Story of BA and BSH with gut microbiota

Chunyu Zhao

20180125

Bile salt biotransformations by human intestinal bacteria [2] - [1]

Jason M. Ridlon, Dae-Joong Kang, and Phillip B. Hylemon

2005

Bile acids are saturated hydroxylated C-24 cyclopentanephenanthrene sterols synthesized from cholesterol 胆固醇 in hepatocytes 肝细胞 (liver).

* cholesterol := chole (bile胆汁) + stereos (solid) ; It is a sterol (modified steroid 类固醇).
* cyclopentanephenanthrene : structure common to all steroids

Two **primary/unconjugated** bile acids synthesized in the human liver are: **cholic acid (CA)** and **chenodeoxycholic acid (CDCA)**.

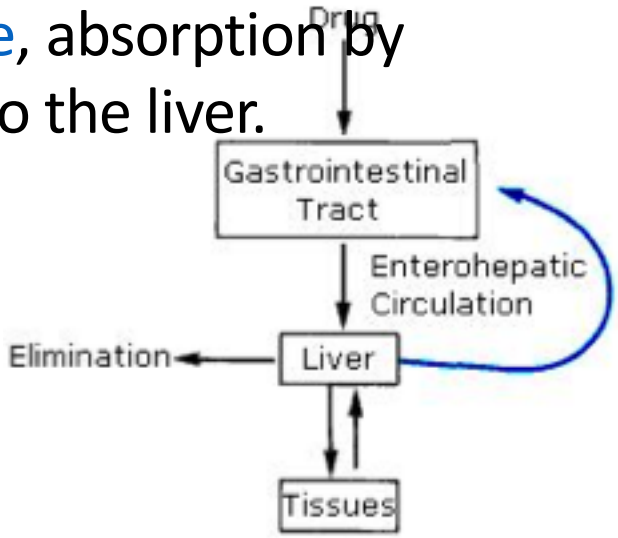
Bile acids are further metabolized by the **liver via conjugation** to **glycine or taurine** e.g. Tauro-CA/CDCA, Glyco-CA/CDCA.

Thus, at physiological pH, **conjugated bile acids (又名bile salts)** are almost fully ionized [6].

* **bile salts:** highly effective **detergents**

Secondary bile acids, produced **solely** by **intestinal bacteria**, in the human **large intestine**, can accumulate to high levels in the **enterohepatic circulation** of some individuals and may contribute to the pathogenesis of colon cancer, gallstones, and other gastrointestinal (GI) diseases.

* **Enterohepatic circulation** refers to the circulation of biliary acids, bilirubin, drugs, or other substances from the liver to the bile, followed by entry into the small intestine, absorption by the enterocyte 肠上皮细胞 and transport back to the liver.



During the enterohepatic circulation肠肝的, bile salts (**conjugated** bile acids produced in liver) encounter populations of **facultative anaerobic bacteria** 兼性厌氧的 of relatively low numbers and diversity in the **small bowel:**

- **bile salt metabolism** by small bowel microbes: deconjugation and hydroxy group oxidation

Ileal 回肠 bile salt transport (Fig2 C 段) is highly efficient (~95%), but 5% bile salts escapes the enterohepatic circulation and becomes **substrate** 基质, 酶作用物 for significant microbial biotransforming reactions in the **large bowel** [6].

Fecal bile acid composition ⇢ **secondary bile acids** [deoxycholic acid (DCA) and lithocholic acid (LCA)], solely produced by microbial biotransforming reactions in the human large intestine.

The major bile salt modifications in the human **large intestine** includes:

{liver} bile salts conjugated BA

{small bowel; completion in large bowel} deconjugation

{large intestine} secondary BA

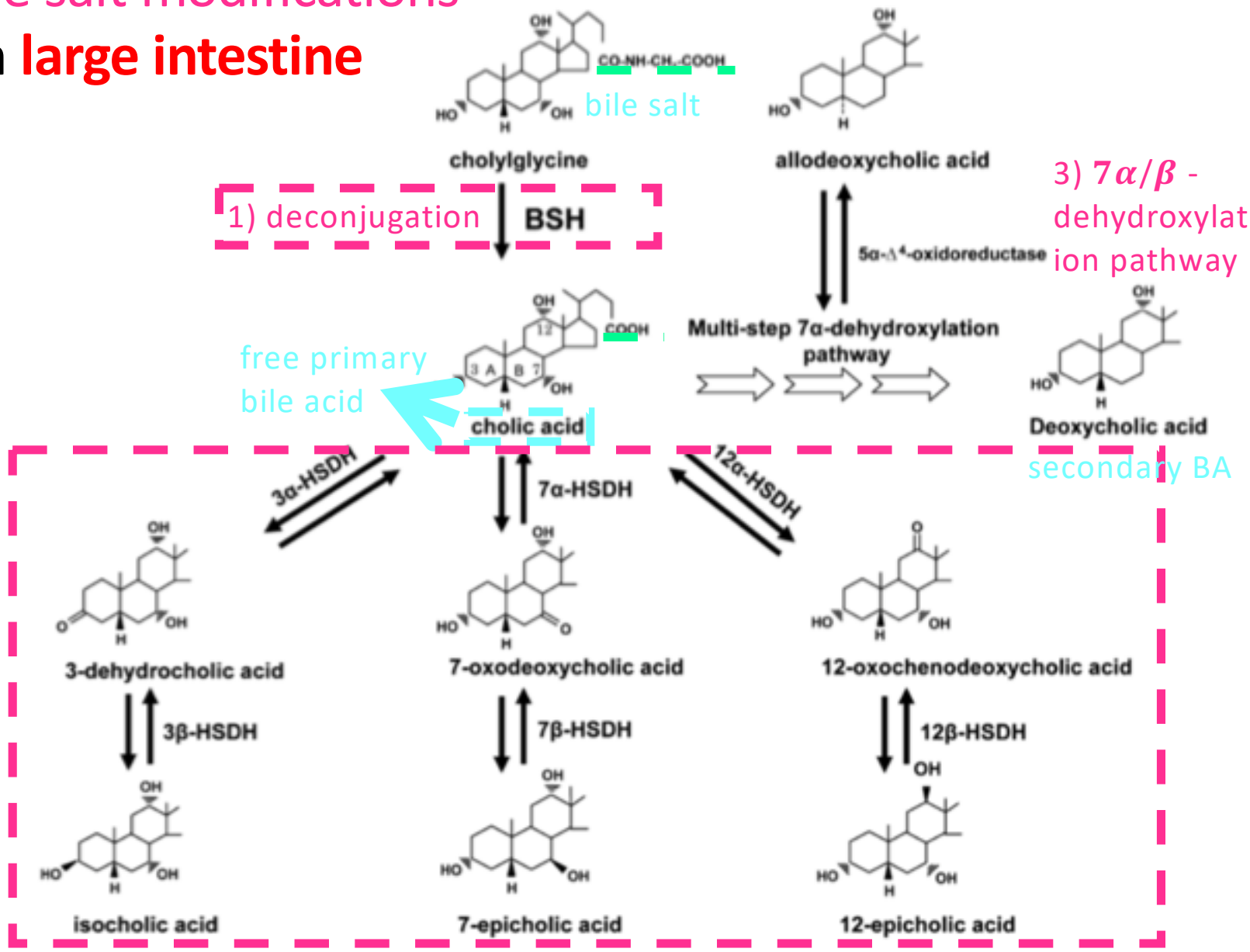


Fig. 1. Bacterial bile salt-biotransforming reactions in the human intestinal tract. Hydroxy group carbons of cholate are numbered and the AB rings are identified. The 3, 7, and 12 carbons of cholic acid (CA) are numbered. Nomenclature is that of Hofmann et al. (160). BSH, bile salt hydrolase; HSDH, hydroxysteroid dehydrogenase.

Bile salt hydrolysis and hydroxyl group dehydrogenation reactions are carried out by a broad spectrum of intestinal anaerobic bacteria,

whereas

Bile salt 7-dehydroxylation appears restricted to a limited number of intestinal anaerobic representing a small fraction of the total colonic flora.

Deconjugation and $7\alpha/\beta$ - dehydroxylation pathway of bile salts:

- increases their hydrophobicity and Pk_a;
- therefore, is associated with increased toxic and metabolic effects

deconjugation: the enzymatic hydrolysis of the amide bond linking bile acids to their amino acid conjugates.
small bowel microbes

BSHs are in the choloyglycine hydrolase family (EC 3.5.1.24):

Bacteroides fragilis
Bacteroides vulgatus
Clostridium perfringens MCV 185
Clostridium perfringens 13
Lactobacillus johnsonii 100-100
  Isozyme A
  Isozyme B
  Isozyme C
  Isozyme D
Lactobacillus plantarum 80
Lactobacillus acidophilus
Bifidobacterium longum BB536
Bifidobacterium longum SBT2928
Bifidobacterium bifidum ATCC 11863
Bifidobacterium adolescentis
Listeria monocytogenes

Microbial enzymes modifying **bile salts** differ between species, with respect to pH optima, enzyme kinetics, substrate specificity, cellular location, and possibly physiological function.

BSHs share a high degree of amino acid sequence similarity with the penicillin V amidase of *Bacillus sphaericus* (Fig 4).

Genes encoding BSHs was cloned, and homologs and putative *bsh* genes have also been identified recently through microbial genome analysis (*blastp*).

The organization and regulation of genes encoding BSH differ between species and genera.

Benefits of BSHs to the bacterium

- detoxification of bile salts
- accumulation free bile acids

[46, 47]: diet in high meats has been shown to significantly increase bother the levels of taurine conjugation to bile acids.

Secondary bile acids (DCA and LCA) predominate in human feces,

therefore, 7α-dehydroxylation most quantitative,

which is restricted to free bile acids.

So, removal of glycine/taurine bile acid conjugates vis BSH enzymes is a prerequisite for intestinal bacteria.

7β-dehydroxylation is more of a luxury than a necessity.

