Alimentary Pharmabiotic Centre

Interfacing Food & Medicine

http://apc.ucc.ie

*Valencia, 2015*

*Bile Research Group*

Dr. Susan Joyce

# Bacterial Bile Salt Hydrolase in the Regulation of Host Lipid Metabolism & Circadian Rhythm: A Role in Probiotic Function?


## Cormac Gahan

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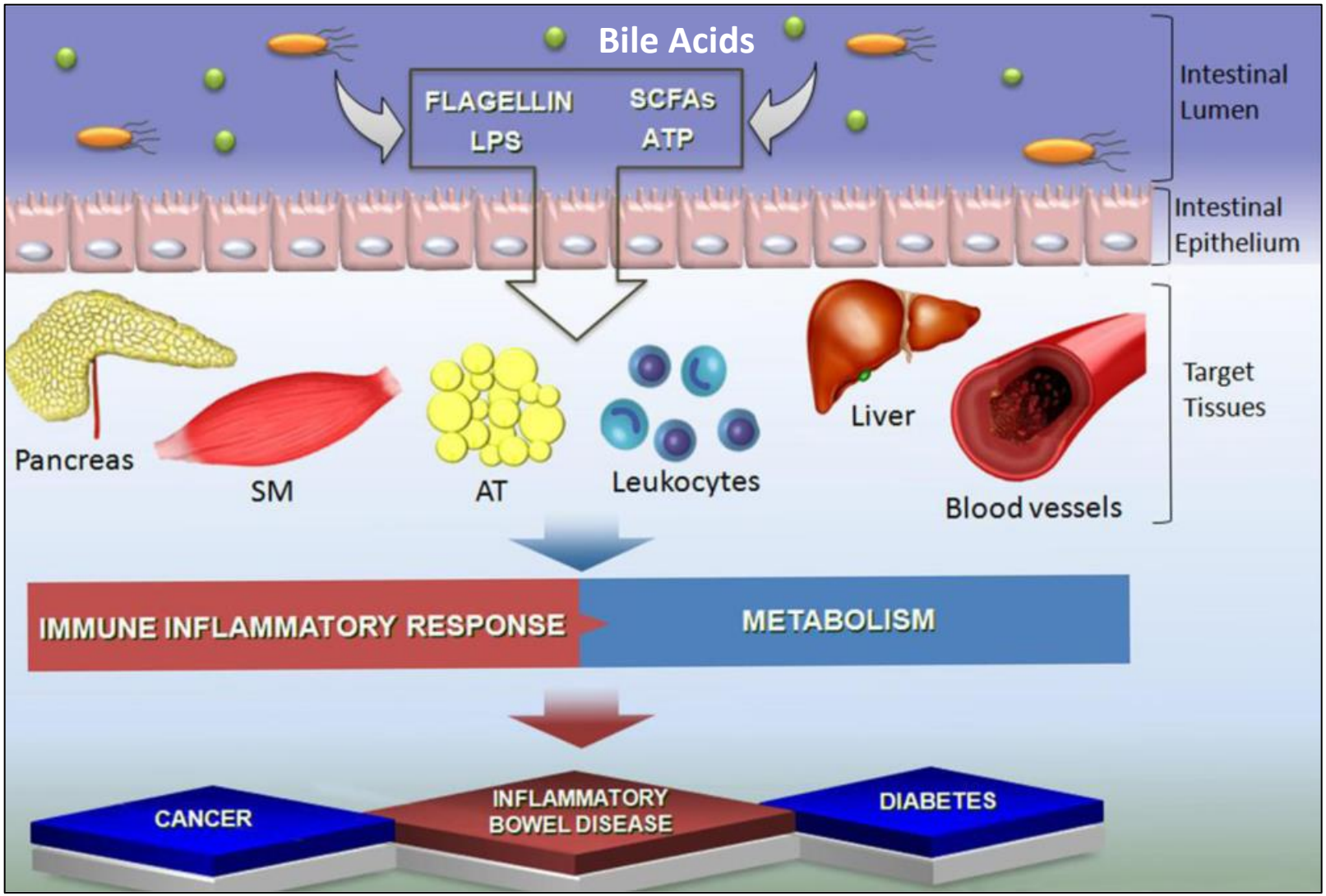
UCC University College Cork, Ireland Coláiste na hOllscoile Corcaigh

eagasc Agriculture and Food Development Authority

CIT Institiúid Teicneolaíochta Chorcaí Cork Institute of Technology

sfi Science Foundation Ireland

# Microbe:Host Interactions




Vinolo et al. *Nutrients* **2011**, *3*(10), 858-876

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# Bile in Systemic Microbe-Host Signalling

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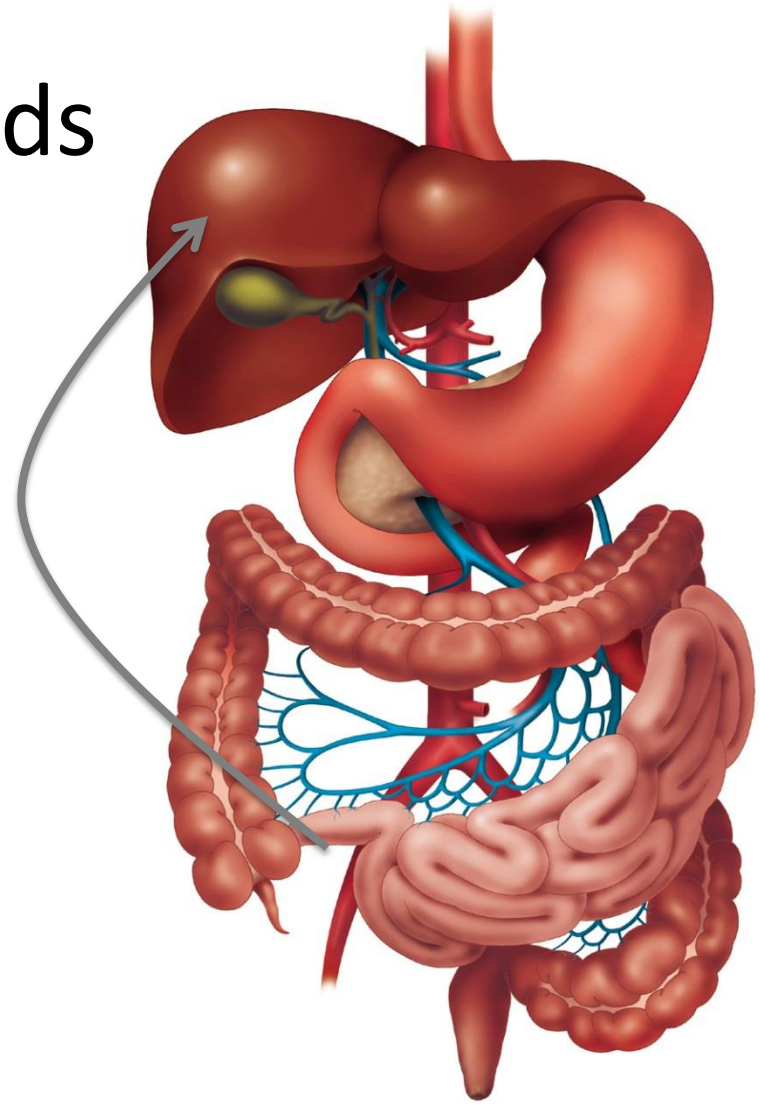
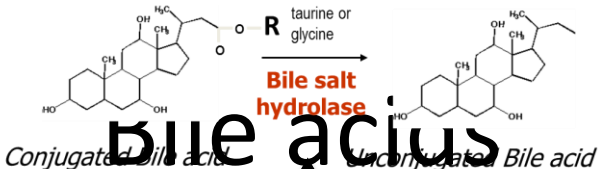
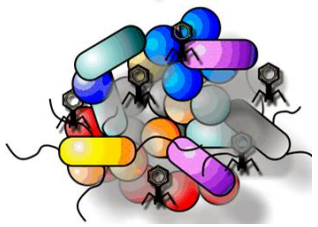
& Bile acids act here



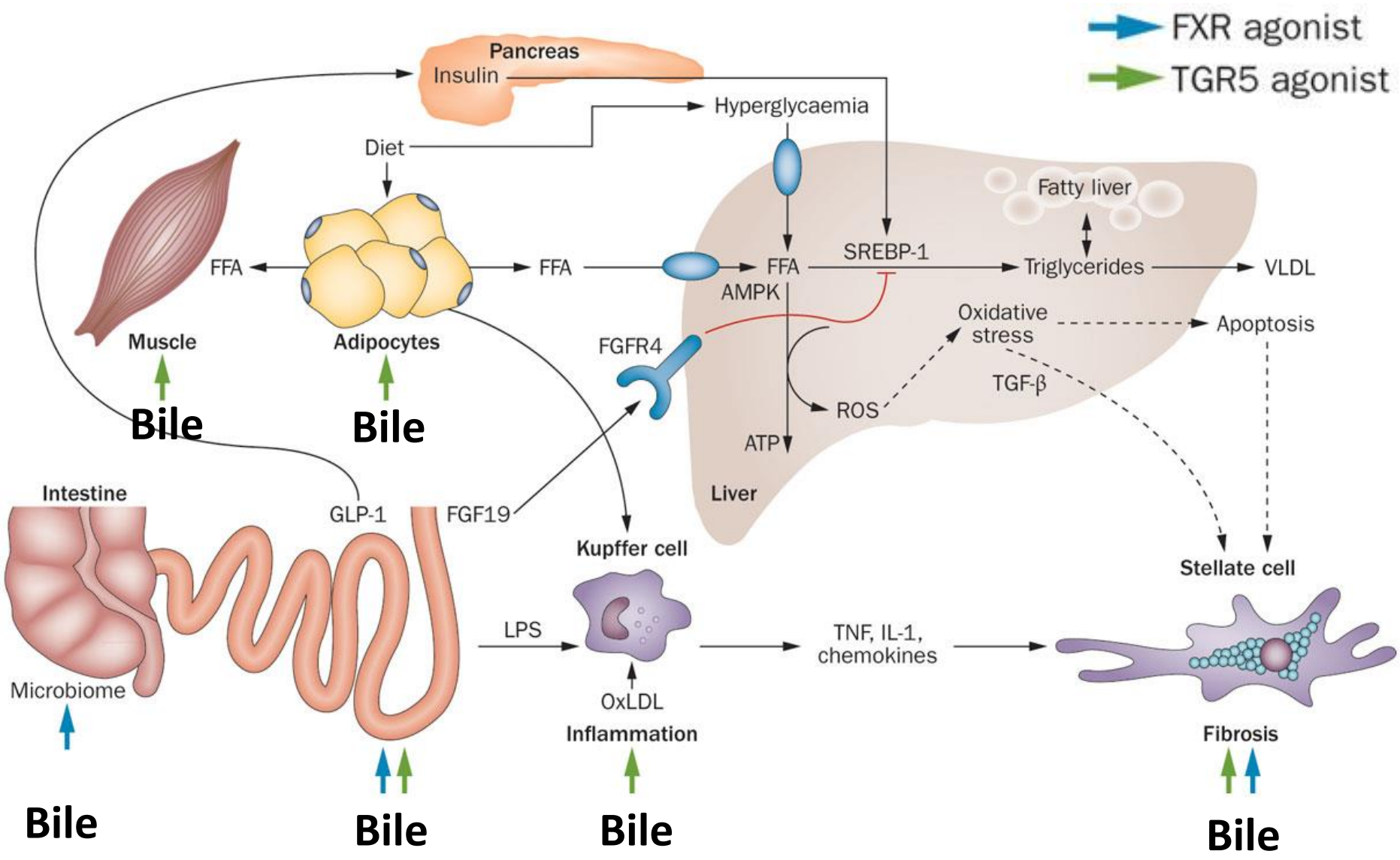



Bile acids

a



# Endocrine function: energy metabolism



# BSH function: a probiotic effector?

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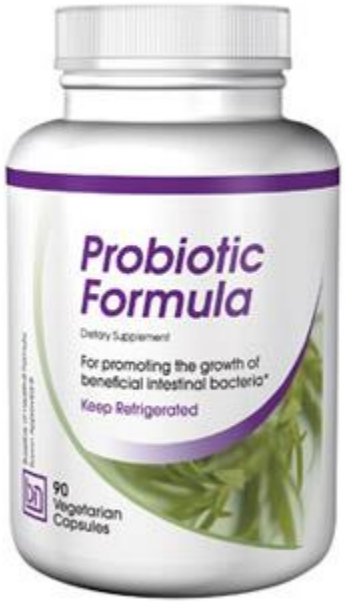


Probiotics: Potential to change this signalling landscape?

# Bacterial bile acid modifications in the gut:

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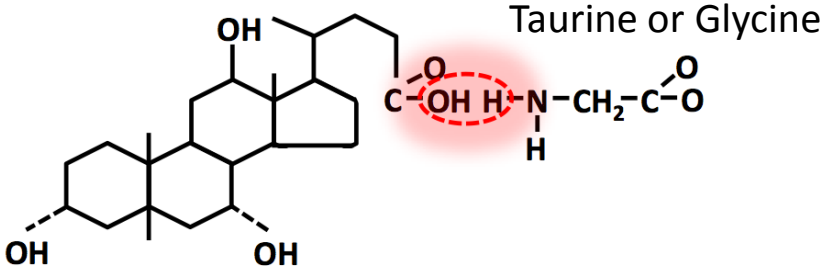
**Conjugated bile acids**

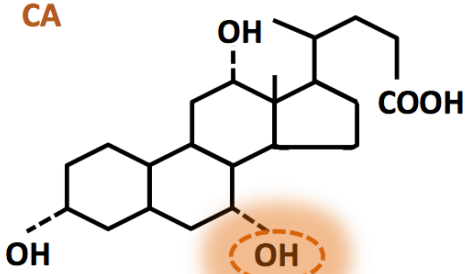
GCA
TCA
GCDCA
TCDCA



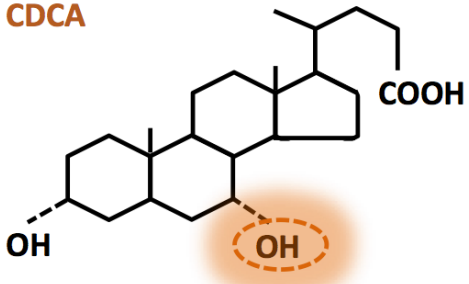

**Bile salt hydrolase**

**Free primary bile acids**

CA



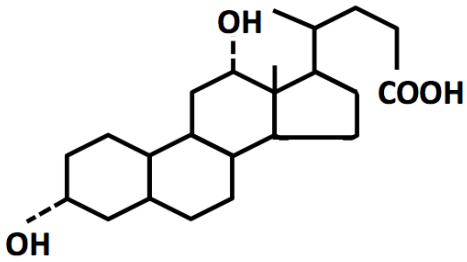

CDCA



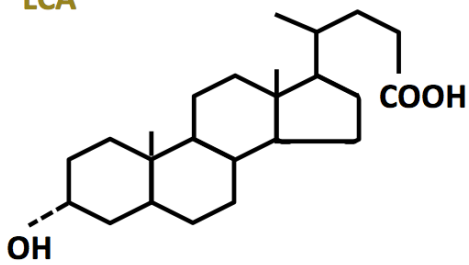

**7-α-dehydroxylase**

**Secondary bile acids**

DCA




LCA

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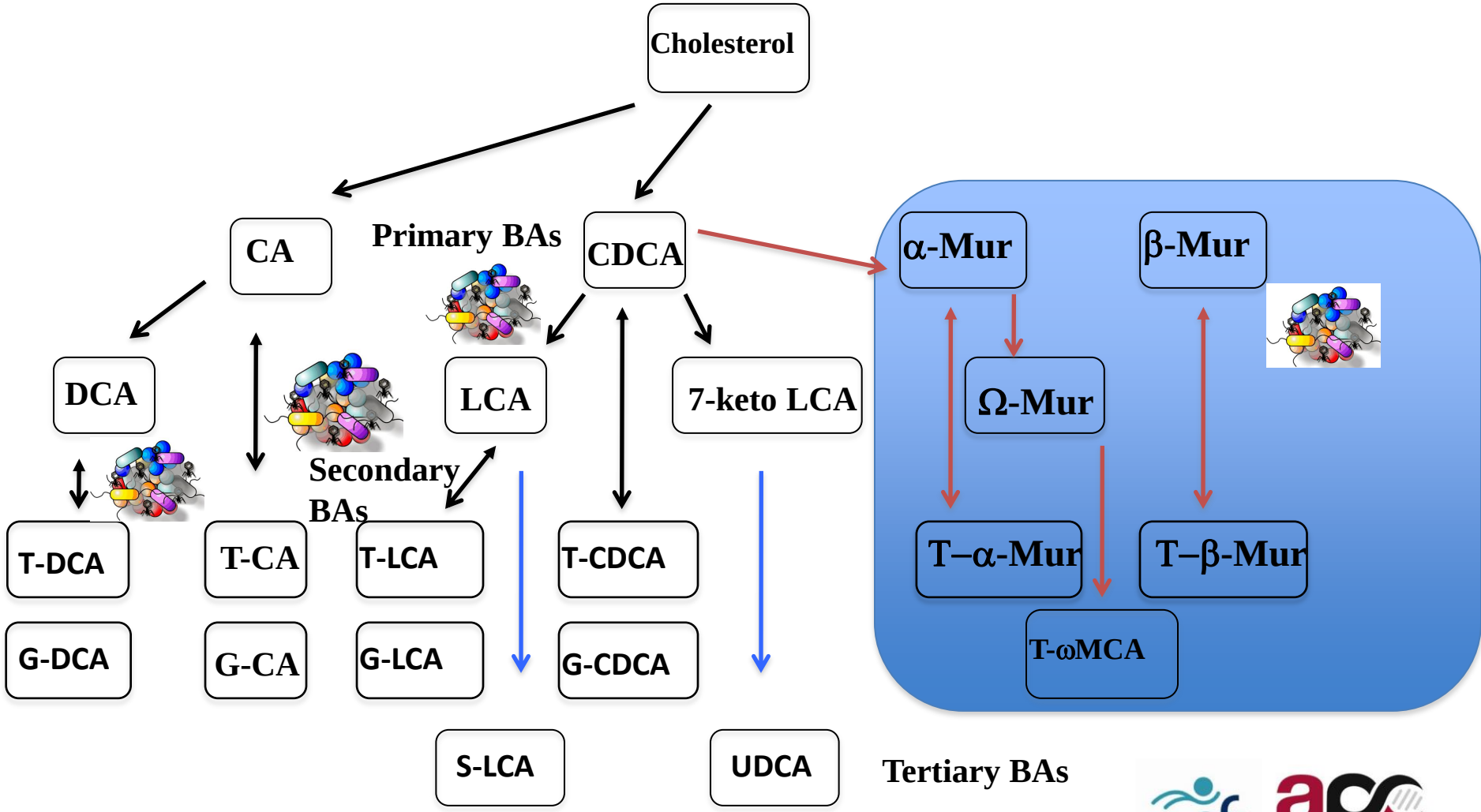
# Complexity:


Cholesterol
CA
Primary BAs
CDCA
DCA
LCA
7-keto LCA
Secondary BAs
T-DCA
T-CA
T-LCA
T-CDCA
G-DCA
G-CA
G-LCA
G-CDCA
S-LCA
UDCA
α-Mur
β-Mur
Ω-Mur
T–α-Mur
T–β-Mur
T-ωMCA
Tertiary BAs

# Bile Acids: *Wider potential significance*

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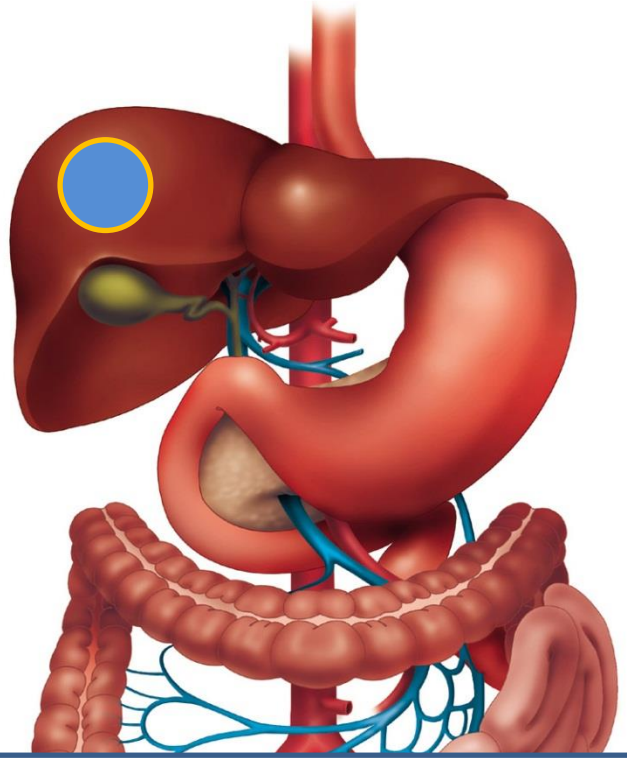
2. Reabsorbed bile salts as signalling molecules regulating endocrine functions (obesity)

Watanabe et al. 2006 Nature, 439: 484-489



Cell Metabolism

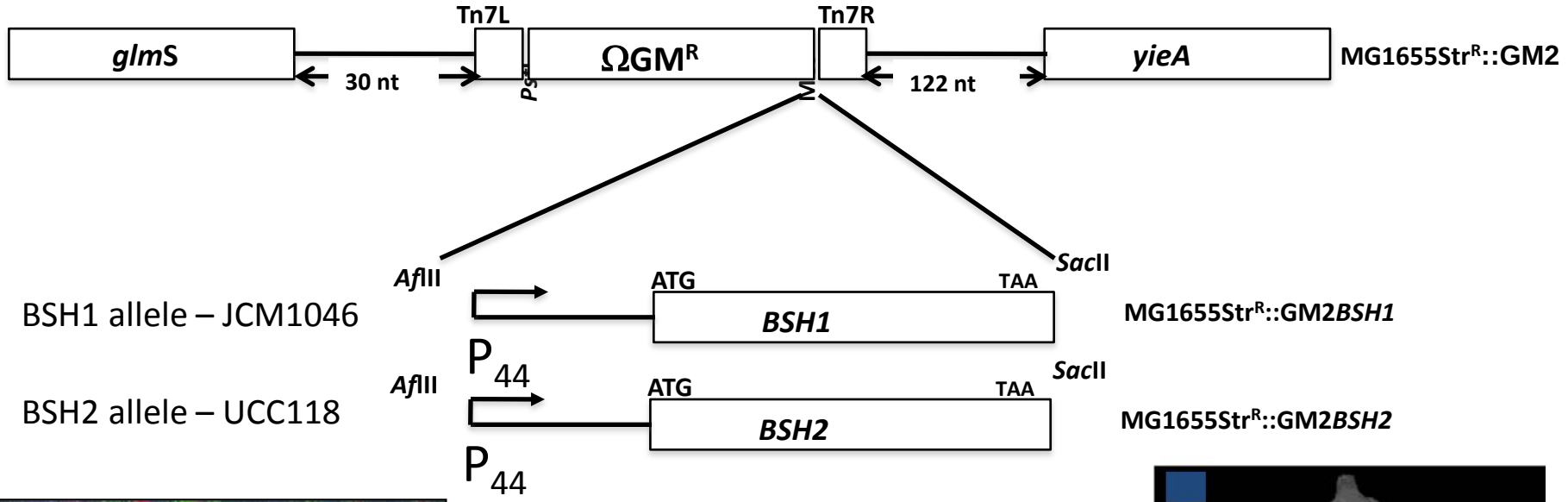
Article

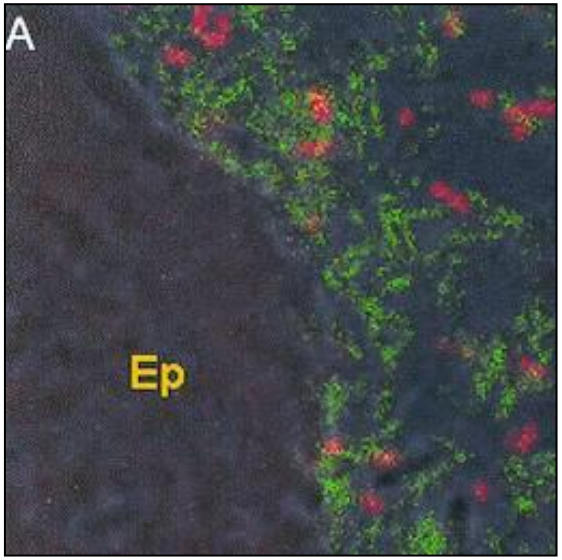
Cell PRESS

## Gut Microbiota Regulates Bile Acid Metabolism by Reducing the Levels of Tauro-beta-muricholic Acid, a Naturally Occurring FXR Antagonist

Sama I. Sayin,[1] Annika Wahlström,[1] Jenny Felin,[1] Sirkku Jäntti,[2] Hanns-Ulrich Marschall,[1] Krister Bamberg,[3] Bo Angelin,[4] Tuulia Hyötyläinen,[2] Matej Orešič,[2] and Fredrik Bäckhed[1,5,*]

# Cloning of BSH in *E. coli MG1655*:

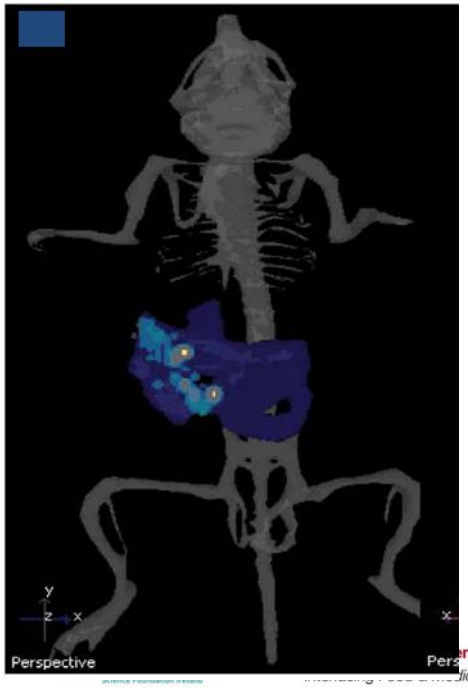



MG1655 (red)
Eubacteria (green)

Miranda *et al.* 2004. *Infect Immun* 72:1666-1676

Strep-treated mice

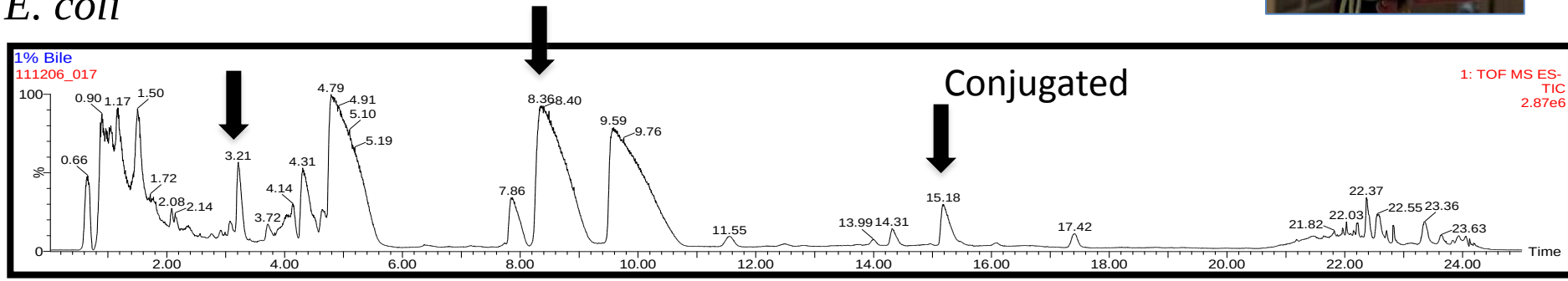


# *In vitro* human bile assays

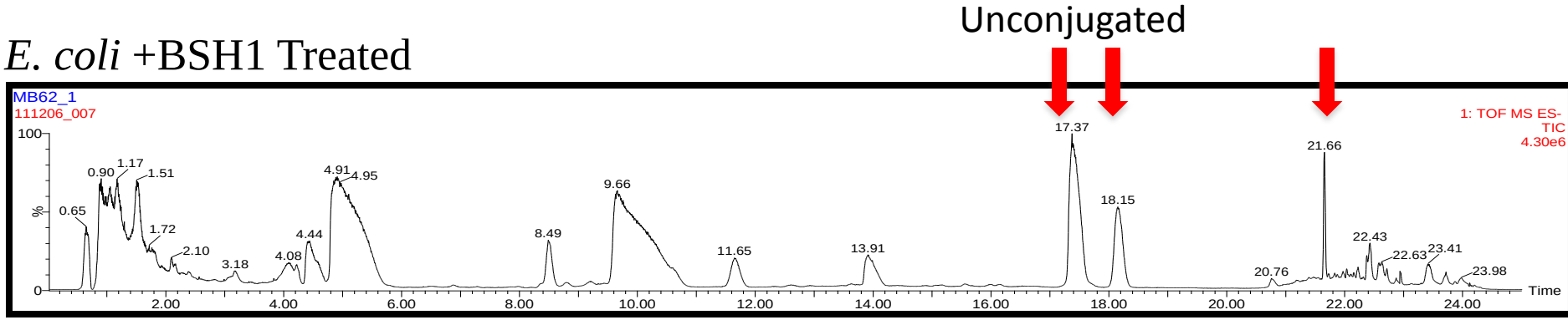
BSH in *E. coli* is working……

*ex vivo* bile acid incubation…..

*E. coli*




*E. coli* +BSH1 Treated

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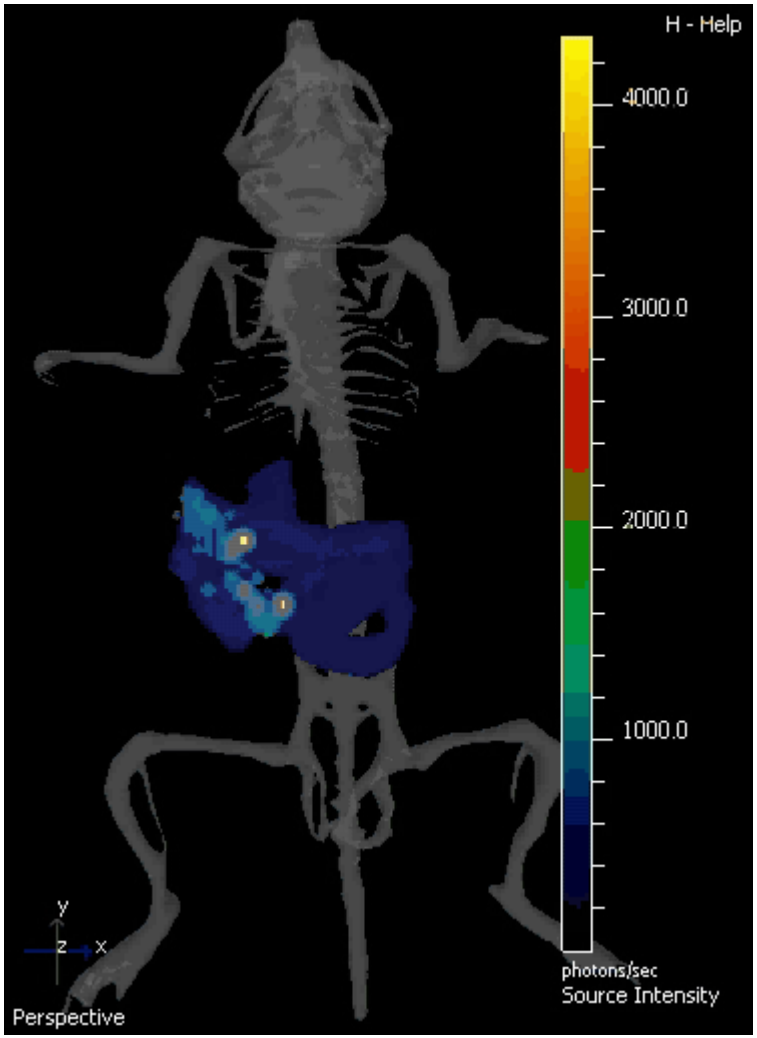
# *E. coli* strain colonises the mouse gut

Cronin M. et al. 2012. PLoS One. 2012;7(1):e30940.



