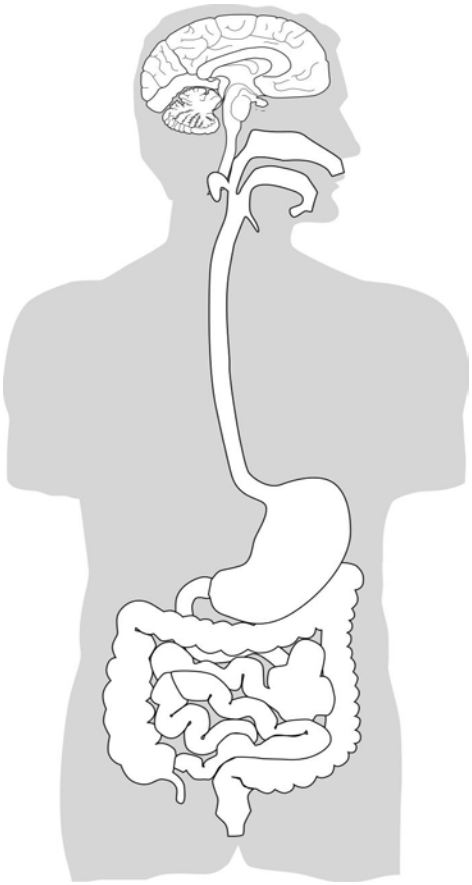
Metabolite cross-feeding among the human gut microbiota



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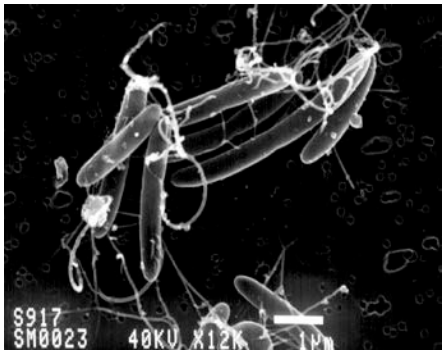

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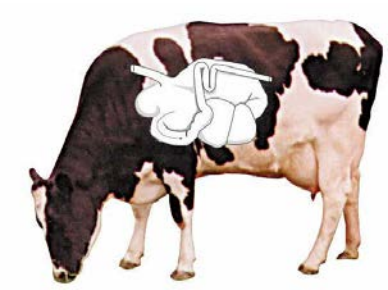
Metabolite cross-feeding in anaerobic gut communities – the rumen



Fermentation products –

Hydrogen (and formate) transfer

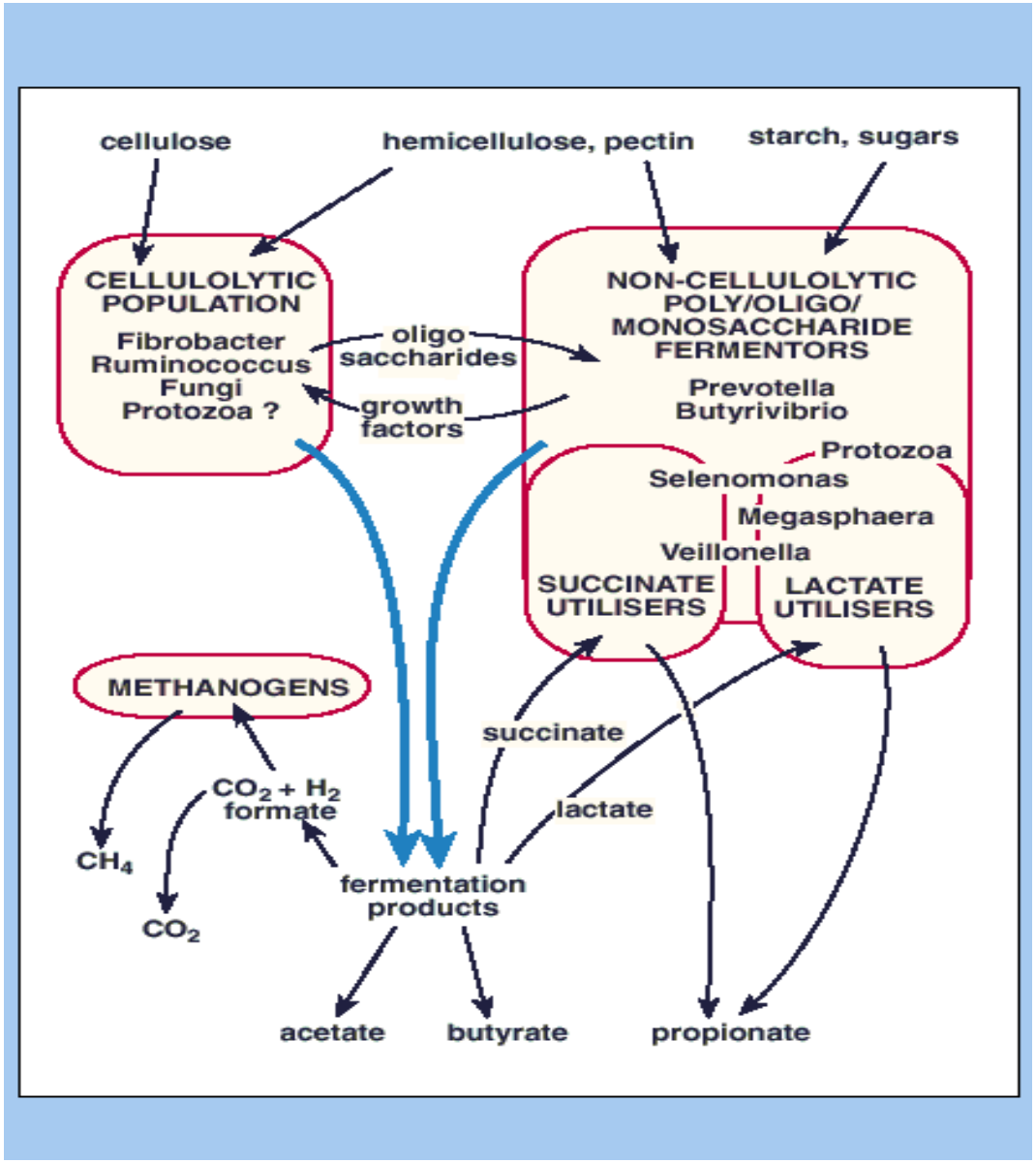
Lactate, succinate, acetate

Products of substrate degradation –

Polysaccharides, oligosaccharides, sugars, phenolic compounds

Vitamins, cofactors, biosynthetic precursors

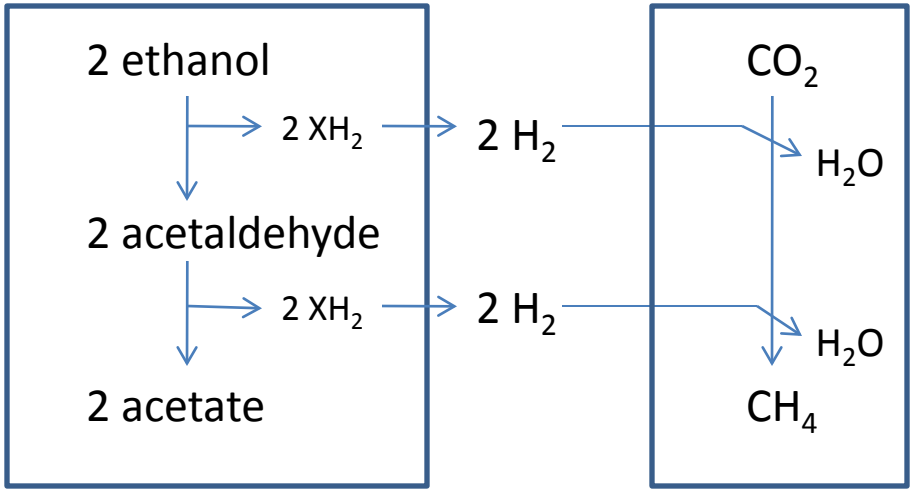
eg. vitamins, branched chain fatty acids, haem



(Flint HJ 1997 TIM 5, 483-488)

Interspecies hydrogen transfer - Mike Wolin, Marvin Bryant

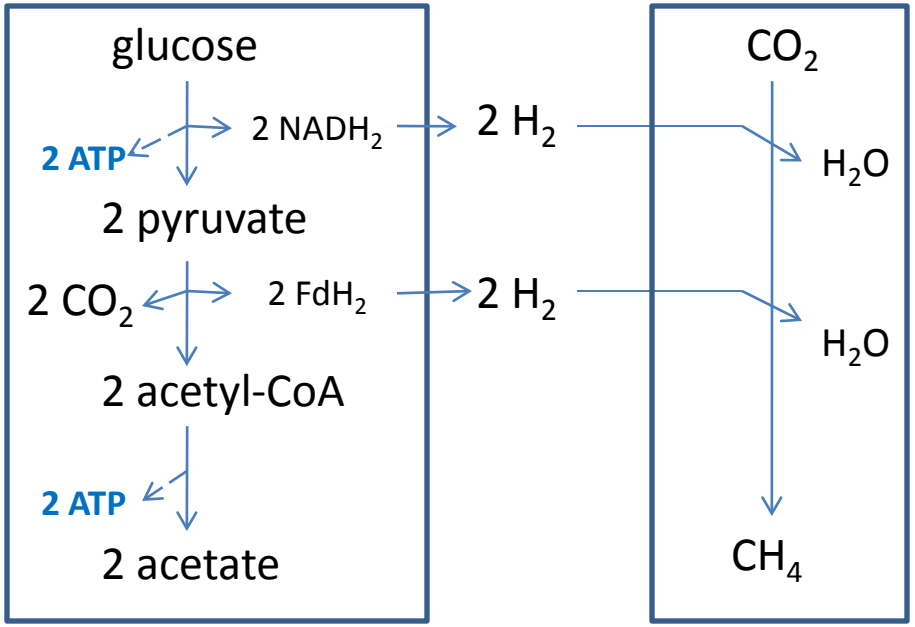
1.



'S organism'

methanogen

2.



Ruminococcus albus

methanogen

Syntrophy dependent on H (or formate) consumption -

'S organism' – first example of an obligate syntroph, ie. growth thermodynamically impossible without the second organism.

2 ethanol to 2 acetate:- **ΔG +19 kJ (no methanogen)** **ΔG -112 kJ (+ methanogen)**

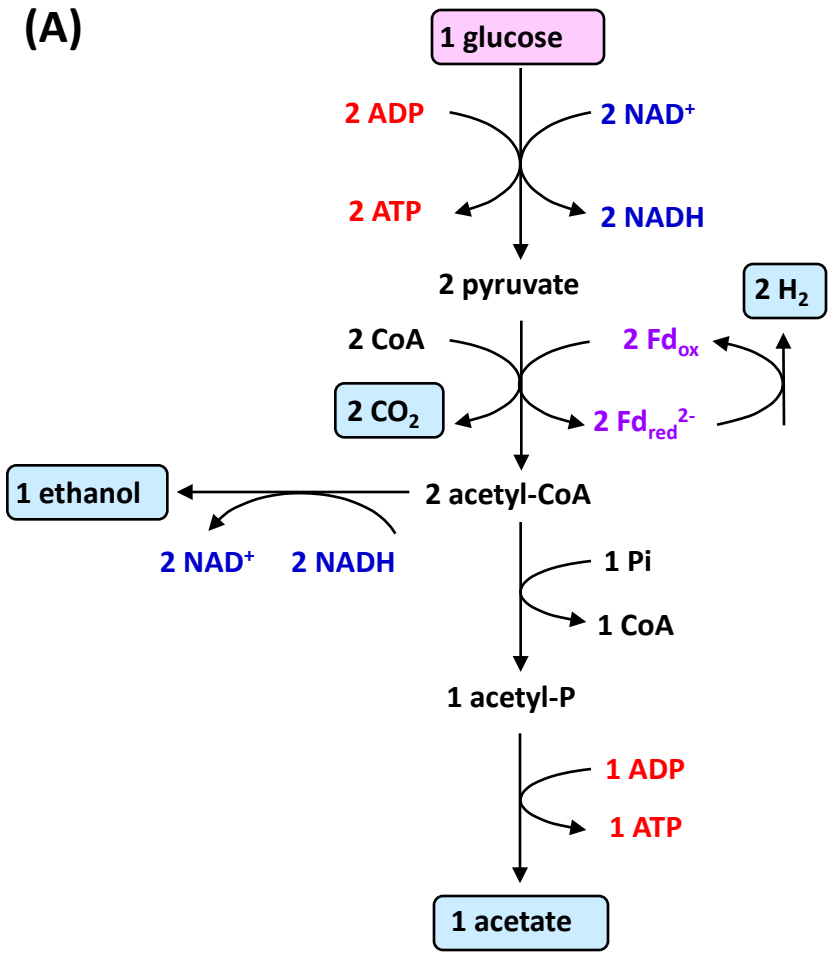
[First isolated as a coculture - *Methanobacillus omelianski* in 1939]