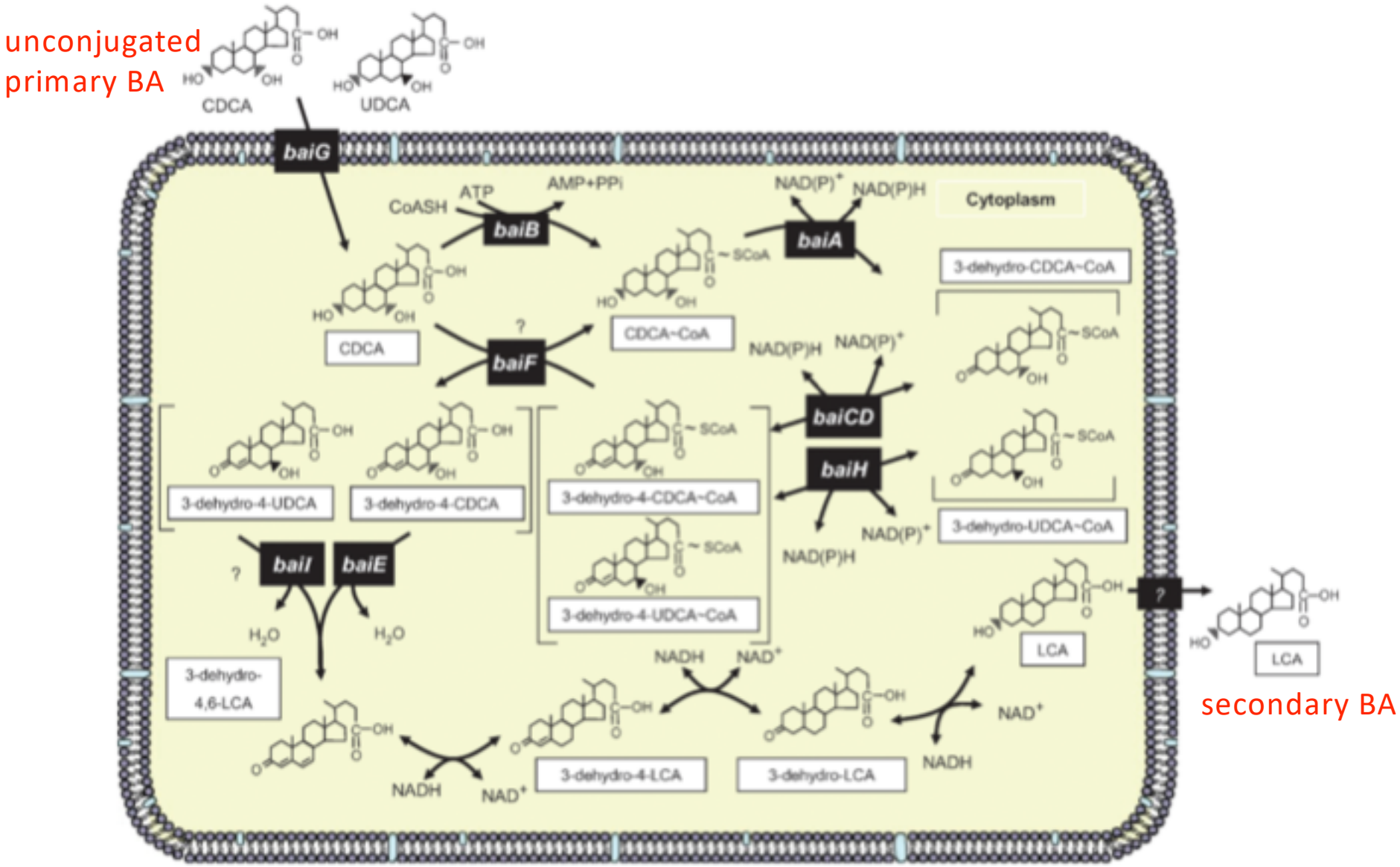
bile acid 7α/βdehydroxylation pathway

- multiple steps
- multiple *bai* genes

Fig. 7. Gene organization of the bile acid-inducible (*bai*) 7α/β-dehydroxylation operons characterized in *C. scindens* VPI 12708. P indicates the promoter region.

end product: toxic secondary bile acid

Secondary bile acids and disease


unconjugated primary BA
CDCA
UDCA
baiG
ATP
CoASH
AMP+PPi
baiB
NAD(P)+
NAD(P)H
baiA
Cytoplasm
CDCA
CDCA~CoA
baiF
?
NAD(P)H
NAD(P)+
baiCD
baiH
3-dehydro-CDCA~CoA
3-dehydro-4-UDCA
3-dehydro-4-CDCA
3-dehydro-4-CDCA~CoA
3-dehydro-4-UDCA~CoA
3-dehydro-UDCA~CoA
NAD(P)+
NAD(P)H
baiI
baiE
?
H2O
H2O
3-dehydro-4,6-LCA
NADH
NAD+
3-dehydro-4-LCA
3-dehydro-LCA
NAD+
NADH
LCA
?
LCA
secondary BA

Complex carbohydrates, which are intrinsically indigestible or which escape digestion and absorption in the proximal gut, are fermented by colonic bacteria to **yield** short-chain fatty acids.
It had been estimated that these short-chain fatty acids constitute 3-9% of our daily caloric intake [4].

Functional and comparative metagenomic analysis of bile salt biotransformation by human intestinal bacteria [28]

Brian V Jones, and Julian R. Marchesi

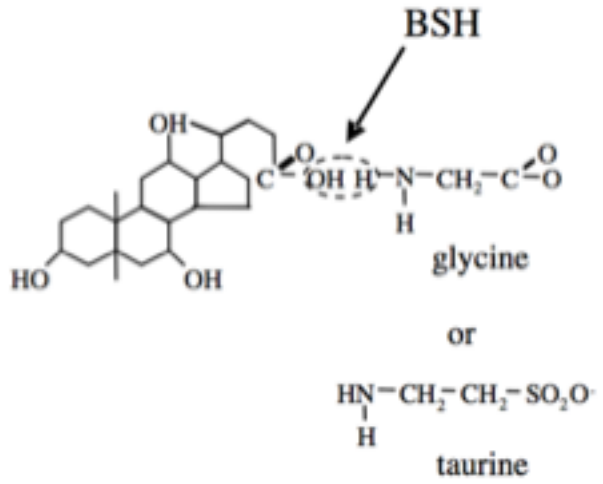
2008

- this paper should tell me which genome has BSH genes

The function, distribution, and abundance of BSH enzymes in the gut community are unknown.

conjugated primary bile acid (bile salt) → (BSH) → free/ unconjugated primary bile acid

repress bacterial growth in small intestine



Gut microbiota can modulate host bile acid synthesis.