# EC-BSH1 significant *in vivo* activity

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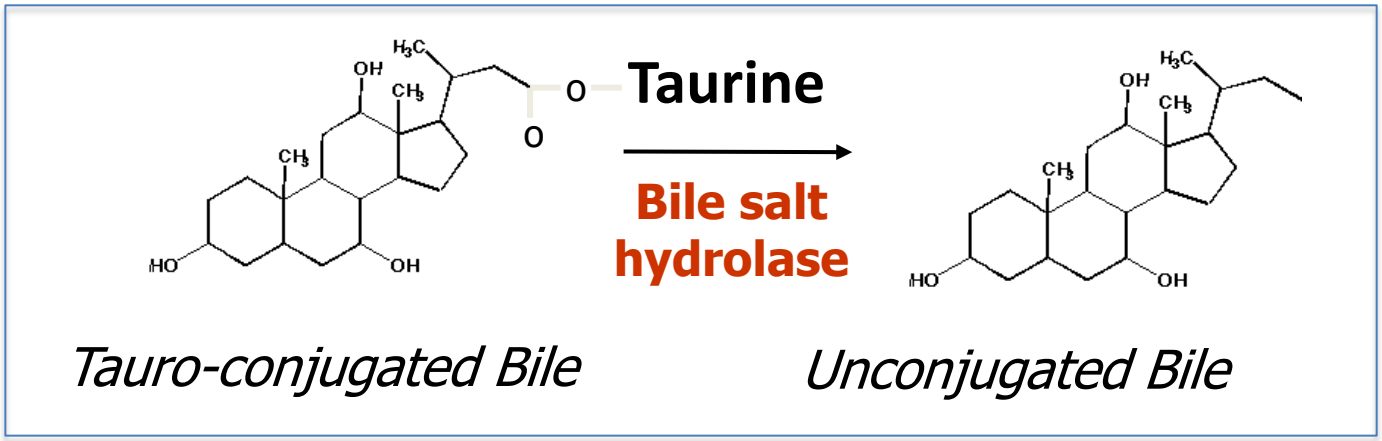
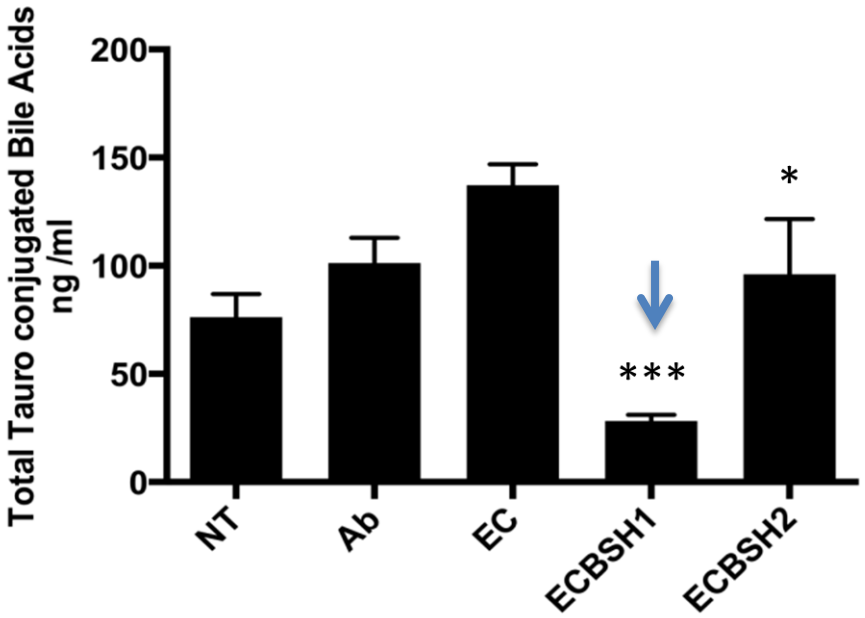
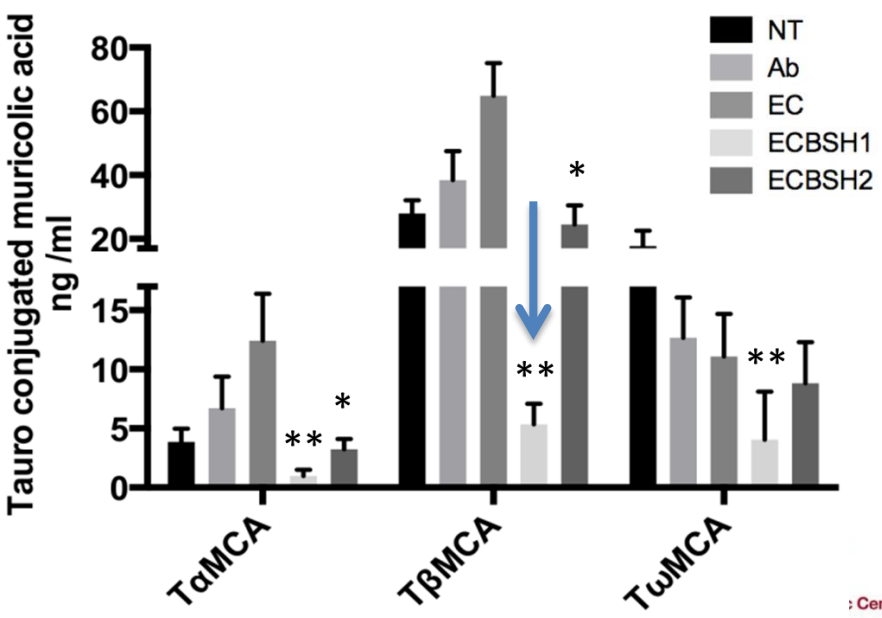


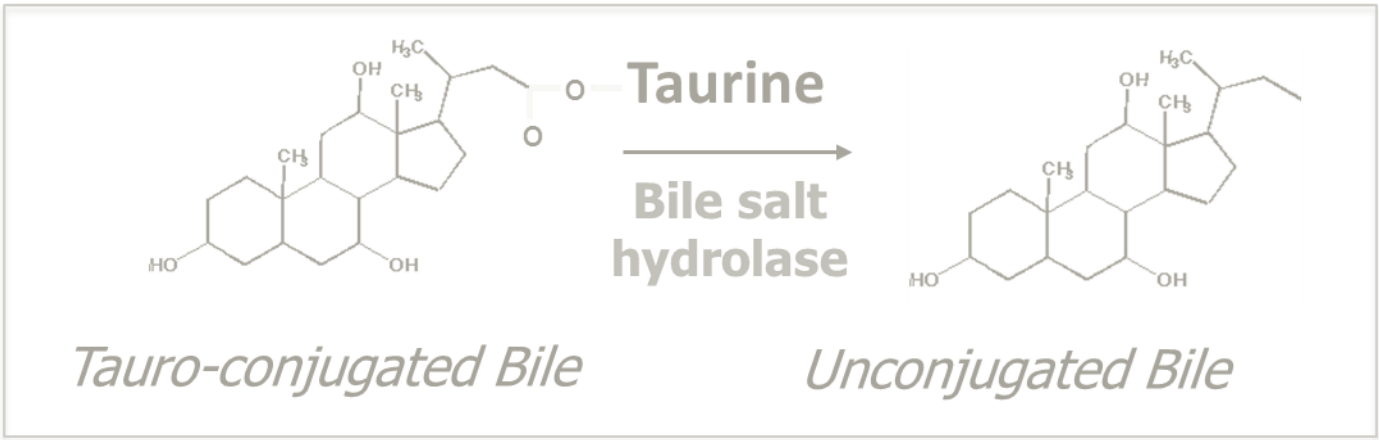

Total Plasma Tauro-Bile Acids



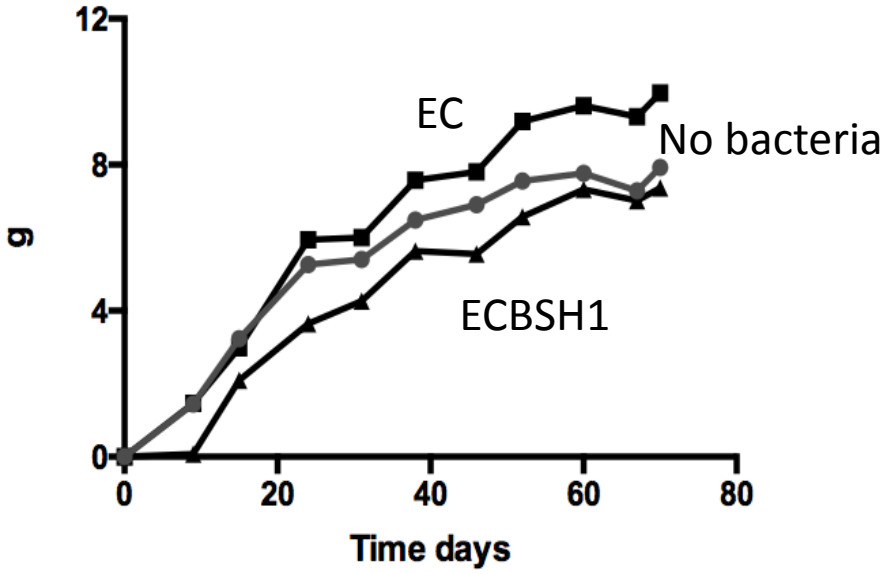

Reduction in TβMCA



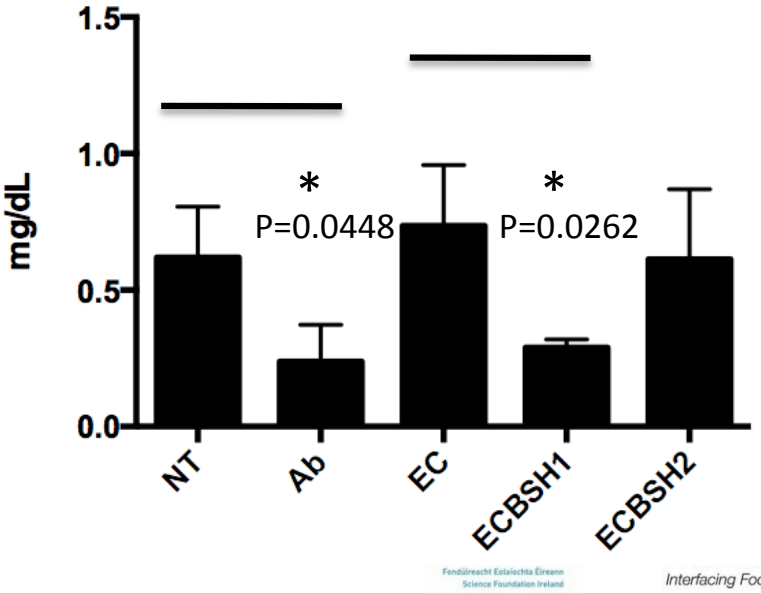
# EC-BSH1 reduced weight gain




## Weight Gain




## Cholesterol



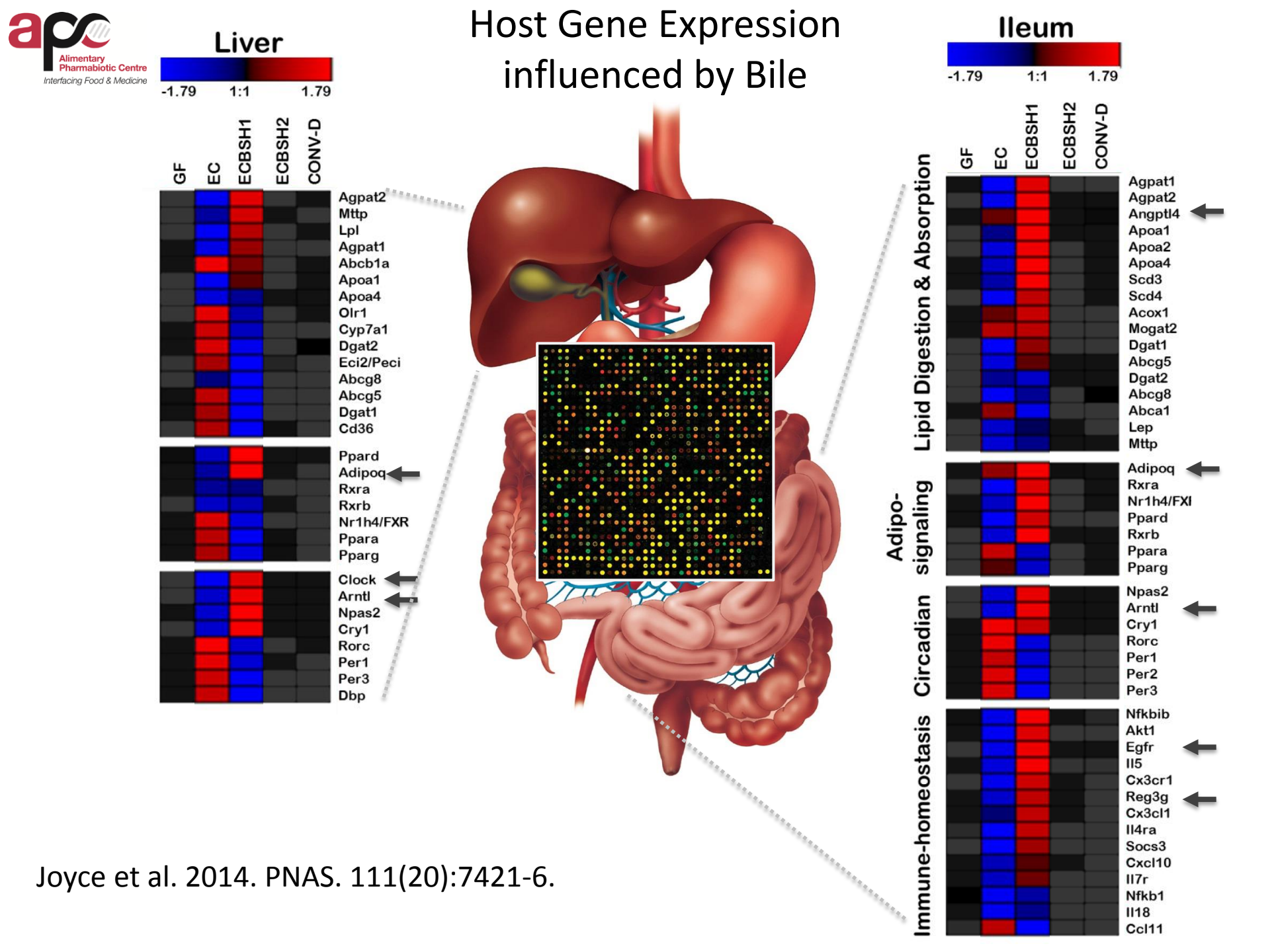
# Mono-colonize Gnotobiotic Mice with BSH+ or BSH-

## Mono-colonise Germ-free mice

all n=5

| Germ-free | EC | ECBSH1 | ECBSH2 | Conv-D |
|---|---|---|---|---|
|  |  |  |  |  |

# Host Gene Expression influenced by Bile




Joyce et al. 2014. PNAS. 111(20):7421-6.

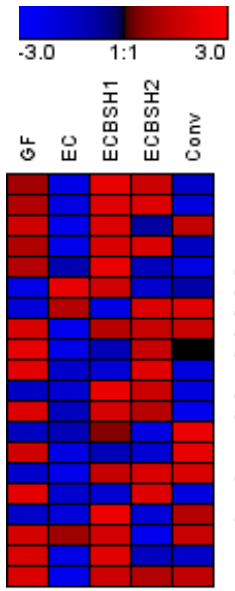
# Role in Reduced Cholesterol & Weight

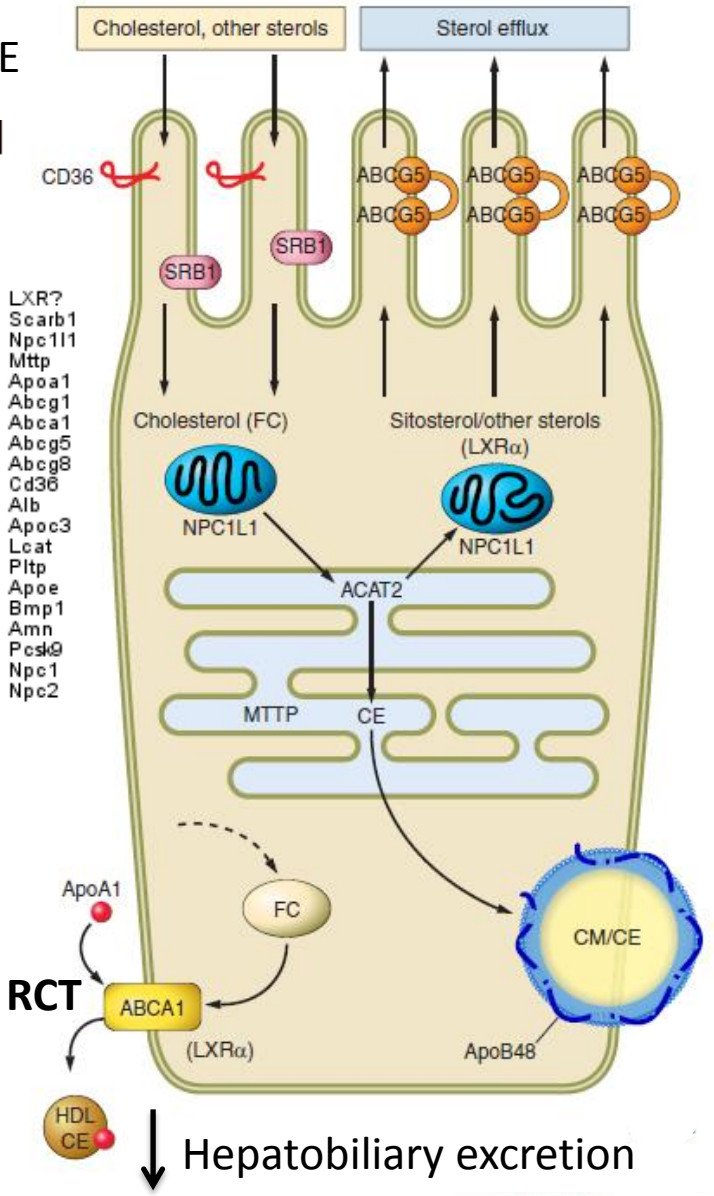
*Targets in Conventional Mice*

## BSH:

- Trans-intestinal cholesterol excretion (TICE)
- Reverse Cholesterol transport (RCT)
- Reduces lipid biosynthesis

