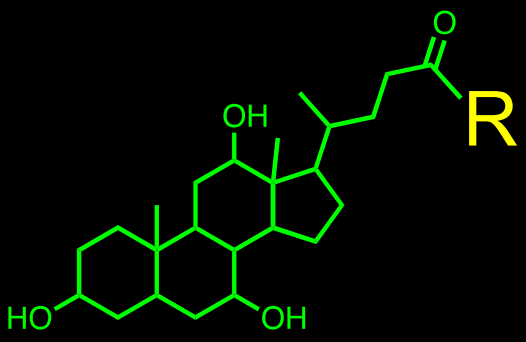
Novel Conjugated Bile Acids from Gut Bacteria



Robert Quinn, PhD
Michigan State University
Department of Biochemistry and Molecular Biology

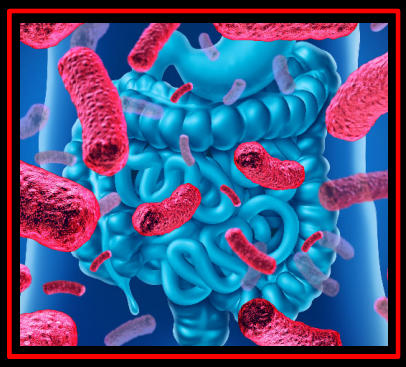
1

Our Research Areas

Integrated multi-omics approach

Lungs

Guts



Reefs

All mucosal associated microbial communities.

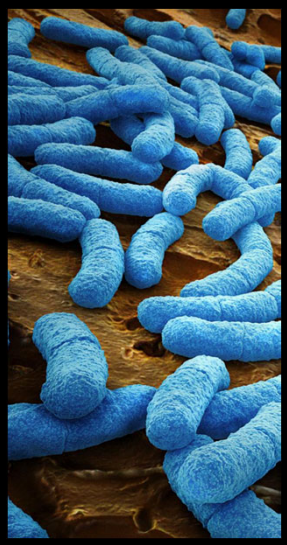
2

We are a walking ecosystem

~30 trillion cells

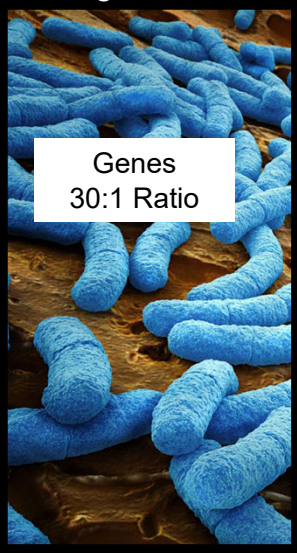
Cells 1:1 Ratio

~40 trillion bacteria



~20 thousand genes

~600 thousand genes



Genes 30:1 Ratio

3

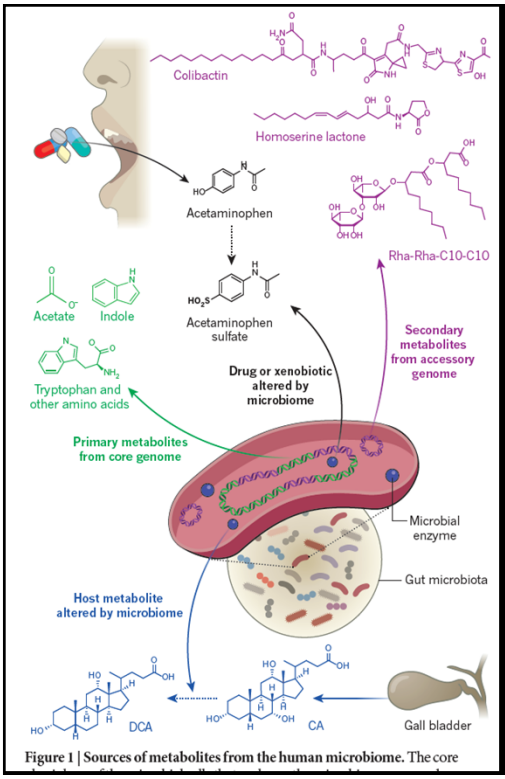


Figure 1 | Sources of metabolites from the human microbiome. The core

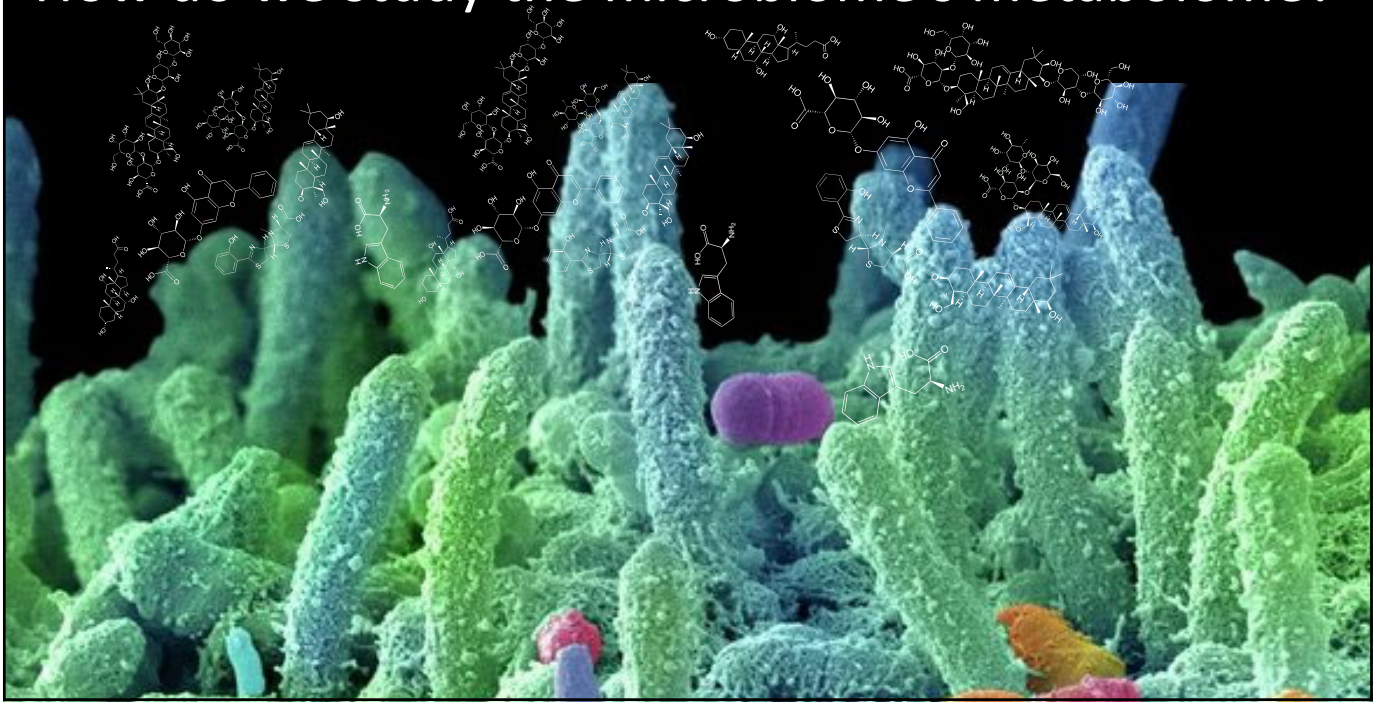
The Microbiome Interacts Through Chemistry

1. Primary Metabolite Production
2. Specialized Metabolites
3. Modification of host metabolites
4. Modification of xenobiotics

Gilbert, Quinn,….Dorrestein, Knight. *Nature*. 2016

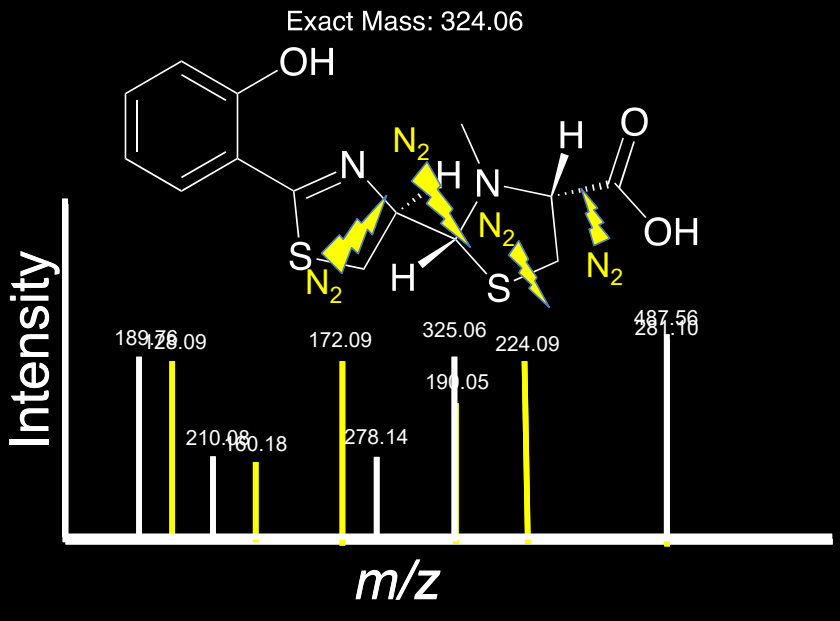
4

How do we study the microbiome's metabolome?



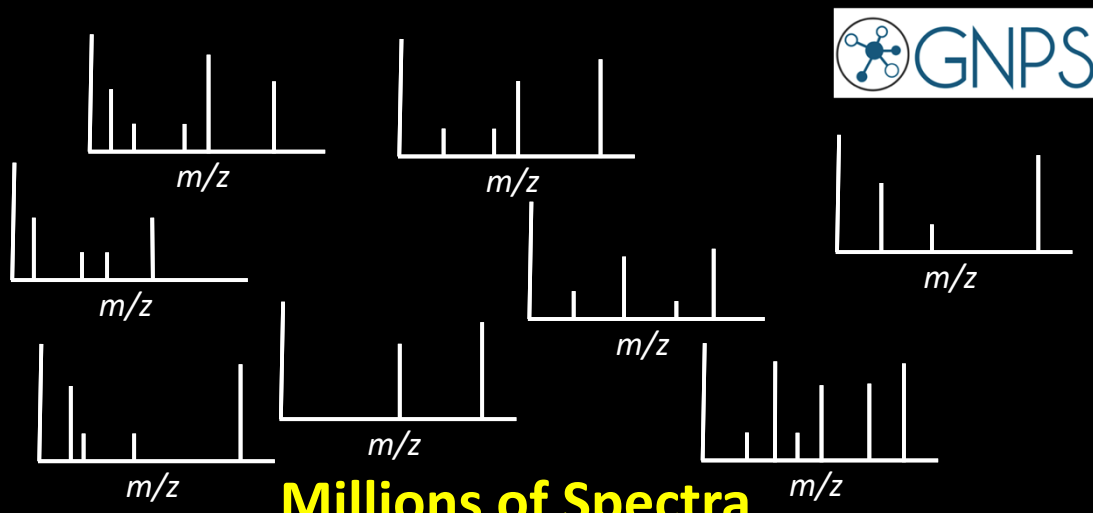
5

Tandem Mass Spectrometry



6

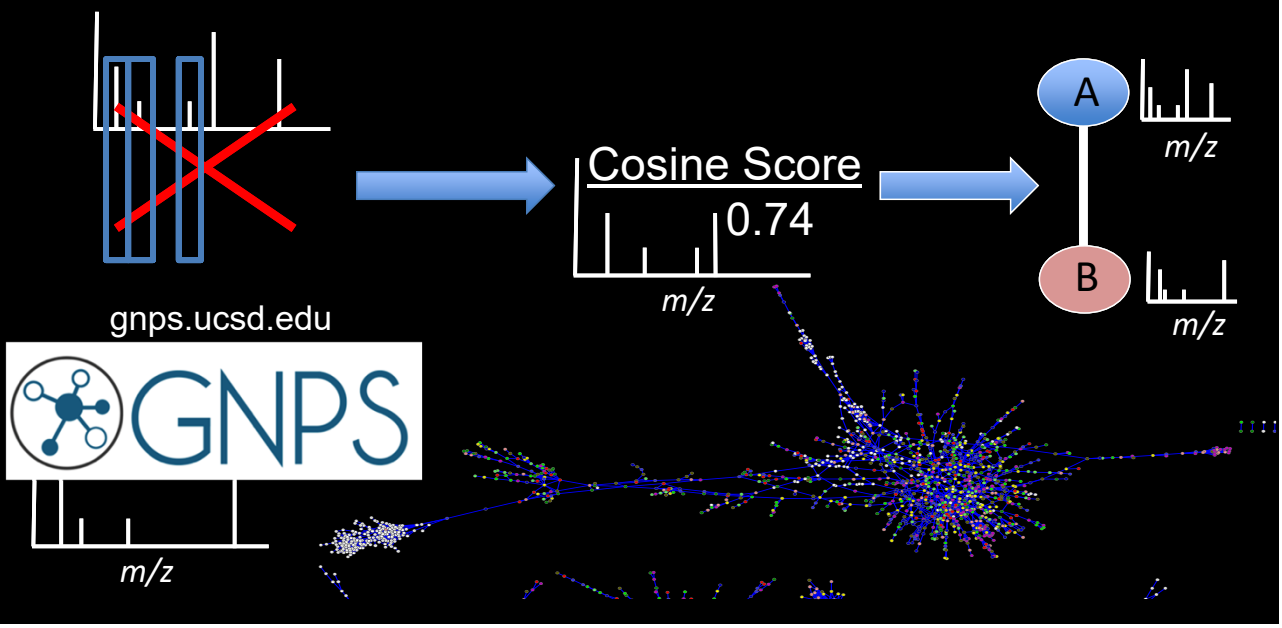
LC-MS/MS Metabolomics



7

Molecular Networking

Watrous et al. PNAS. 2012



8

How does the microbiome impact the chemistry of an entire organism?