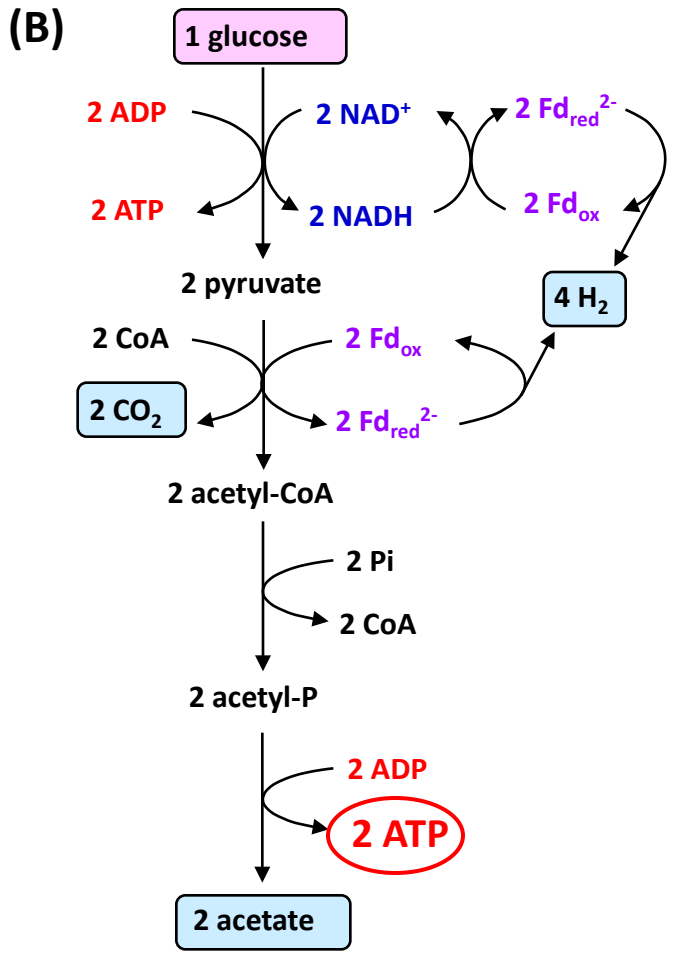
Some other examples of syntrophy (diverse habitats)*:-

	substrates used in co-culture	...in pure culture
Obligate syntrophs –		
Syntrophomonas sapovorans	Fatty acids (C4-C18, C16:1, C18:1, C18:2)	None?
Pelotomaculum schinkii	Propionate (C3)	None?
Non-obligate syntrophs –		
Syntrophobacter wolinii	Propionate (C3)	C3 + sulfate; fumarate
Syntrophomonas bryantii	Fatty acids (C4-C11)	crotonate (C4:1)
Sporotomaculum syntrophicum	Benzoate	crotonate; crotonate + benzoate

*[McInerney *et al* (2008) Ann NY Acad Sci 1125: 58-72]

Fermentative metabolism of *Ruminococcus albus* (Wolin & Miller, 1983)





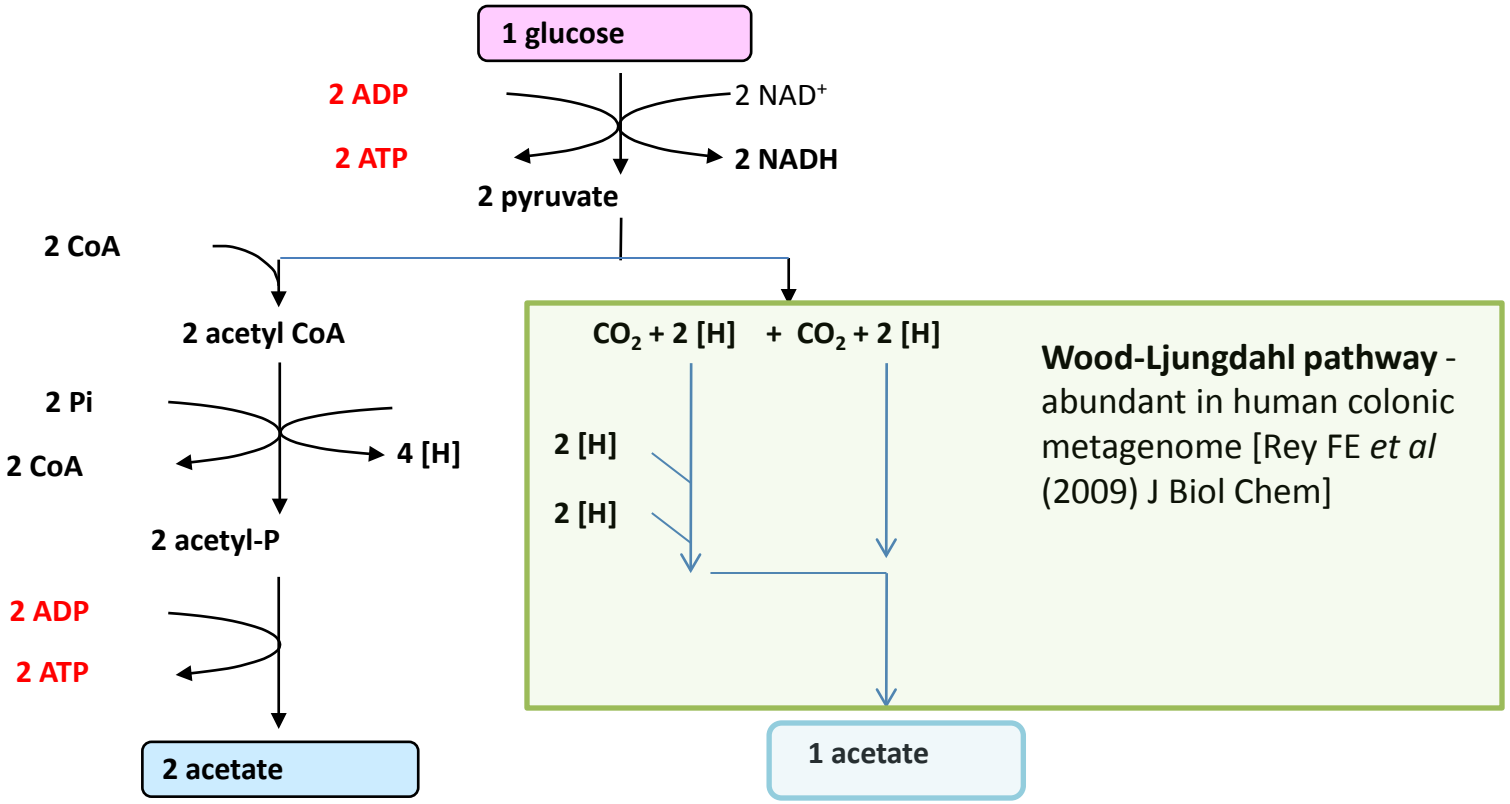
No hydrogen consumption

hydrogen consumption

Slide - courtesy of Petra Louis

Acetogenesis –

[1 glucose to 3 acetate]



eg. Blautia hydrogenotrophica

(formerly *Ruminococcus hydrogenotrophicus*) [Bernalier A *et al* (1996) Arch Microbiol]

Hydrogen utilizers in the human colon

Methanogens (eg. *Methanobrevibacter smithii* – Archaea)

Approx. 1 in 3 of people are methanogenic[1] (breath H_2):
CH_4 producers harbour $>10^8$ methanogens/ ml faeces[2]
CH_4 non-producers harbour 10^2-10^3/ ml faeces[2]

Acetogens (eg. *Blautia hydrogenotrophica* – Lachnospiraceae)

Acetogenesis can contribute 30% of acetate C ($^{13}CO_2$)[3].

Sulphate-reducers (eg, *Desulphovibrio piger* - Proteobacteria)

Possible association with colitis?

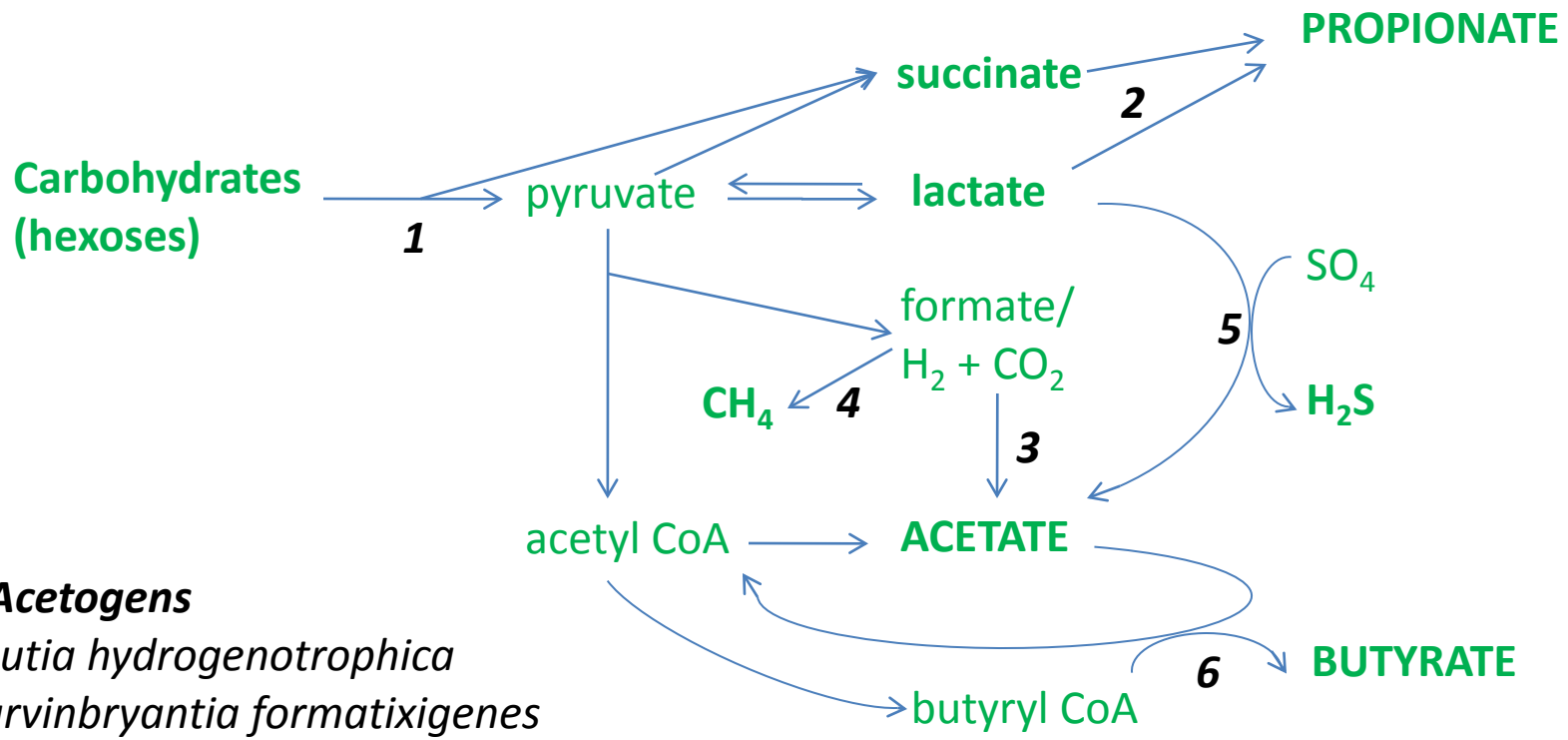
These three groups compete for hydrogen, but may also co-exist[2,4,5,6]

[[1]Levitt *et al.,* 2000, Am J. Gastr; [2]Dore *et al.,* 1995, FEMS ME; [3]Miller & Wolin 1996, AEM; [4]Bernalier *et al.,* 1996, FEMS ME; [5]Gibson *et al.,* 1993, FEMS ME; [6]Nava *et al.,* ISME J]

