

Impact of diet upon intestinal microbiota and microbial metabolites

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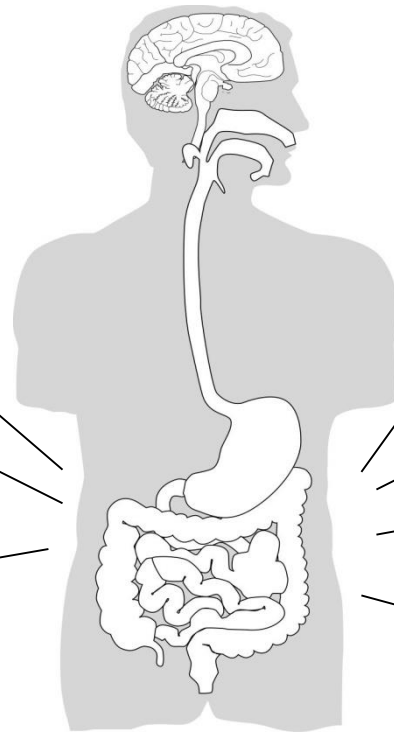


Impact of the gut microbiota on human nutrition and health

metabolism of non-digestible dietary components

supply of additional energy, vitamins

release of phytochemicals, enterohepatic circulation of xenobiotics, drugs



modification of host secretions (mucin, bile, gut receptors..)

immune function, inflammation

pathogenesis

barrier function

Gut function, gut disorders:

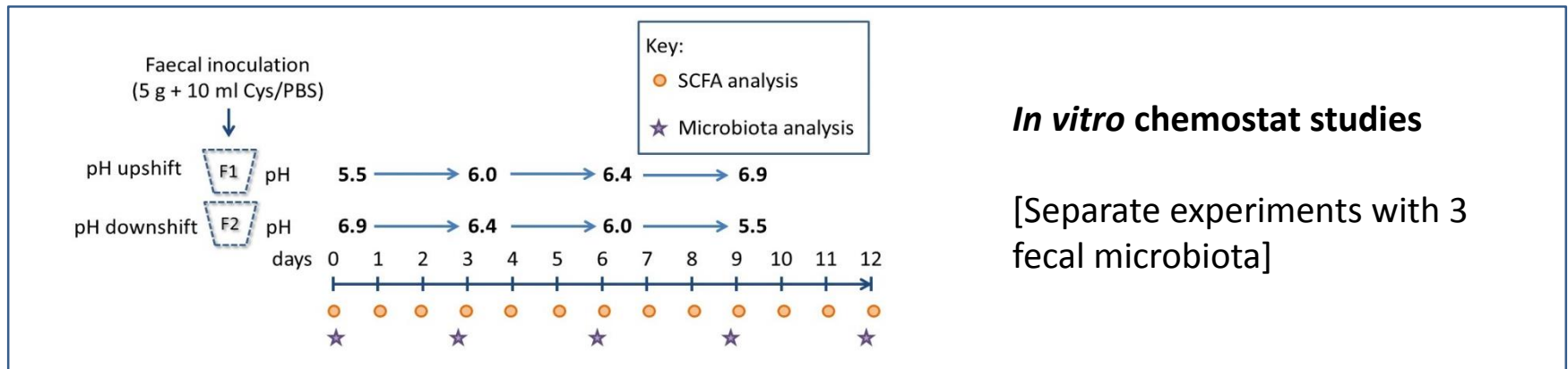
Colitis; Irritable bowel syndrome
Colorectal cancer
Infections

Systemic effects:

Energy supply, satiety
Diabetes
Heart disease
Autoimmune disorders

Modulation of the human gut microbiota by dietary fibres occurs at the species level

