *G. vaginalis*, *P. bivia*, and others possess hippuricase (HipO) activity, but *L. crispatus* does not.
- These results explain microbiota-linked patterns observed in human vaginal metabolomic data.
- HipO expression appears to be inducible in several species.
- Genomic analysis identified putative *hipO* genes in multiple species; the enzymatic activity was confirmed experimentally.
- Hippurate inhibits bacterial growth at high concentrations but promotes *P. bivia* growth within a specific range, suggesting a metabolic role.

FUTURE DIRECTION

- Characterize hippuricase activity in additional vaginal bacterial strains and species.
- Expand genomic analysis for putative *hipO* homologs in the vaginal microbiota.
- Use mass spectrometry to better characterize hippuricase activity.
- Characterize determinants of *hipO* expression.
- Interrogate hippurate and hippuricase contributions to bacterial growth and metabolism.

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