Sarkis Mazmanian
CalTech

Germ Free

29 Organs

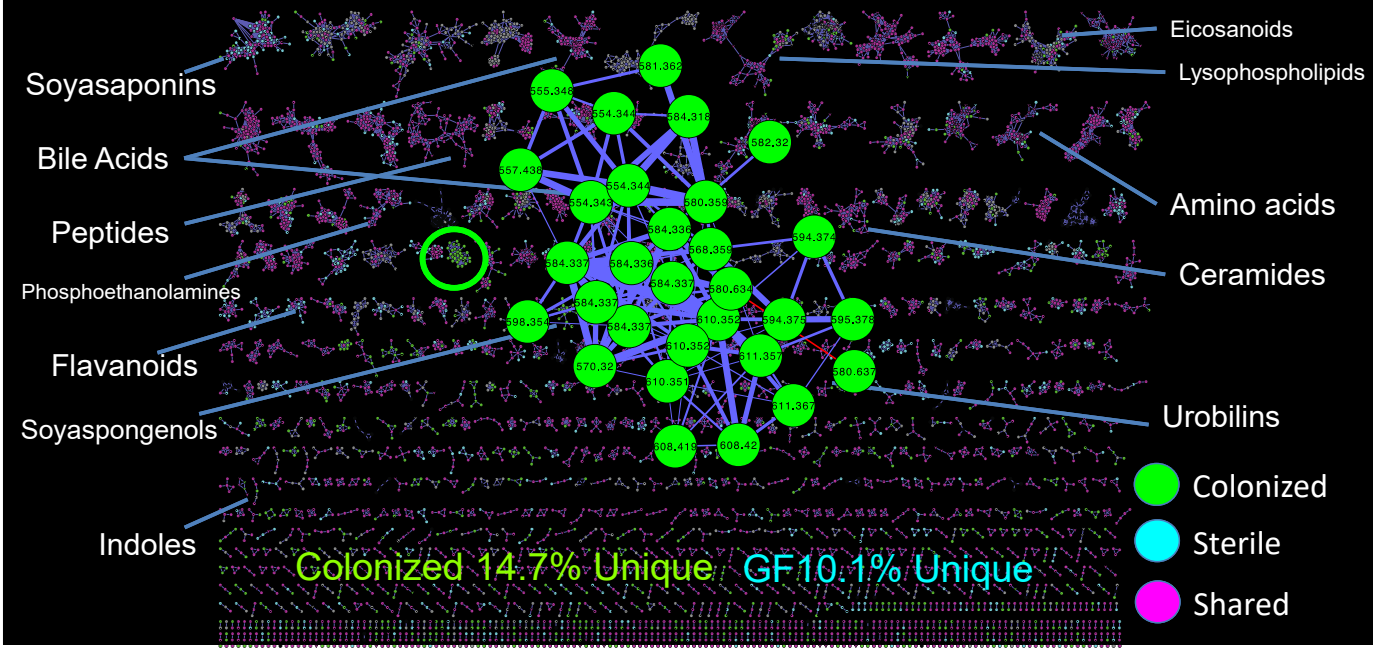
96 samples/ animal

n=4

Colonized

9

Map of the Microbiome Chemical Space



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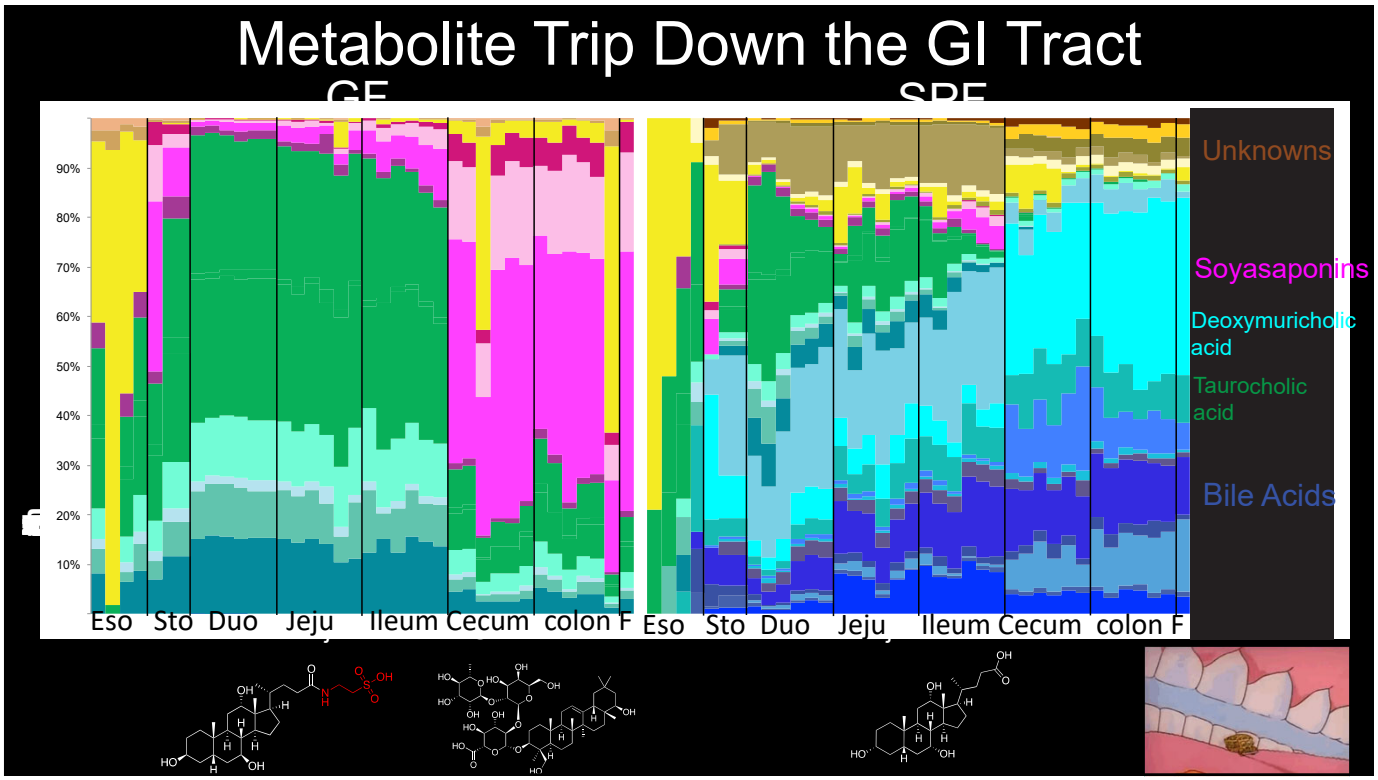
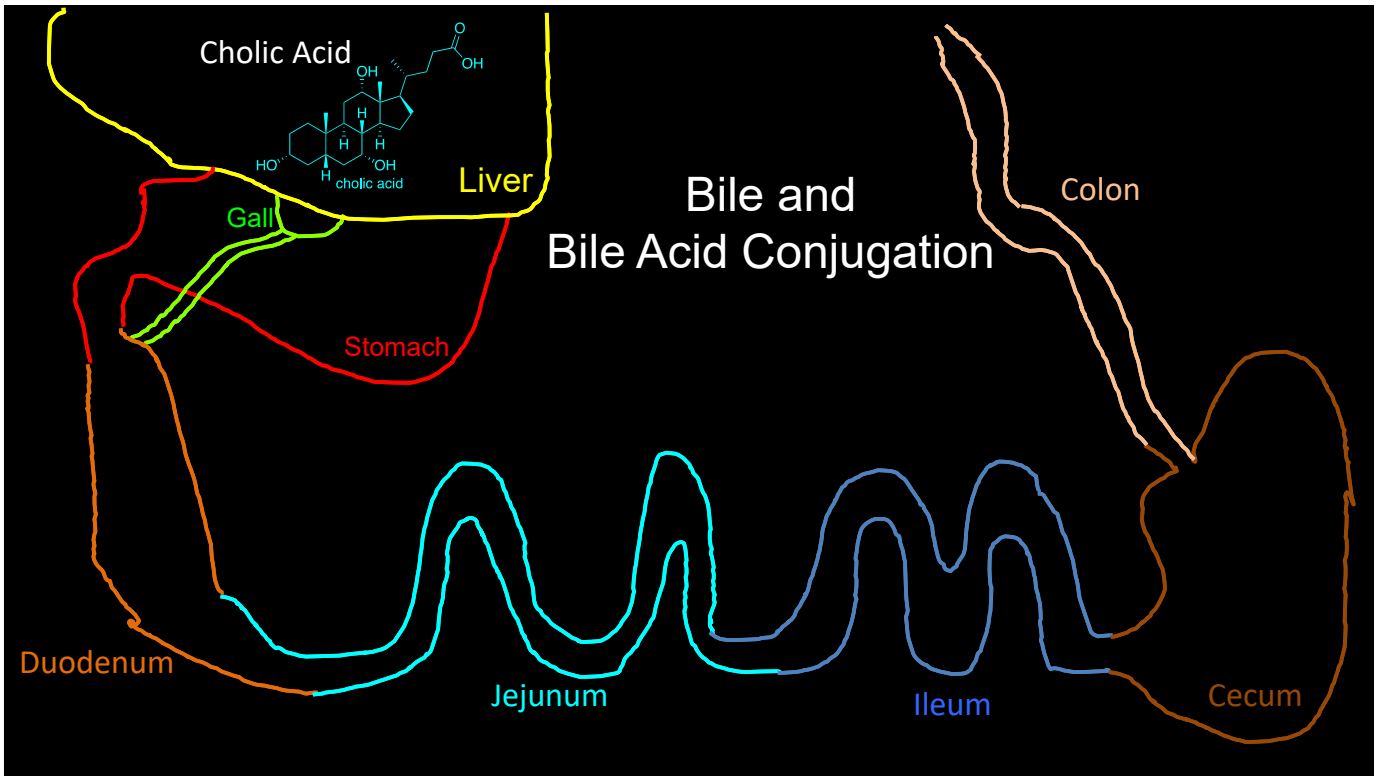
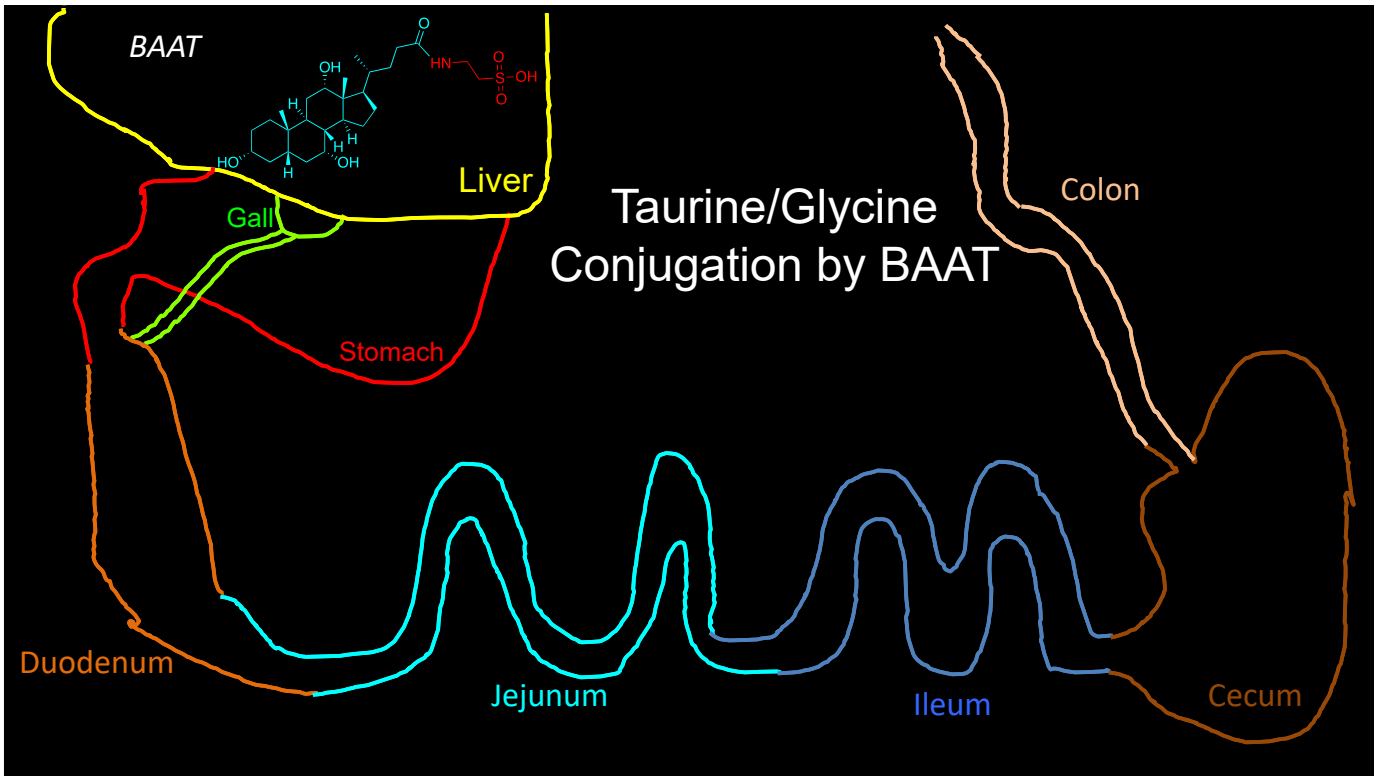

Metabolite Trip Down the GI Tract
GF
SPF
90%
80%
70%
60%
50%
40%
30%
20%
10%
Eso Sto Duo Jeju Ileum Cecum colon F
Eso Sto Duo Jeju Ileum Cecum colon F
Unknowns
Soyasaponins
Deoxymurichollic acid
Taurocholic acid
Bile Acids

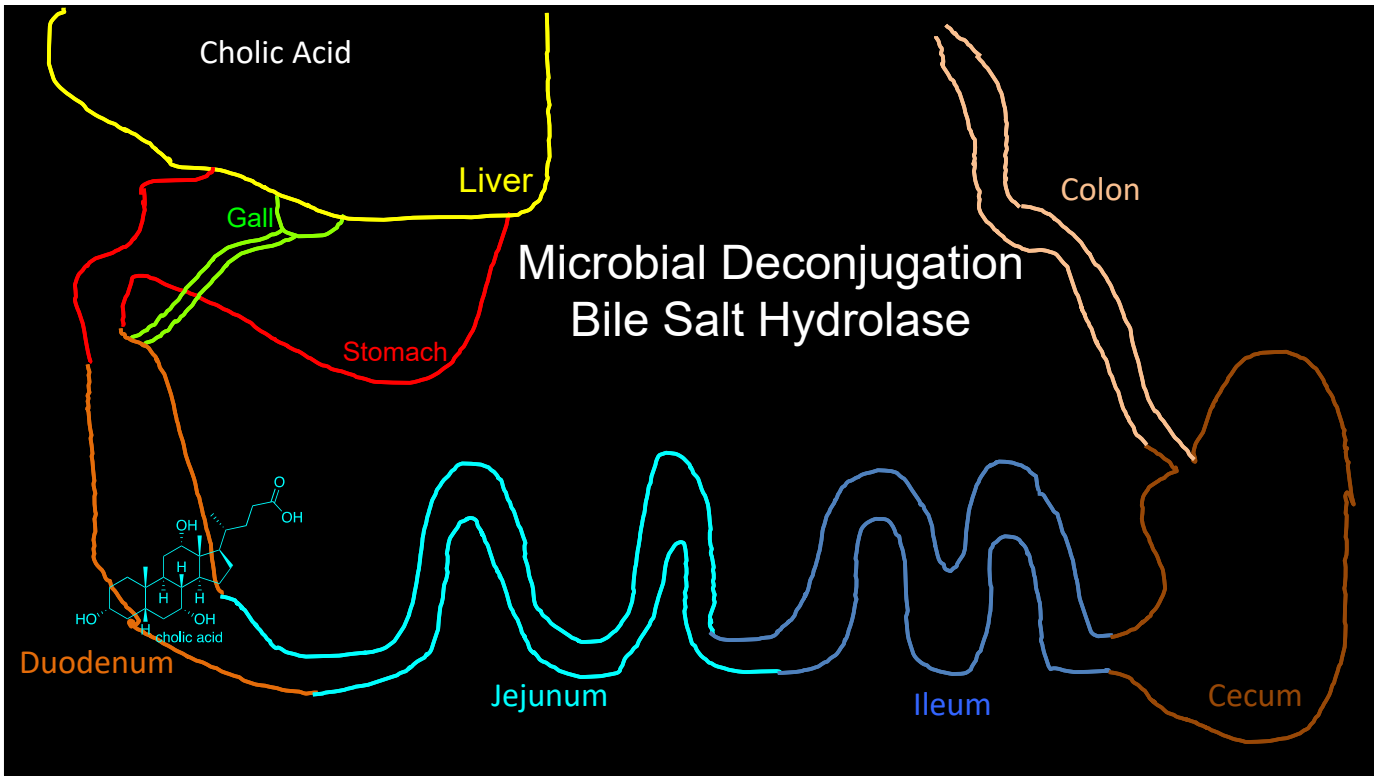
11


Cholic Acid
cholic acid
Liver
Gall
Stomach
Bile and
Bile Acid Conjugation
Colon
Duodenum
Jejunum
Ileum
Cecum

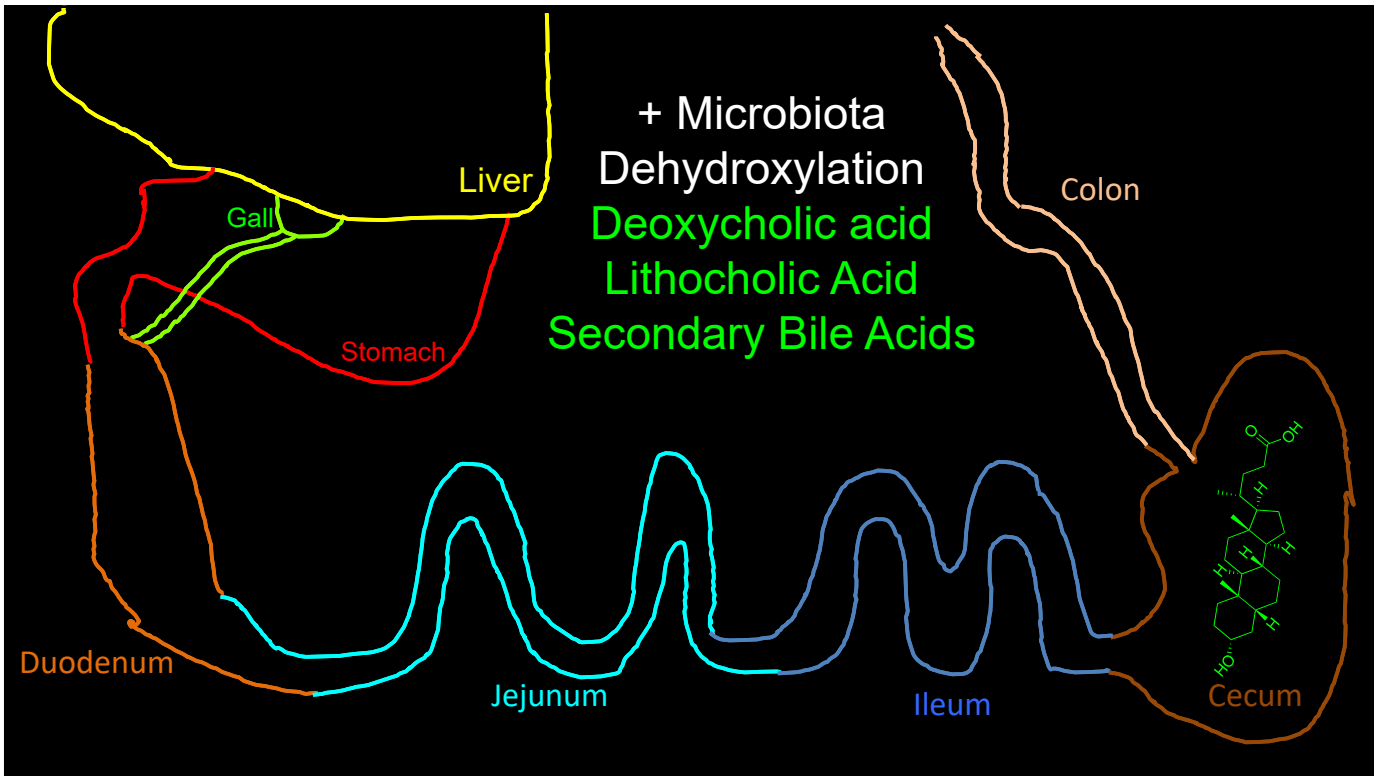
12


BAAT
Liver
Gall
Stomach
Taurine/Glycine
Conjugation by BAAT
Colon
Duodenum
Jejunum
Ileum
Cecum

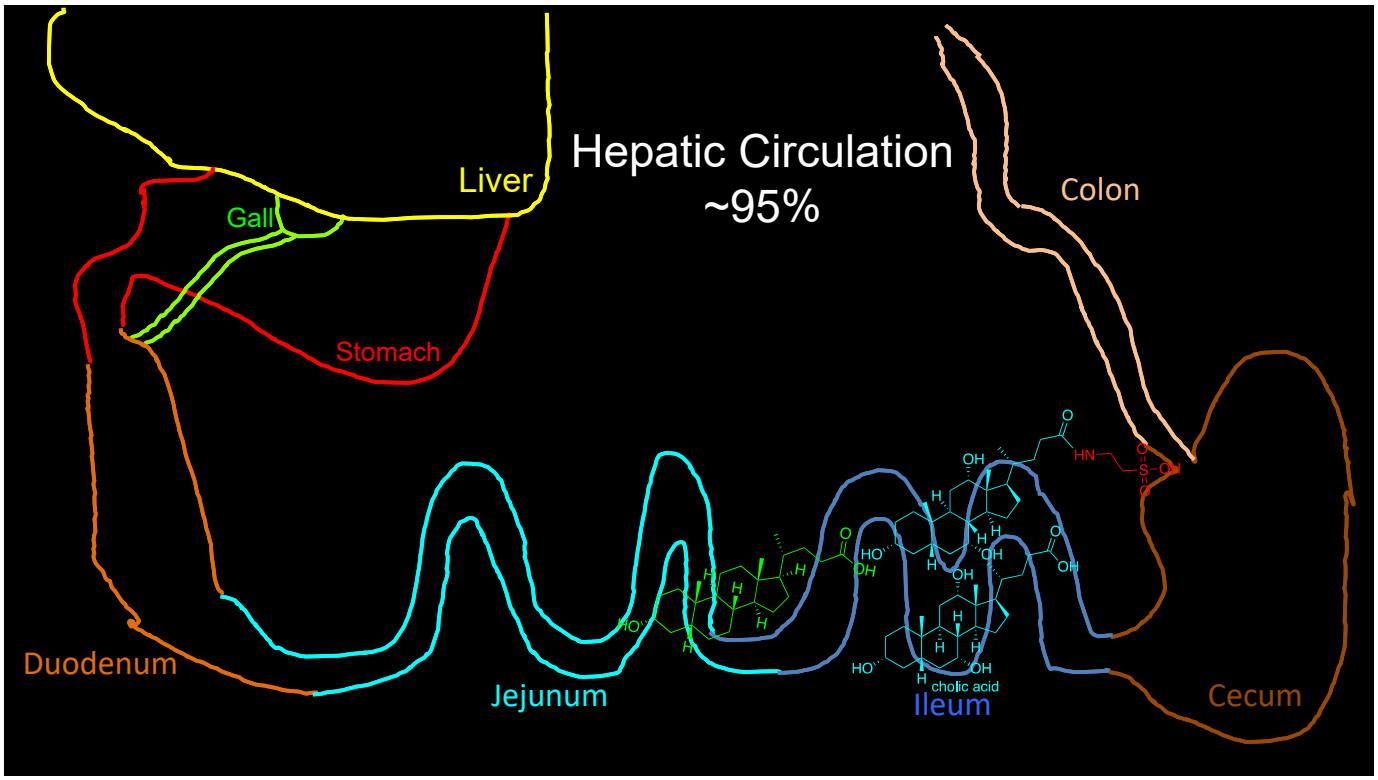
13


Cholic Acid
Liver
Gall
Stomach
Microbial Deconjugation
Bile Salt Hydrolase
Colon
cholic acid
Duodenum
Jejunum
Ileum
Cecum