Cross-feeding in short chain fatty acid metabolism

1. Carbohydrate fermentors
widespread - Bacteroidetes, Firmicutes, Actinobacteria

2. Propionate producers
Bacteroidetes
Veillonellaceae



3. Acetogens
Blautia hydrogenotrophica
Marvinbryantia formatixigenes
(Firmicutes)

4. Methanogens
Methanobrevibacter smithii
(Archaea)

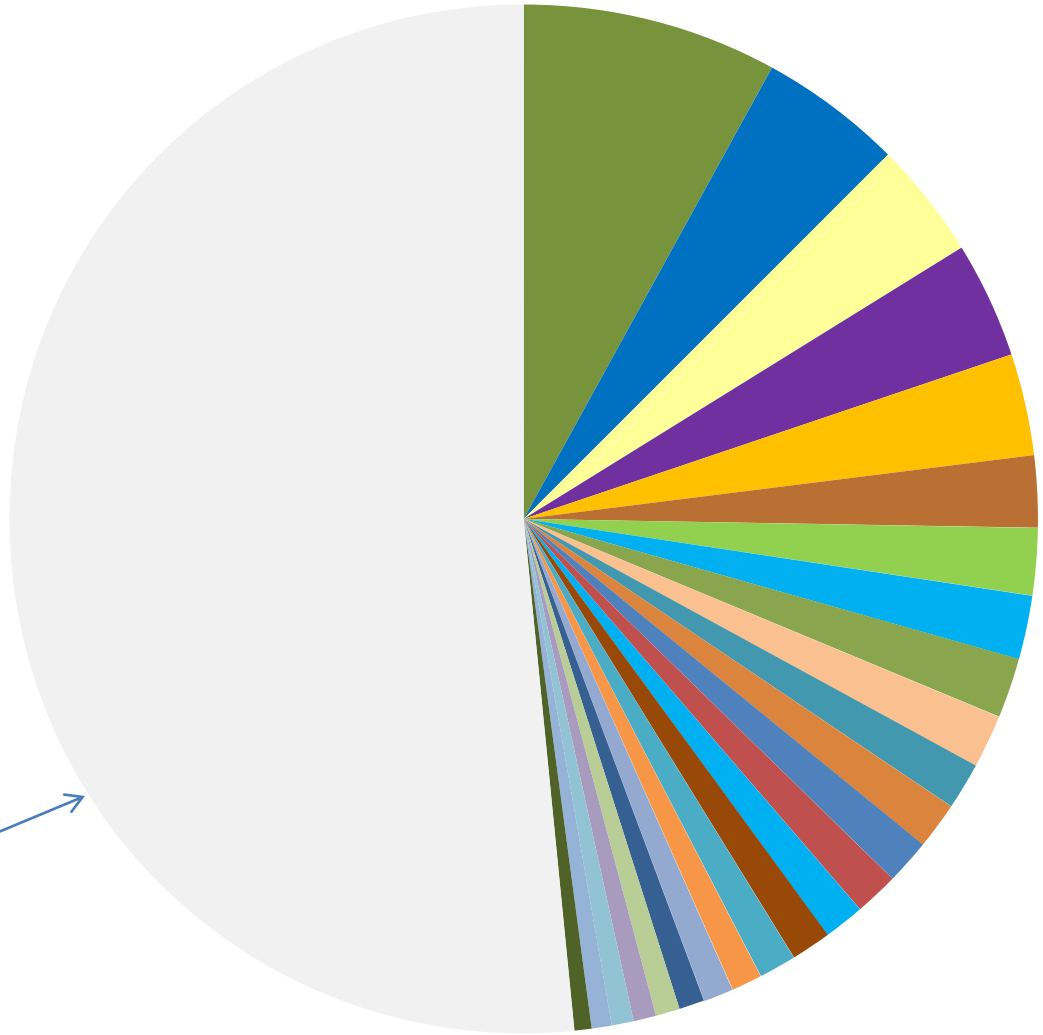
5. Sulfate reducers
Desulfovibrio piger
(Proteobacteria)

6. Butyrate producers
Faecalibacterium prausnitzii
Eubacterium rectale, Roseburia spp.
Eubacterium hallii, Anaerostipes spp.
(Firmicutes)

Dominant bacterial species

25 cultured species accounted for approximately 50% of 16S rRNA sequences :-

Walker AW *et al* ISME J (2011) - 26 faecal samples from 6 obese males

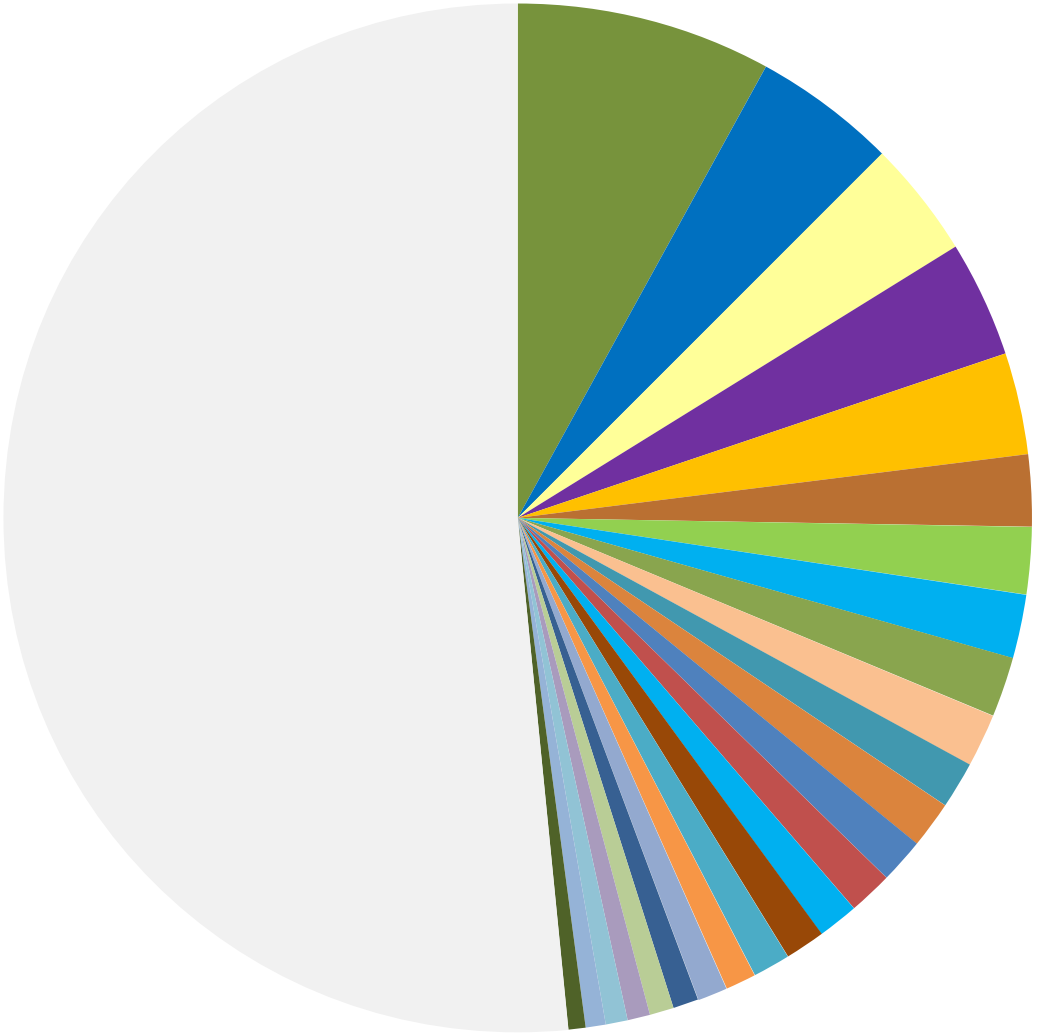


295 other phylotypes (72% uncultured)

(from - Flint HJ *et al* Gut Microbes, 2012)

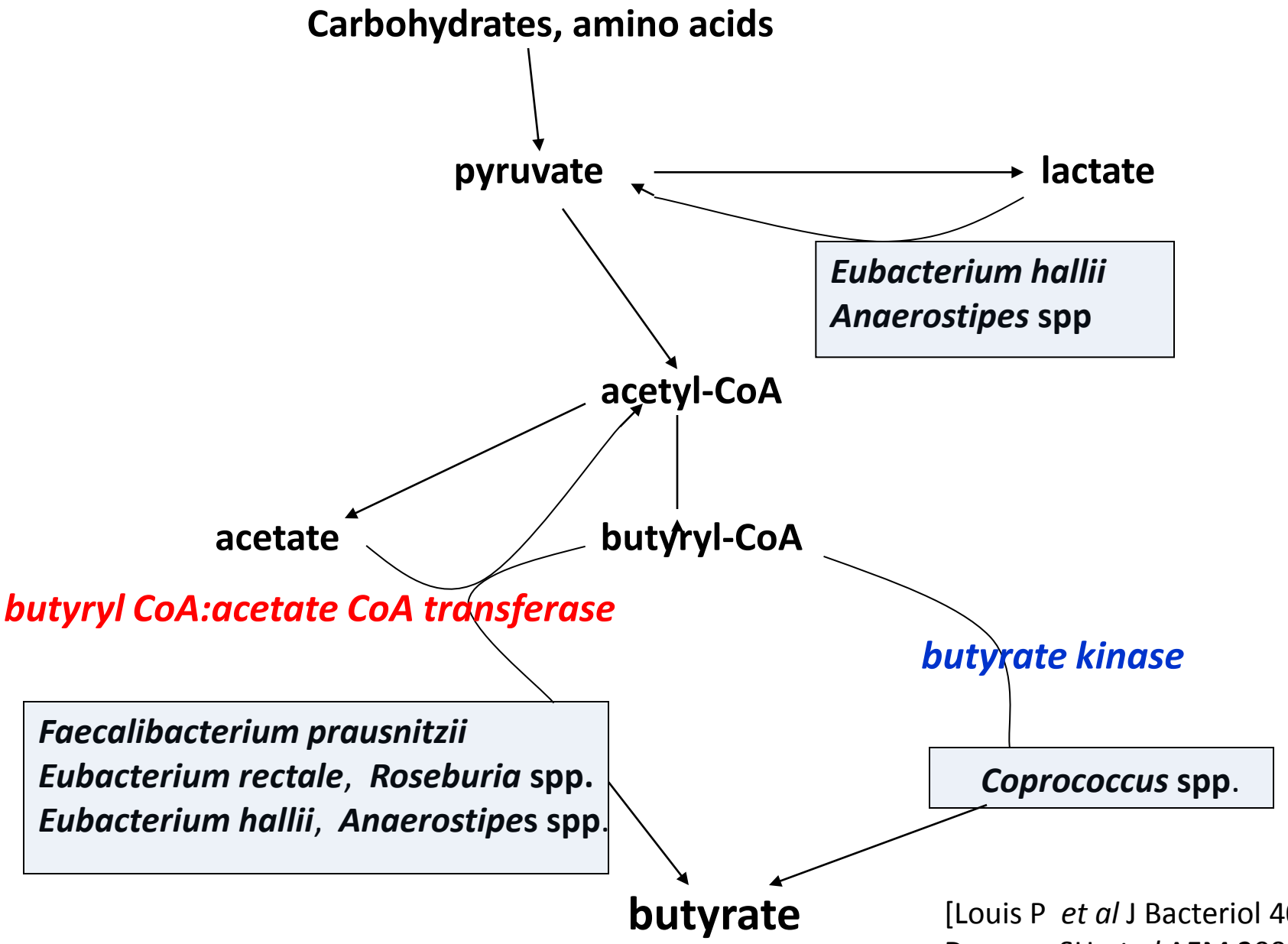
Dominant bacterial species – butyrate-producers

Walker AW *et al* ISME J (2011) - 26 faecal samples from 6 obese males



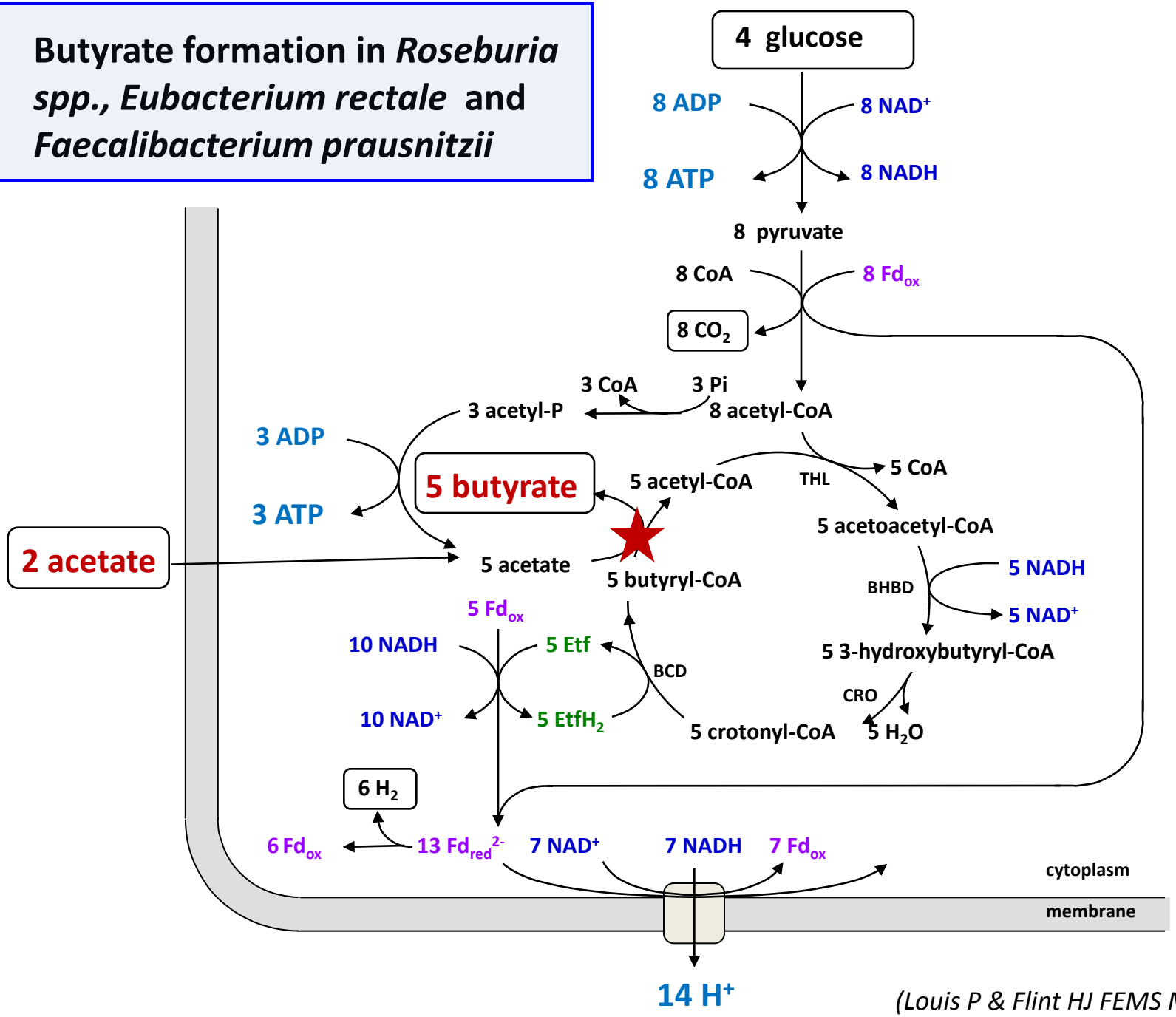
- Faecalibacterium prausnitzii
- Eubacterium rectale
- Colinsella aerofaciens
- Clostridium clostridioforme
- Bacteroides vulgatus
- Anaerostipes hadrus
- Ruminococcus bromii
- Eubacterium hallii
- Blautia wexleri
- Bacteroides dorei
- Roseburia faecis
- Dorea longicatena
- Subdoligranulum variabile
- Bacteroides uniformis
- Ruminococcus obeum
- Bacteroides ovatus
- Blautia luti
- Parabacteroides distasonis
- sp nov A2-166
- sp nov SR1/5
- Lachnospira pectinoschiza
- sp nov 80/3
- Dialister invisus
- Roseburia inulinivorans
- Ruminococcus callidus
- others