Distribution of BSH Activity in the Human Gut Microbiota

Clones assigned as *Firmicutes* (30%) and *Actinobacteria* (8.9%) were capable of degrading all tested CBAs and human bile, whereas clones assigned to *Bacteroidetes* (14.4%) were generally only active against taruo-CBA.

Comparison of amino acid sequences of complete ORFs encode BSH genes revealed 9 distinct BSH types.

Table S3. Identification of sequences homologous to functional BSH obtained through function-driven metagenomic analysis

Division*	BSH type†	Homologous sequences‡	Identity§	Alignment to Ntn_PVA-conserved domains¶, %
Bacteroidetes	A (1 clone)	*Bacteriodes ovatus* ATCC8483, hypothetical protein, BACOVA_03057 ZP_2066063	80% (287/355)	100
	B (2 clones, 100%)	*Bacteroides uniformis* ATCC8429, hypothetical protein, BACUNI_02933 ZP_02071494.1	100% (361/361)	100
	C (1 clone)	*Bacteroides uniformis* ATCC8429, hypothetical protein, BACUNI_02933 ZP_02071494.1	67% (241/358)	100
Firmicutes	D (5 clones, 99–100%)	*Eubacterium ventriosum* ATCC27560, hypothetical protein, EUBVEN_02567 ZP_02027297.1	99% (326–327/329)	100
Unclassified	E (2 clones, 100%)	*Eubacterium ventriosum* ATCC27560, hypothetical protein, EUBVEN_02567 ZP_02027297.1	70% (234/333)	100
	F (3 clones, 99–100%)	*Ruminococcus obeum* ATCC29174, hypothetical protein, RUMOBE_03454 ZP_01965714.1	99–100% (302–322/322)	100
	G (1 clone)	*Ruminococcus obeum* ATCC29174, hypothetical protein,RUMOBE_00028 ZP_01962315.1	76% (250/325)	100
Actinobacteria	H (1 clone)	*Collinsella aerofaciens* ATCC25986, hypothetical protein, COLAER_00574 ZP_01771587	71% (219/307)	98.35
	I (3 clones, 99–100%)	*Bifidobacterium adolescentis* L2–32, bile salt hydrolase, BIFADO_01120 ZP_02028683	99% (344–345/347)	100%

Comparative Metagenomic Analyses of BSHs in Human Gut and Other Environmental Metagenomes

Goal: to identify BSH and homologous enzymes

Methods: aa seqs representative of each of our functional BSH types A-I were compared with several gut metagenomes using *tblastn*:
- hits with significant e values (1e-8 or lower) AND a length of 30 aa or more were retrieved, and homology compared with BSH and related proteins in our extensive in-house database.

Different BA pools in human and murine species: murine bile is composed predominantly of tauro-CDA, and murocholic acid, which is not synthesized in humans.

Evolution of the Ntn_CGH-like Family of Proteins to BSH Activity in the Gut

penicillin V amidase (PVA) is closely related to BSH and exhibits conservation of putative critical catalytic amino acids [23].

- 29% sequence similarity

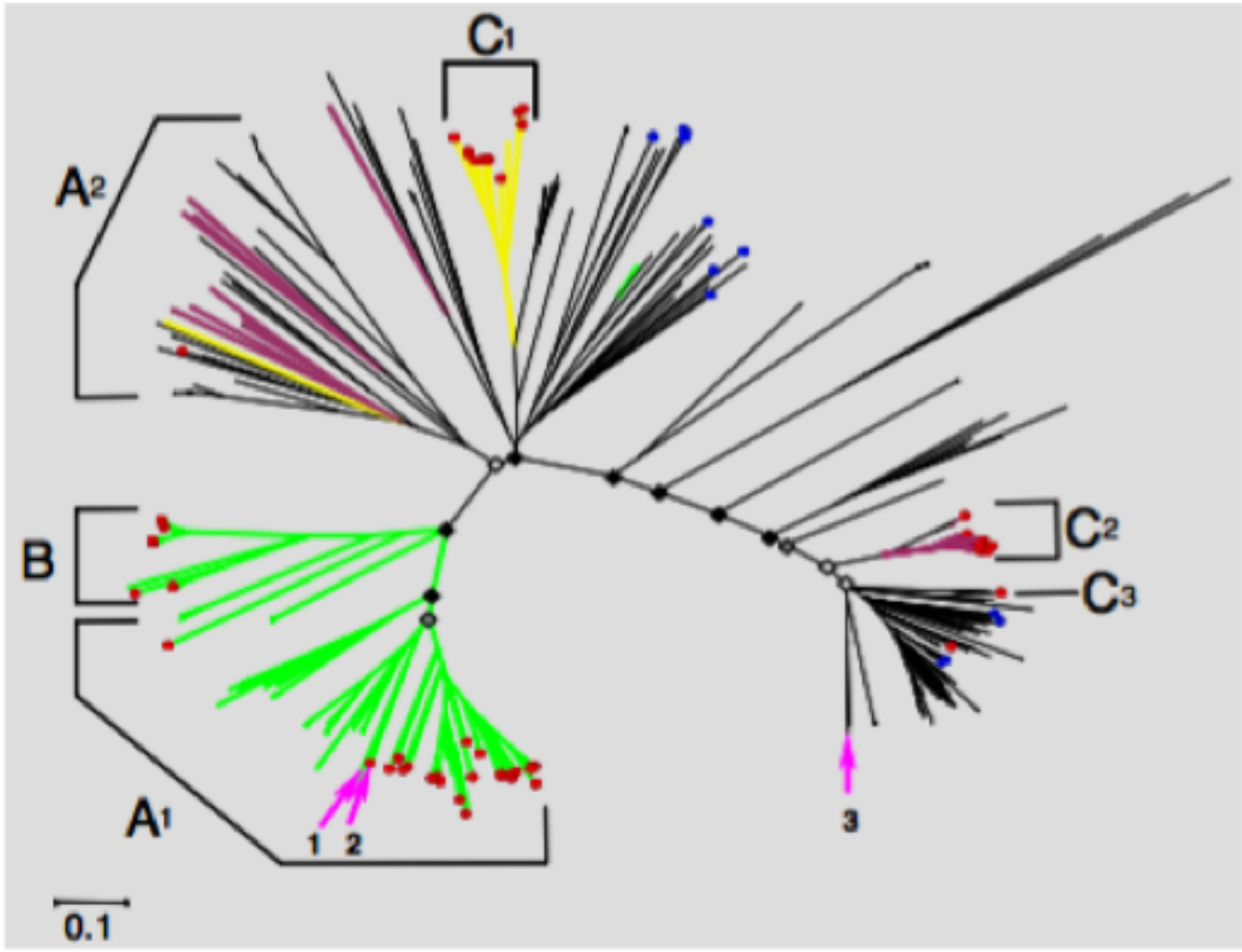
methods: phylogenetic analysis using amino acid sequences exhibiting residues conserved among BSH and PVA