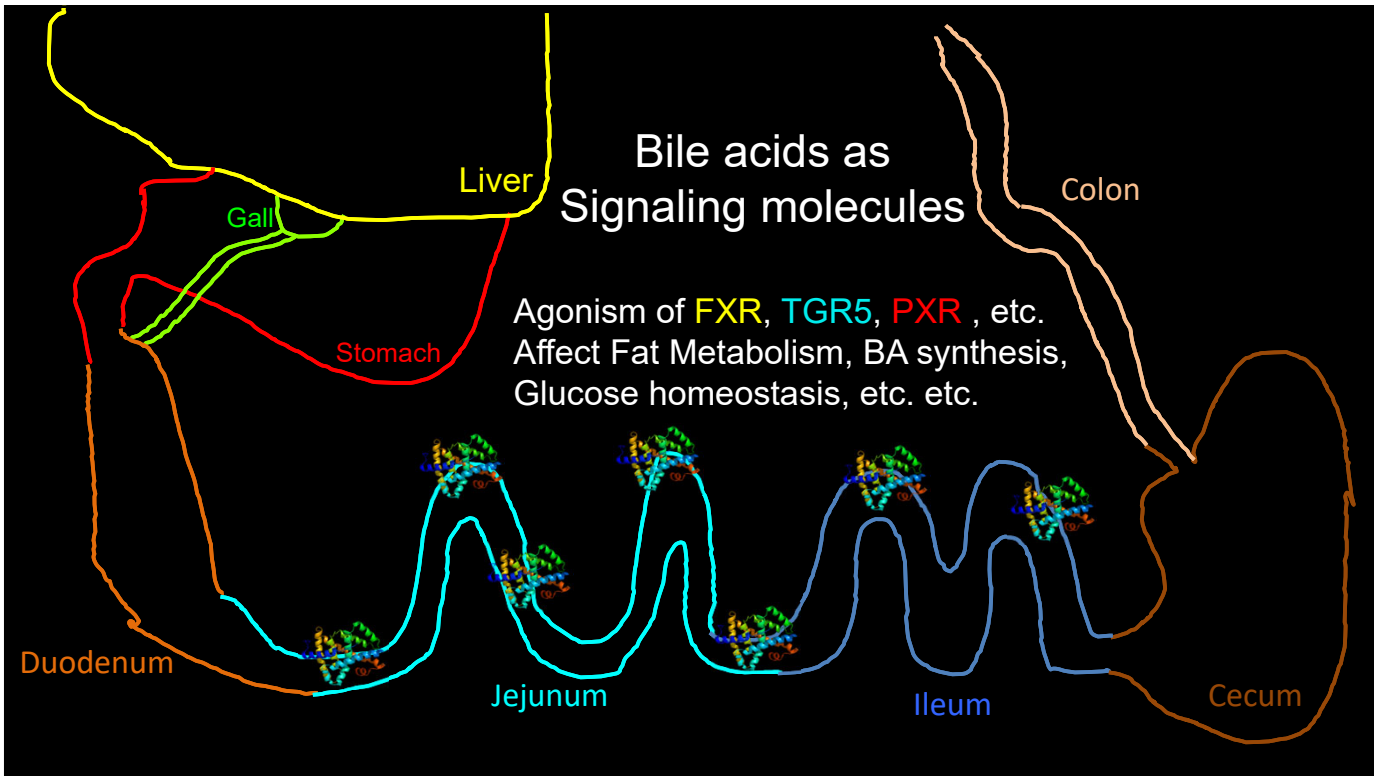
14


+ Microbiota
Dehydroxylation
Deoxycholic acid
Lithocholic Acid
Secondary Bile Acids
Liver
Gall
Stomach
Colon
Duodenum
Jejunum
Ileum
Cecum

15

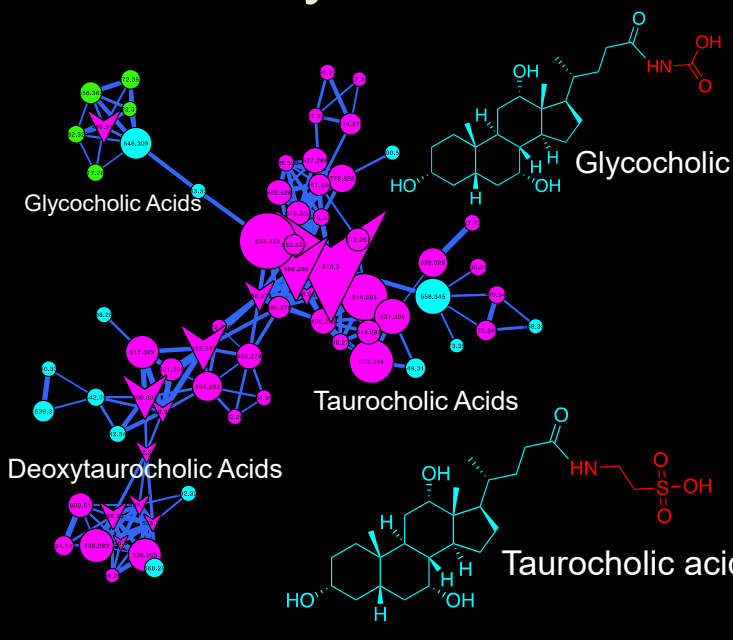

Hepatic Circulation
~95%
Liver
Gall
Stomach
Colon
Duodenum
Jejunum
Ileum
Cecum
cholic acid

16

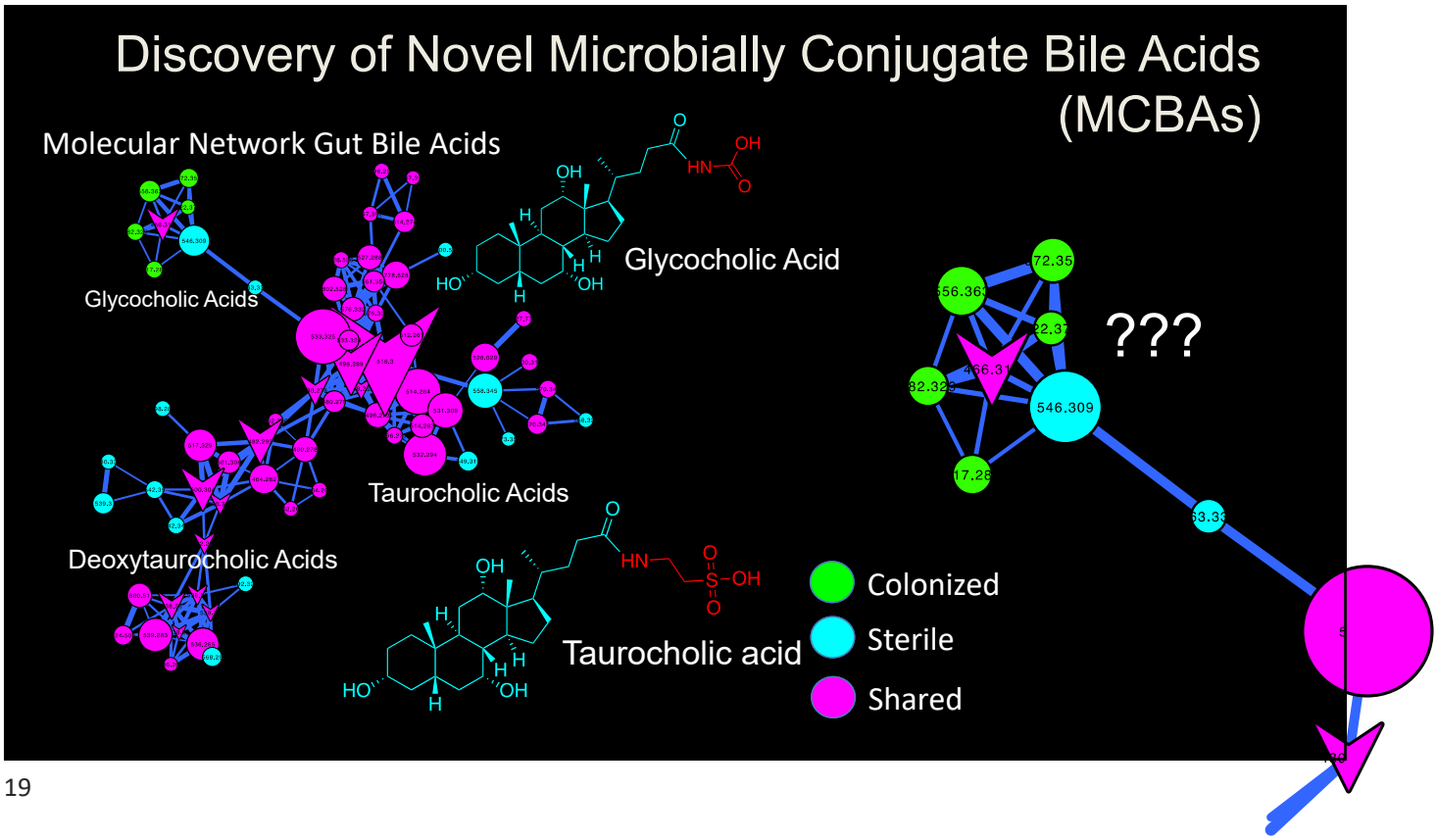


17

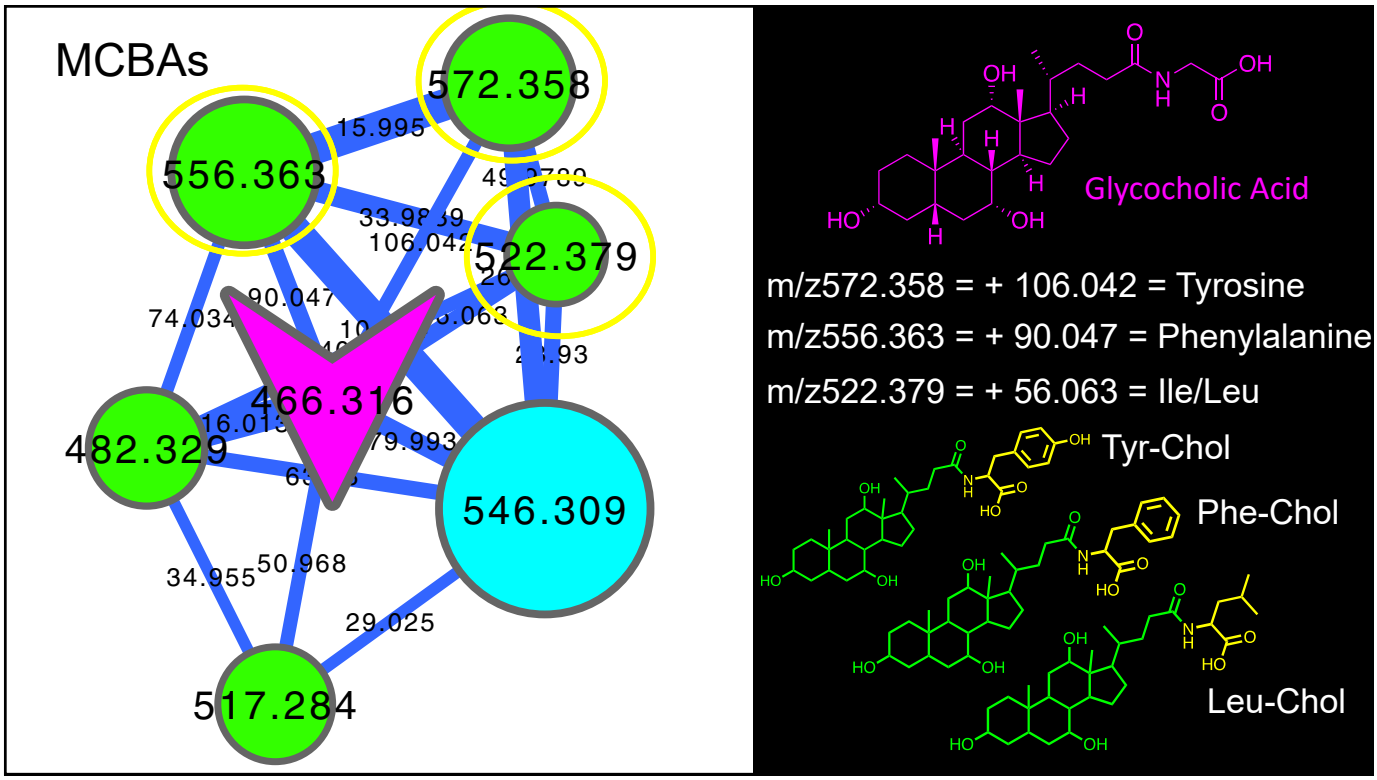
Discovery of Novel Microbial Bile Acids



18


Discovery of Novel Microbially Conjugate Bile Acids (MCBAs)
Molecular Network Gut Bile Acids
Glycocholic Acids
Glycocholic Acid
Taurocholic Acids
Deoxytaurocholic Acids
Taurocholic acid
???
546.309
Colonized
Sterile
Shared

19


MCBAs
572.358
556.363
522.379
466.316
482.329
546.309
517.284
15.995
74.034
90.047
34.955
50.968
29.025
Glycocholic Acid
m/z572.358 = + 106.042 = Tyrosine
m/z556.363 = + 90.047 = Phenylalanine
m/z522.379 = + 56.063 = Ile/Leu
Tyr-Chol
Phe-Chol
Leu-Chol

20

HEINRICH O. WIELAND

The chemistry of the bile acids

Nobel Lecture, December 12, 1928

Scientific chemistry occupied itself with the constituent substances of the bile at an early stage. L. Gmelin, Thénard and also Berzelius already did work on the acids present in bile, and several publications of the Liebig Laboratory in Giessen dealt with this subject. But it was only in 1848 that A. Strecker succeeded in isolating from ox-gall, the two most wide-spread acids, *taurocholic acid* and *glycocholic acid*, two conjugated substances of cholic acid $C_{24}H_{40}O_5$, condensed amide-like with taurine and glycine at the carboxyl group.

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Article | Published: 26 February 2020

Global chemical effects of the microbiome include new bile-acid conjugations

Robert A. Quinn, Alexey V. Melnik, [...] Pieter C. Dorrestein

Nature **579**, 123–129 (2020) | Cite this article

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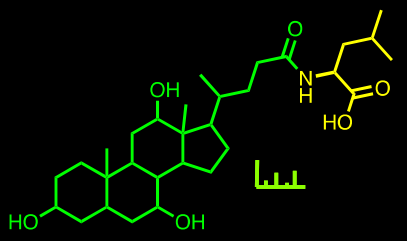
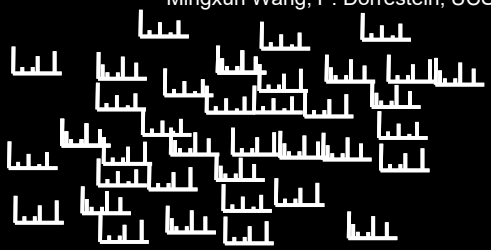
MASST Search: They are also found in humans

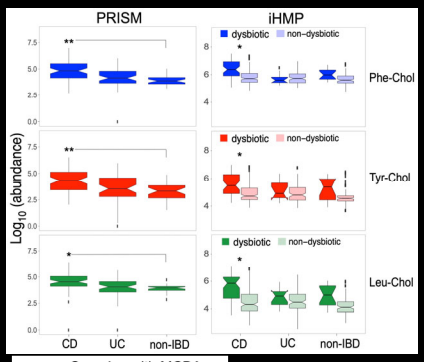


Mingxun Wang, P. Dorrestein, UCSD

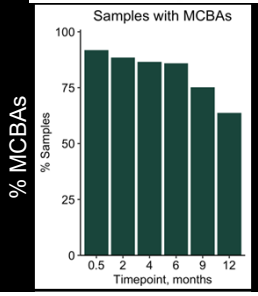


GNPS

Elevated in Crohn's Disease



Curtis Huttenhower, Harvard



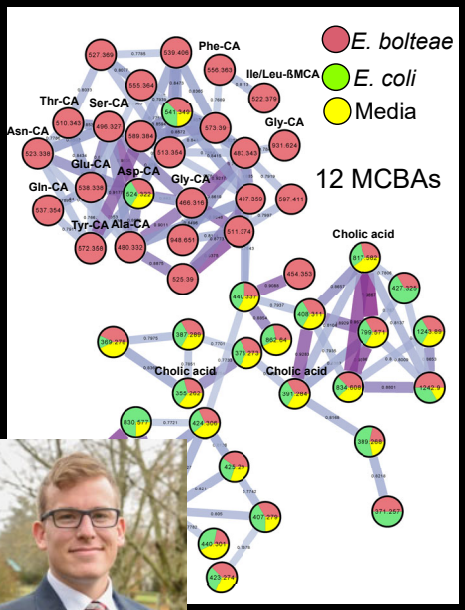
ABC Baby fecal study
n = 1362 samples

- 92% of samples
- 129 of 272 babies
- 13 different conjugates

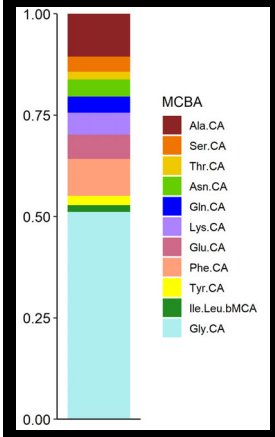
Julie Lumeng, U Michigan

22

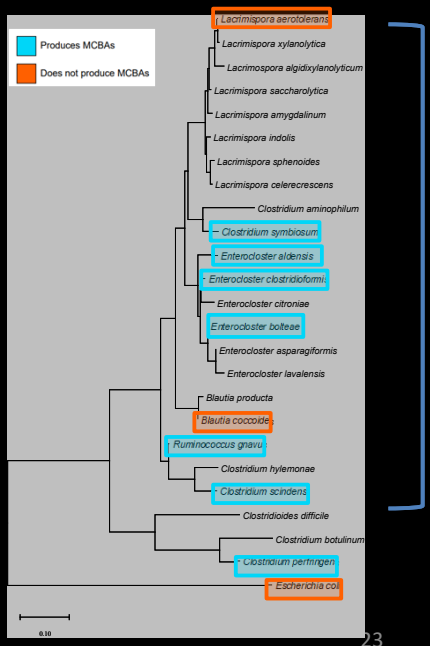
In vitro MCBA production by gut bacterium *Enterocloster bolteae*