Butyrate metabolism



[Louis P *et al* J Bacteriol 4004
Duncan SH *et al* AEM 2004]

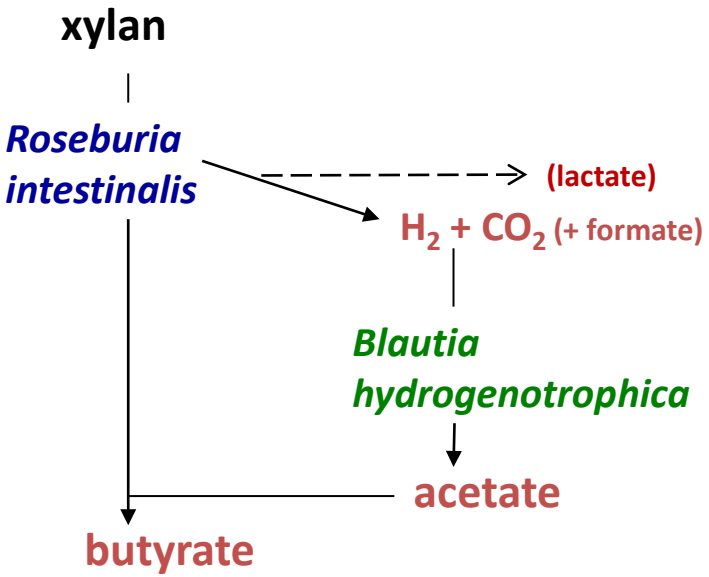
Butyrate formation in *Roseburia spp., Eubacterium rectale* and *Faecalibacterium prausnitzii*

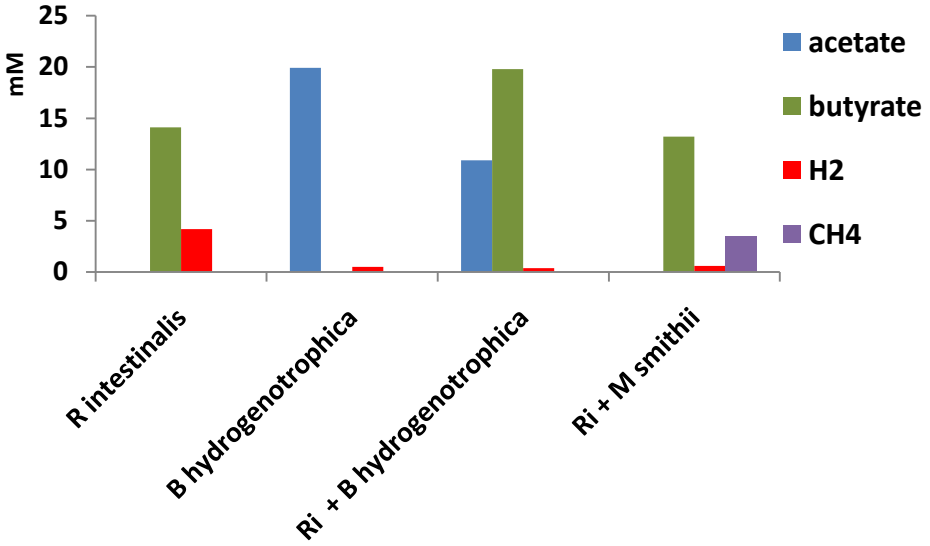


(Louis P & Flint HJ FEMS ML 2009)

Co-culture with an acetogen

Chassard *et al.*, 2006 FEMS ML





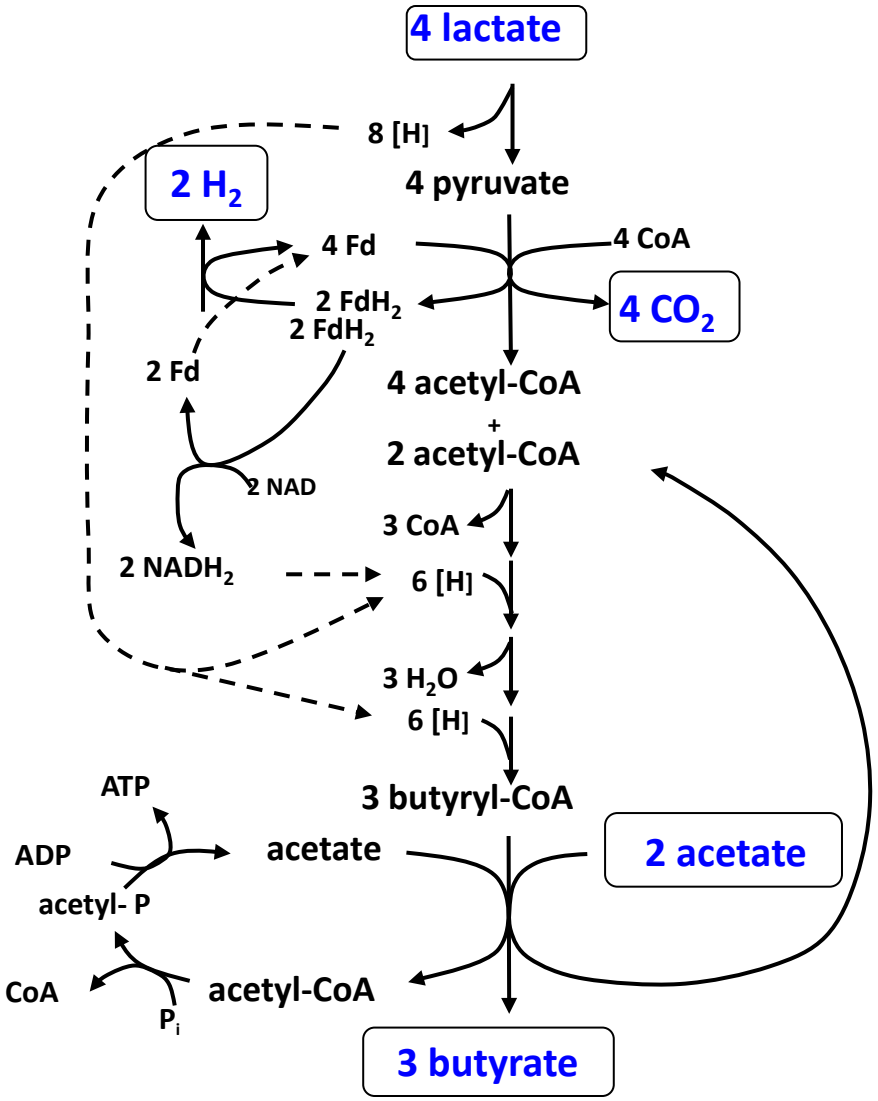
Co-culture with acetogen ↓ H_2 ↑ acetate ↑ butyrate

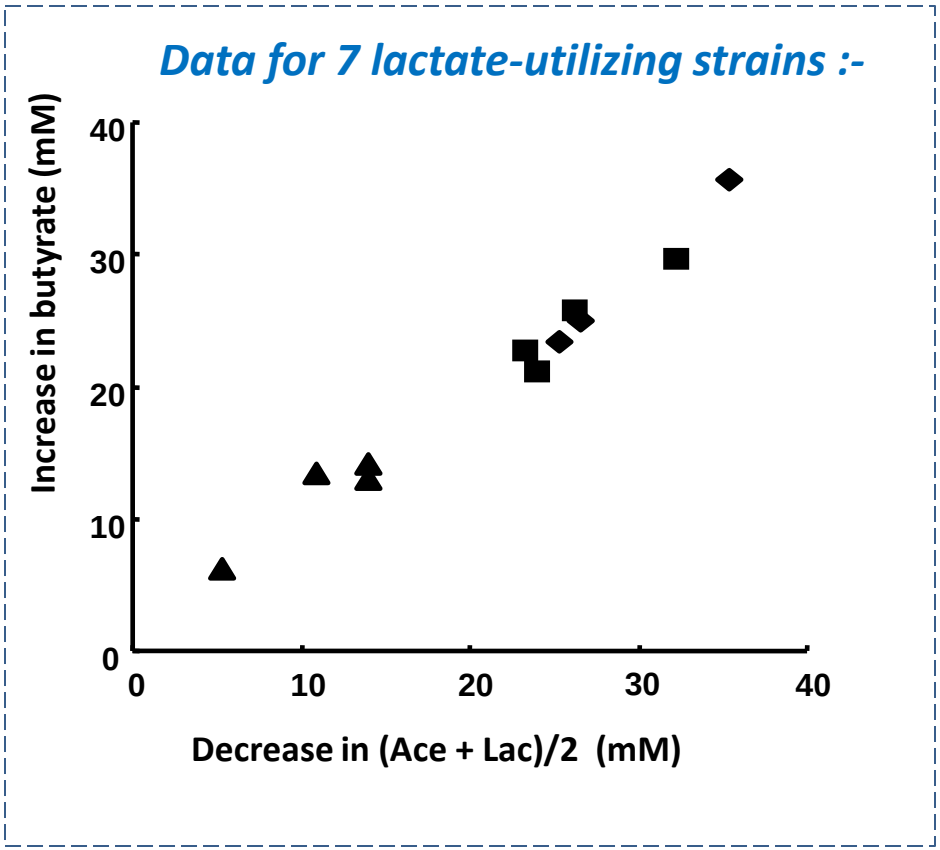
Co-culture with methanogen ↓ H_2 ↑ CH_4

Consequences for energy harvest in the complete ecosystem?