C1: *Bacteroidetes*
tauro - CBA

A2: Firmicutes
tauro - CBA
no BSH, so PVA

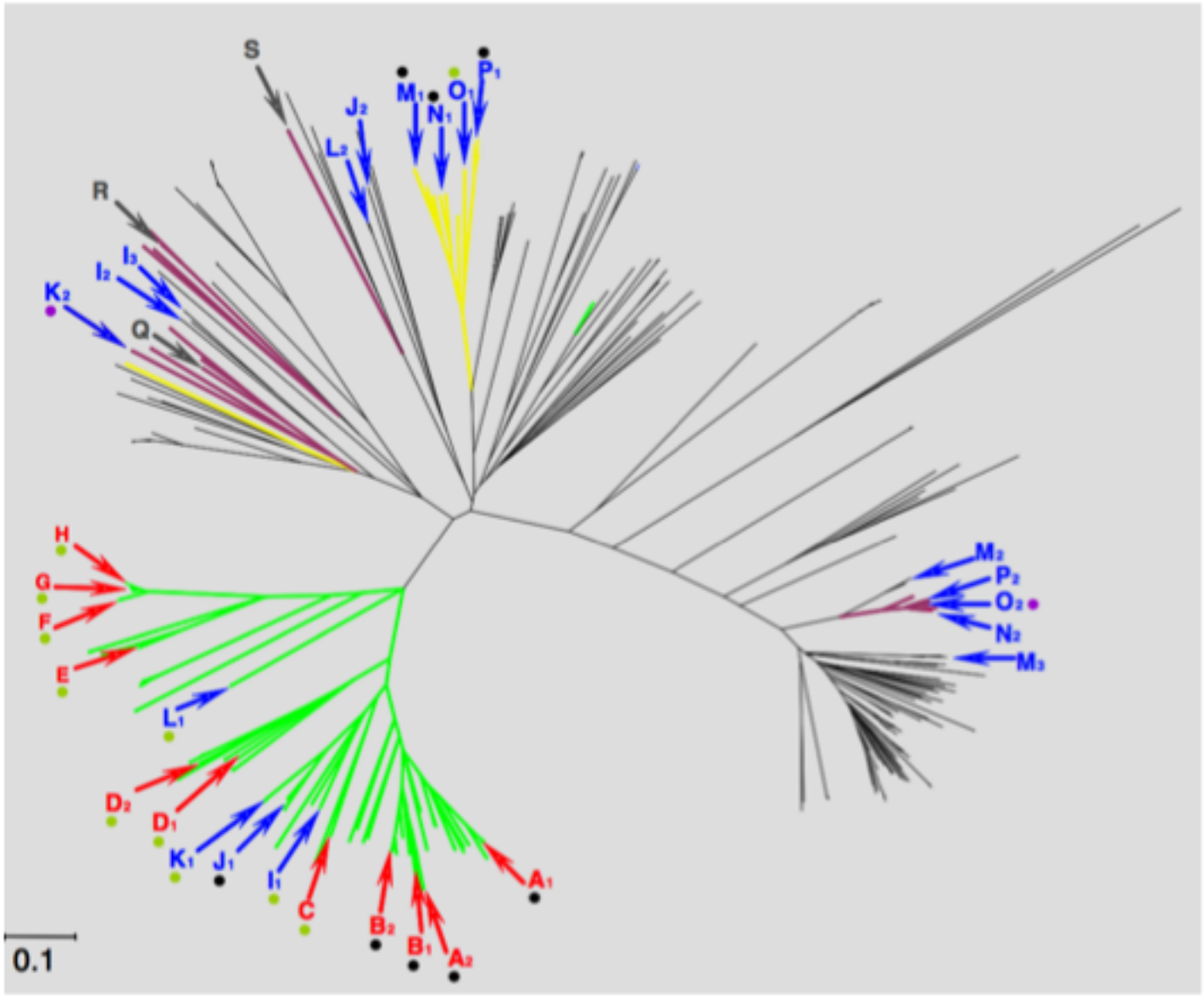
B: Actinobacteria
glyco/tauro - CBA

A1: Firmicutes
glyco/tauro – CBA



Blue arrow: both BSH and PVA

Red arrow: BSH only



Observations from Fig 2 and Fig S4:

- majority are BSH
- few are PVA – primarily from gut-associated *Bacteroidetes* (C2)
- multiple but only identical copies of PVA/BSH proteins: some species encode both activities

Conclusion: identified BSH as a conserved microbial adaptation to the human GI tract

Analysis of the Role of Microbial BSH Activity

CBAs exhibit direct antimicrobial activity and during colonization of the human GI tract microbes are exposed to inhibitory levels of CBA.