Doug Guzior



RCM Media
24hr Culture, 37°C
Synthesis requires live cells



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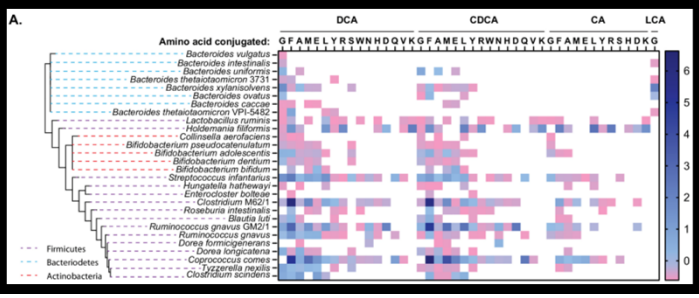
RESEARCH ARTICLE
July/August 2021 Volume 6 Issue 4 e00805-21
https://doi.org/10.1128/mSystems.00805-21

Dominant Bacterial Phyla from the Human Gut Show Widespread Ability To Transform and Conjugate Bile Acids

L. N. Lucas[a,b], K. Barrett[a,b], R. L. Kerby[a], Q. Zhang[a], L. E. Cattaneo[a,b], D. Stevenson[a], F. E. Rey[a], and D. Amador-Noguez[a]

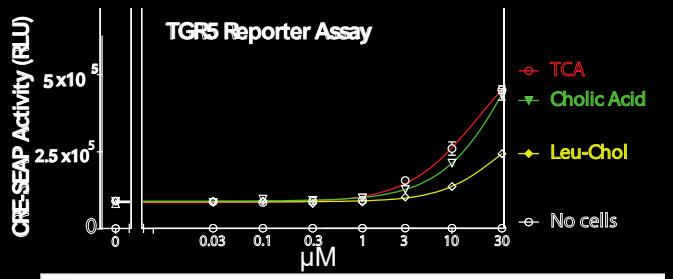
[a]Department of Bacteriology, University of Wisconsin—Madison, Madison, Wisconsin, USA
[b]Microbiology Doctoral Training Program, University of Wisconsin—Madison, Madison, Wisconsin, USA

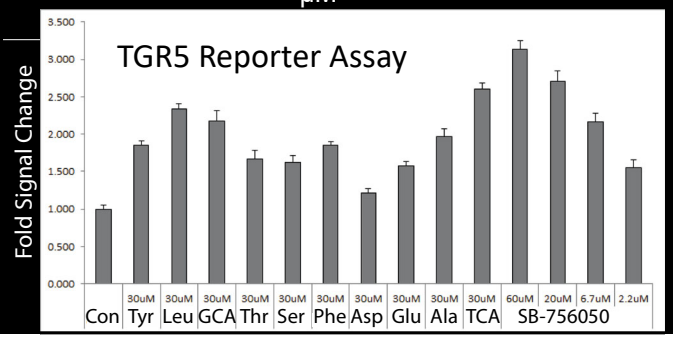
- Discovery of 44 new MCBAs
- 28 of 72 isolates tested
- Covering Actinobacteria, Firmicutes and Bacteroidetes



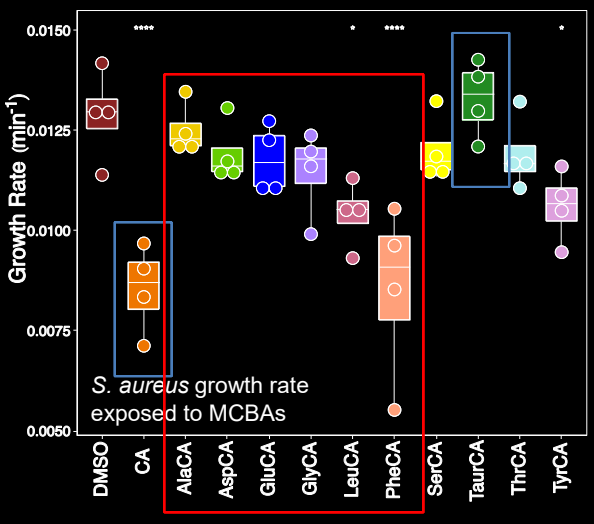
So cool! But what do they do?

Alter Bile Acid Signaling but AA-Dependent





MCBAs are Less Antimicrobial but AA dependent



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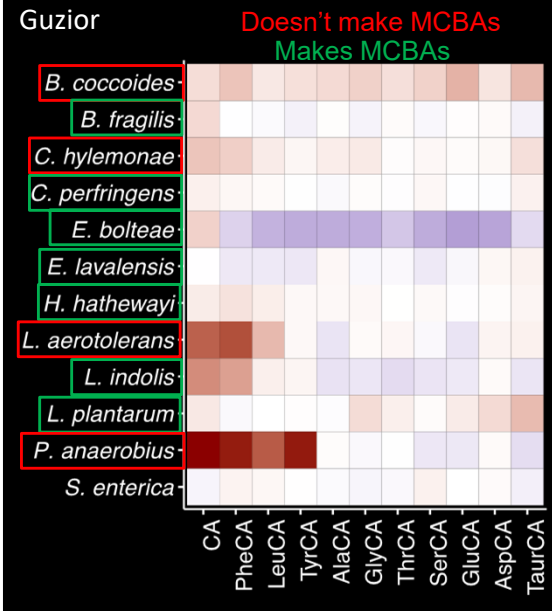
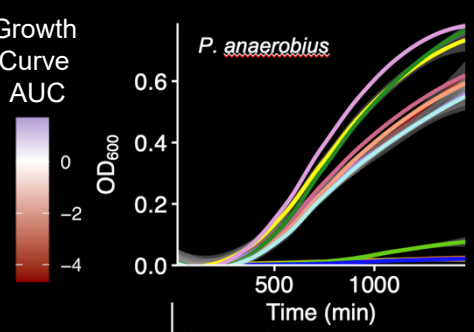
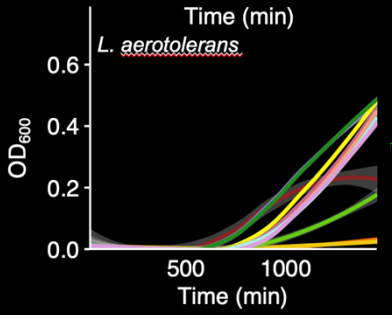
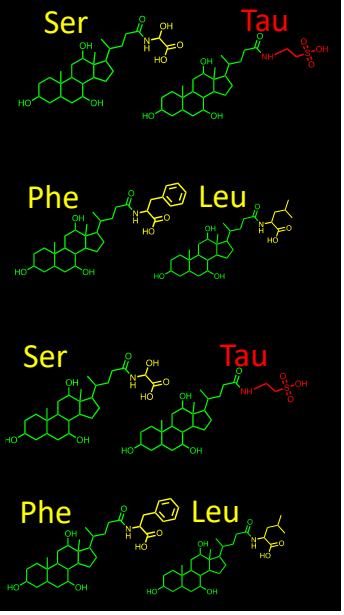
MCBAs have Antimicrobial Properties Depending on the Amino Acid Conjugated

Doug Guzior

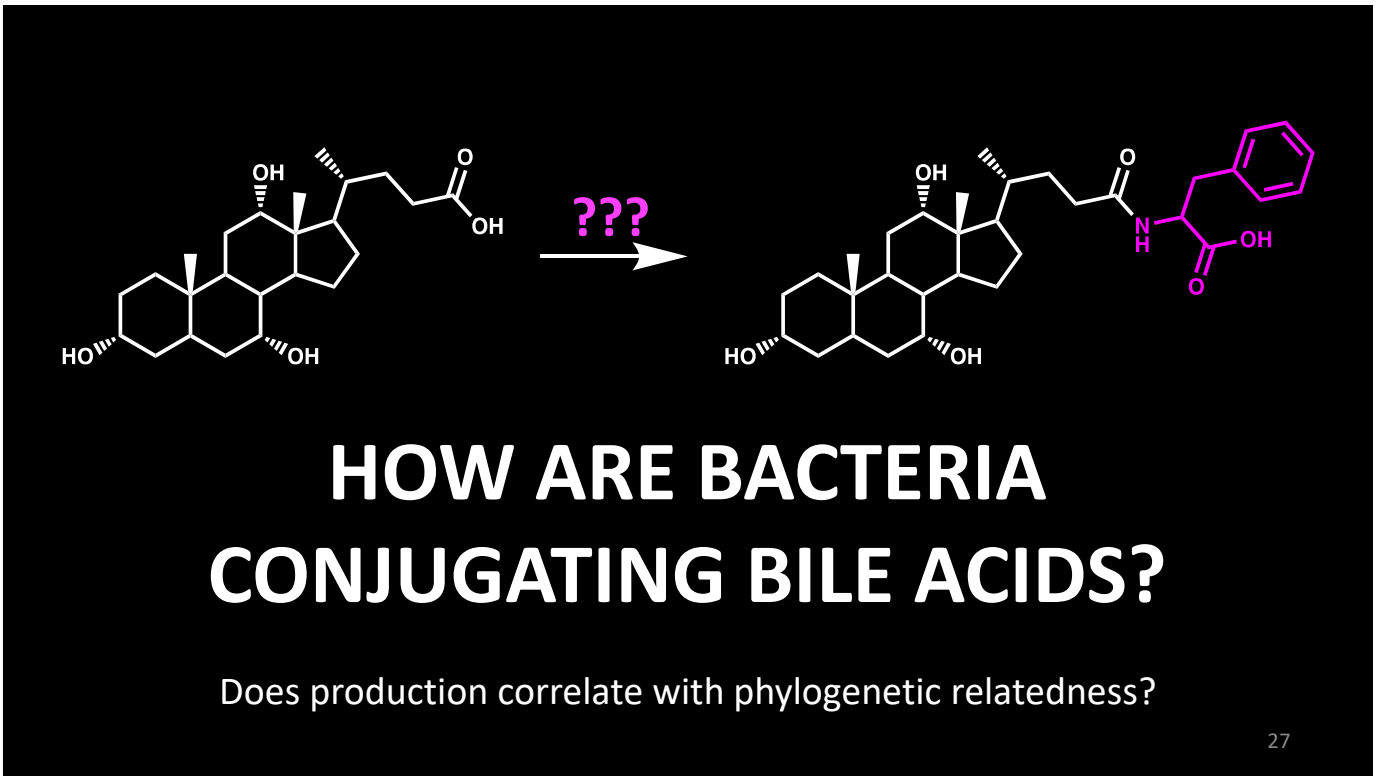




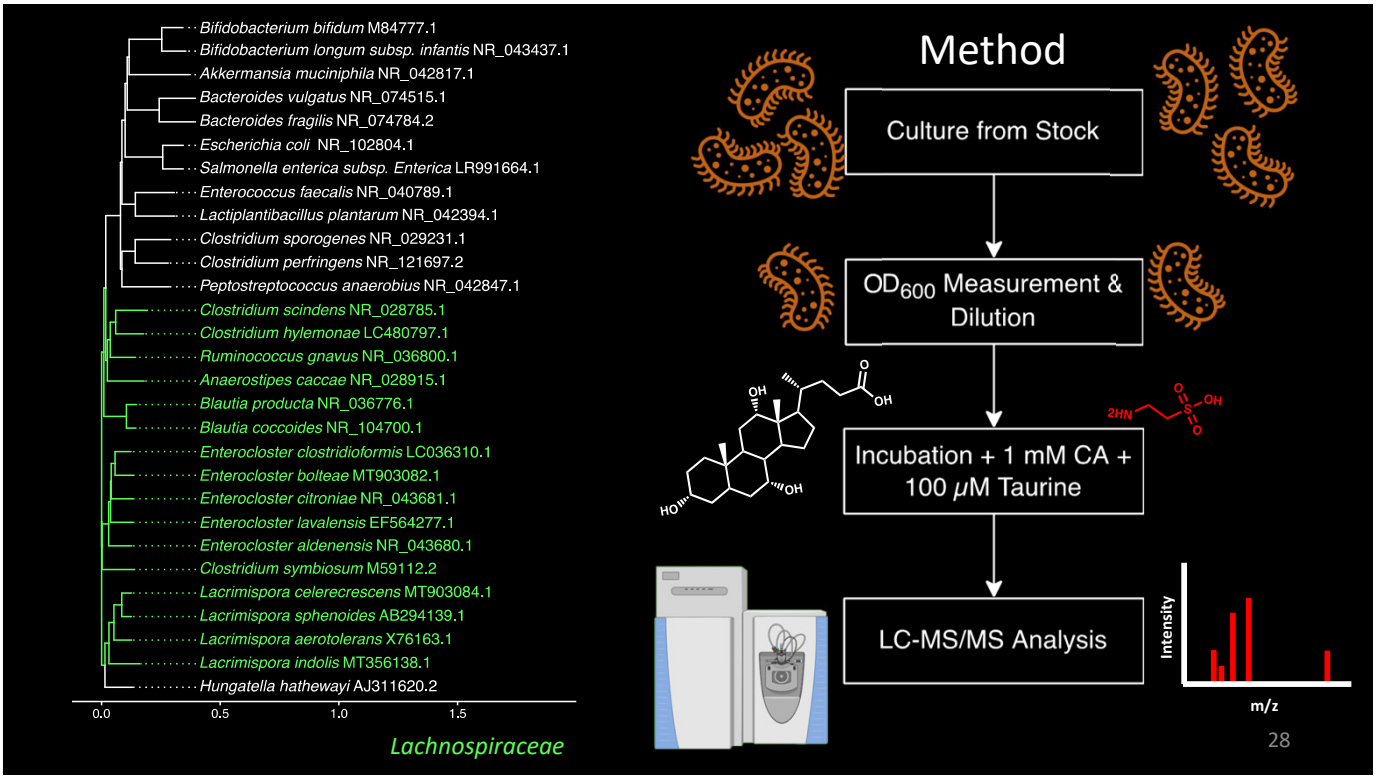




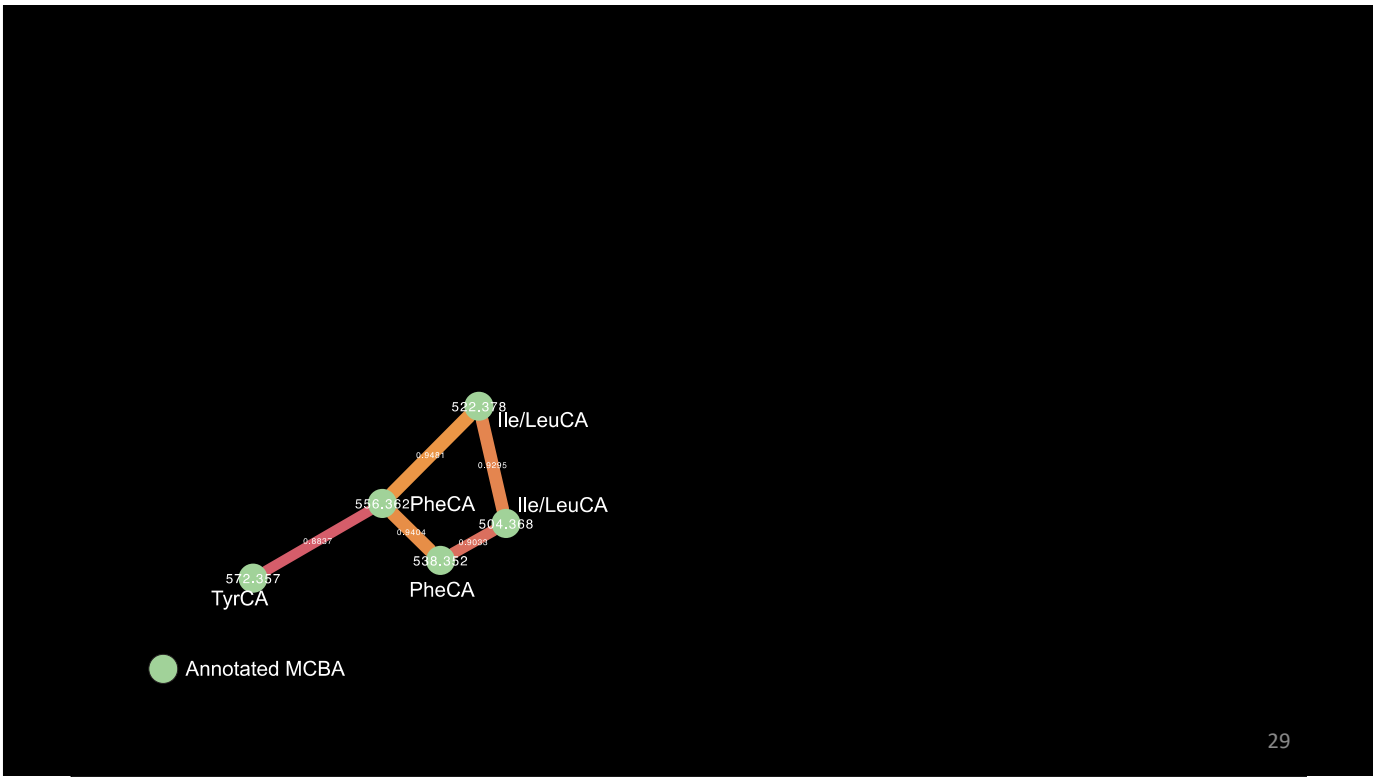
26


OH
O
OH
HO
OH
???
OH
O
N
H
OH
O
HO
OH
HOW ARE BACTERIA CONJUGATING BILE ACIDS?
Does production correlate with phylogenetic relatedness?
27