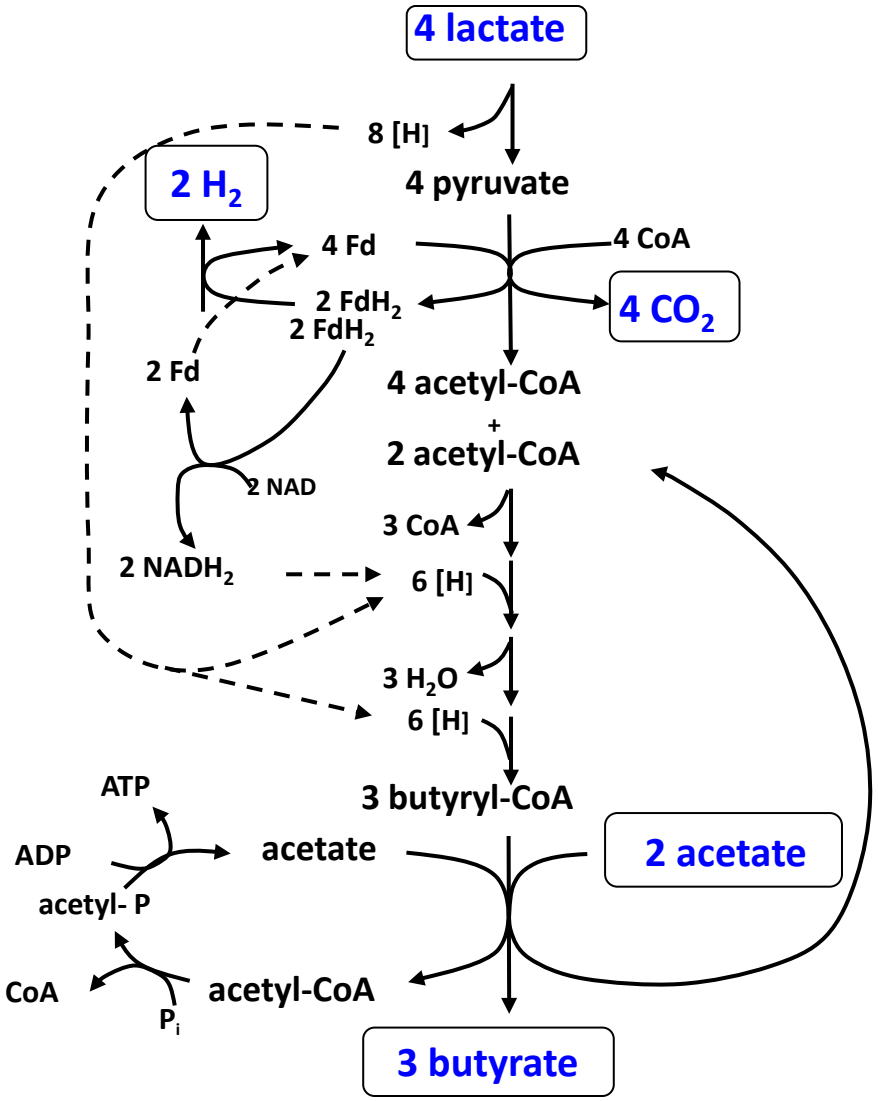
[nb. methanogens can coexist with acetogens, but may also slow gut transit]

Butyrate formation from lactate and acetate by *Eubacterium hallii and Anaerostipes spp.* [Duncan *et al* 2004 AEM 70, 5810-5817]

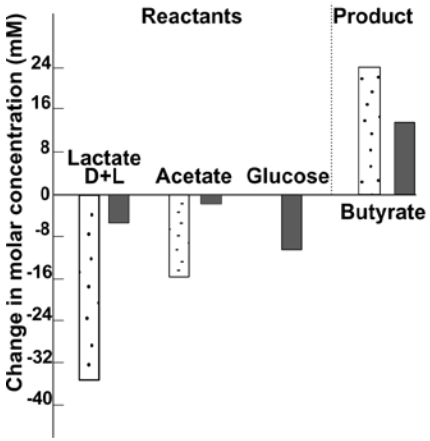




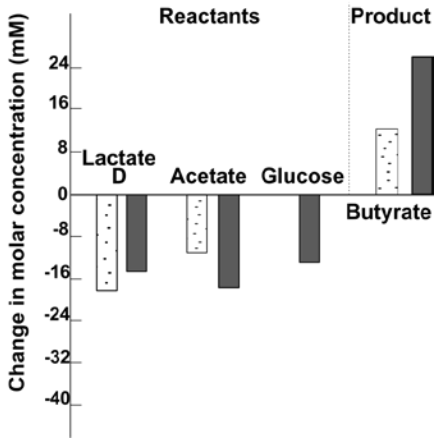
Butyrate formation from lactate and acetate by *Eubacterium hallii* and *Anaerostipes* spp. [Duncan *et al* 2004 AEM 70, 5810-5817]



E. hallii L2-7



A. hadrus SS2/1



[Munoz-Tamayo R *et al* 2011, FEMS Microbiol Ecol]

- *E. hallii* L2-7 uses both D- and L– lactate.
- *A. hadrus* SS2/1 uses only D- lactate*.

- *E. hallii* uses less lactate when glucose present.
- *A. hadrus* less affected by glucose.

* D- lactate is a neurotoxin at high concentrations

Interactions between lactate-utilizing bacteria

Bifidobacterium adolescentis

↓

L- lactate + acetate

↓

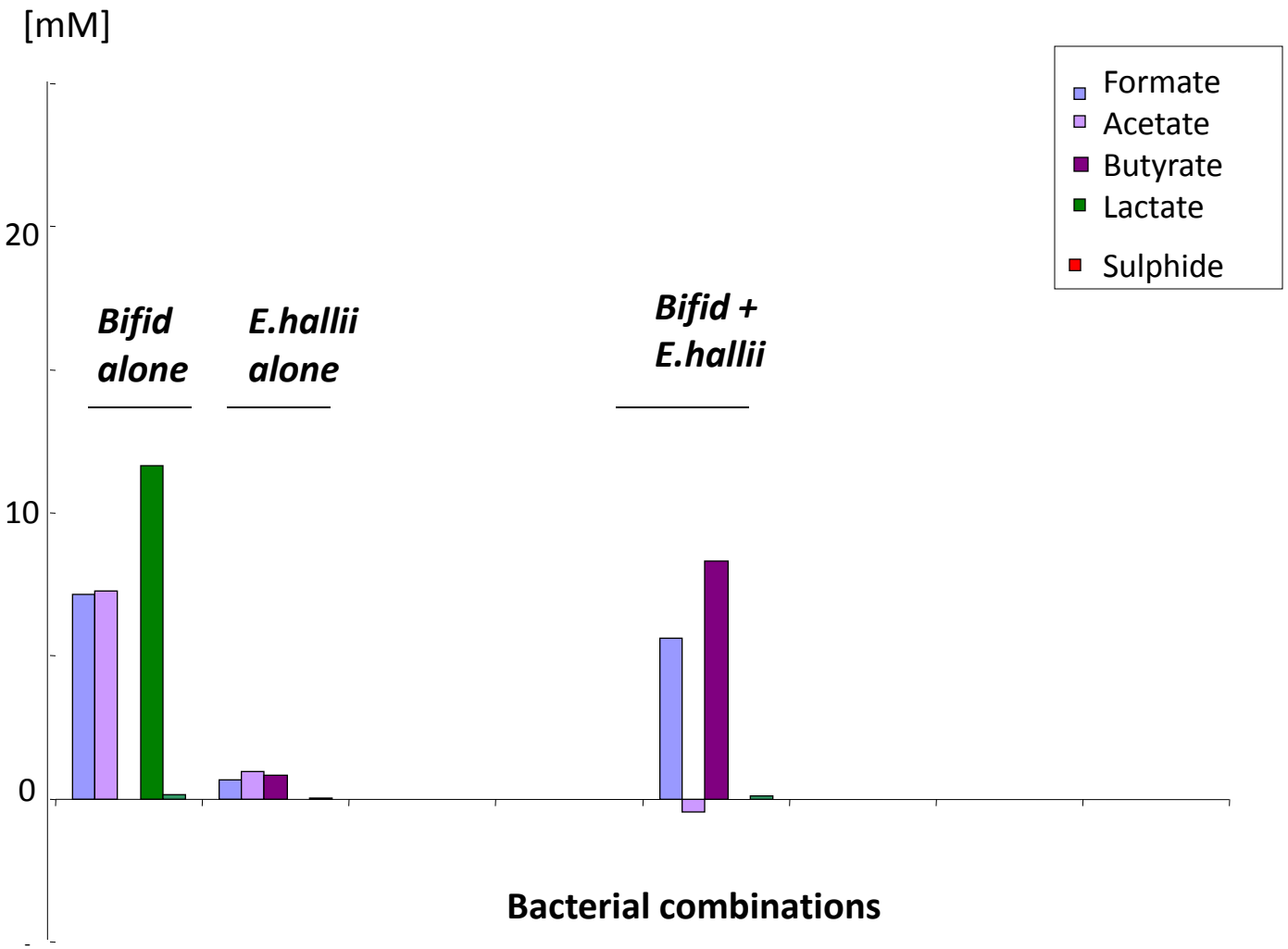
Eubacterium hallii

↓

butyrate

(beneficial)

[**Starch** supplied as energy source]



Interactions between lactate-utilizing bacteria

Bifidobacterium adolescentis

↓

L- lactate + sulphate

↓

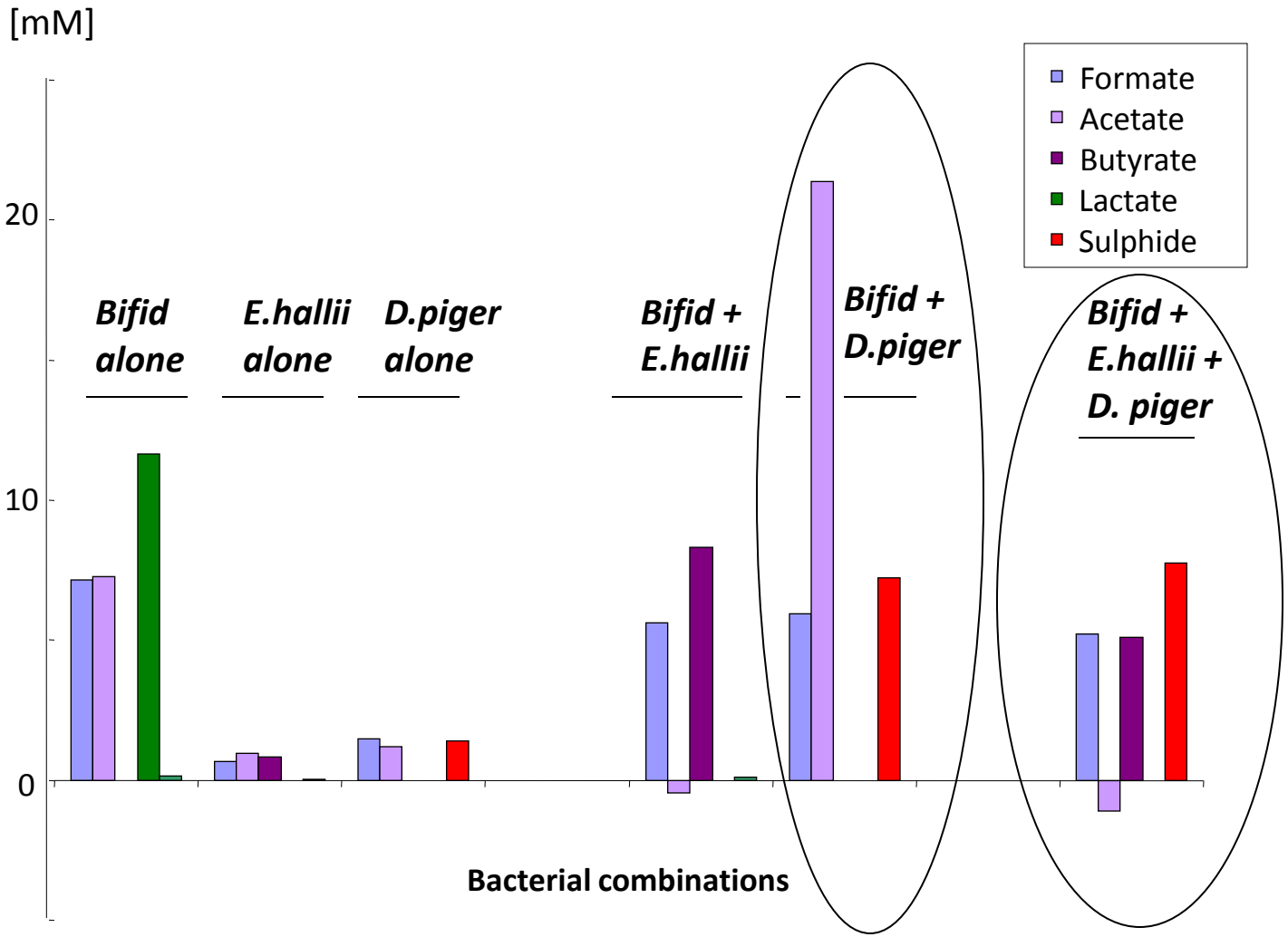
Desulfovibrio piger

↓

acetate + H_2S

(toxic)

[Starch supplied as energy source]

