

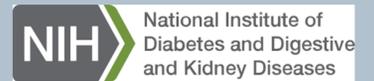
# Detection of novel specialized metabolites produced by *Bacteroides vulgatus*



THE UNIVERSITY  
of NORTH CAROLINA  
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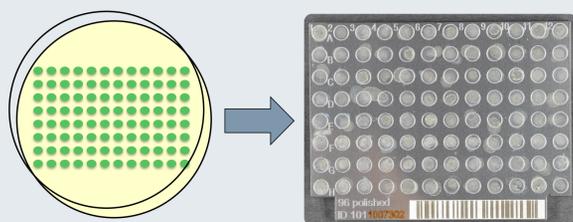
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## Abstract

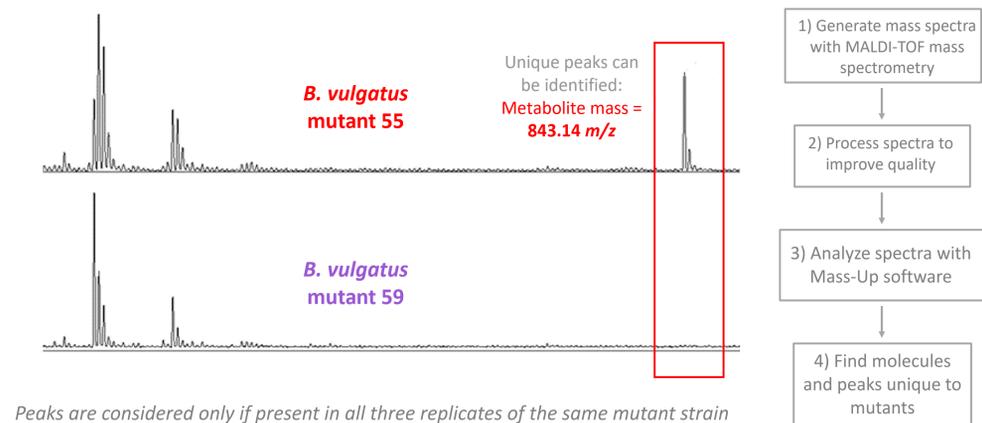
Gut colonization by the microbe *Bacteroides vulgatus* has been linked to type II diabetes in humans. Diabetes can cause serious health consequences by causing the body to resist insulin or by inhibiting its production. The long-term goal of our research is to determine how specialized metabolites produced by *B. vulgatus* impact the human microbiome to better treat chronic diseases like diabetes. In this study, we sought to establish a pipeline to detect unique molecules made by *B. vulgatus* and gain a better understanding of *B. vulgatus* biology as it relates to human health. To do this, we coupled Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry with IDBac and Mass-Up, programs for analyzing metabolite data. We selected *B. vulgatus* mutants from a transposon library and generated mass spectra of their metabolites. We then analyzed the data to infer the relationships between multiple *B. vulgatus* strains based on their metabolite profiles. So far, this analysis indicates we can rapidly identify *B. vulgatus* transposon mutants that produce small molecules with unique masses using this pipeline. So far, we have screened ~1400 transposon mutants in triplicate and identified 284 mutants with altered metabolite profiles. In doing so, we have validated Mass-Up's utility for metabolite screening of transposon mutants, as well as identified strains for follow-up studies. In addition to screening additional mutants, we intend to analyze strains with altered metabolite profiles to link genes to molecule production to explore *B. vulgatus* physiology and its potential impact on the host.