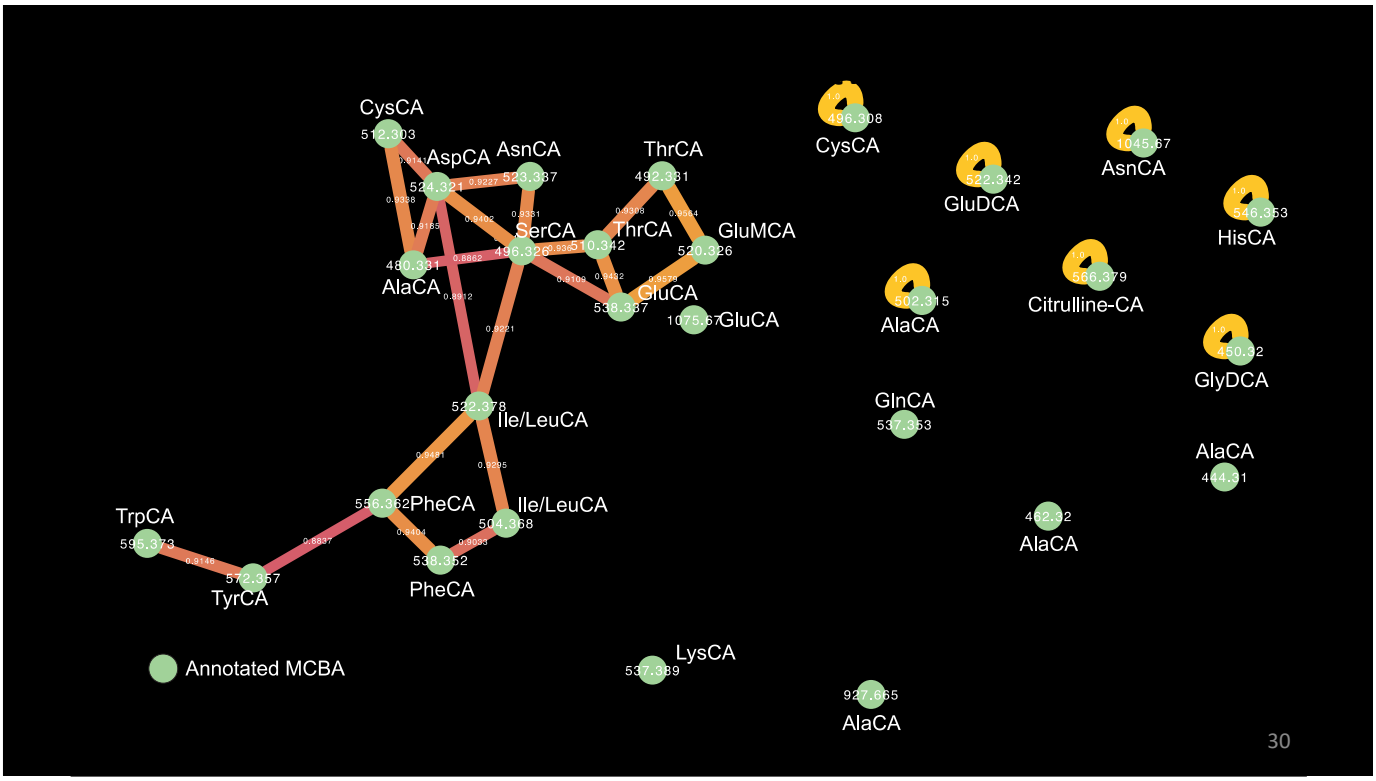
27


Bifidobacterium bifidum M84777.1
Bifidobacterium longum subsp. infantis NR_043437.1
Akkermansia muciniphila NR_042817.1
Bacteroides vulgatus NR_074515.1
Bacteroides fragilis NR_074784.2
Escherichia coli NR_102804.1
Salmonella enterica subsp. Enterica LR991664.1
Enterococcus faecalis NR_040789.1
Lactiplantibacillus plantarum NR_042394.1
Clostridium sporogenes NR_029231.1
Clostridium perfringens NR_121697.2
Peptostreptococcus anaerobius NR_042847.1
Clostridium scindens NR_028785.1
Clostridium hylemonae LC480797.1
Ruminococcus gnavus NR_036800.1
Anaerostipes caccae NR_028915.1
Blautia producta NR_036776.1
Blautia coccoides NR_104700.1
Enterocloster clostridioformis LC036310.1
Enterocloster bolteae MT903082.1
Enterocloster citroniae NR_043681.1
Enterocloster lavalensis EF564277.1
Enterocloster aldenensis NR_043680.1
Clostridium symbiosum M59112.2
Lacrimispora celerecrescens MT903084.1
Lacrimispora sphenoides AB294139.1
Lacrimispora aerotolerans X76163.1
Lacrimispora indolis MT356138.1
Hungatella hathewayi AJ311620.2
0.0
0.5
1.0
1.5
Lachnospiraceae
Method
Culture from Stock
OD600 Measurement & Dilution
Incubation + 1 mM CA + 100 μM Taurine
LC-MS/MS Analysis
Intensity
m/z
28

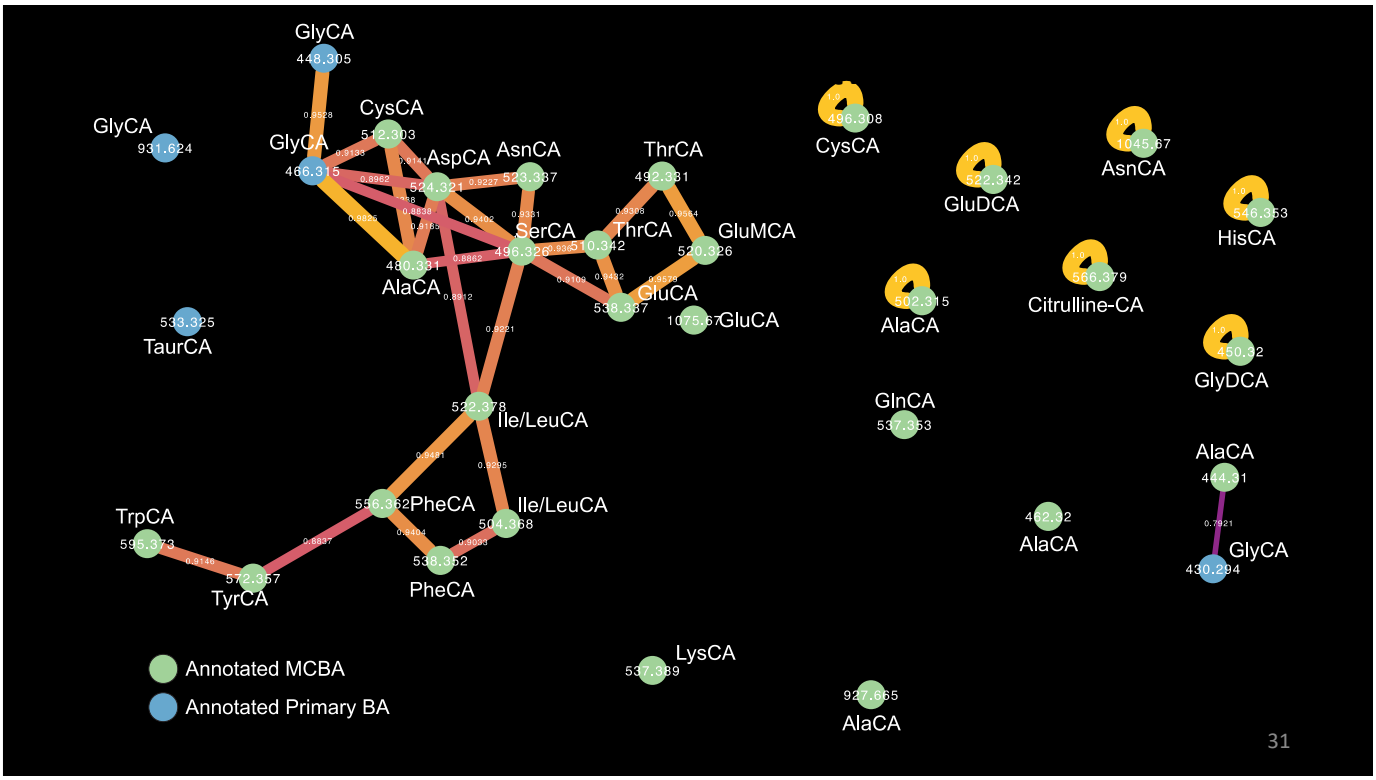
28


522.378
Ile/LeuCA
556.362
PheCA
Ile/LeuCA
504.368
538.352
PheCA
572.357
TyrCA
Annotated MCBA
29

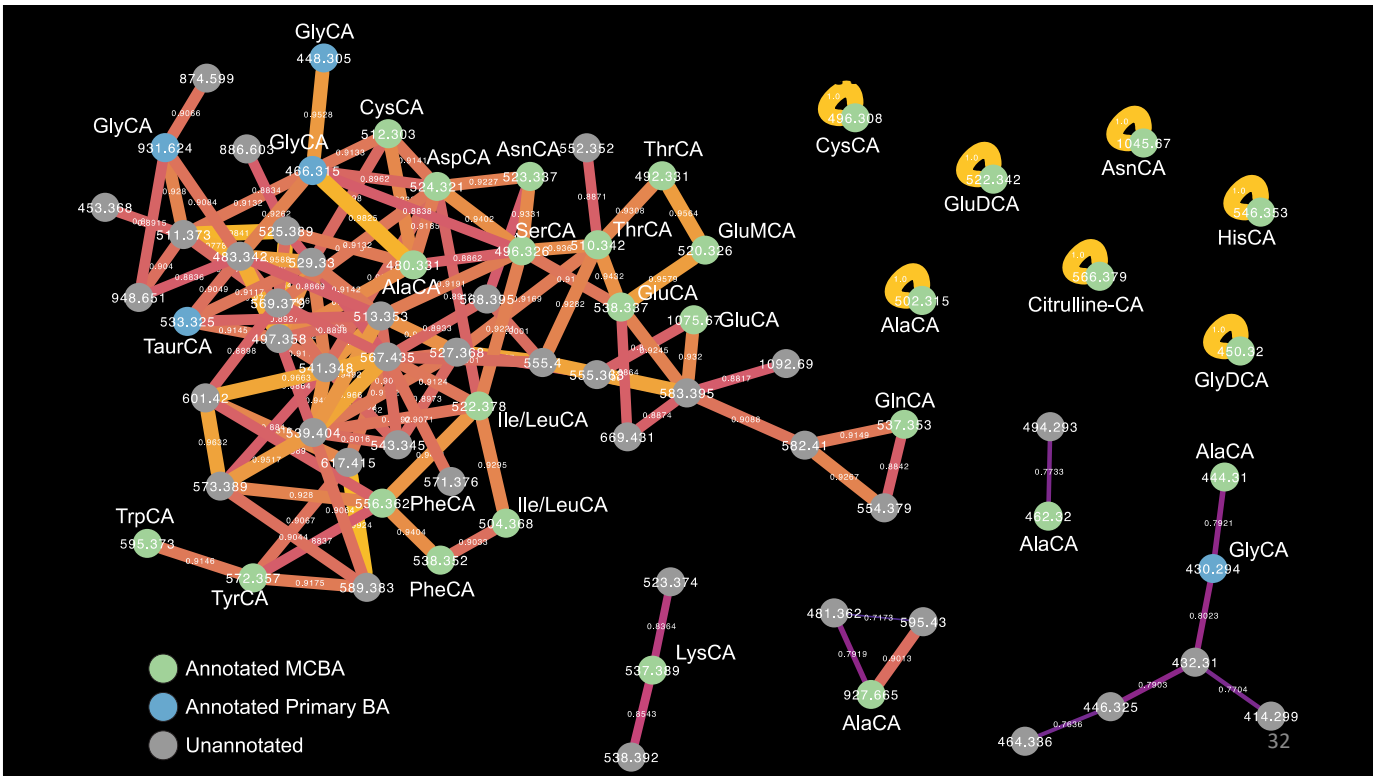
29


CysCA
512.303
AspCA
AsnCA
523.387
524.321
ThrCA
492.381
SerCA
ThrCA
GluMCA
496.326
510.342
520.326
480.381
AlaCA
GluCA
538.387
1075.67
GluCA
522.378
Ile/LeuCA
556.362
PheCA
Ile/LeuCA
504.368
538.352
PheCA
TrpCA
595.373
572.357
TyrCA
Annotated MCBA
LysCA
537.389
927.665
AlaCA
496.308
CysCA
522.342
GluDCA
1045.67
AsnCA
546.353
HisCA
566.379
Citrulline-CA
502.315
AlaCA
450.92
GlyDCA
GlnCA
537.353
AlaCA
464.31
462.32
AlaCA
30

30


GlyCA
448.305
GlyCA
931.624
GlyCA
466.315
CysCA
512.303
AspCA
524.321
AsnCA
523.387
ThrCA
492.381
SerCA
496.326
ThrCA
510.342
GluMCA
520.326
AlaCA
480.381
GluCA
538.387
GluCA
1075.67
TaurCA
533.325
522.378
Ile/LeuCA
PheCA
556.362
Ile/LeuCA
504.368
PheCA
538.352
TrpCA
595.373
TyrCA
572.357
CysCA
496.308
GluDCA
522.342
AsnCA
1045.67
HisCA
546.353
AlaCA
502.315
Citrulline-CA
566.379
GlyDCA
450.32
GlnCA
537.353
AlaCA
464.31
GlyCA
430.294
AlaCA
462.32
LysCA
537.389
AlaCA
927.665
Annotated MCBA
Annotated Primary BA
31

31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Annotated Primary BA
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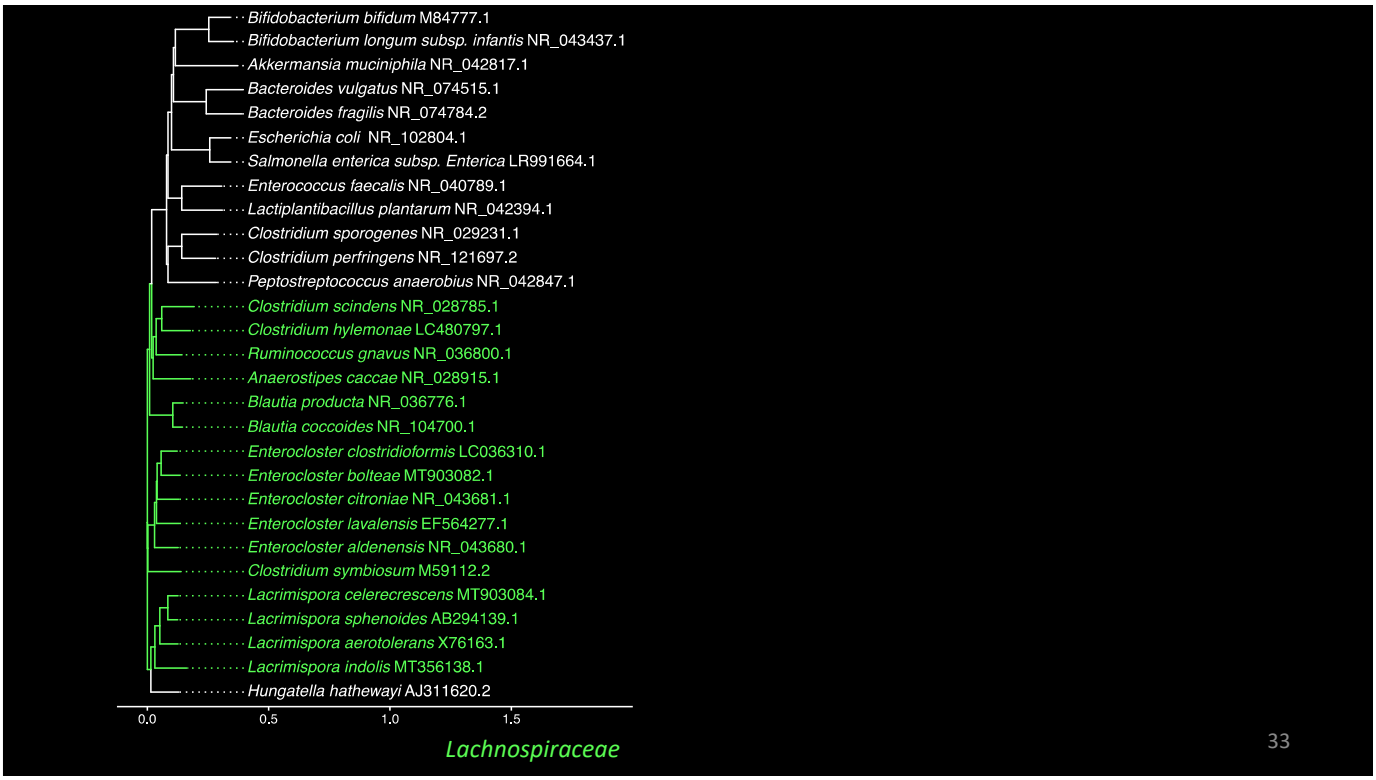
32

32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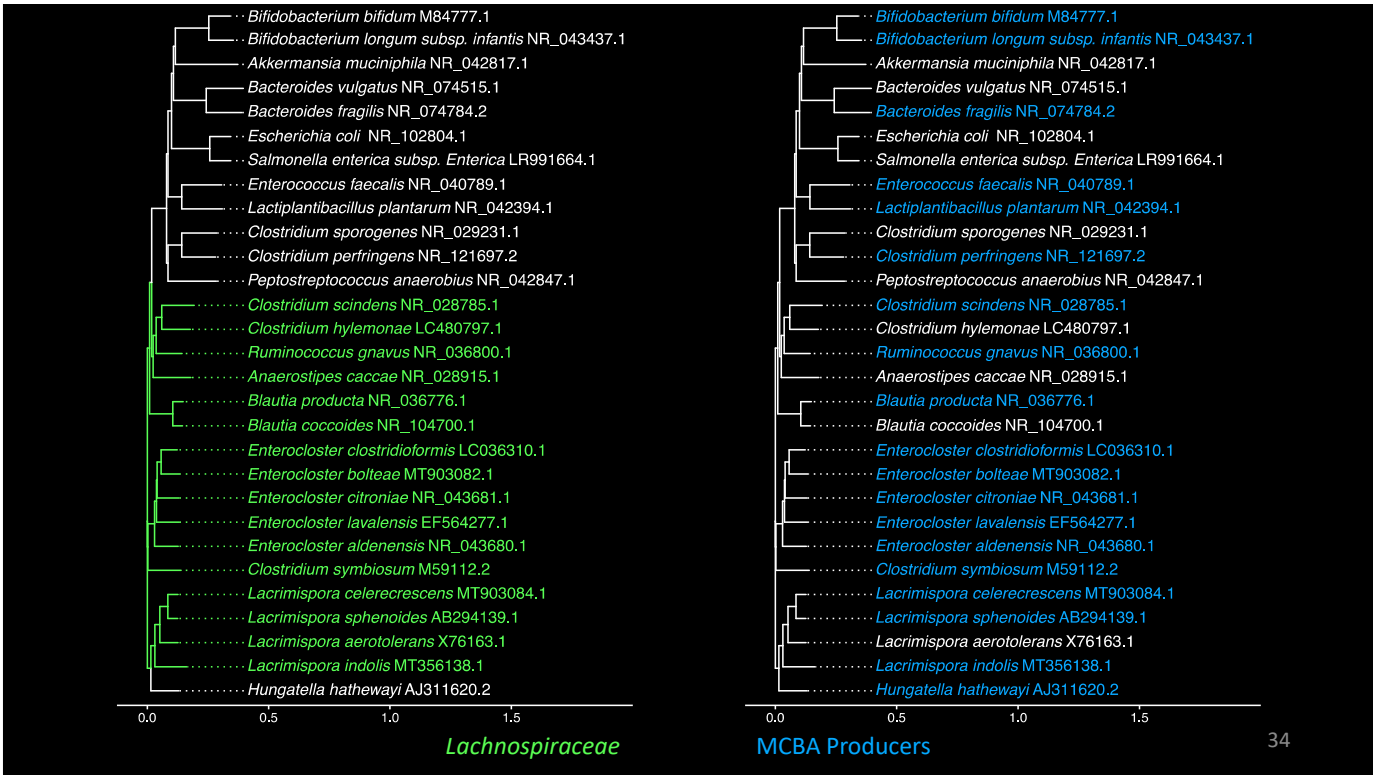
Lachnospiraceae
33