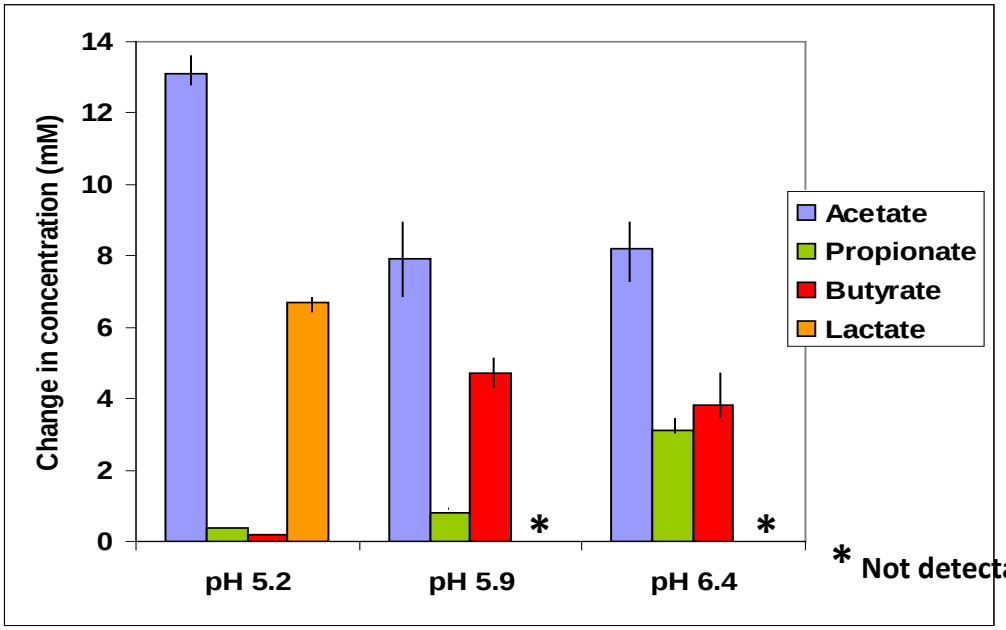


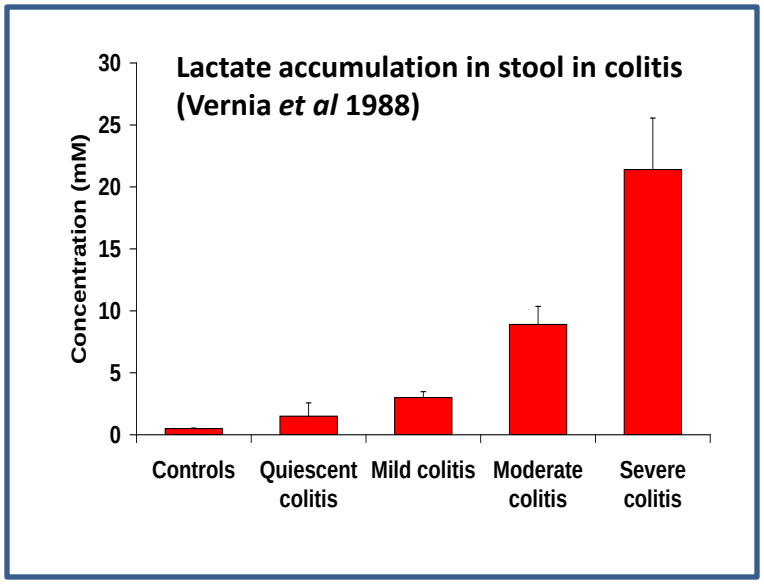
(Marquet P et al FEMS ML 2009)

pH-induced dysbiosis *in vitro* - fermentation patterns

+ polysaccharides →

[means of 24h incubations with four different faecal inocula]





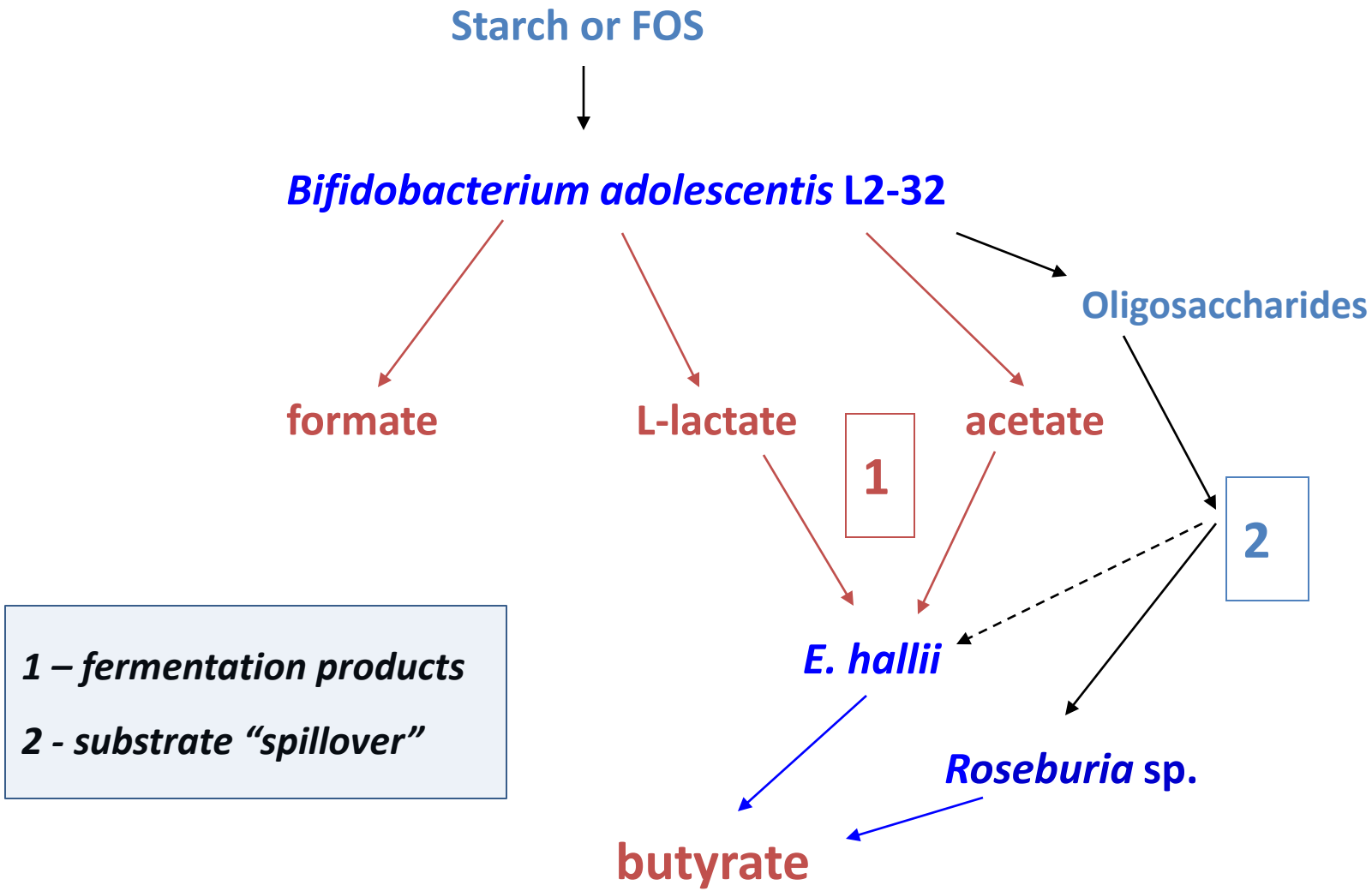
Lactate accumulation shown to be due mainly to reduced lactate utilization by other bacteria at pH 5.2 (^{13}C lactate)

[Belenguer *et al* AEM 2007; also Walker *et al* AEM 2005, Duncan *et al* EM 2009]

Conclusions

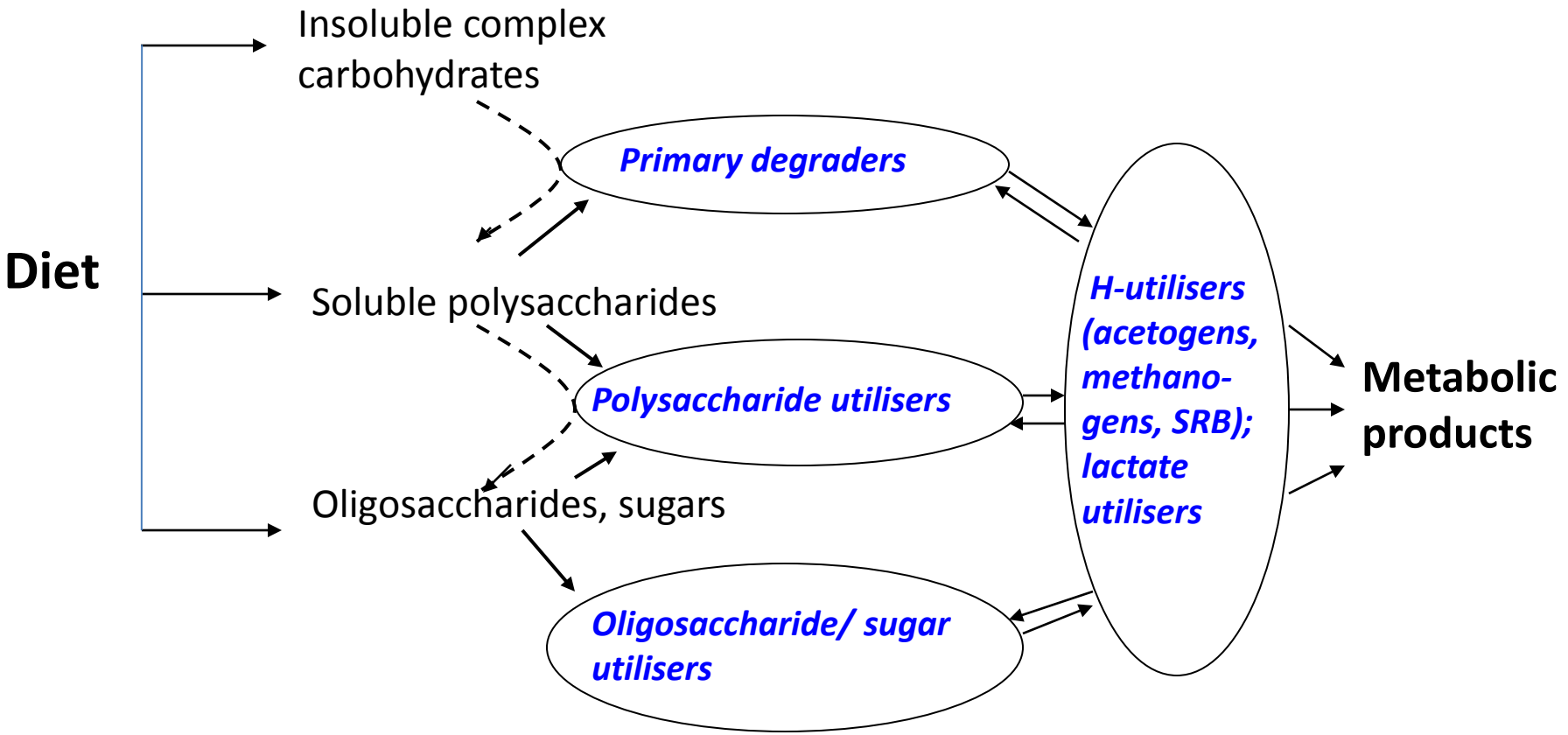
- Cross-feeding must be assumed to occur widely in any complex microbial community
- A few syntrophs may not be capable of isolation in pure culture
- Hydrogen consumption can profoundly affect the energetics and metabolism of hydrogen-producing organisms, and therefore also community composition and metabolic outputs
- Cross-feeding of acetate, lactate and succinate plays a major role in short chain fatty acid production
- Cross-feeding of fermentation products can be expected to broaden the impact of probiotics and prebiotics on the gut microbiota

Two distinct mechanisms of cross-feeding can be involved in stimulating butyrate formation in cocultures*



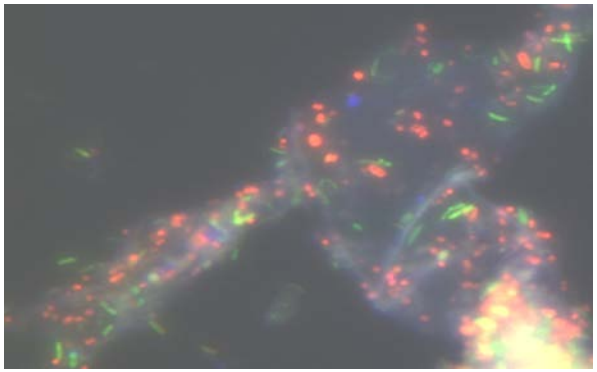
[* Belenguer A *et al.,* AEM 2006 - also Falony G *et al.,* AEM 2006)