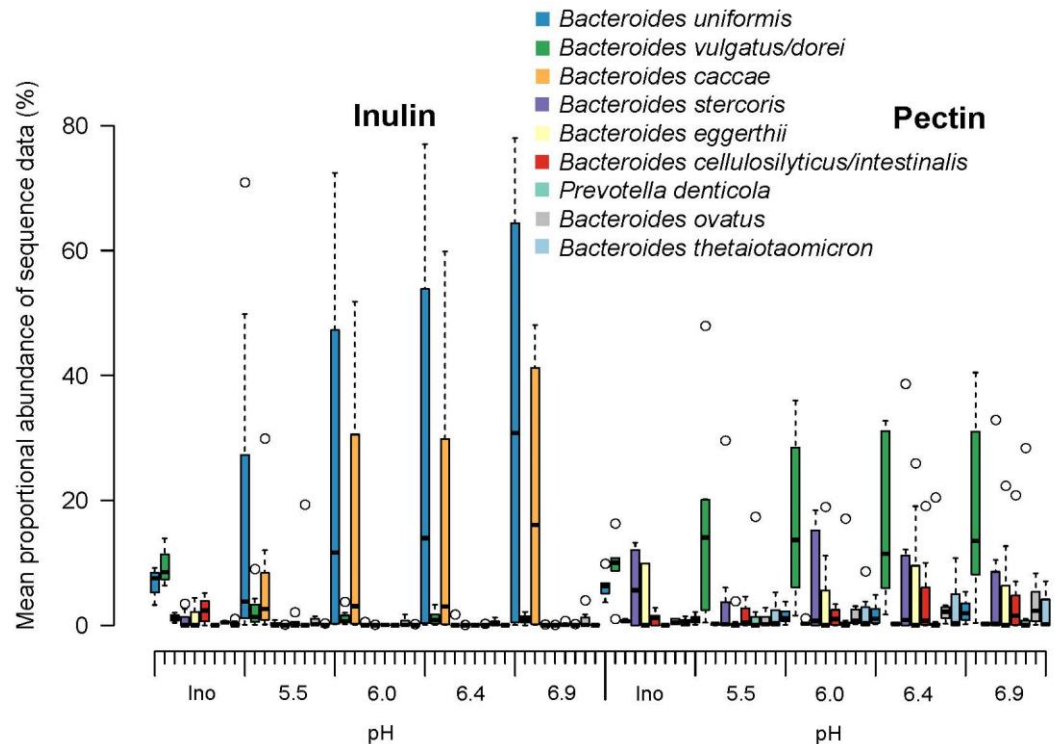
[Chung WSF *et al* BMC Biology, 2016]



In vitro chemostat studies

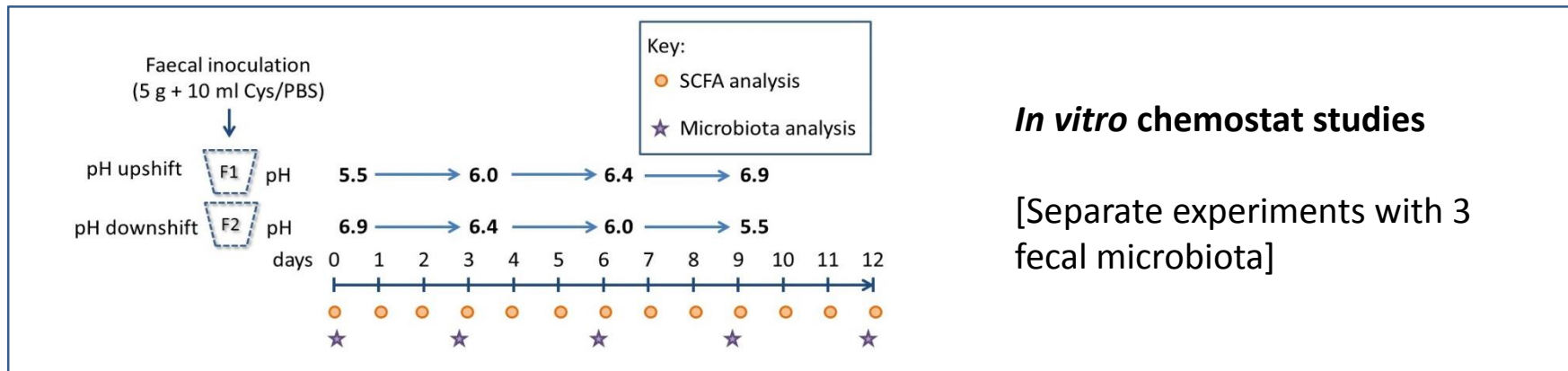
[Separate experiments with 3 fecal microbiota]

Bacteroides spp.