We hypothesized that BSH may facilitate colonization of the GI tract by mediating resistance to CBA.

Our data clearly demonstrate that BSH activity benefits bacteria by enhancing resistance to CBA and increasing survival in the GI tract, and we propose that this facilities colonization and development of the gut microbiota.

However, the proportion of species that encode this activity remains to be established, and it is likely that many well adapted members of this community do not encode BSH.

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Personalized modeling of the human gut microbiome reveals distinct bile acid deconjugation and biotransformation potential in healthy and IBD individuals

Almut Heinken, and Ines Thiele
2017

constraint-based modeling

AGORA: [16] matlab toolbox

metabolic potential

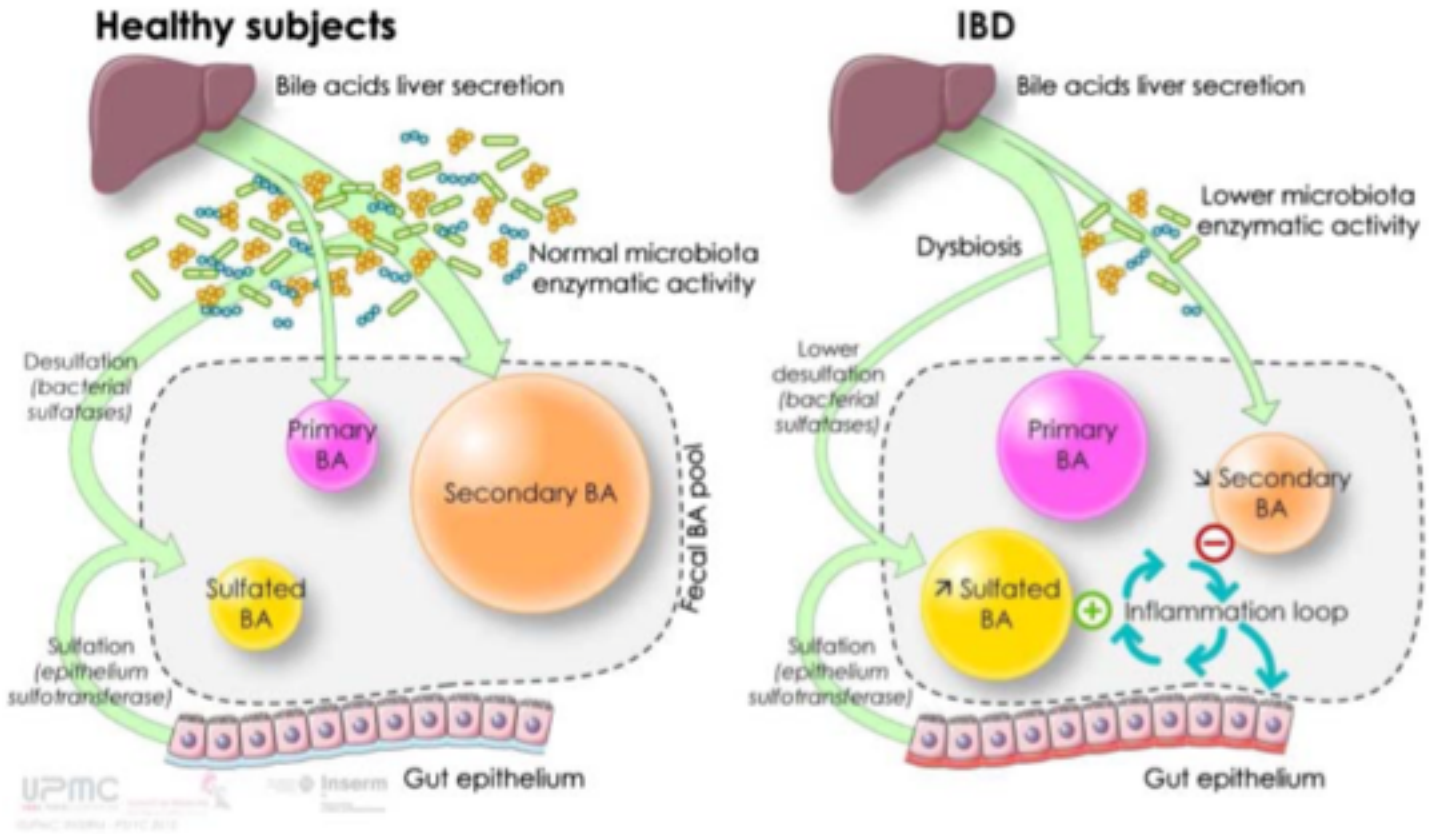
flux balance analysis: to compute functional states [17]

The microbiomes of pediatric IBD patients were significantly depleted in their bile acid production potential compared with controls.

IBD microbiomes were depleted in contributions of *Bacteroidetes* strains but enriched in contributions of *Proteobacteria*.

[6]: reduced abundance of the bile salt hydrolase (*bsh*) gene in Firmicutes in four CD patients and an increased abundance of the *bsh* gene in the Actinobacteria phylum in UC patients.

Figure 7 Physiopathological model of luminal bile acid dysmetabolism in inflammatory bowel diseases. This figure is only reproduced in colour in the online version.