RCT and TICE




**TICE**

# DCA and CDCA induce lipid signalling genes in Caco2 cells

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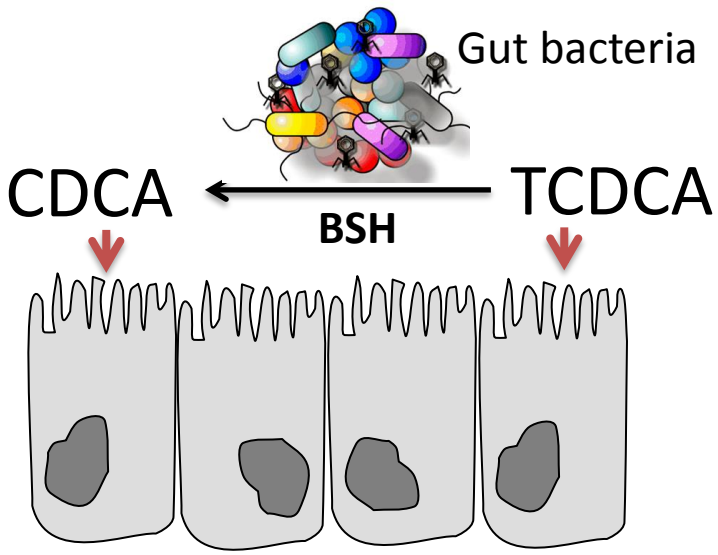
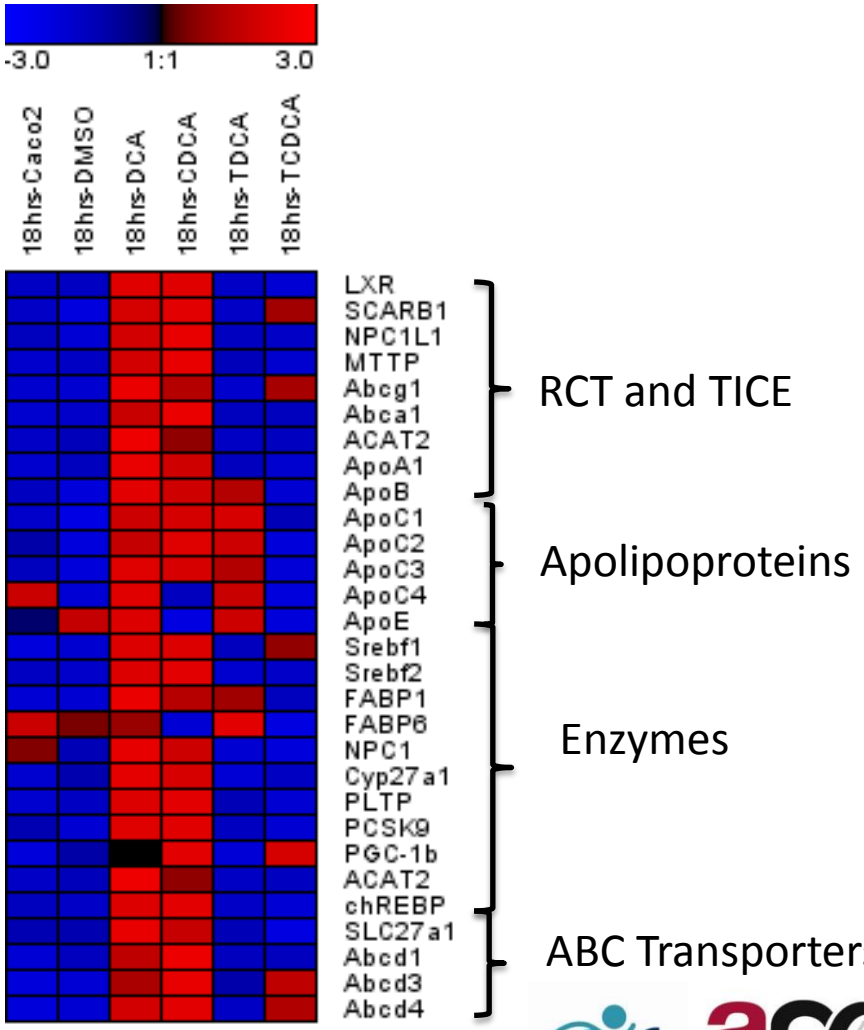
## RT-PCR




Currently examining functional lipid transport using a transwell system

## 18 hours

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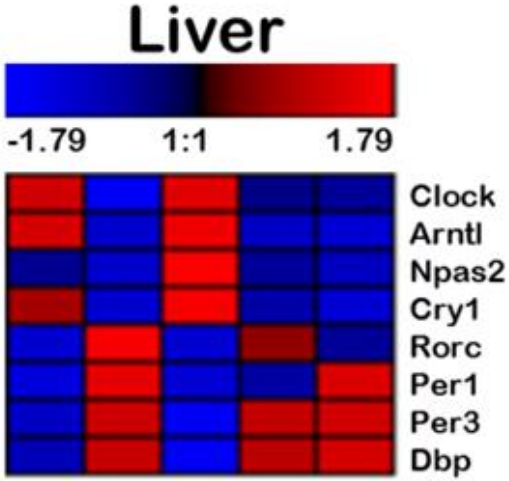
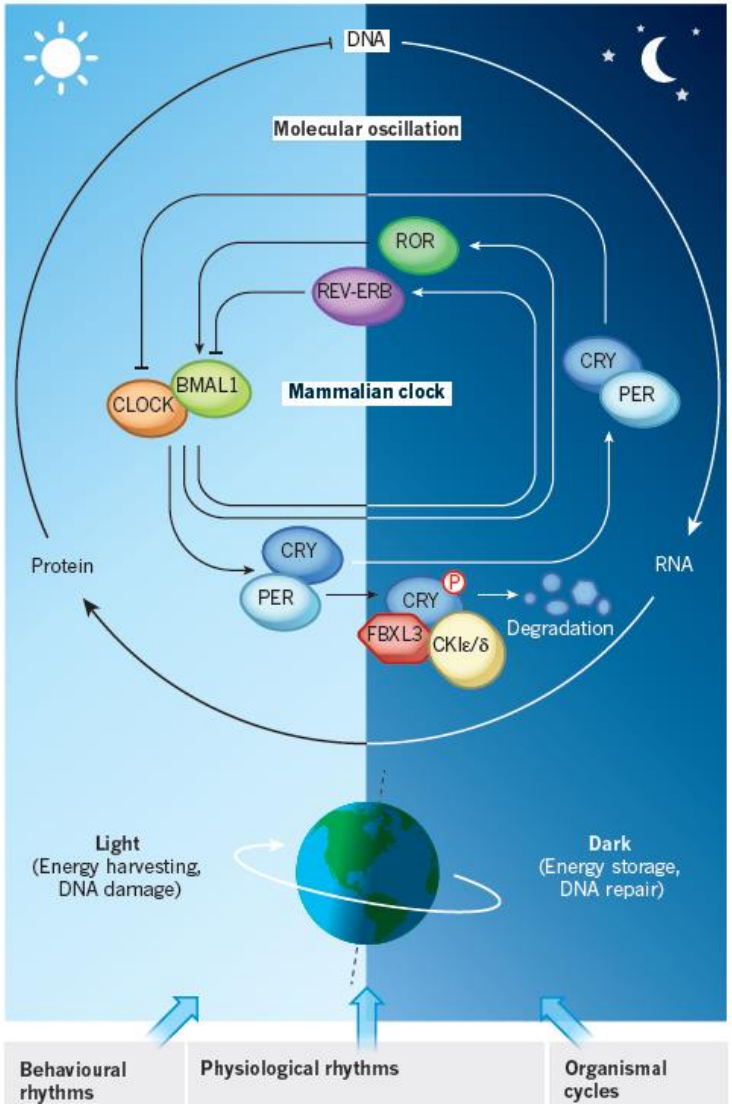
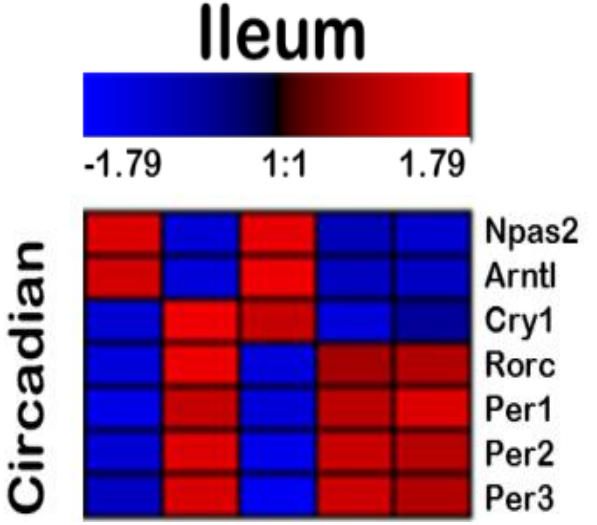
# Circadian Genes Switch by BSH1

*in mice*




**EC BSH1**




**EC BSH1**

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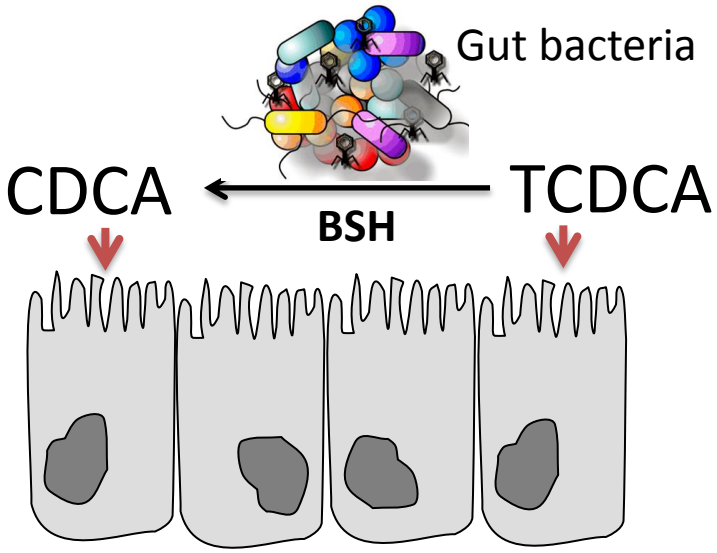
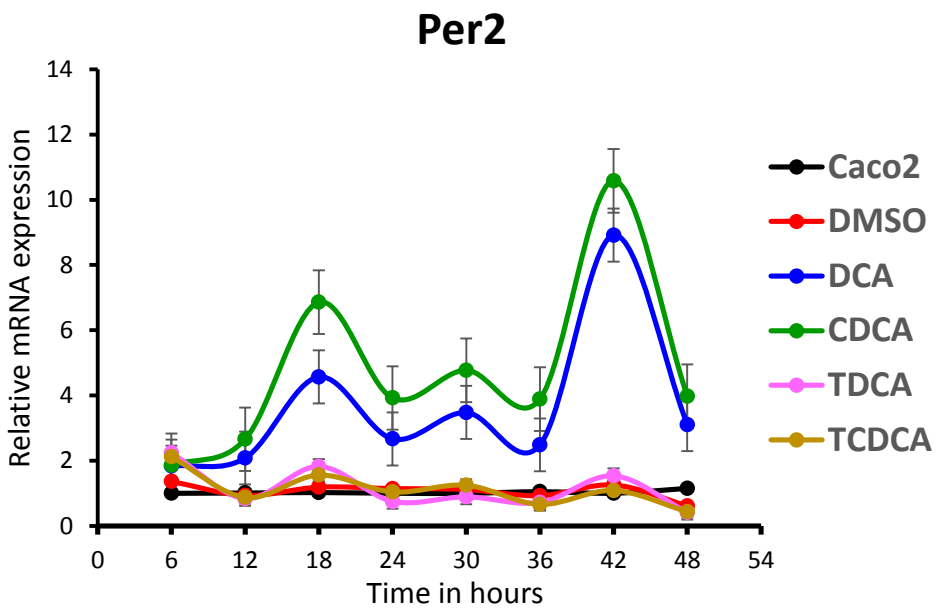
# CACO-2 Circadian Genes Influenced *in vitro*

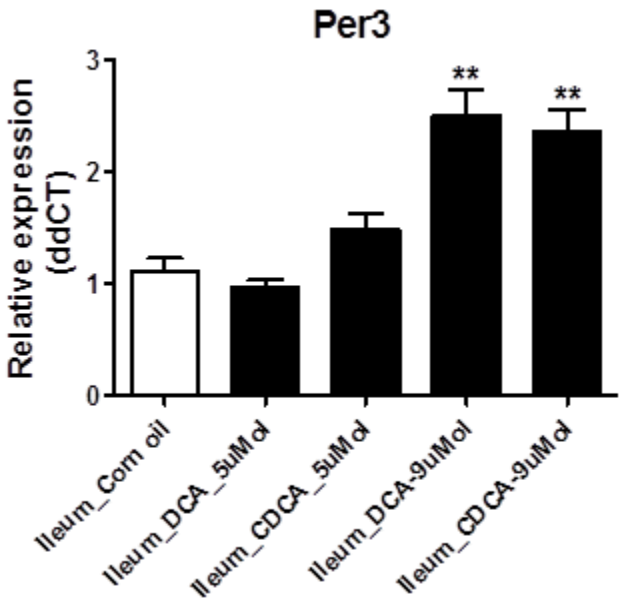


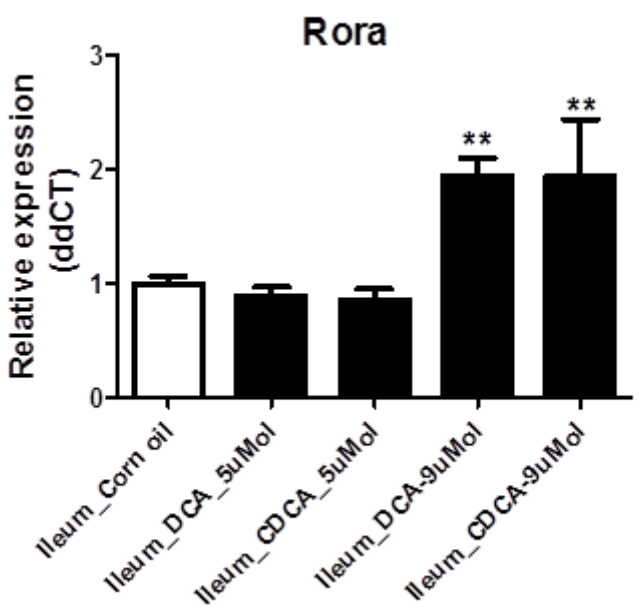




Unconjugated bile acids enhance periodic expression of circadian genes *in vitro*

*Dr. Kalai Govindarajan*

# Oral gavage alters ileal circadian gene expression



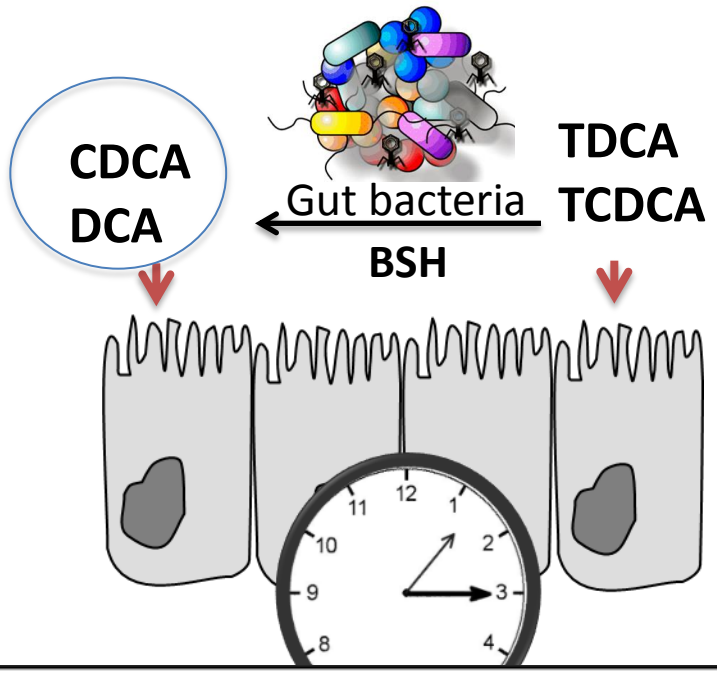



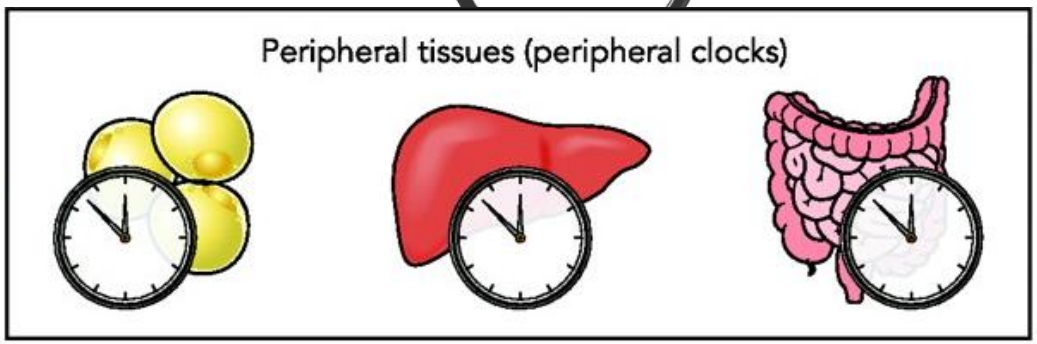

Unconjugated bile acids enhance periodic expression of circadian genes *in vivo*

*Dr. Kalai Govindarajan*

# Hypothesis: BSH activity alters peripheral circadian clock

- Well known that bile synthesis is governed by circadian rhythm
- We show that bacterial activity alters the signalling potential of bile with an influence upon peripheral rhythms
- Potential for probiotic intervention?
- Thaiss CA et al 2014. Cell. Diurnal oscillations in gut bacteria!