33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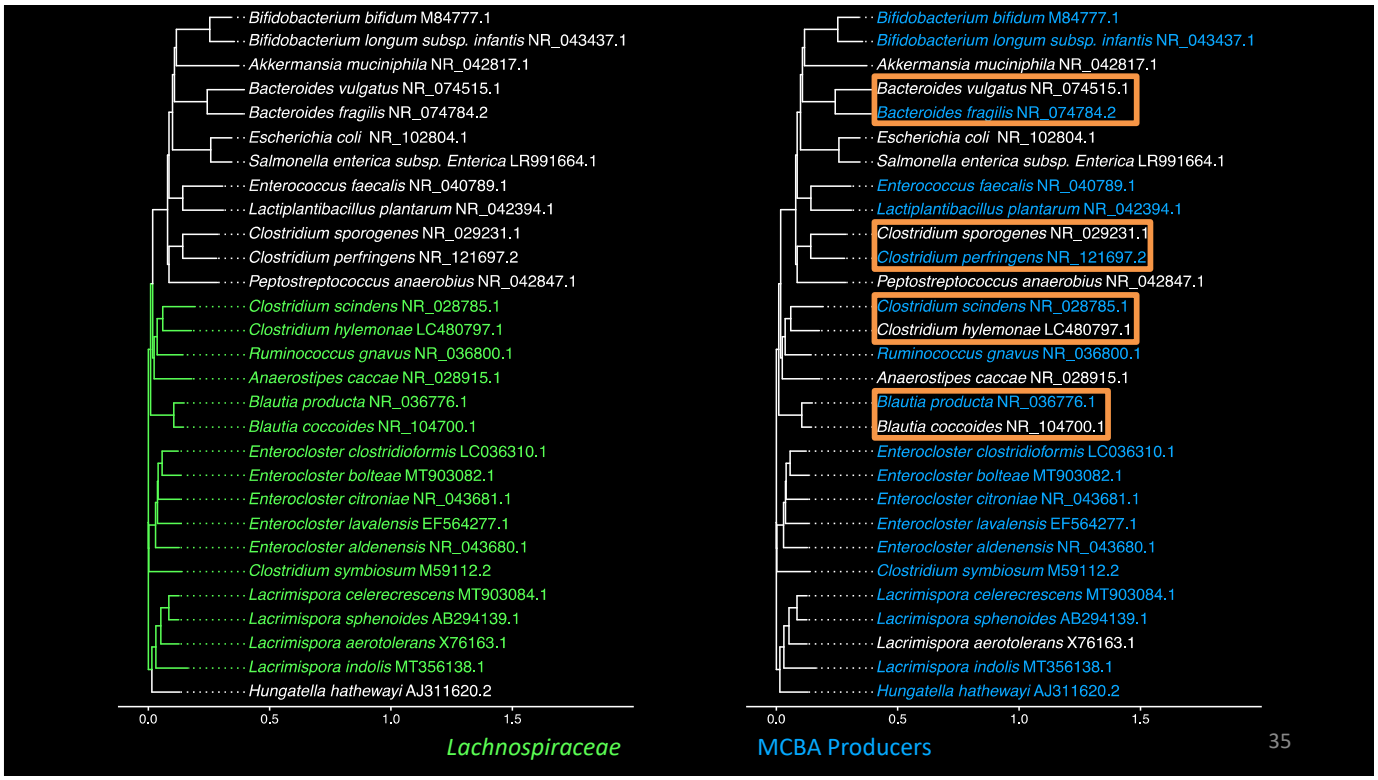
MCBA Producers
34

34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Lachnospiraceae
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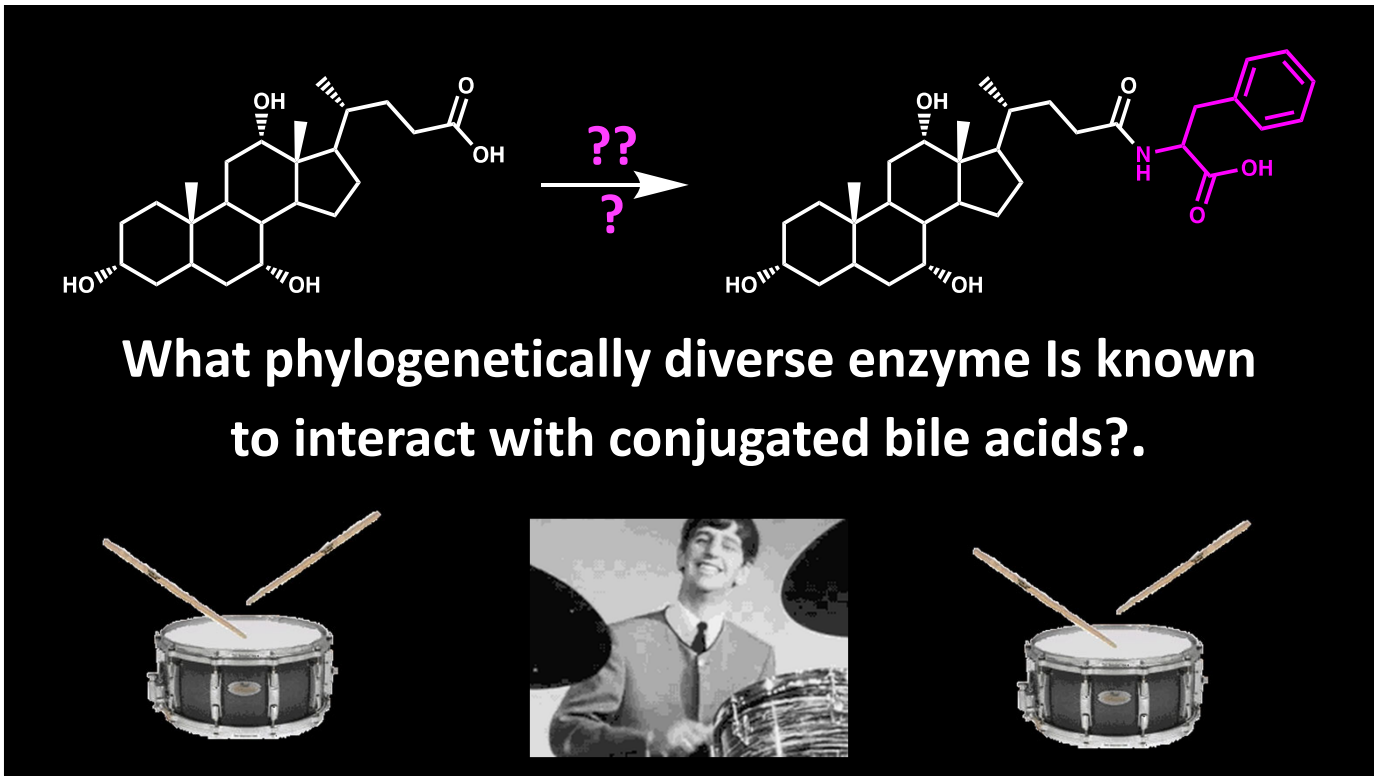
MCBA Producers
35

35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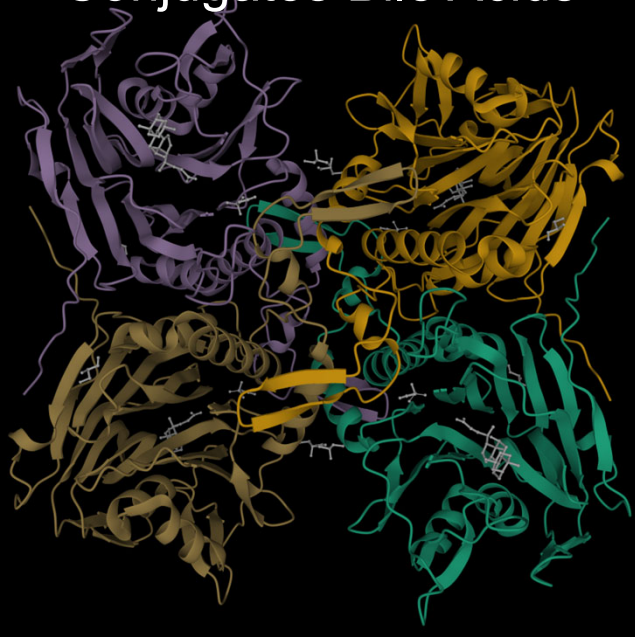
What phylogenetically diverse enzyme Is known to interact with conjugated bile acids?.

36

Bile Salt Hydrolase Previously Unknown Amino-transfer Conjugates Bile Acids



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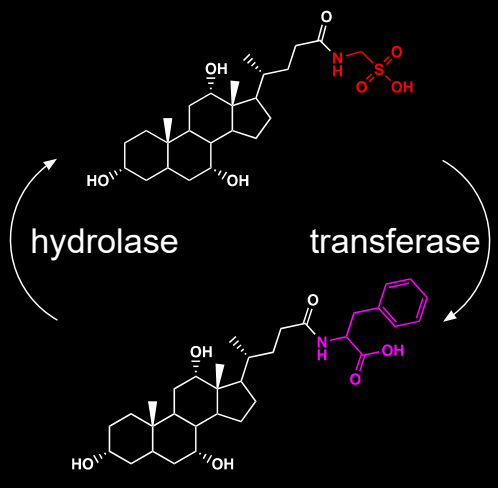
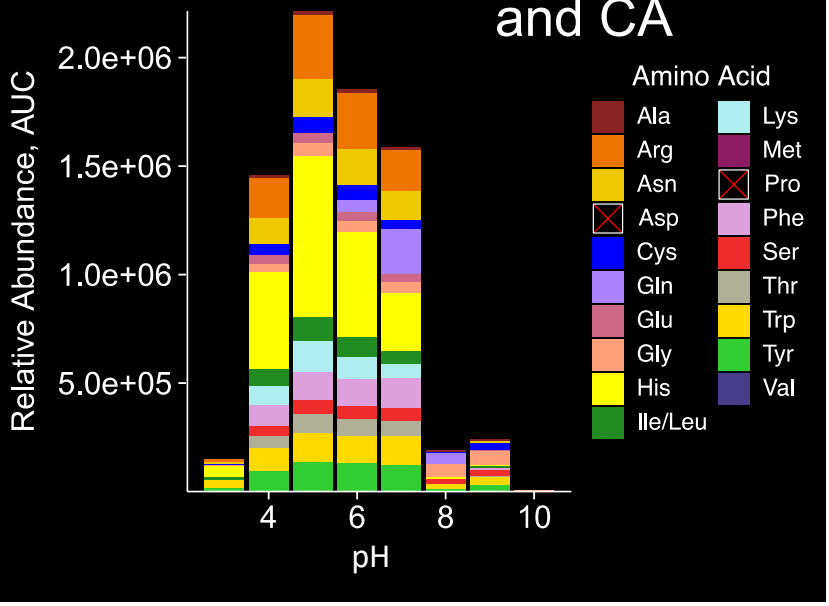
*Cp*BSH Transfers Amino Acids to TaurCA and GlyCA and CA





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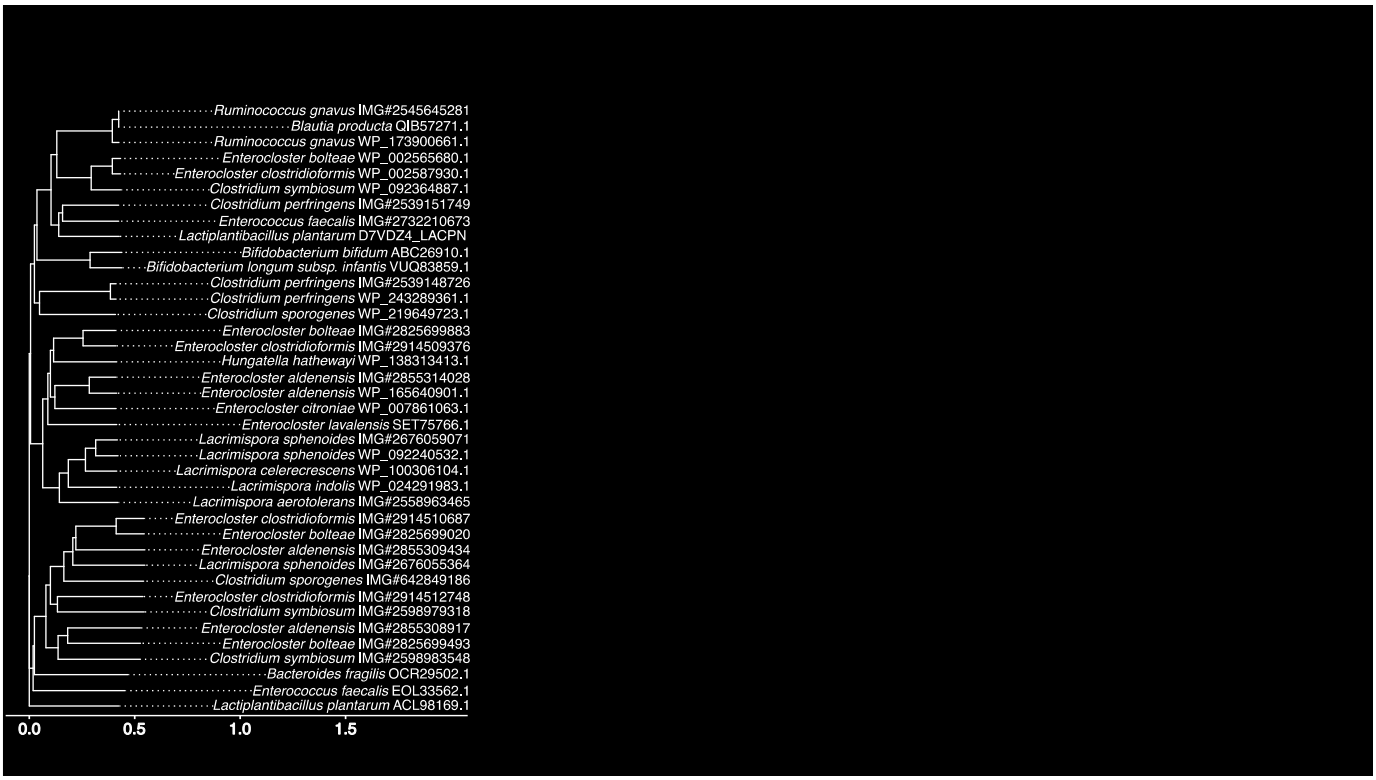

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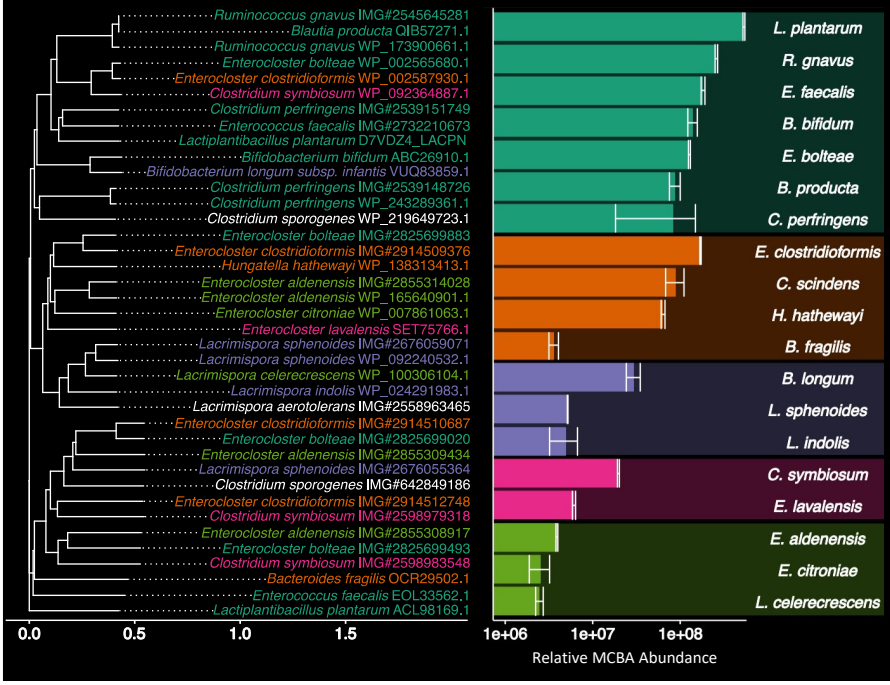
MCBA producers cluster based on abundance and diversity



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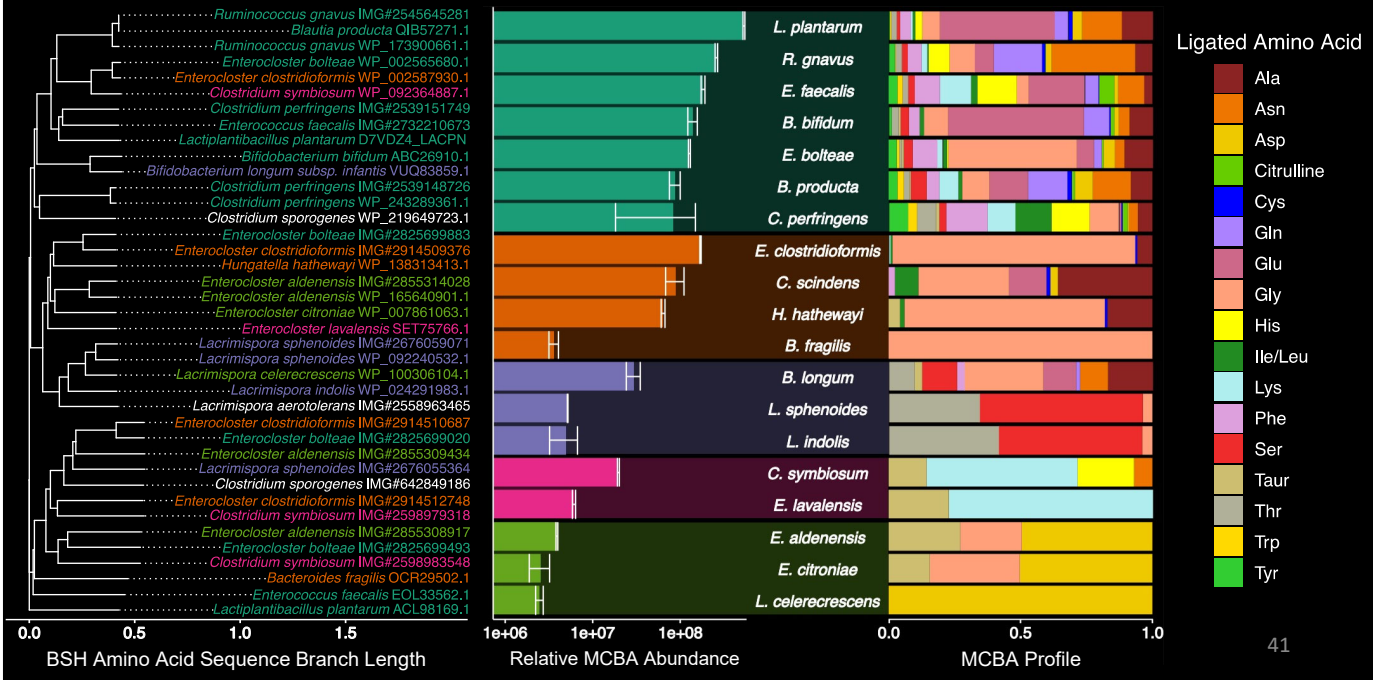
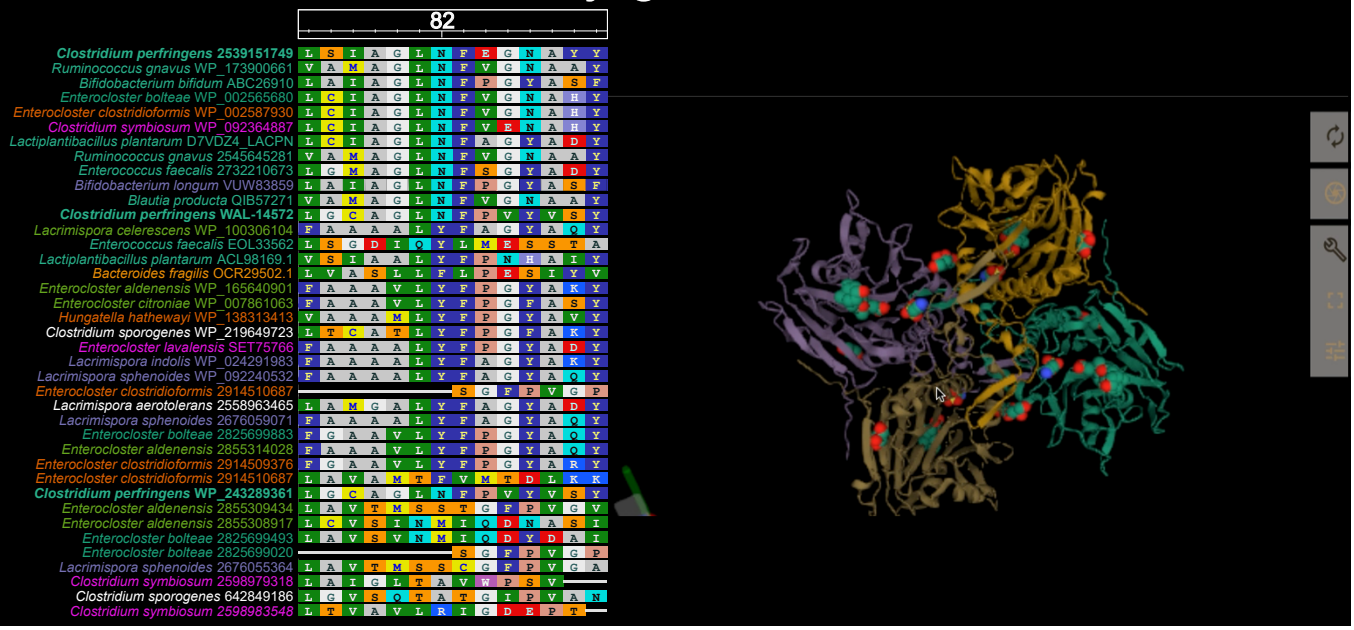
MCBA producers cluster based on abundance and diversity