Cross-feeding from the breakdown of complex carbohydrate substrates

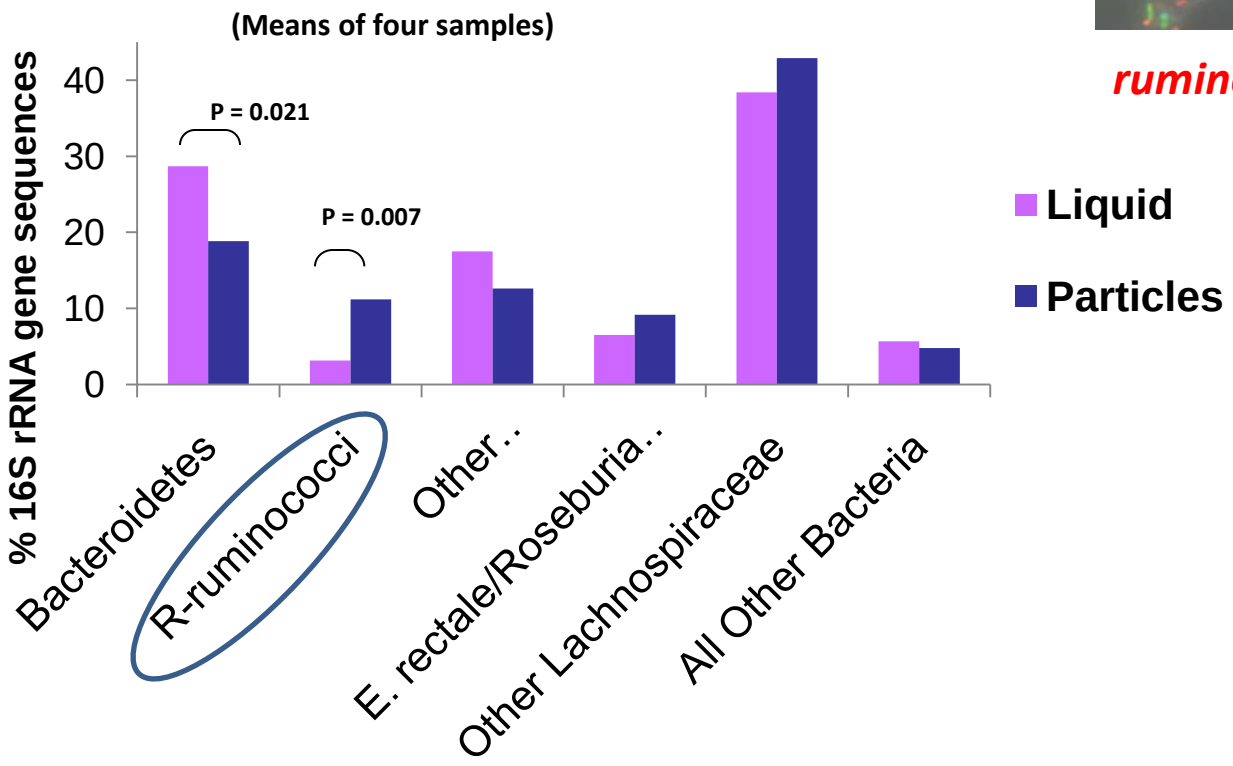


(Flint HJ *et al* Env Micro 2007)

Partitioning of bacterial 16S rRNA sequences between liquid and particulate fractions of human faecal samples



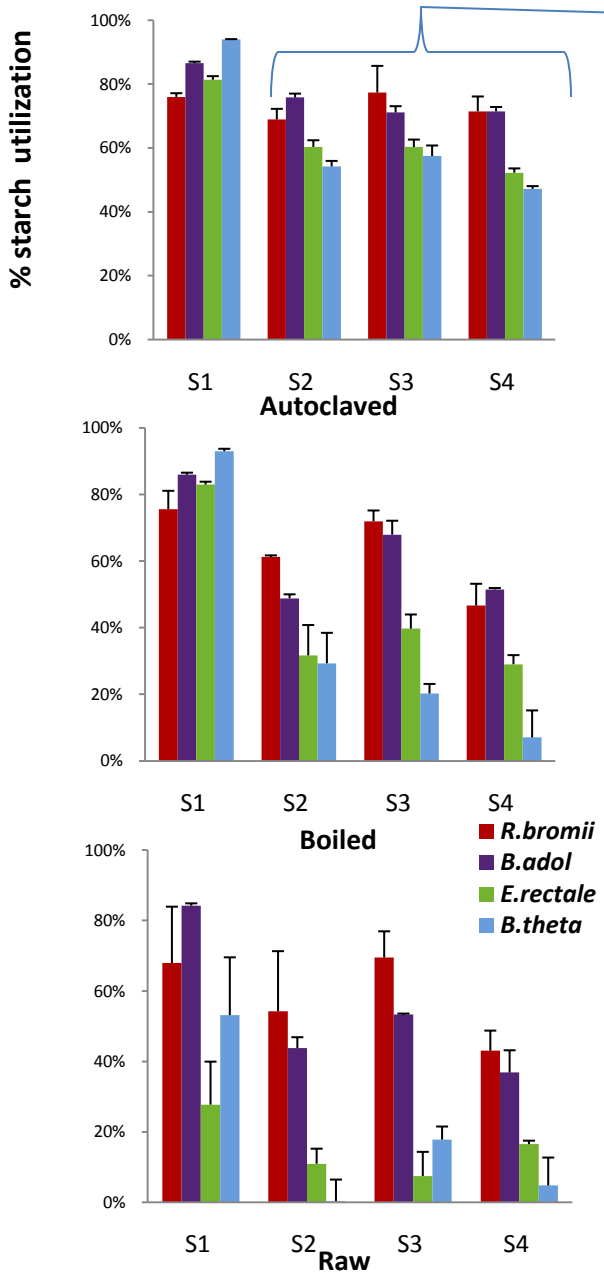
ruminococci *on faecal fibre*



- **R-ruminococci are preferentially associated with fibre particles in stool samples.**
- **Bacteroidetes partition more into the liquid phase**

[Walker AW *et al* Env Microbiol (2008)]

Degradation of four types of corn starch by four amylolytic human gut bacteria



Resistant starches (RS2 – S2,S3; RS3 – S4)

Ruminococcus bromii **(Firmicutes)**

Bifidobacterium adolescentis **(Actinobacteria)**

Eubacterium rectale **(Firmicutes)**

Gram-positive

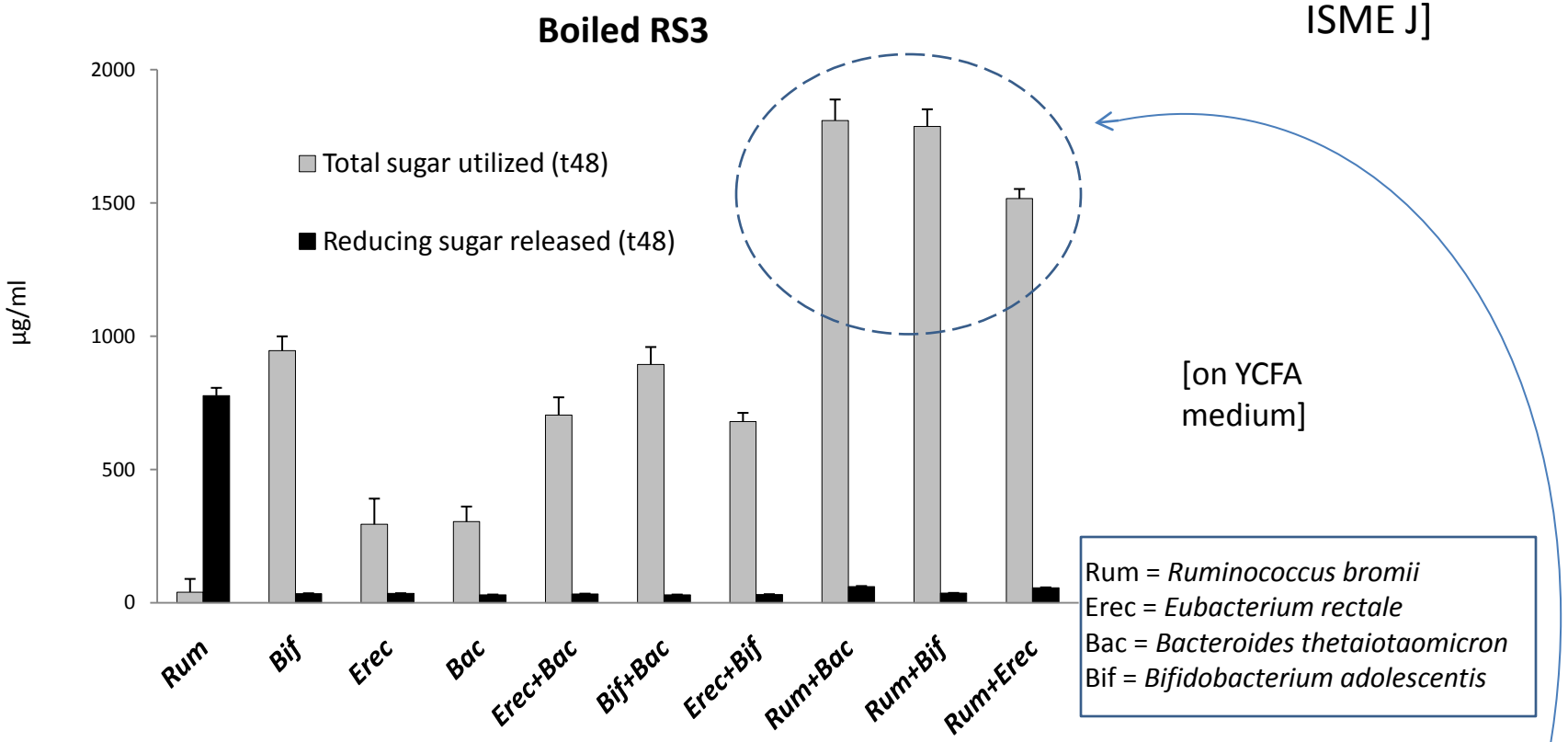
Bacteroides thetaiotaomicron **(Bacteroidetes)**

Gram-negative

[Ze X *et al* 2012 ISME J]

Stimulation of starch utilization *in vitro* by co-incubation with *R. bromii* L2-63

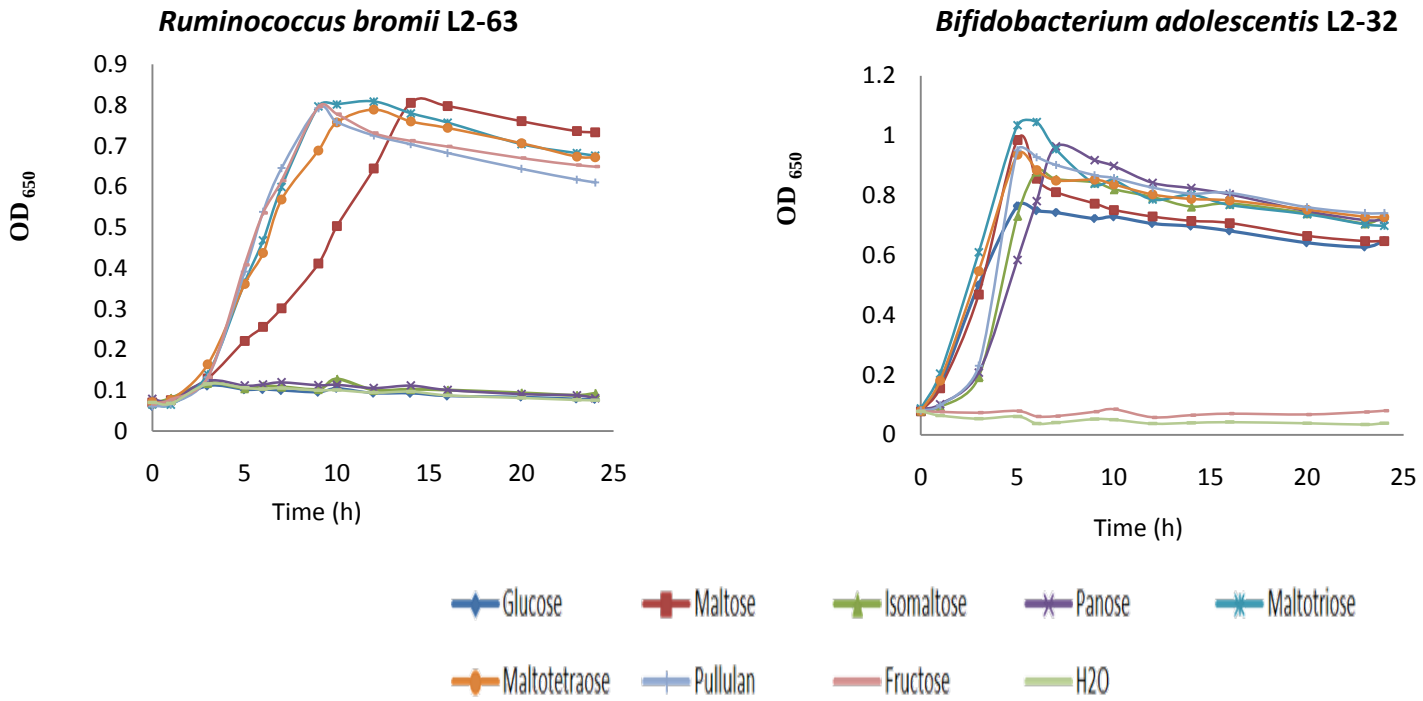
[Ze X *et al* 2012 ISME J]

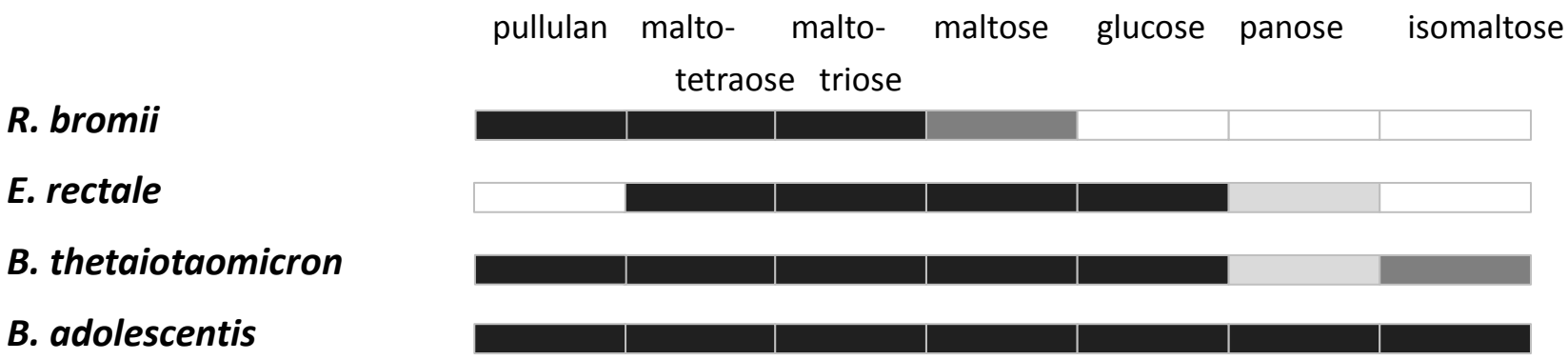


R. bromii (Rum) is unable to grow on YCFA medium, but its enzymes still degrade RS, mainly to maltose and glucose (reducing sugars).