# BSH role in local homeostasis?

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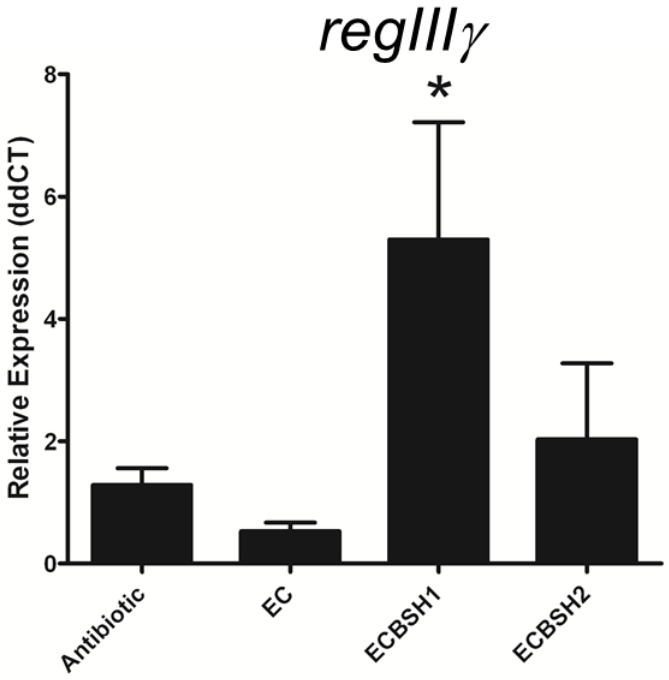
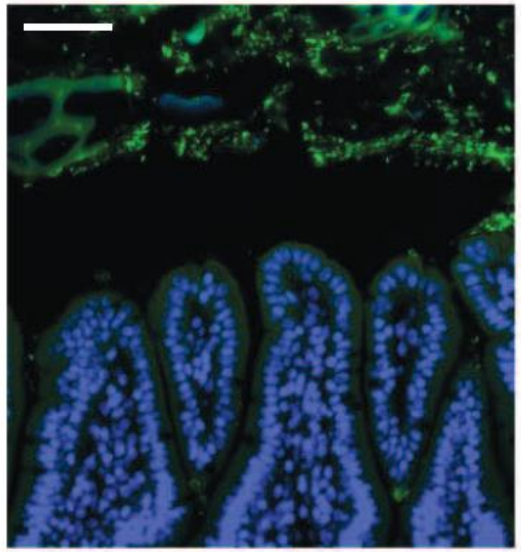
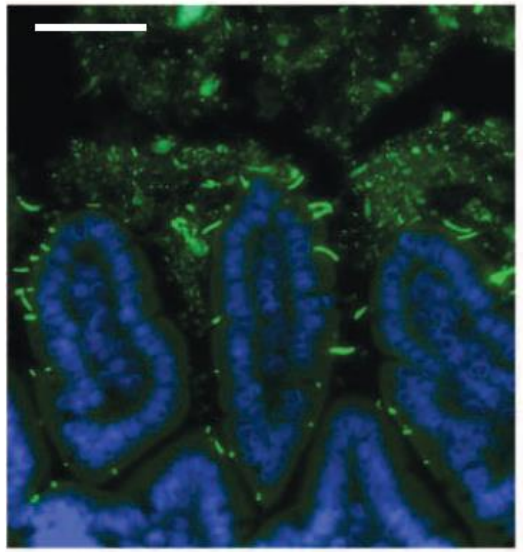
Conventionally-raised mice




wild-type



RegIIIγ$^{-/-}$



**The Antibacterial Lectin RegIIIγ Promotes the Spatial Segregation of Microbiota and Host in the Intestine**

Shipra Vaishnava,[1] Miwako Yamamoto,[1] Kari M. Severson,[1] Kelly A. Ruhn,[1] Xiaofei Yu,[1] Omry Koren,[3] Ruth Ley,[3] Edward K. Wakeland,[1] Lora V. Hooper[1,2]*

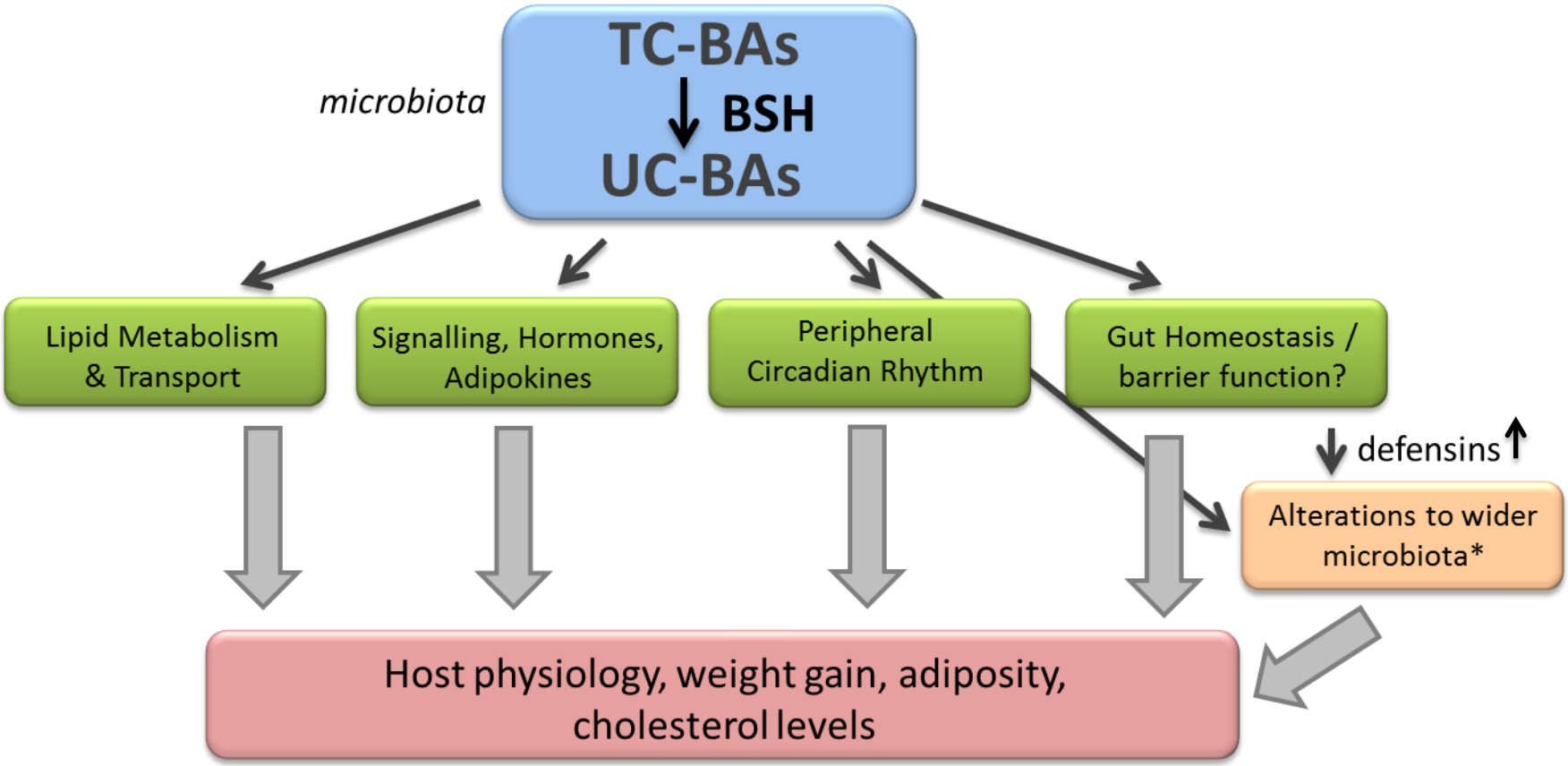
SCIENCE VOL 334 14 OCTOBER 2011

# Conclusions

- BSH important for bacterial colonisation (mutualism?)
- Expression of BSH (single gene/function) in the gut significantly influences local and systemic responses
- Allelic variation was significant (BSH1 versus BSH2)
- Expression of BSH1 influences body weight and serum cholesterol - possible pathways identified

Joyce et al. 2014. PNAS. 111(20):7421-6.

# Bile conversions: Multiple Effects Upon the Host




Joyce et al. 2014. PNAS. 111(20):7421-6.

# Functional Annotation of the Microbiome

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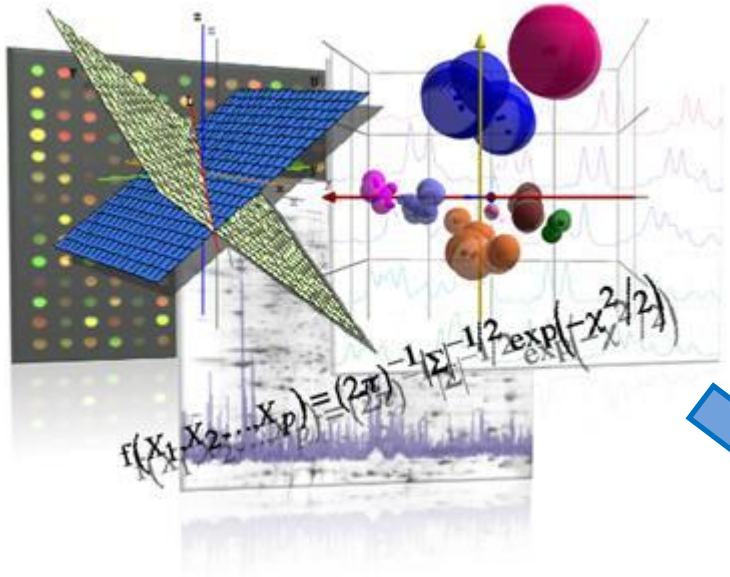
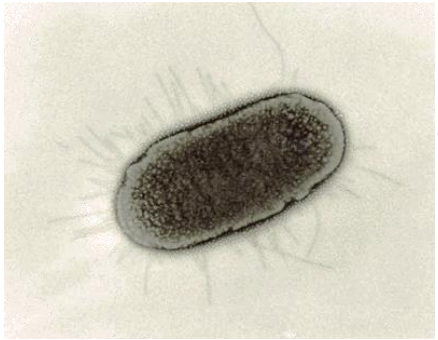
Systems Approach: What's there?



**Culture-independent (sequencing, proteomics)**

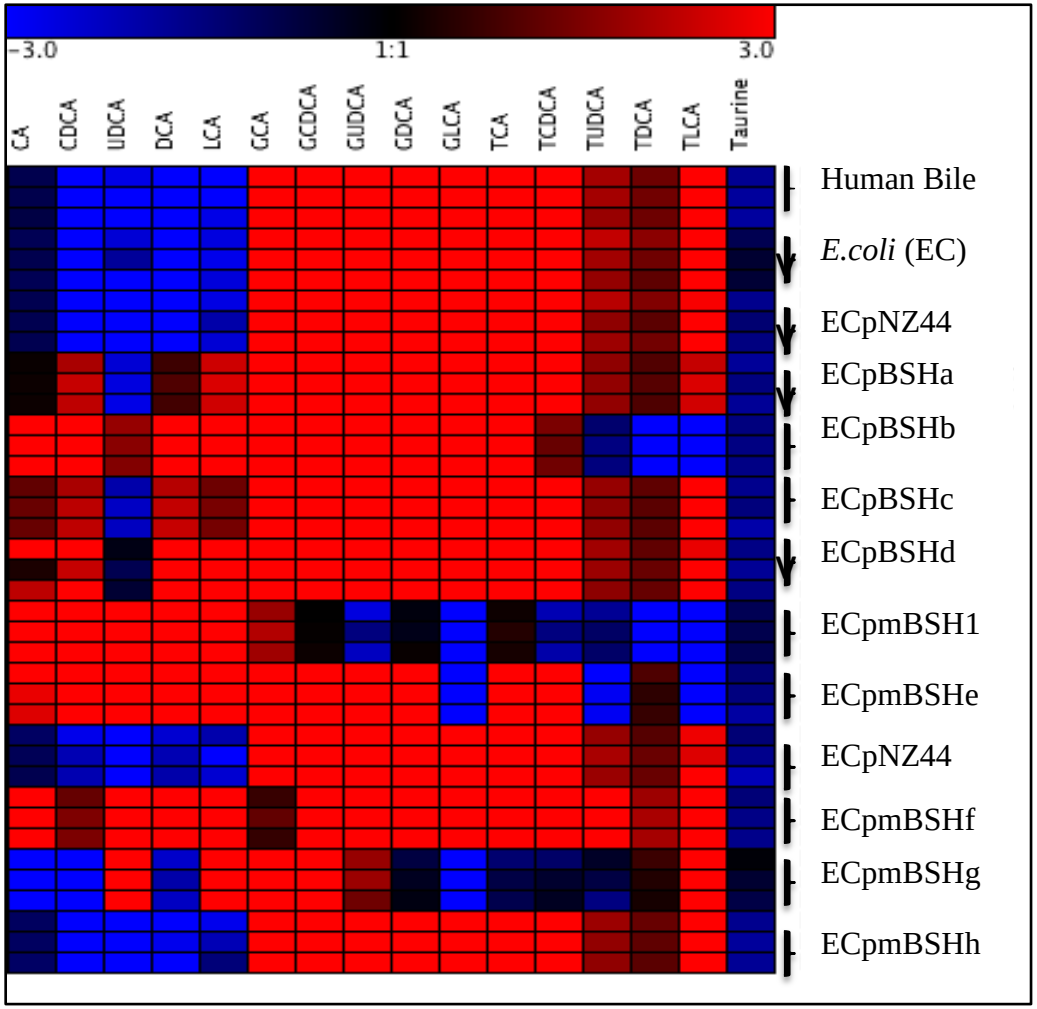
Reductionist: What are they doing?



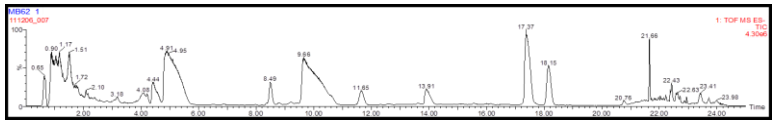
Single protein effects on system

**Single organism / protein**

# Future? Selecting Probiotics




UPLC-MS analysis of bile conversions



Profiling cloned BSHs and whole probiotics (left)

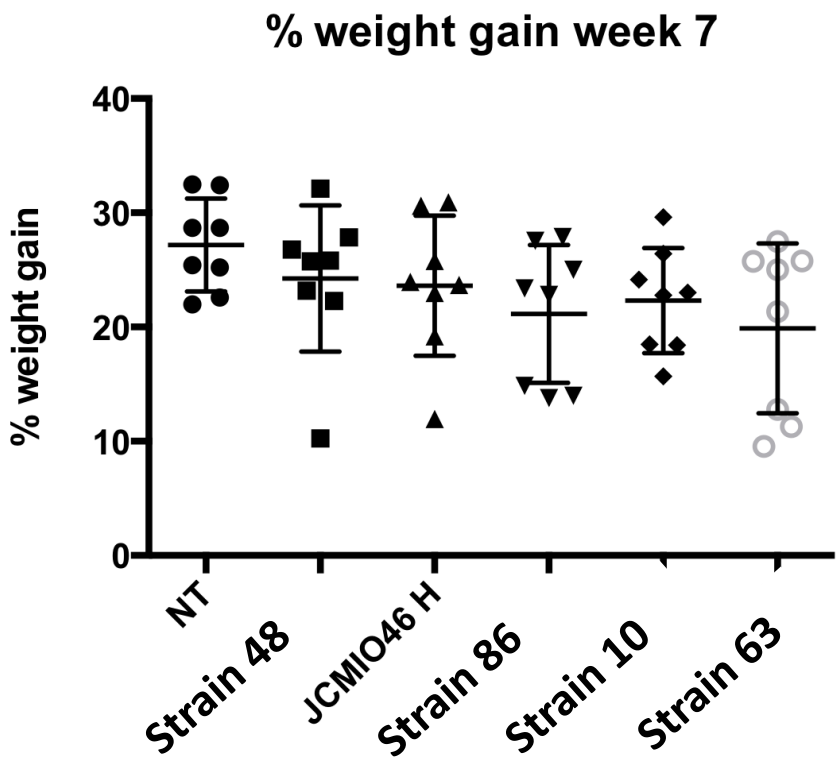
Some probiotics have BSH but no demonstrable activity

*Sarah Louise Long*

# Correlate probiotic BSH activity with effects on adiposity?

Probiotics selected on the basis of BSH activity show a trend towards inducing weight loss in mice

Currently testing in a diet-induced obesity model



# On-going work:

- Mechanisms/pathways/circadian rhythm
- Relating BSH structure to function & selection of probiotics
- Analysis of bile acid metabolism in human disease states