## Mutants selected from a transposon library

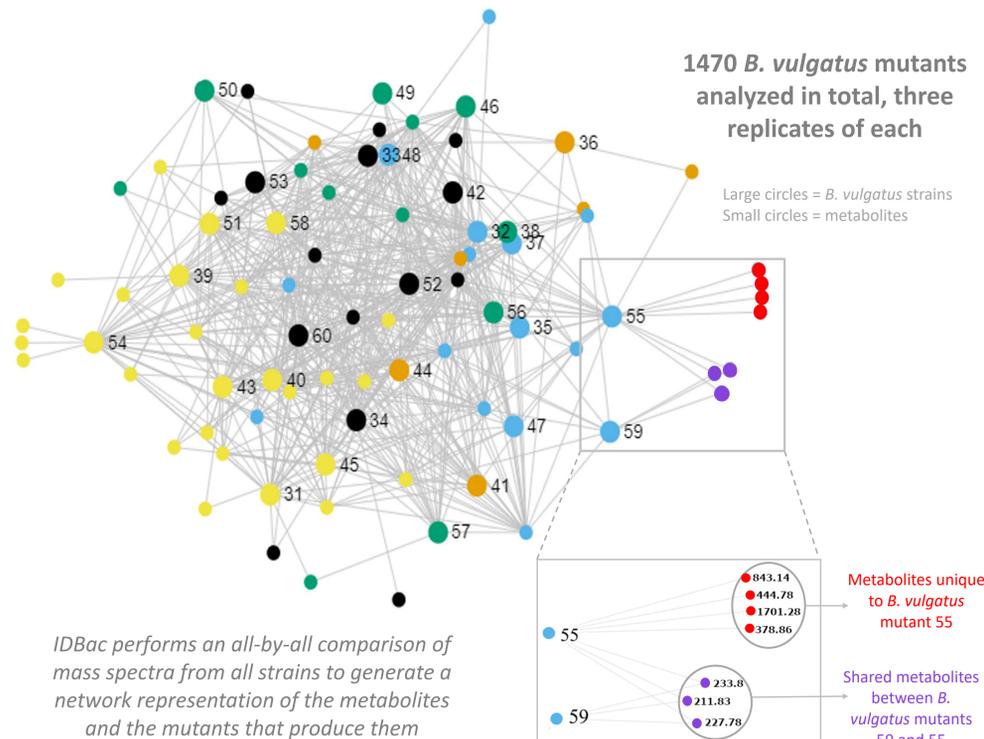


*B. vulgatus* mutants arrayed in sets of 96 are transferred to 96-well polished MALDI-TOF-MS plate

## MALDI-TOF mass spectrometry analysis



## Metabolite Association Network (MAN)

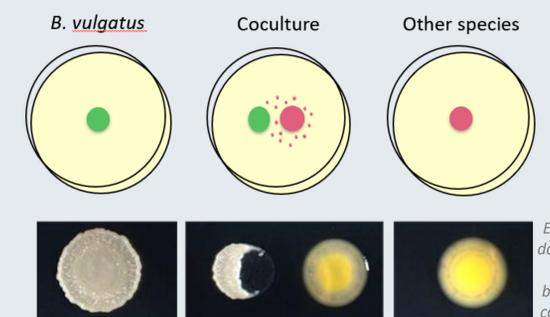


## Results

- ❖ Established a methodology for rapidly profiling metabolite production of *B. vulgatus* using MALDI-TOF MS
- ❖ Collected mass spectra from 1440 mutant strains in triplicate
- ❖ Significantly improved the quality of mass spectra
- ❖ Detected 284 potentially altered metabolites in mutant library
- ❖ Working to identify genes linked to metabolite production

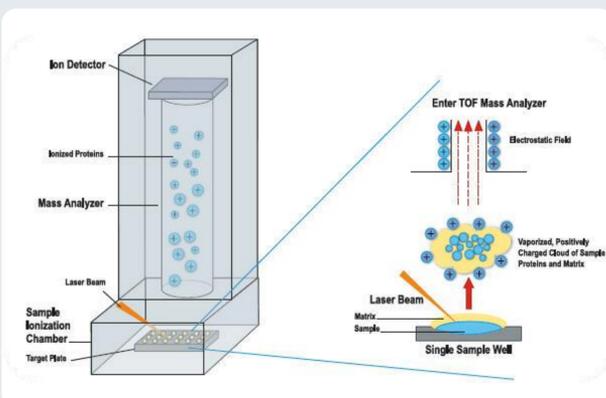
## Future work

- ❖ Cross reference masses with publicly available mass spectrometry data to search for potential molecule matches
- ❖ Conduct LC-MS/MS on mutants with altered metabolite profiles to obtain accurate metabolite masses and identify metabolites
- ❖ Fluorescence- and phenotype-based coculture screen to identify chemically mediated microbial interactions



Results from mutant *B. vulgatus* strains will be compared with wildtype to identify phenotypic differences

## Generating mass spectra with MALDI-TOF MS



Specialized metabolites (200–2,000 Da) are collected in reflectron mode

## Peak occurrence matrix generated with Mass-Up

Peak (m/z)	WT	41	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
398.45																			
461.39		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
506.61														1					
551.11																			1
614.63														1					
665.69							1												
674.46							1												
710.03									1										
825.75														1					
926.11														1					
1078.71							1												
1724.37														1					

Green boxes indicate the presence of a metabolite

Red boxes indicate the absence of a metabolite

Peak occurrence matrices can be used to generate hierarchical clustering analysis and PCA plots to identify similarity between mutants

## Acknowledgements

- ❖ Pediatric Obesity Microbiome & Metabolism Study (POMMS) <https://sites.duke.edu/pomms/>
- ❖ Coupling MALDI-TOF mass spectrometry protein and specialized metabolite analyses to rapidly discriminate bacterial function (doi.org/10.1073/pnas.1801247115)
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