As a result, starch utilization by the other three bacteria is greatly stimulated in co-culture with *R. bromii*.

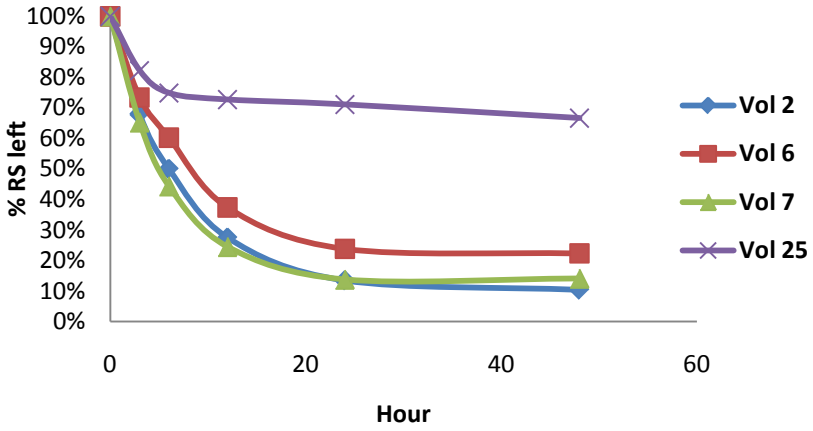
Growth on starch breakdown products -



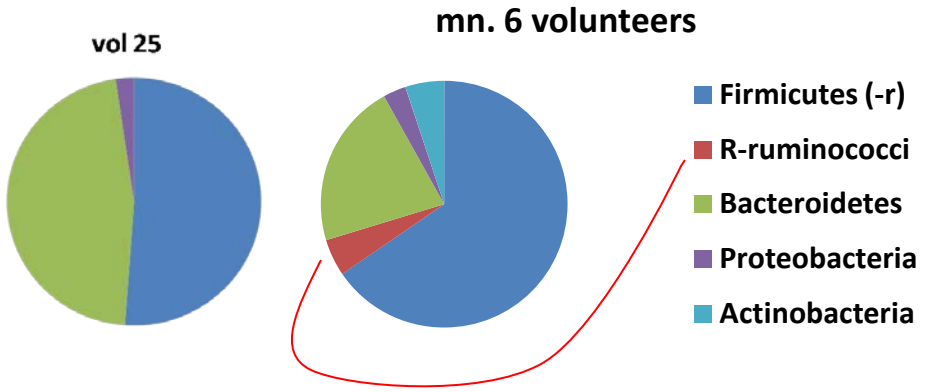


"Keystone" role of *Ruminococcus bromii* in the fermentation of resistant starch

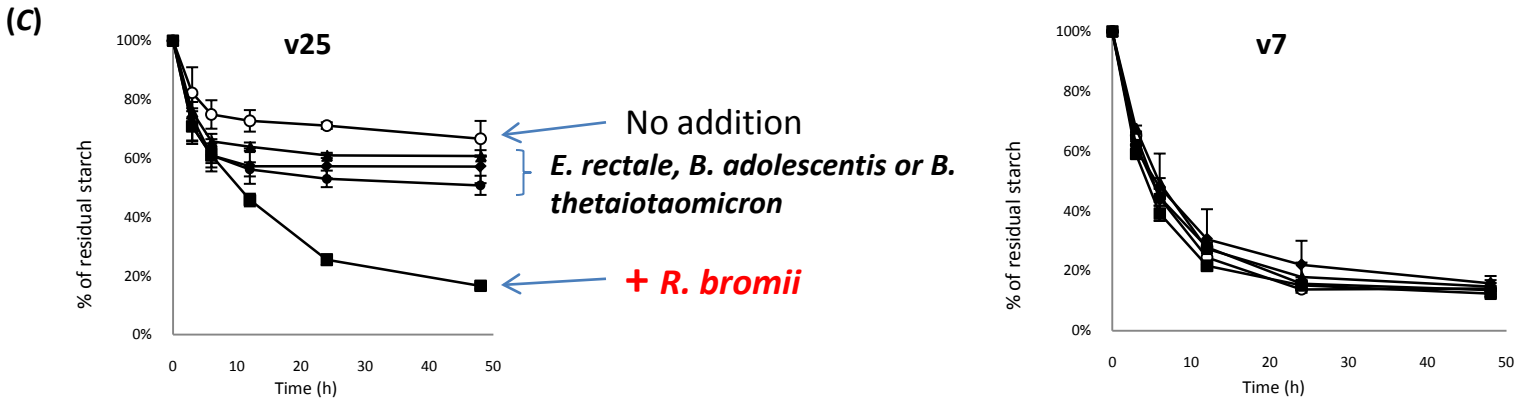
Utilization of Type 3 RS by human faecal bacteria *in vitro* :-



Bacterial community profiles (16S rRNA sequencing)



Effect of adding amylolytic bacteria to faecal incubations *in vitro* :-

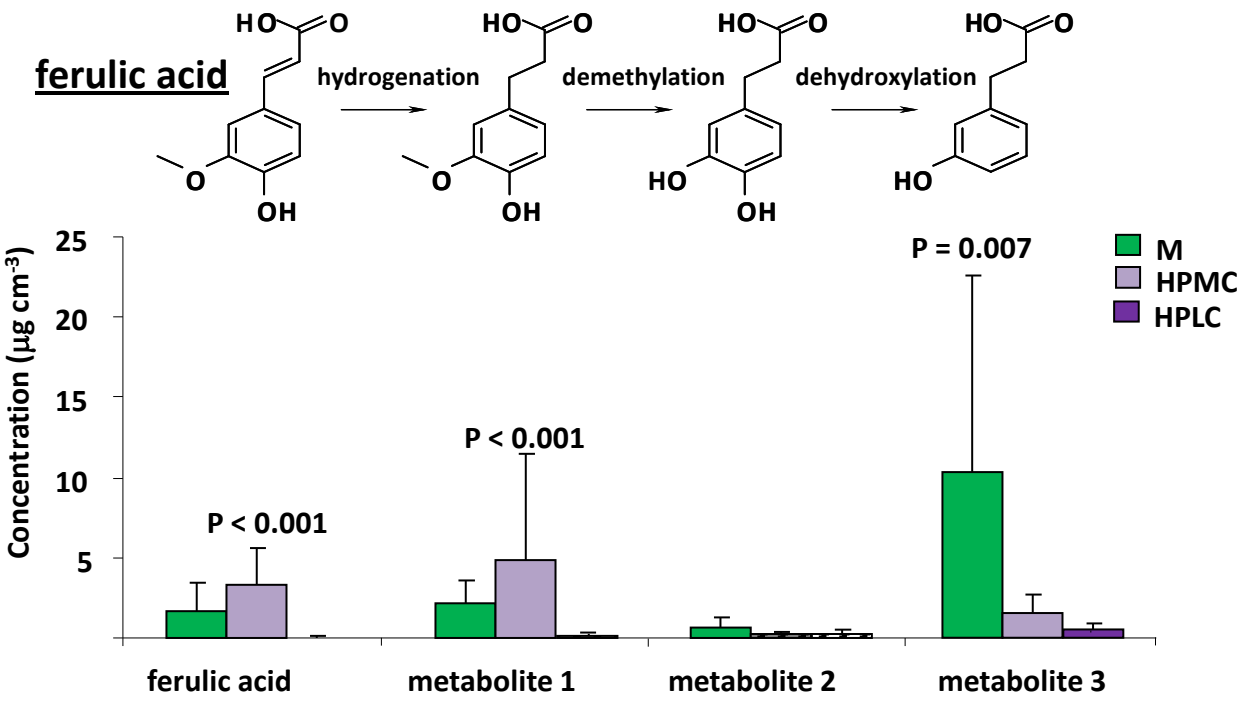


[Ze X *et al* ISME J 2012; Walker AW *et al* ISME J 2011]

Microbial conversion of plant-derived phenolics – 'community pathway' ?

Faecal concentrations
(human dietary study)

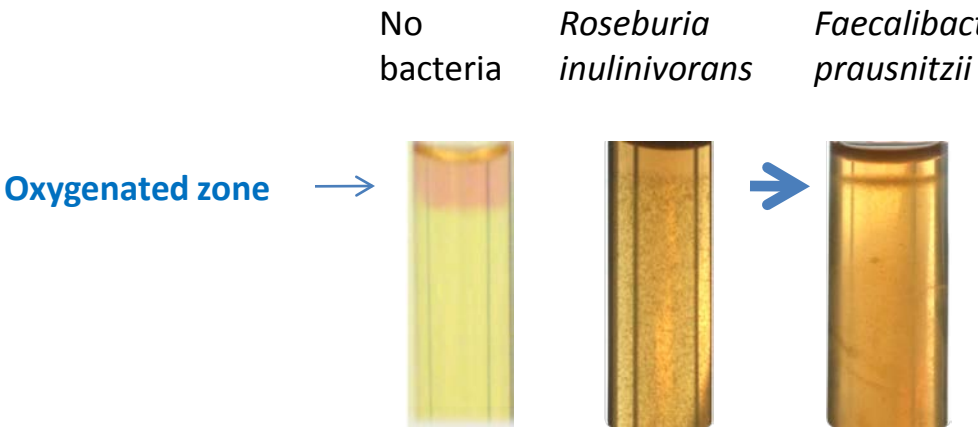
[Russell WR *et al* AJCN 2011]

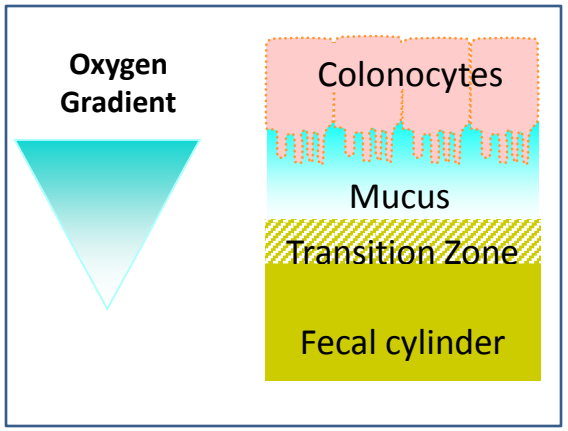


Cross-feeding of growth factors & vitamins

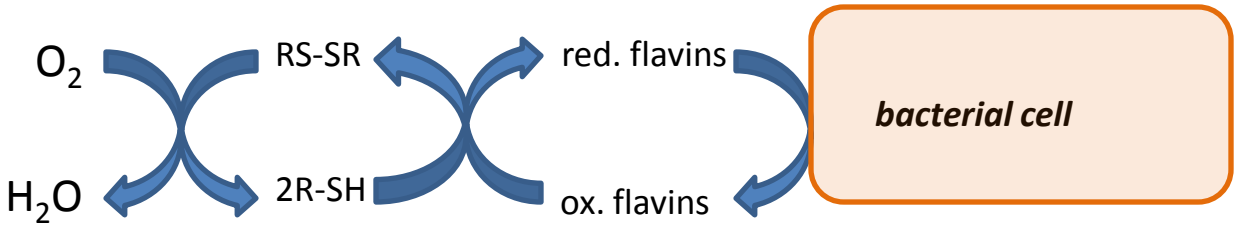
External supply of redox reactants (eg. flavins) ?

★ **MT Khan *et al* The gut anaerobe *Faecalibacterium prausnitzii* uses an extracellular electron shuttle to grow at oxic-anoxic interphases. *ISME J* (2012)**





Dependent on thiols (eg. glutathione) + redox reagent (eg. riboflavin)



Networks of association – suggested from metagenomic output.

But do they indicate - inter-species interactions?

- similar responses to gut environment/diet/host?

Further conclusions

For some recalcitrant substrates (eg. plant cell walls, mucin, resistant starch) specialised 'keystone' species facilitate utilization by the rest of the community

'Cross-feeding' can refer to almost any interaction whereby a product of one organism affects the growth and metabolism of another member of the microbial community

The future - is highly interactive!

We already need theoretical modelling to predict the outcomes of such interactions in complex communities

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