[4]: http://gut.bmj.com/content/gutjnl/62/4/531.full.pdf

[7]: method section was printed: two blast search

The amino acid sequences of 24 functional BSH 'types' were used to search human gut metagenomes constituting the MetaHIT (Metagenomics of the Human Intestinal Tract) dataset,[6] which comprises the gut microbiomes of 124 individuals of varying disease status (99 healthy, 21 ulcerative colitis, 4 Crohn's disease). Sequences in the MetaHIT dataset producing valid hits (tBlastn: minimum 35% identity ≥50 amino acids, $1e^{-5}$) to these functional BSH types were retrieved, and encoded BSH-like homologues were affiliated with a phylogenetic division based on top hits (by bit-score) from subsequent BlastX searches of the non-redundant dataset. Affiliated hits were then used to construct non-redundant BSH relative abundance profiles for major phylogenetic divisions in the human gut microbiota (figure 1), as previously described.[5]

[6] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3463861/pdf/gutjnl-2012-302137.pdf

LCA and DCA produced by *Clostridium scindens* have been shown to inhibit the pathogen *Clostridium difficile* in a dose-dependent manner [13].

organisms capable of bile acid deconjugations

Recently, a comprehensive collection of curated genome-scale reconstructions for 773 human gut microbial strains, AGORA, has been published [26].

AGORA has curated for a number of gut-specific subsystems, including fermentation, carbon source biosynthesis, respiration, and vitamin biosynthesis. However, AGORA does not account for bile acid transformations. The present study fills this gap.

Presence of bile acid biotransformation genes in 693 analyzed genomes

238 of 693 analyzed organisms were found to be capable of bile acid deconjugations and biotransformation, including 217 reconstructure AGORA organisms.

The complete reconstructed bile acid biotransformation subsystem contains 38 secondary bile acid metabolites, and 82 reactions.

Bile acid transformation capabilities are complementary

Many strains capable of synthesizing secondary bile acids do not possess the bile salt hydrolase ...

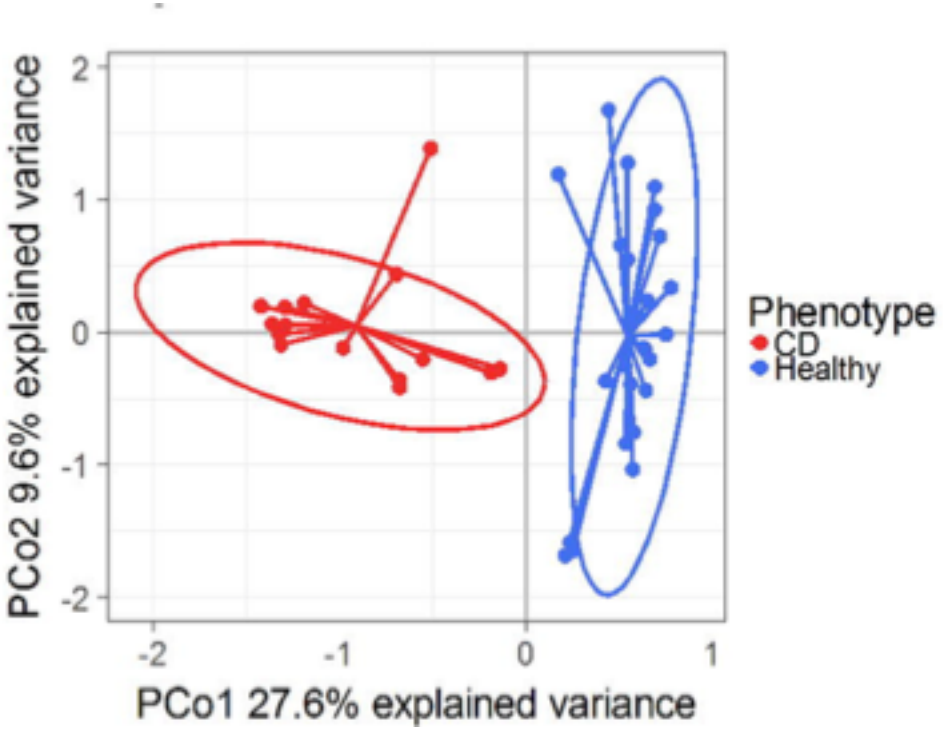
pairwise models

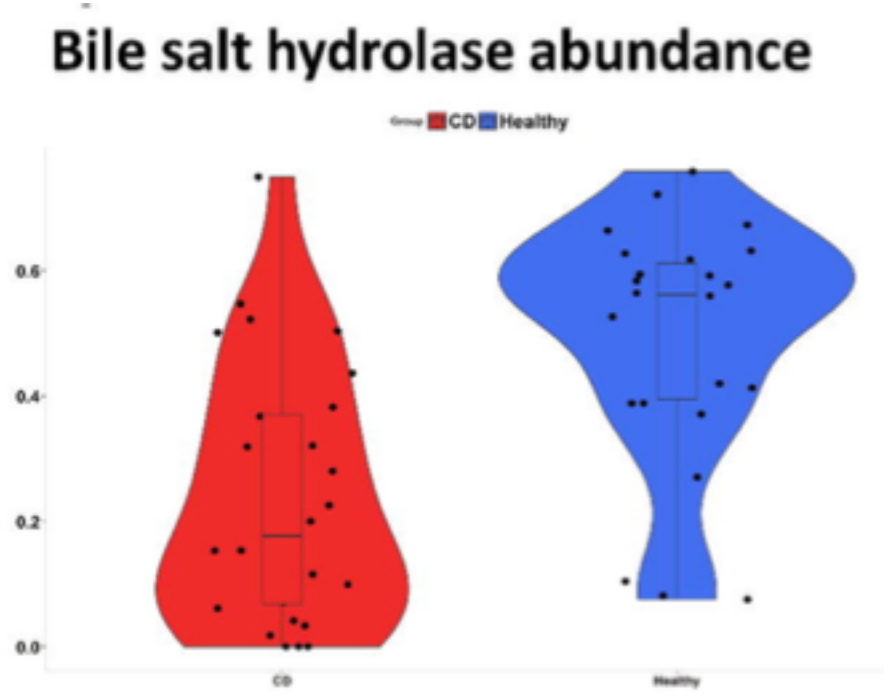
Taken together, these results demonstrate that bile acid biotransformation is a microbial community task and that the synthesis requires specific strain-strain combinations.