41

41

Amino acid sequence of BSH Determines AA Conjugation Profile



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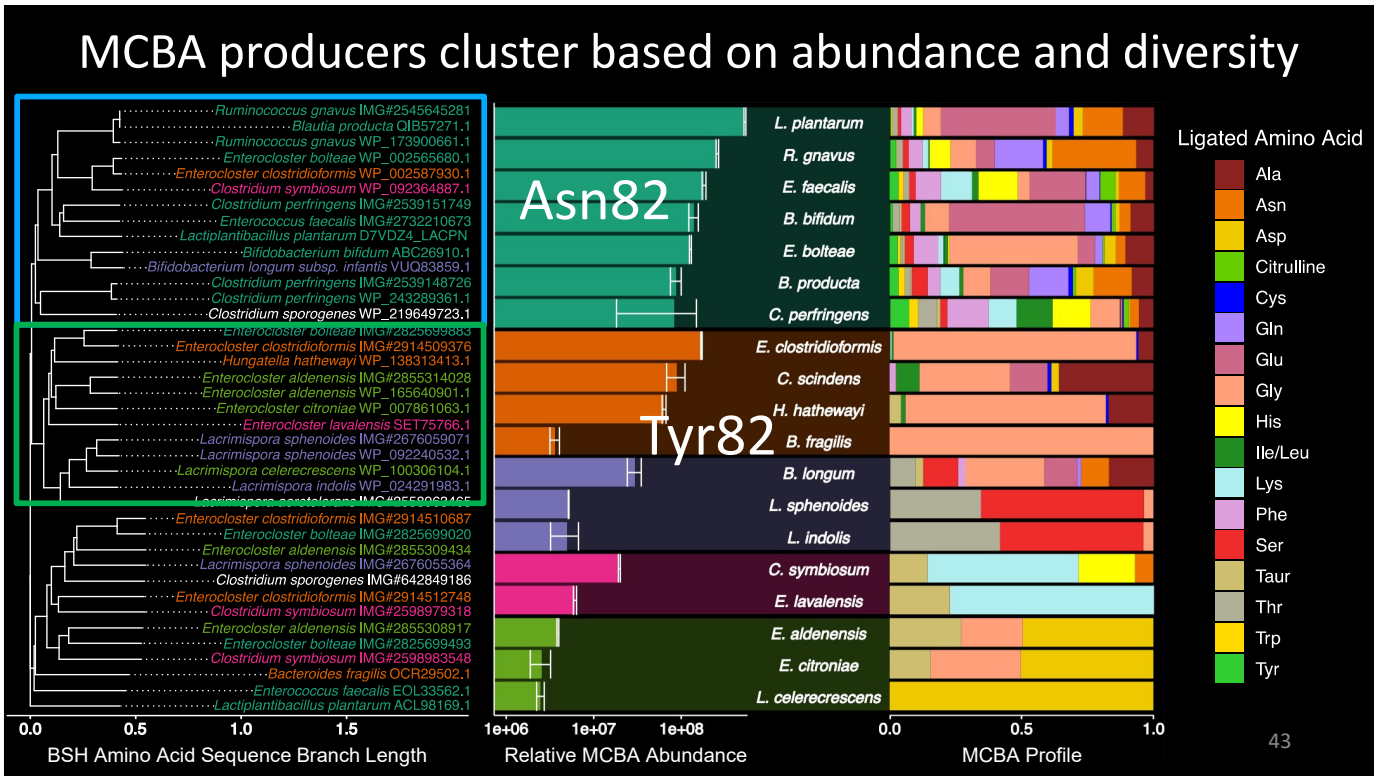
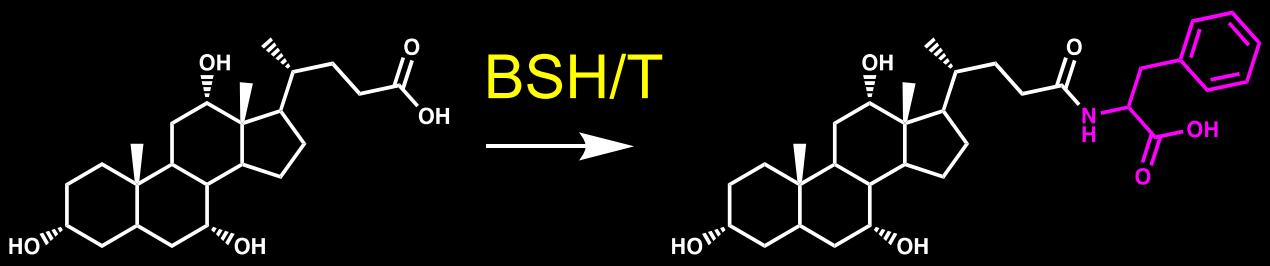


43

So how can we study MCBA properties and function?



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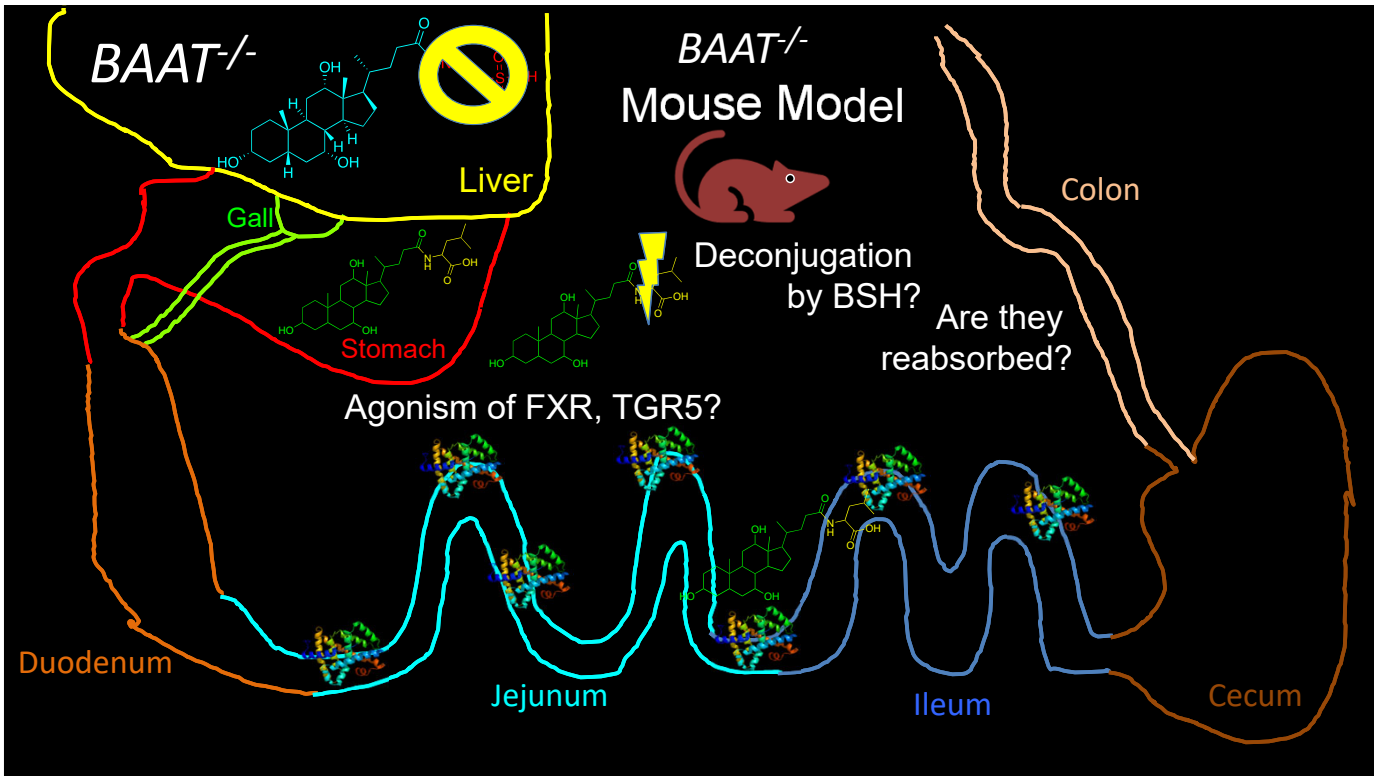
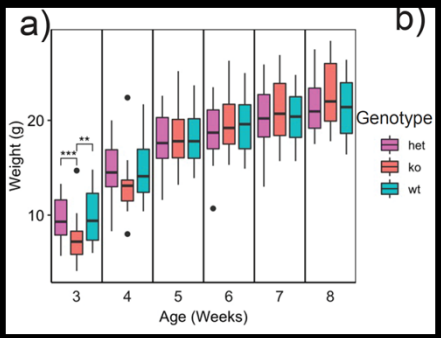
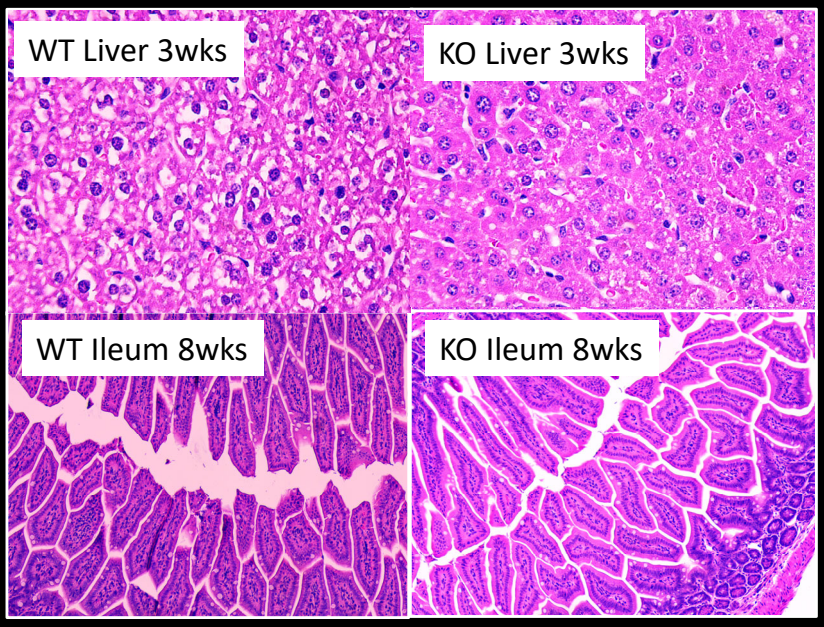


45

BAAT$^{-/-}$ Phenotype

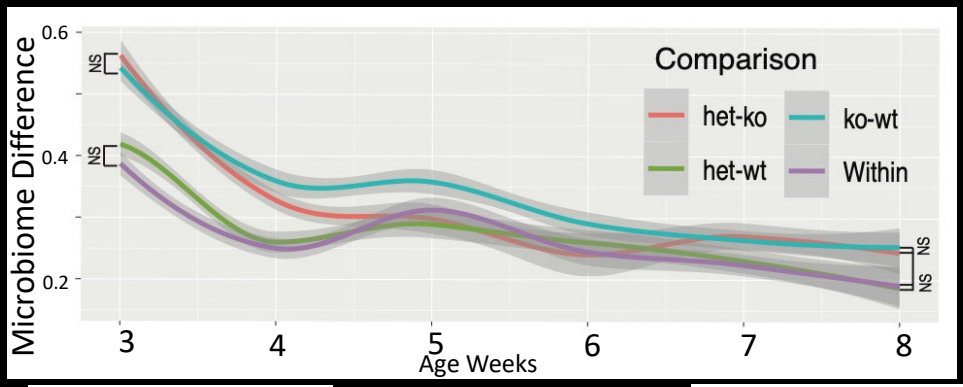


- Pups struggle after weaning
- Exhibit catch-up growth
- Little apparent pathology in adulthood
- Many ko x ko litters fail to thrive or die, mothers sometimes die

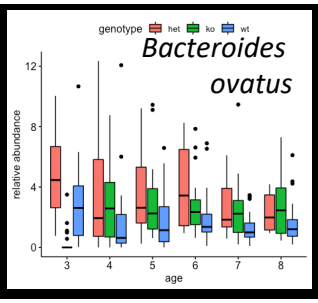


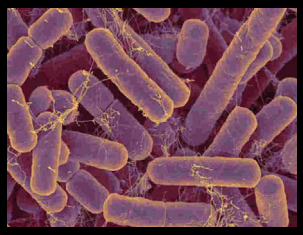
46

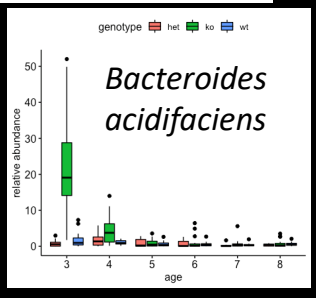
BAAT$^{-/-}$ Mice have Altered Microbiome Early in Life



But it Normalizes!!!







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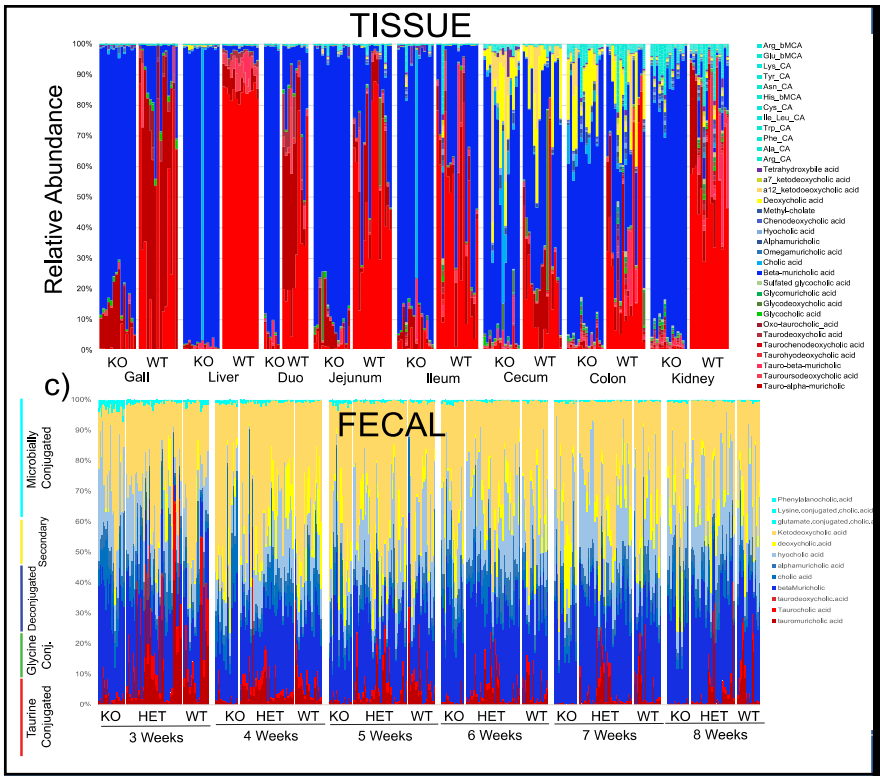