# Gut Microbes Addendum

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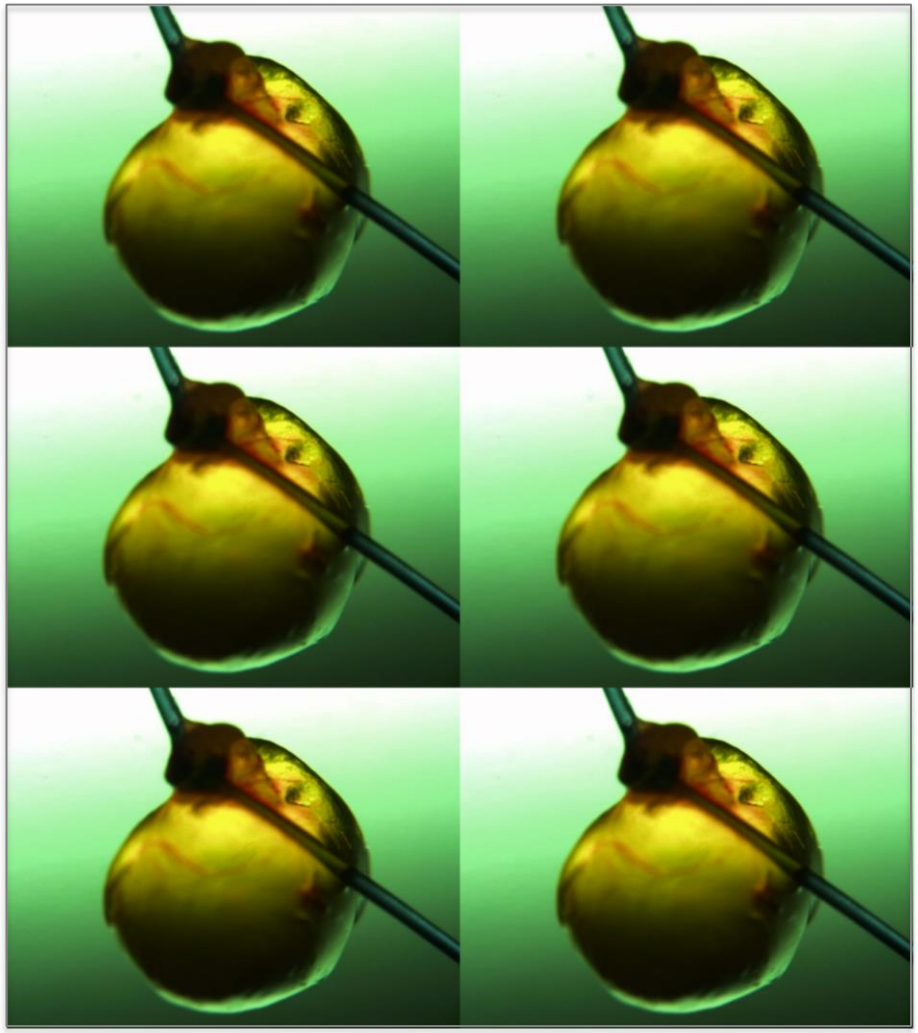
‘bacterial bile salt hydrolase in host metabolism…..’

*Original Photo:* Dr. Debbie Watson

# Acknowledgements

Alimentary Pharmabiotic Centre

http://apc.ucc.ie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Sarah-Louise Long

Dr. Susan Joyce

Prof. Colin Hill

Prof. Fergus Shanahan

Dr. Julian Marchesi

Dr. Brian Jones

Dr. Paul Cotter

Frances O'Brien

& Staff of BSU

Dr. Carthage Moran

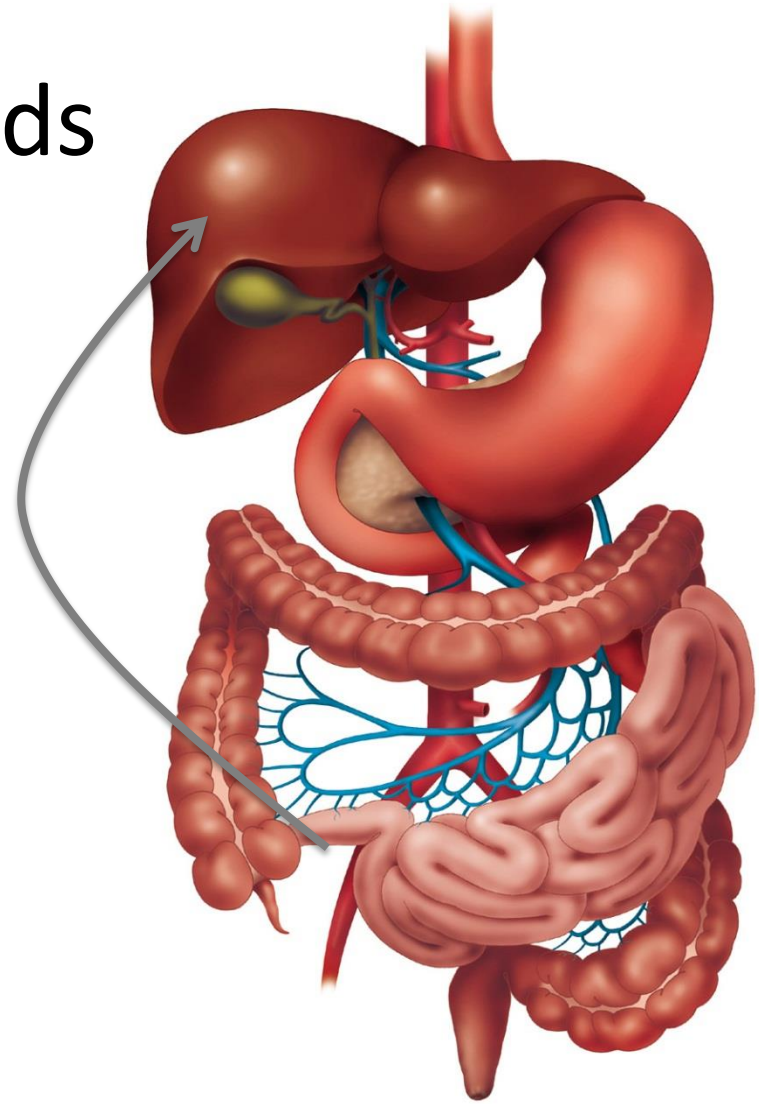
Dr. Marcus Claesson

sfi Science Foundation Ireland

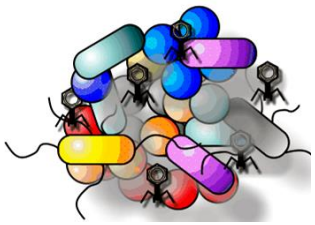
HRB Health Research Board

# Take home message

& Bile acids act here



Intervention?



Bile acids act here

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# Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance

NATURE MEDICINE

Sungsoon Fang[1] et al. 2015

# UPLC-MS

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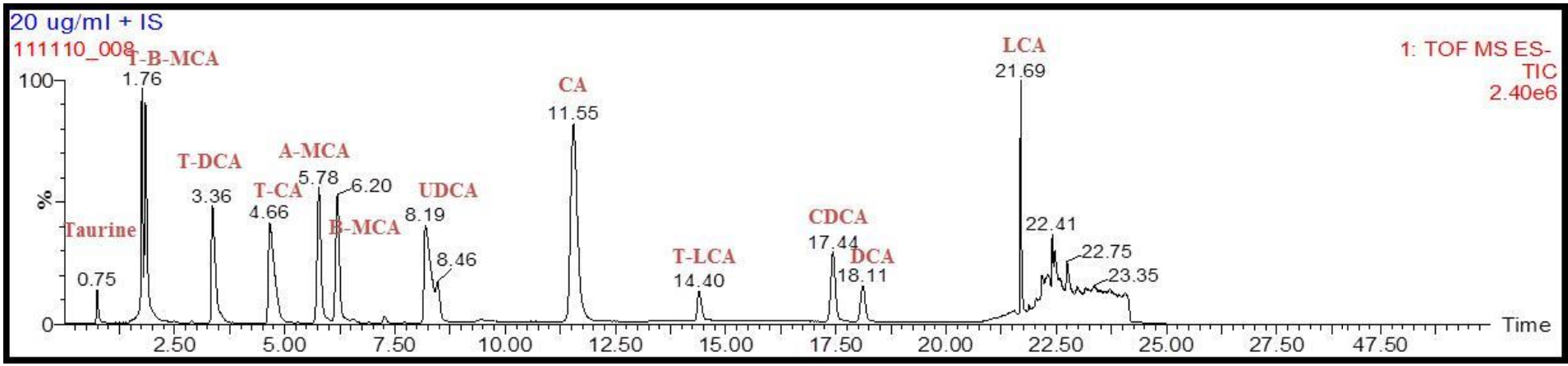
## UPLC-MS Approach to detect 30 Bile Acids

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# Intervention Alters Plasma Bile Acid Profiles in Mono-colonised Mice:

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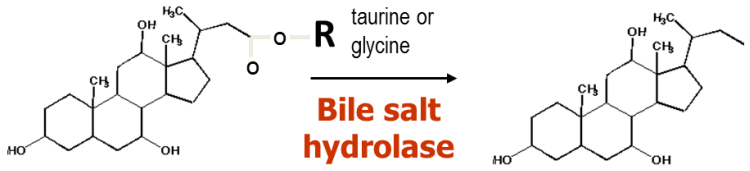
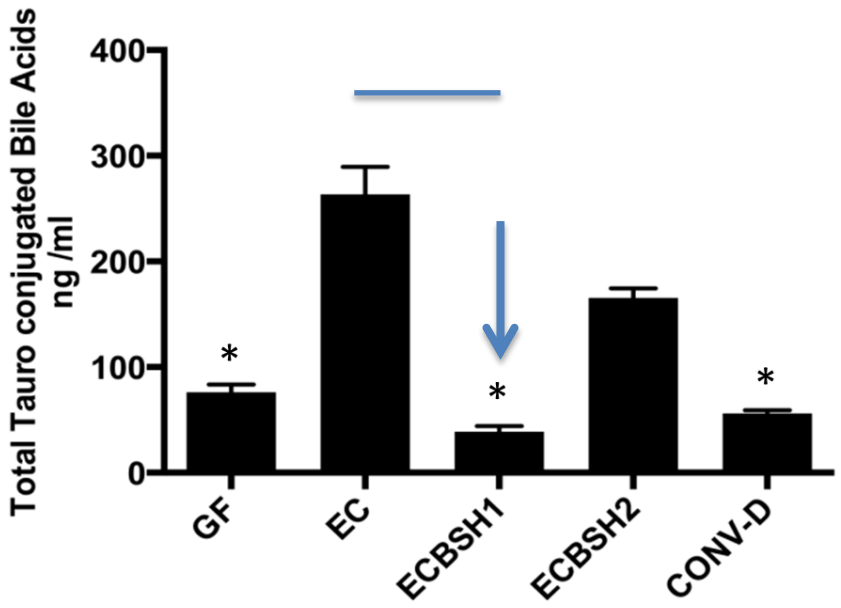
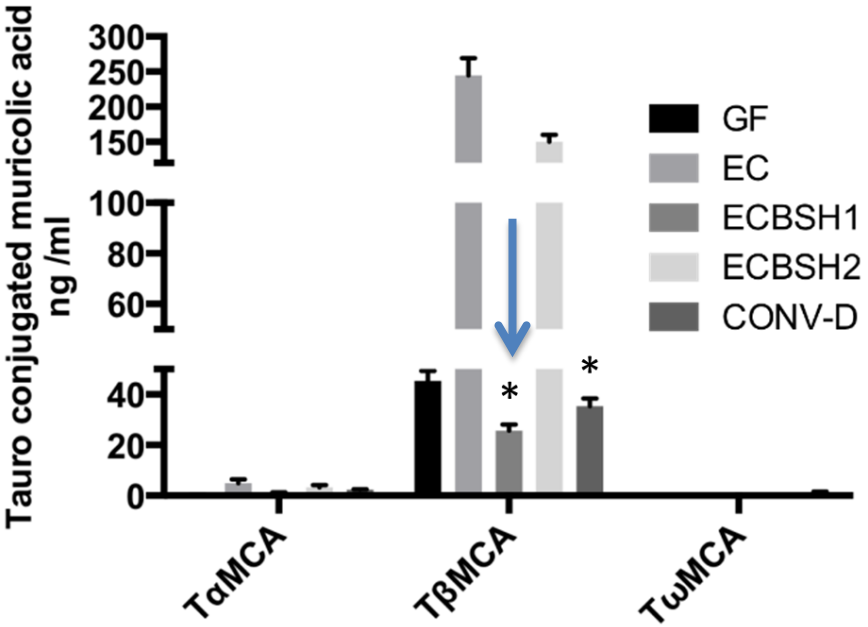
## Total Tauro-Conjugated Plasma Bile Acids




*Conjugated Bile acid* *Unconjugated Bile acid*




## Reduction in TβMCA



# Intestinal Bacteria

*microbiota*

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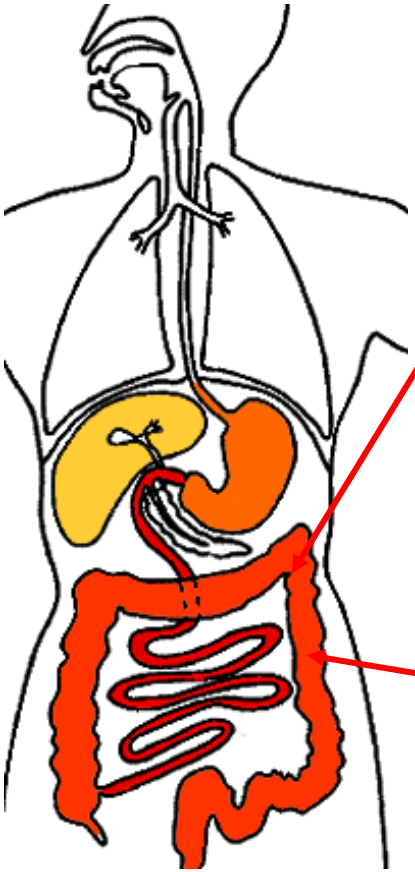
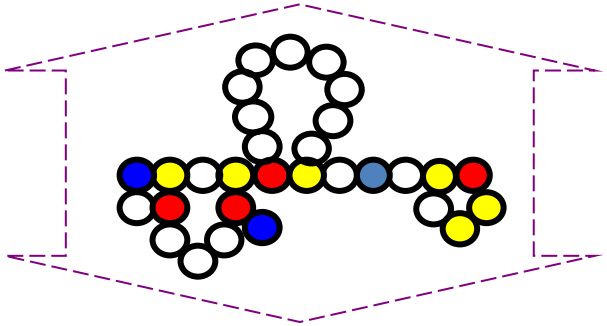
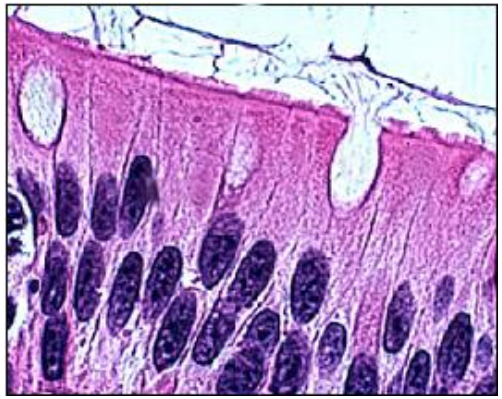
## …a hidden organ