IBD-associated microbiomes are depleted in bile acid deconjugation and transformation capability

PLEASE/COMBO

- total reaction abundance
- reaction abundance on the genus level





The biosynthetic capabilities of a strain need to be viewed in the context of the entire gut microbiome community's metabolic network while also taking metabolic constraints (e.g., substrate availability) into account.

We demonstrated that microbes can complement each other's bile acid pathway (Fig 3a).

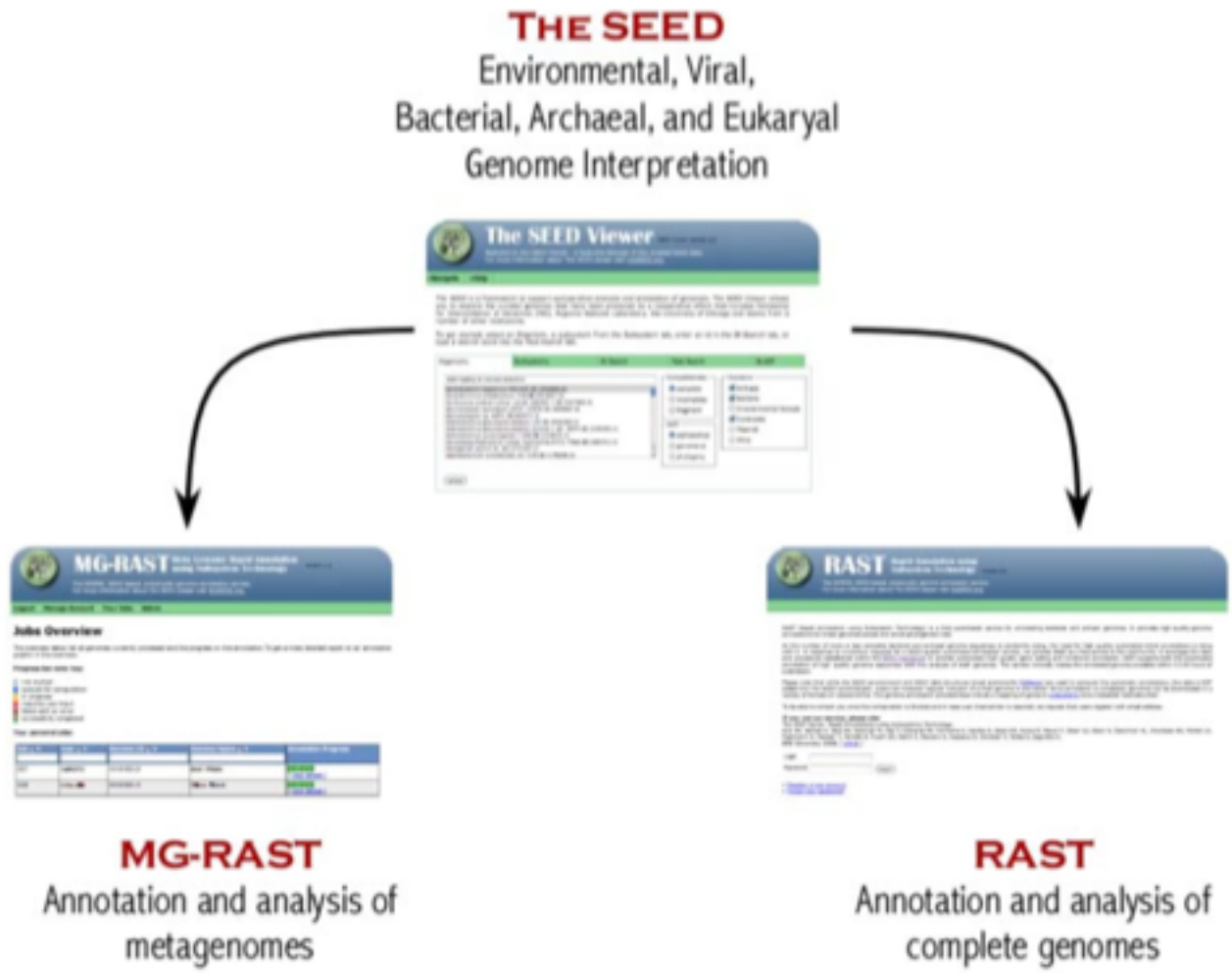


Figure 1 Overview of the SEED family of services. Each member of the family contributes a unique service to microbial genome analysis. The underlying platform, the SEED, integrates complete microbial genomes and data associated with them. The RAST server provides automatic high-quality annotation of complete genomes, while the mg-RAST server provides automatic high-quality annotation of metagenomes.

[5] Gut bacteria use mostly fermentation to generate energy, converting sugars, in part, to short-chain fatty acid, that are used by the host as energy source.

The genes annotated by COG were classified into the 25 COG categories, and genes that were annotated by KEGG were assigned into KEGG pathways.