BAAT$^{-/-}$ mice have significantly altered bile acid profiles

Taurine Conjugated

Glycine Conjugated

Cholic/muricholic

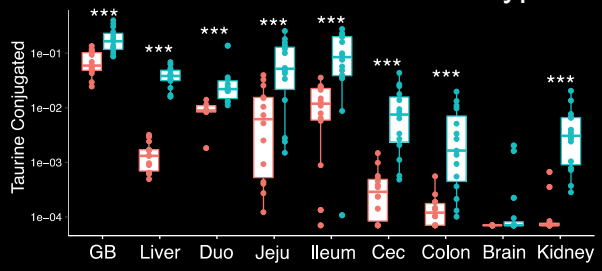
Secondary

MCBAs

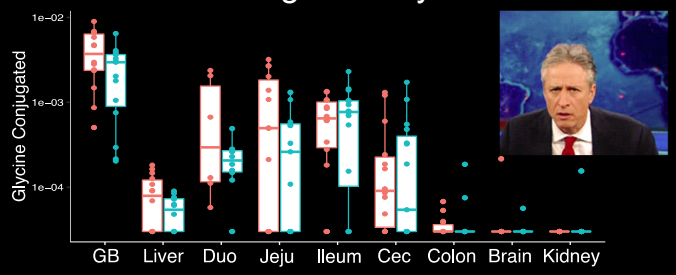
48

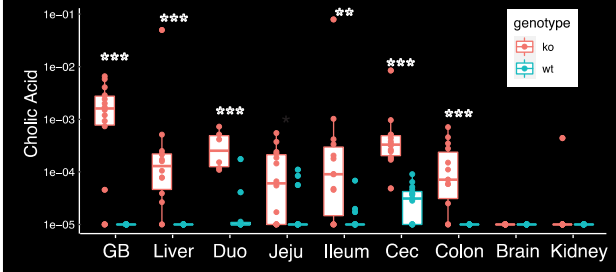
BAAT$^{-/-}$ Bile Acid Profiles

27x times more TCA in wildtype liver

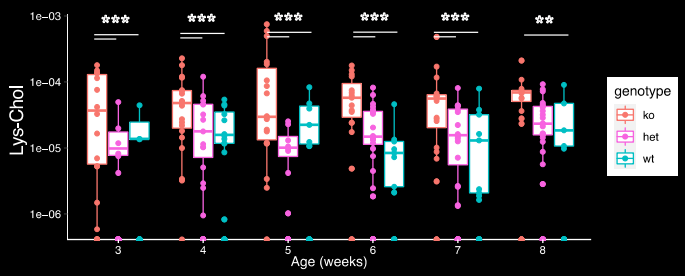


GCA not significantly different





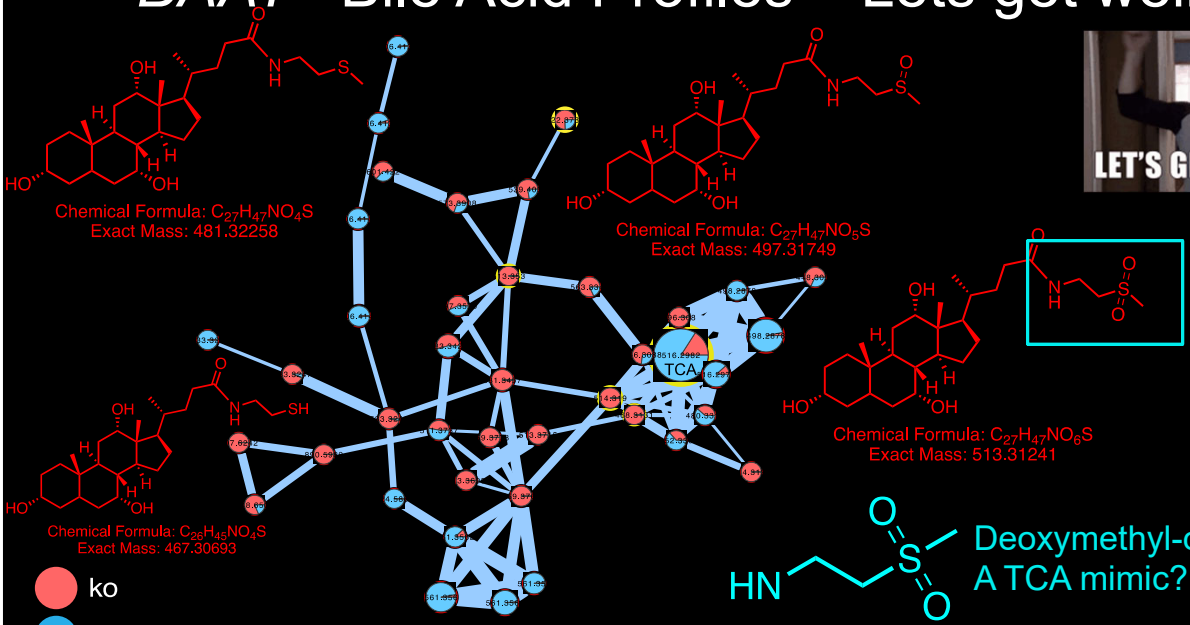
Elevated Cholic acid



Elevated MCBAs in Fecal samples

49

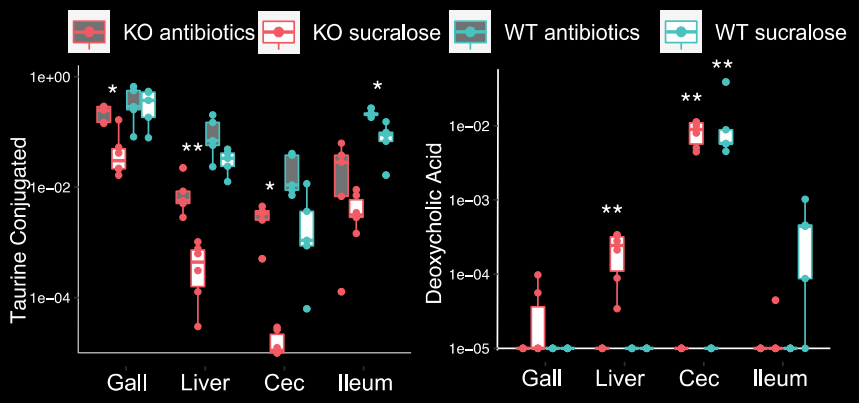
BAAT$^{-/-}$ Bile Acid Profiles – Lets get weird



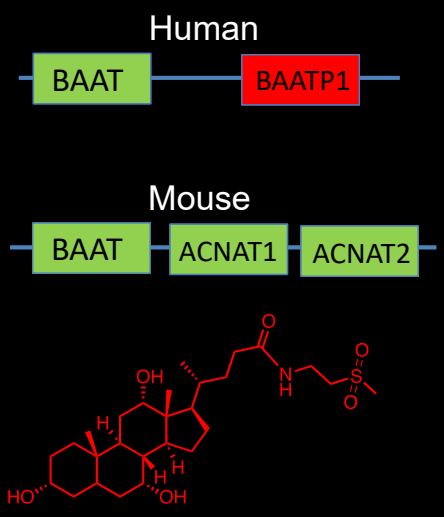
Deoxymethyl-cysteamine
A TCA mimic?

50

Unique Conjugated Bile Acids in *BAAT*$^{-/-}$ Mice are Not Microbial – *ACNAT1* and *ACNAT2*



Antibiotic treatment of KO Mice did not eliminate BA conjugation.



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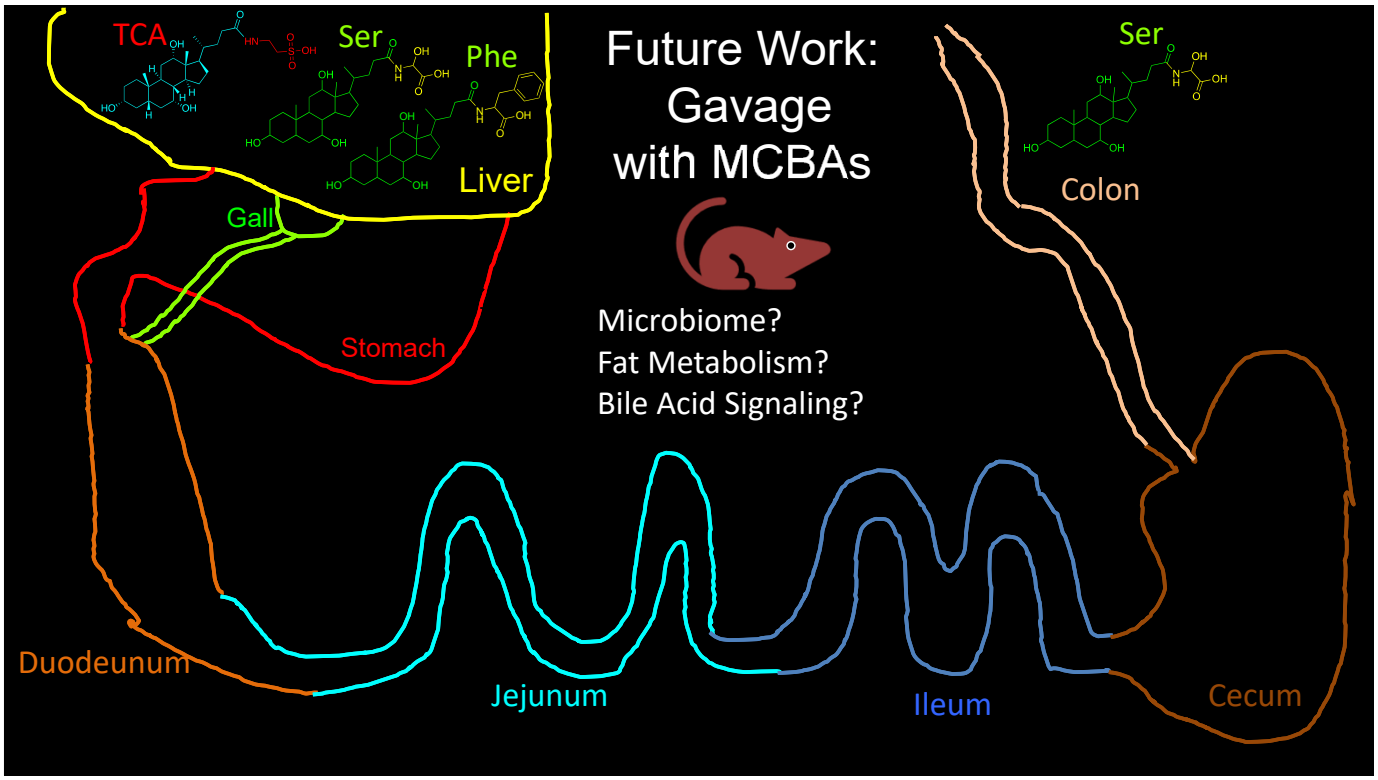

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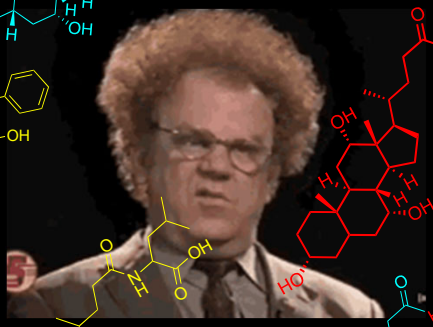
Mammalian Bile Acid Conjugation:



We have a lot of work to do

53

Acknowledgements

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Pieter Dorrestein
Rob Knight

U Michigan
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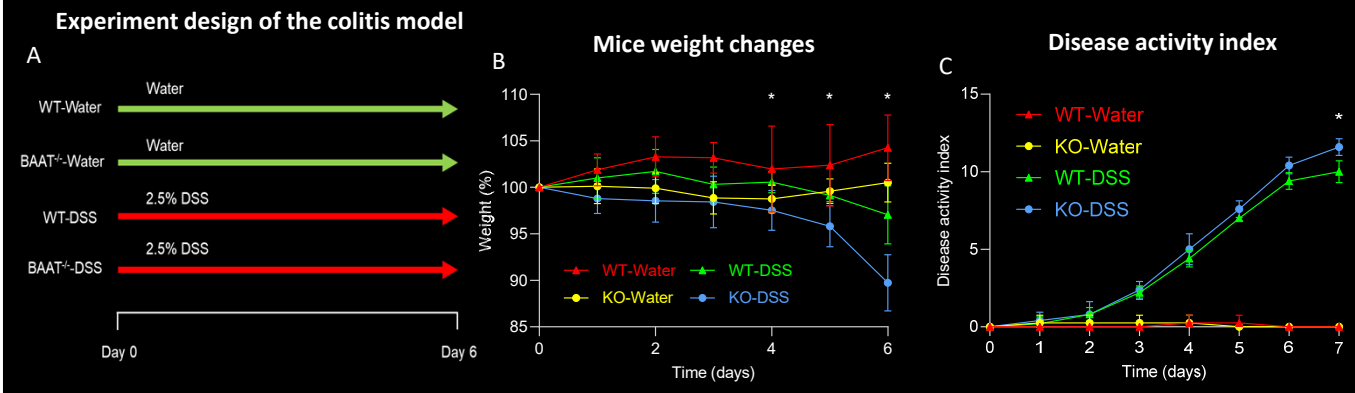
Quinn Lab
Kerri Neugebauer
Doug Guzior
Maxwell Okros
Christian Martin
Jeremy Feiner
Yousi Fu

MSU Mass Spec Core
Dan Jones, MSU
Tony Schilmiller

quinnrob@msu.edu
robertquinnlab.com

54

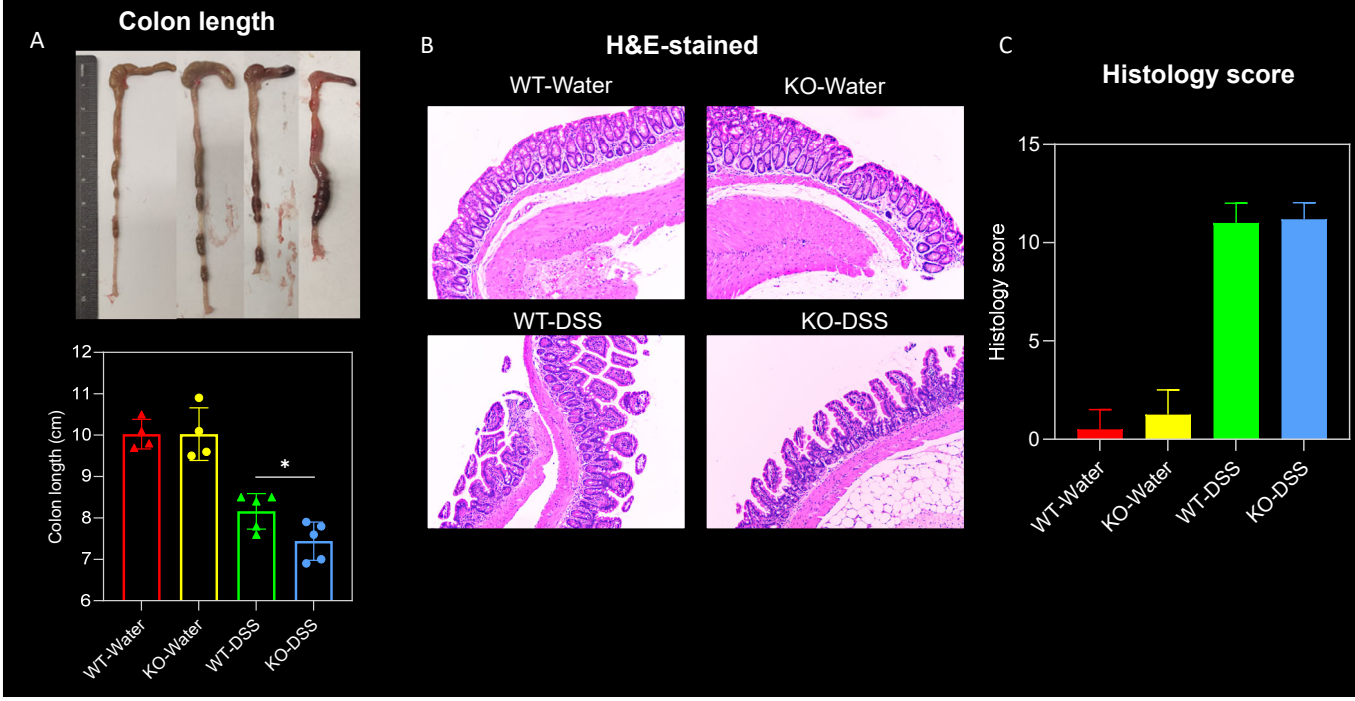
BAAT Knockout Mouse and DSS IBD Model



BAAT KO mice showed more weights loss and higher Disease scores than WT mice

55

BAAT Knockout Mouse and DSS IBD Model



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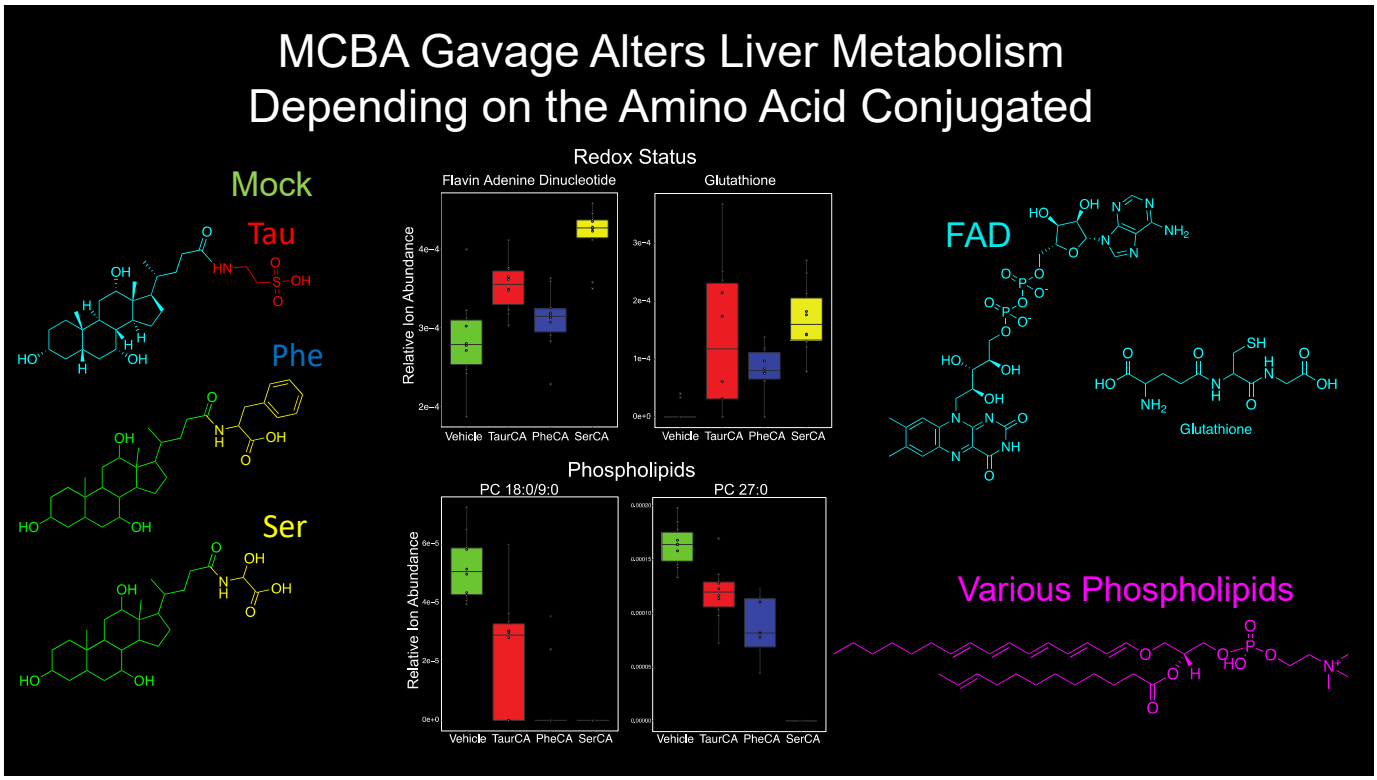
MCBA Gavage Alters Liver Metabolism
Depending on the Amino Acid Conjugated
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Tau
Phe
Ser
Redox Status
Flavin Adenine Dinucleotide
Glutathione
Relative Ion Abundance
Vehicle TaurCA PheCA SerCA
FAD
Glutathione
Phospholipids
PC 18:0/9:0
PC 27:0
Various Phospholipids

57