- More bacteria ($10^{14}$) than human cells ($10^{13}$) in the body
- Approx 2000 bacterial phylotypes in human intestine
- Significant metabolic activity

sfi Fondúireacht Eolaíochta Éireann Science Foundation Ireland

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# The Human Superorganism

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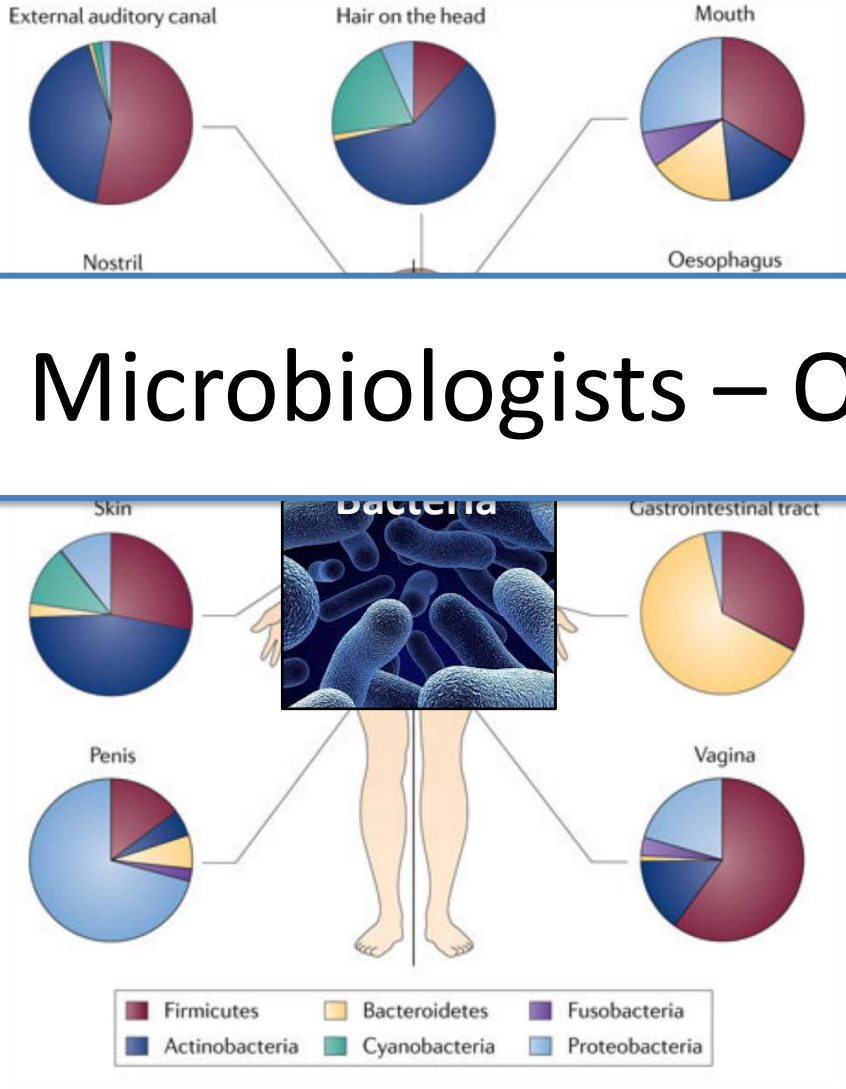
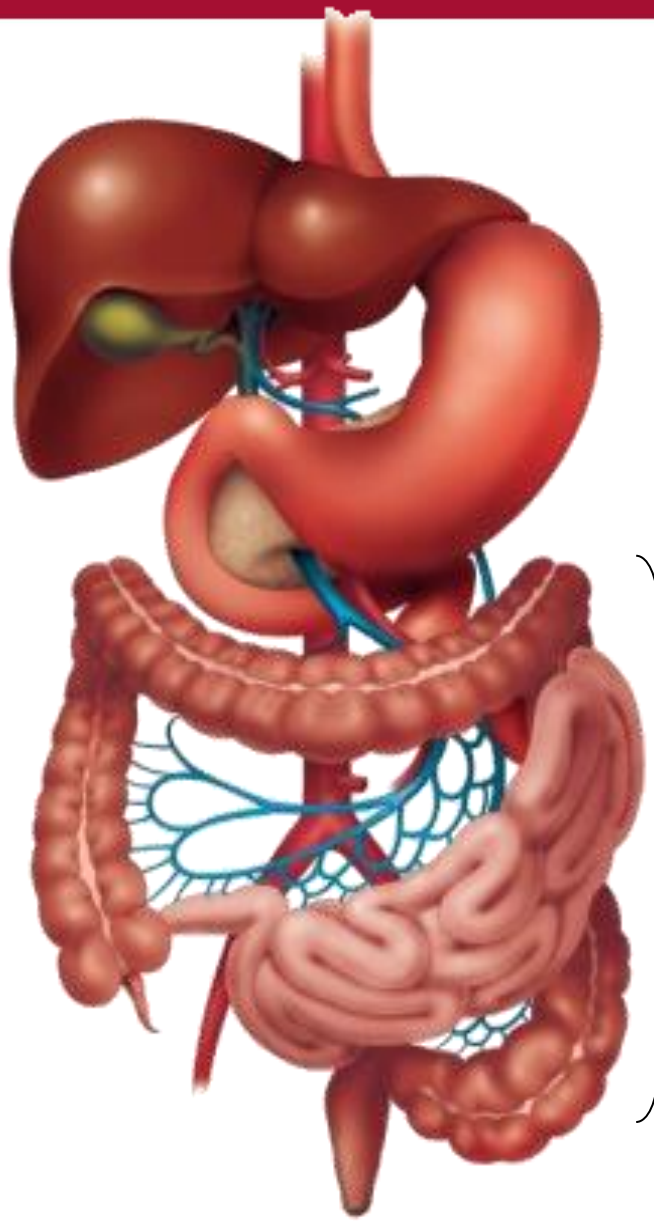
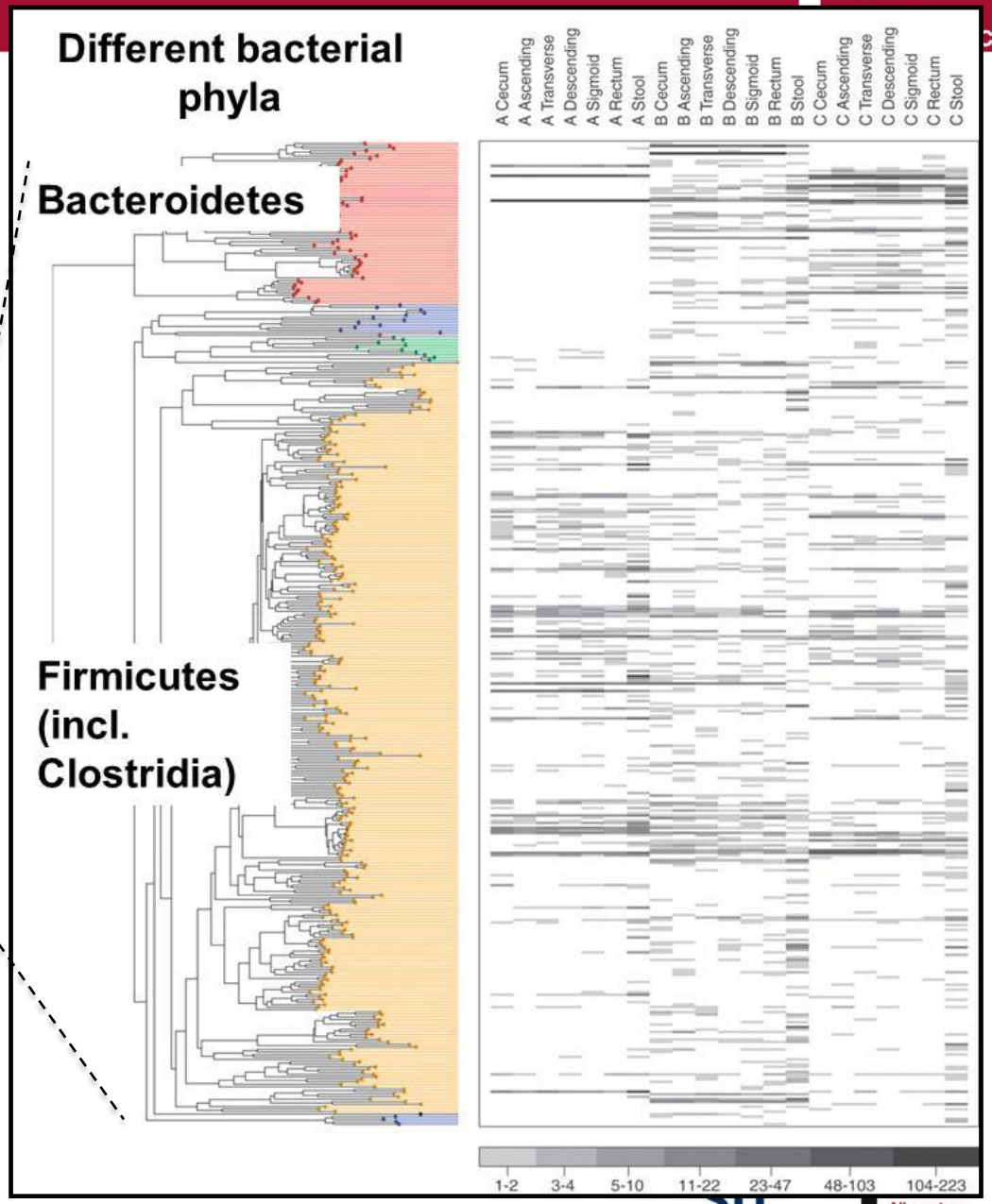



## Microbiologists – Our Time Has Come !

- Metabolism > Liver
- Energy extraction – fat deposition
- Immune stimulation
- Barrier to infection

# *Early study*

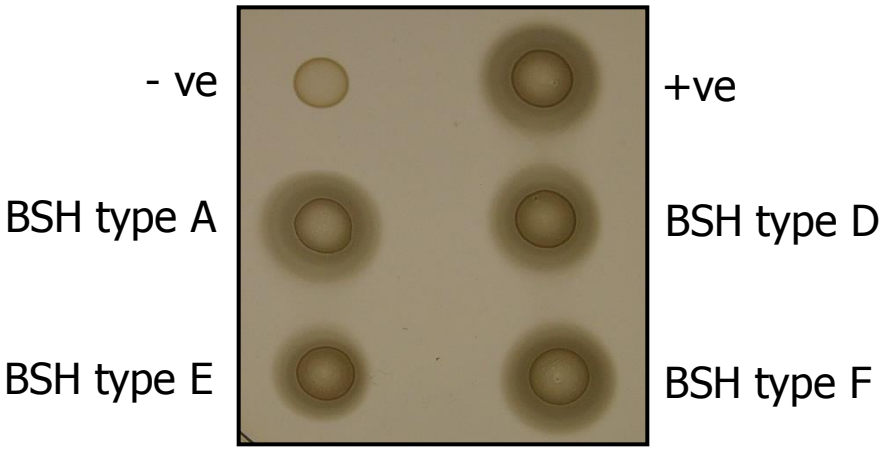




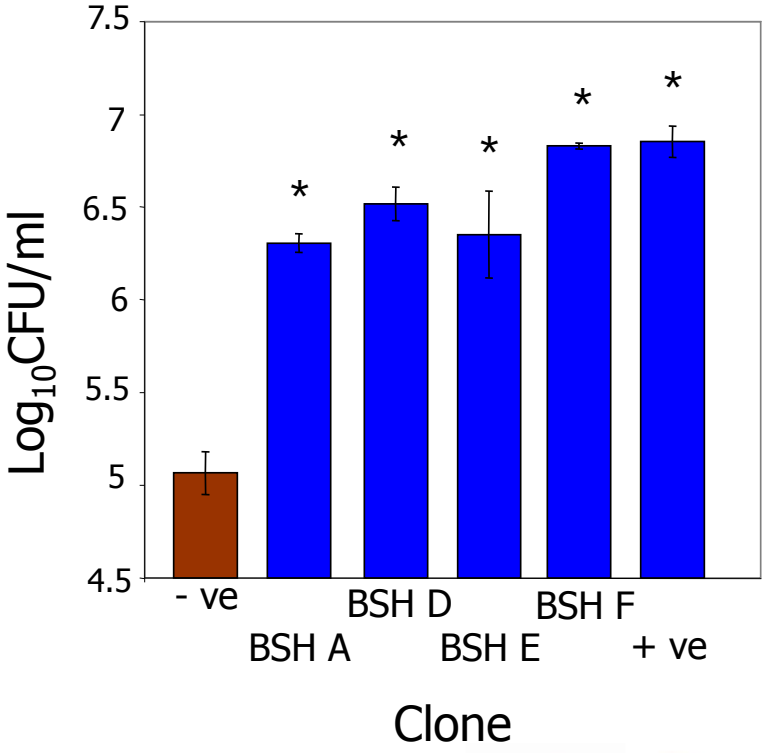

Eckburg PB. *et al.* 2005. Diversity of the human intestinal microbial flora. *Science* 308:1635-1638.

# BSH enhances microbial bile resistance

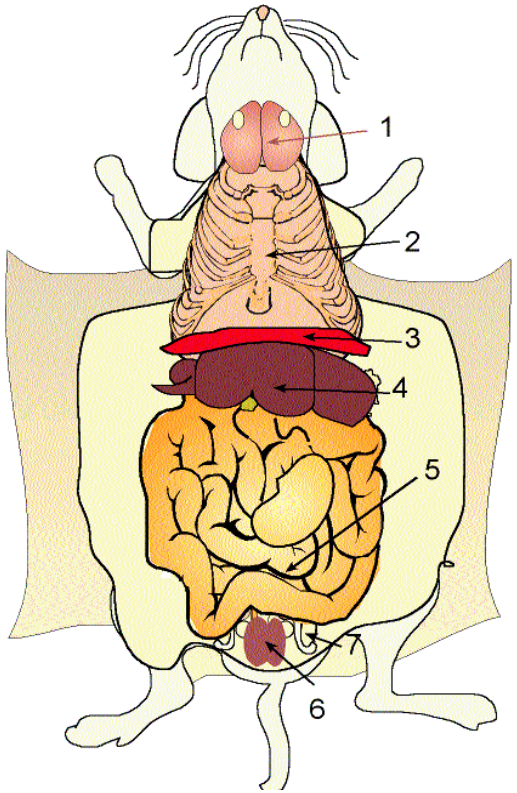
Cloning and expression of gut BSH enzymes in *Listeria innocua:*



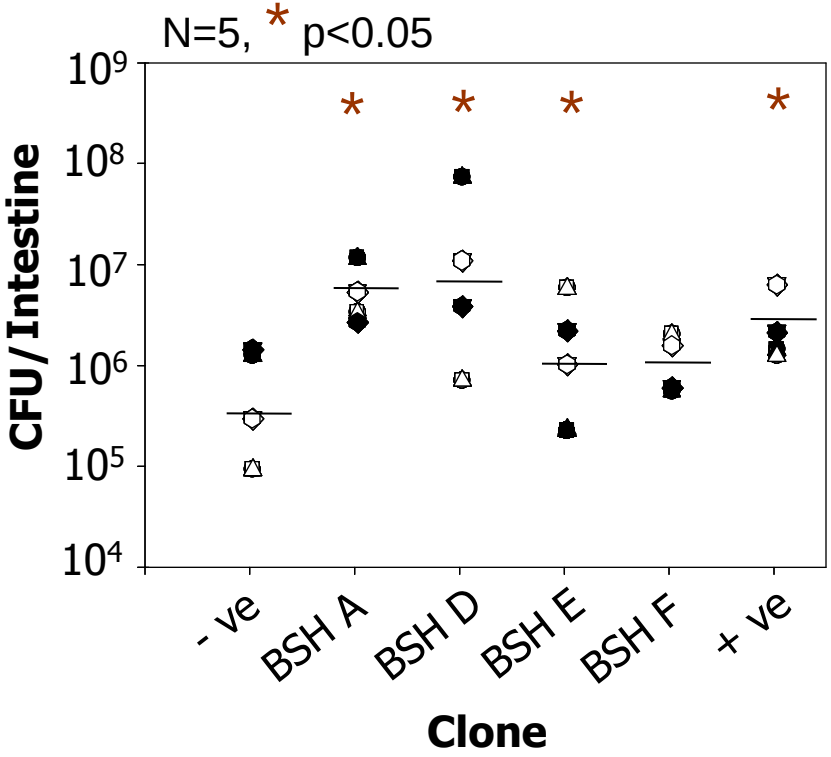

BSH enhances survival of *Listeria innocua* in bovine bile:



# BSH enhances gut colonization in conventional mice (day 3 PI)

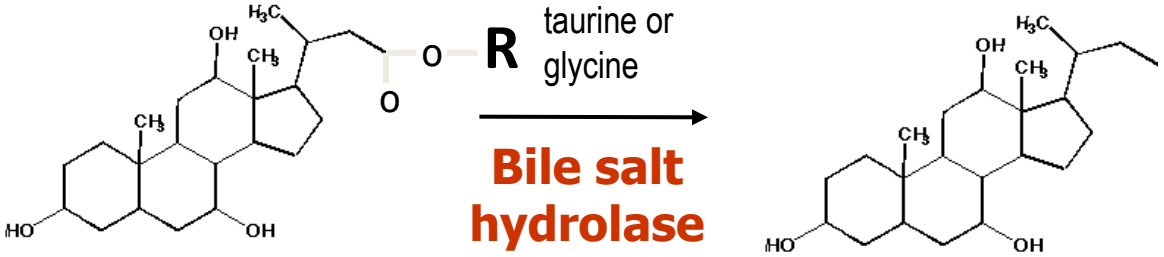



BSH expressed in *Listeria innocua*:




Jones, B, Begley, M *et al,* 2008. *Proc Natl Acad Sci USA*, 105:13580-13585

# Microbial Bile salt hydrolase activity



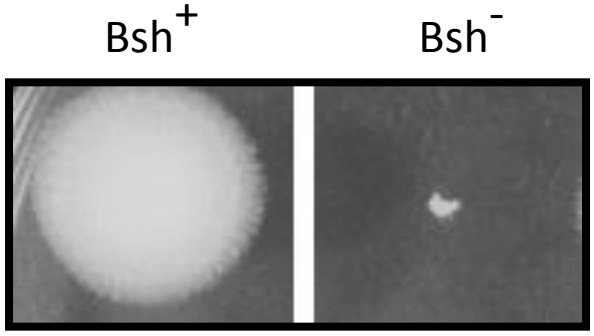

*Conjugated Bile acid*

*Unconjugated Bile acid*

dehydroxylation

dehydrogenation

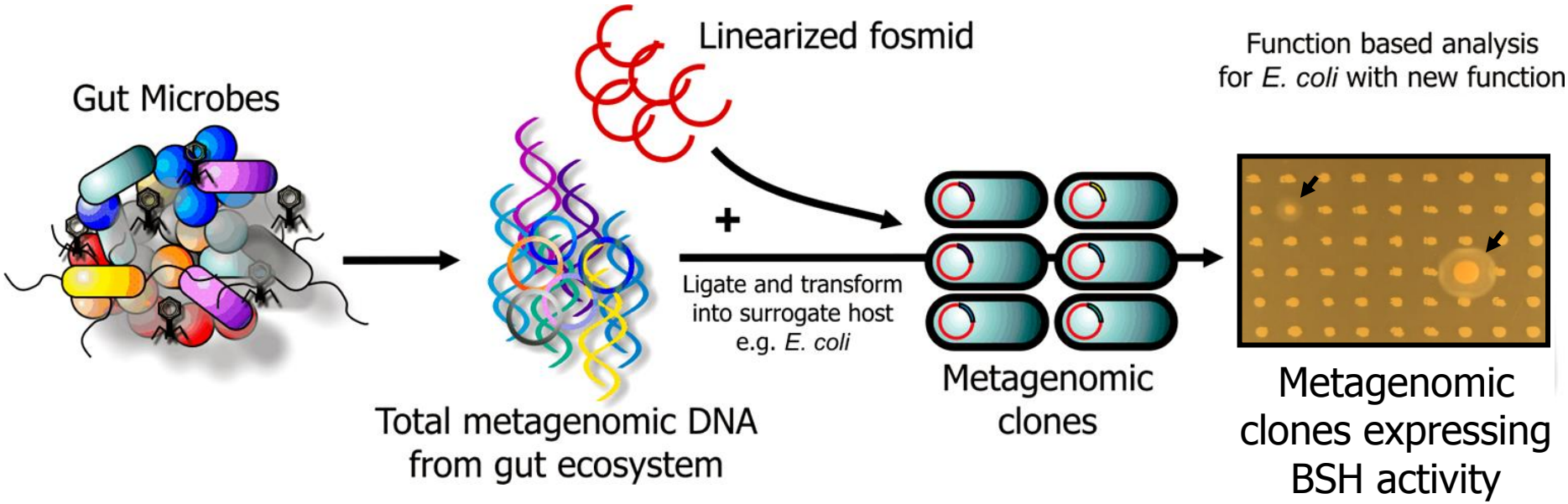
BSH catalyses the
'gateway reaction'




Precipitate on Tauro-Bile plates

# Functional Metagenomics - diversity of BSH activity in gut bacteria

*Dr. Brian Jones & Dr. Julian Marchesi*




- High Mw DNA was extracted from a faecal sample from a healthy male
- Fosmid bank consisting of 89856 metagenomic clones

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# Functional Annotation of the Microbiome

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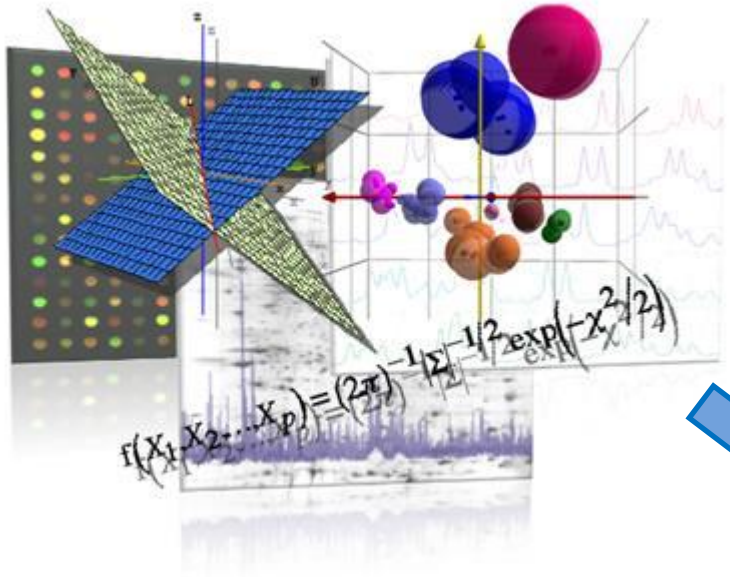
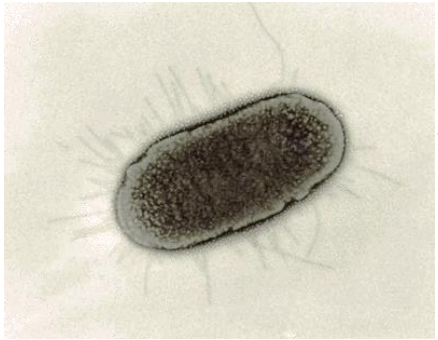
Systems Approach: What's there?



**Culture-independent (sequencing, proteomics)**

Reductionist: What are they doing?



Single protein effects on system

**Single organism / protein**

# Intervention Alters Plasma Bile Acid Profiles in Mono-colonised Mice:

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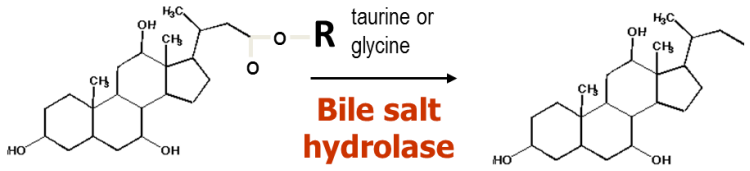
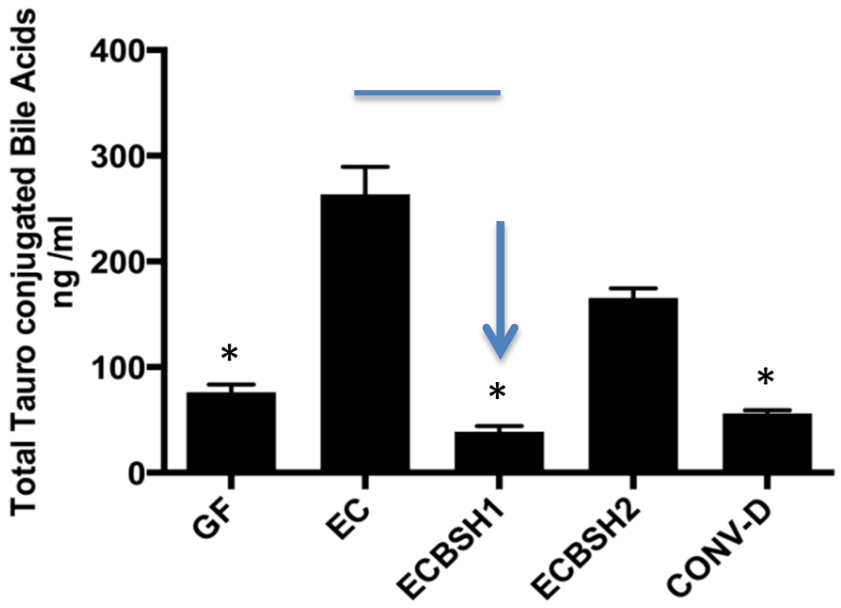
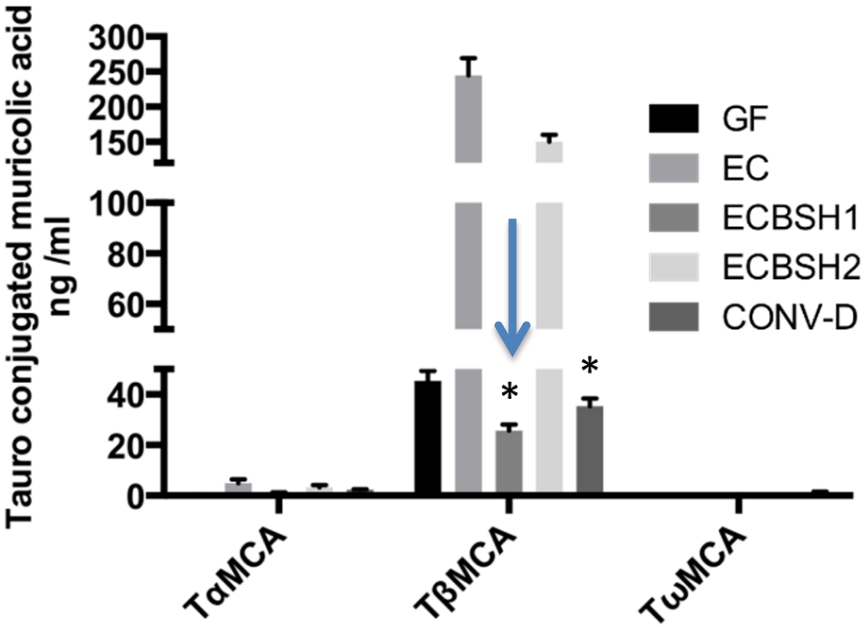
## Total Tauro-Conjugated Plasma Bile Acids



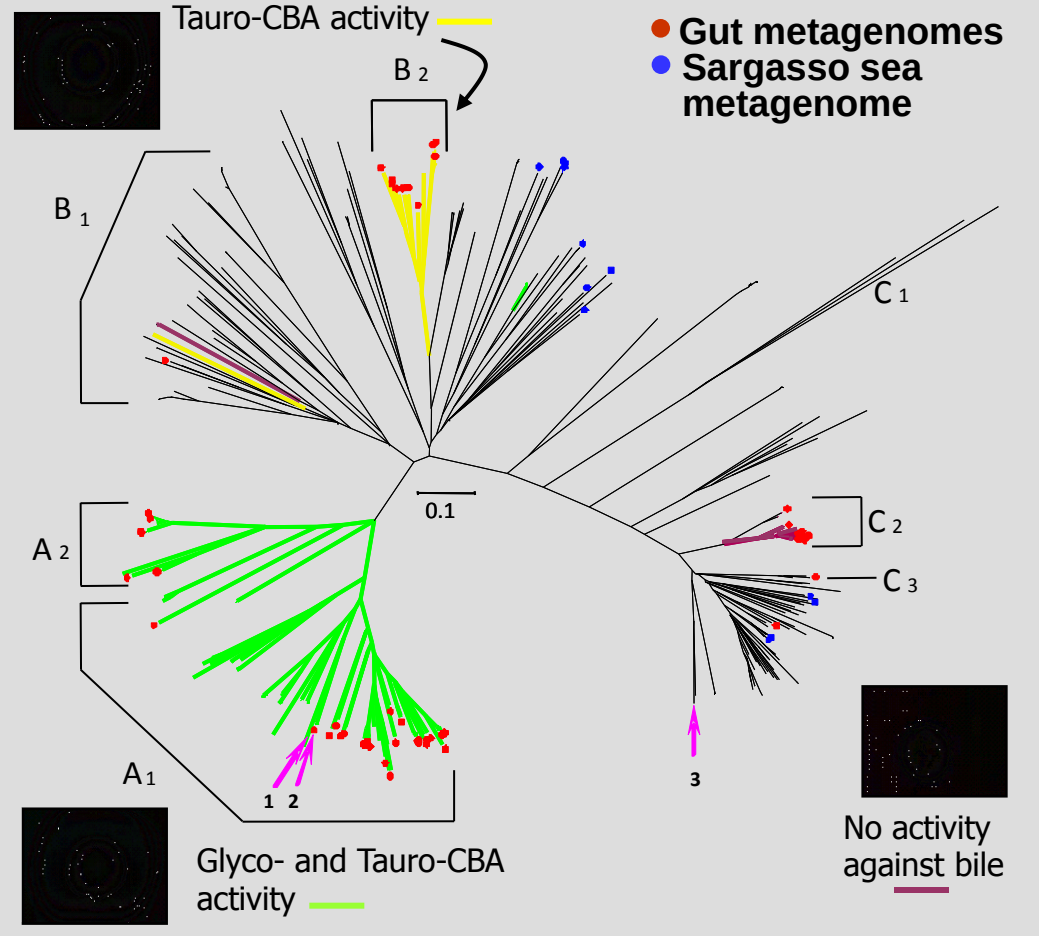

*Conjugated Bile acid* *Unconjugated Bile acid*




## Reduction in TβMCA



# Phylogenetic analysis of 400 *PVA-BSH* family sequences host-level selection in the gut



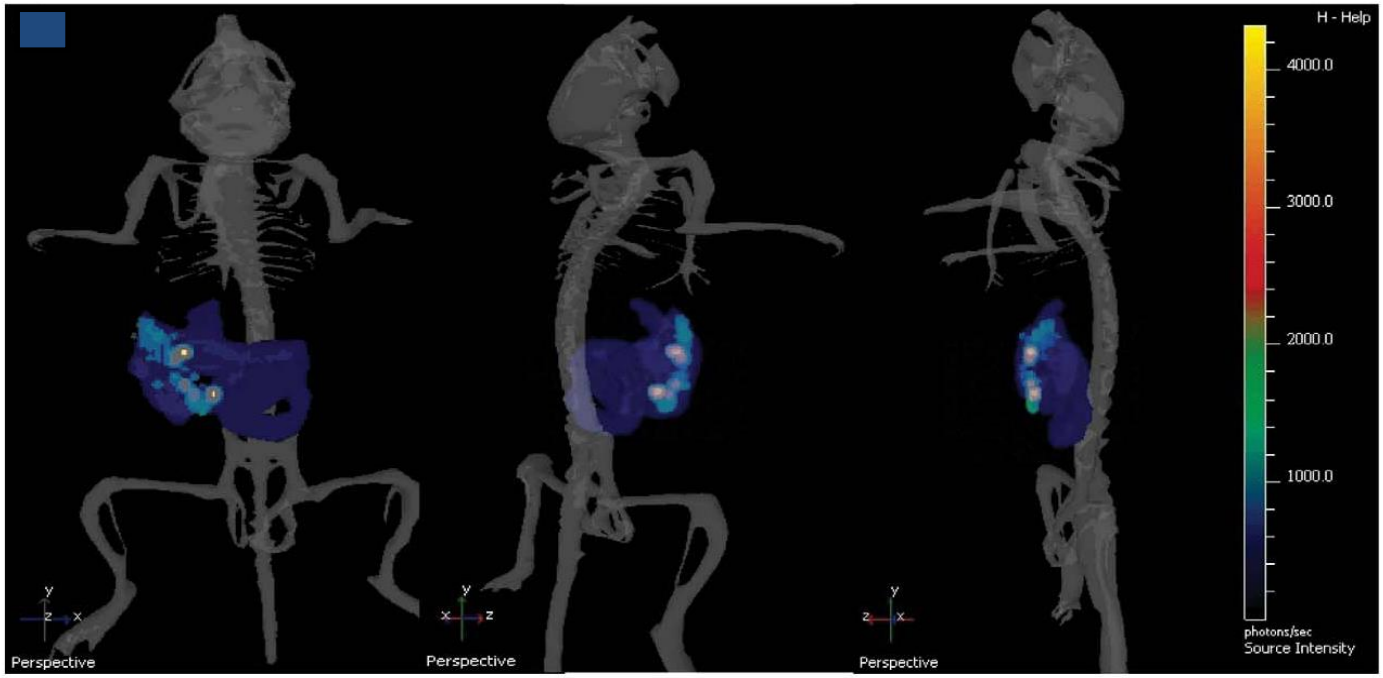

BSH - associated only with bacteria from the gut or gut pathogens (groups A and B)

A related enzyme - PVA is present in gut & non-gut bacteria but does not have BSH activity (group C)

Gut *Archea* (marked 1 and 2) have evolved potent BSH activity whereas non-gut *Archea* (marked 3) do not express active BSH

*Proc Natl Acad Sci U S A. 2008. 105(36):13580-5.*

# *E. coli* MG1655$_{strep^R}$ colonises the mouse GI tract




60 days - Strep

Cronin M. et al. 2012. PLoS One. 2012;7(1):e30940.

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# Quantitative UPLC-MS

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| Analyte | RT | FORMULA | Mol. Wt. |
|---|---|---|---|
| Taurine | 0.75 | $C_2H_7NO_3S$ | 124.0068 |
| Dehydrocholic acid | 1.71 | $C_{24}H_{34}O_5$ | 498.2889 |
| Tauro B Muricholic acid | 1.87 | $C_{26}H_{44}NO_7S$ | 514.28385 |
| Deoxycholic Acid | 1.9 | $C_{24}H_{40}O_4$ | 391.2848 |
| Taurodeoxycholic acid | 3.39 | $C_{26}H_{44}NO_6S$ | 498.2889 |
| Taurocholic acid | 4.82 | $C_{26}H_{45}NO_7S$ | 514.2838 |
| Alpha Muricholic acid | 5.89 | $C_{24}H_{40}O_5$ | 407.27975 |
| Beta Muricholic acid | 6.26 | $C_{24}H_{40}O_5$ | 407.27975 |
| Tauro-Chenodeoxycholic acid | 8.39 | $C_{26}H_{44}NO_6S$ | 498.2889 |
| Ursodeoxycholic acid | 8.57 | $C_{24}H_{40}O_4$ | 391.2848 |
| Cholic acid | 11.76 | $C_{24}H_{40}O_5$ | 407.27975 |
| Taurolithocholic acid | 14.252 | $C_{26}H_{45}NO_5S$ | 482.207 |
| Chenodeoxycholic acid | 17.63 | $C_{24}H_{40}O_4$ | 391.2848 |
| Lithocholic Acid | 21.72 | $C_{24}H_{40}O_3$ | 375.2899 |

**Internal Standards: Deuterated Chenodeoxycholic acid and Cholic acid**