Modulation of the human gut microbiota by dietary fibres occurs at the species level

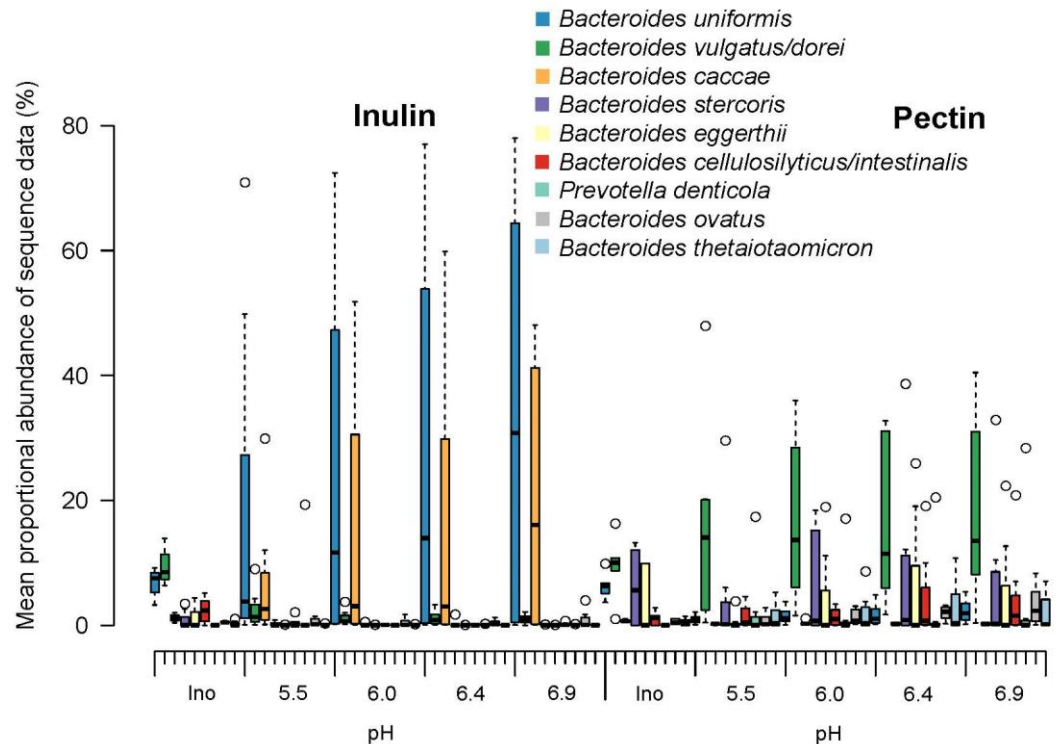
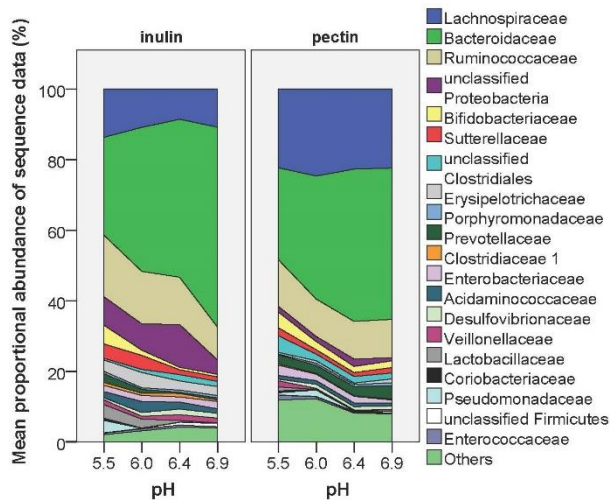
[Chung WSF *et al* BMC Biology, 2016]



In vitro chemostat studies

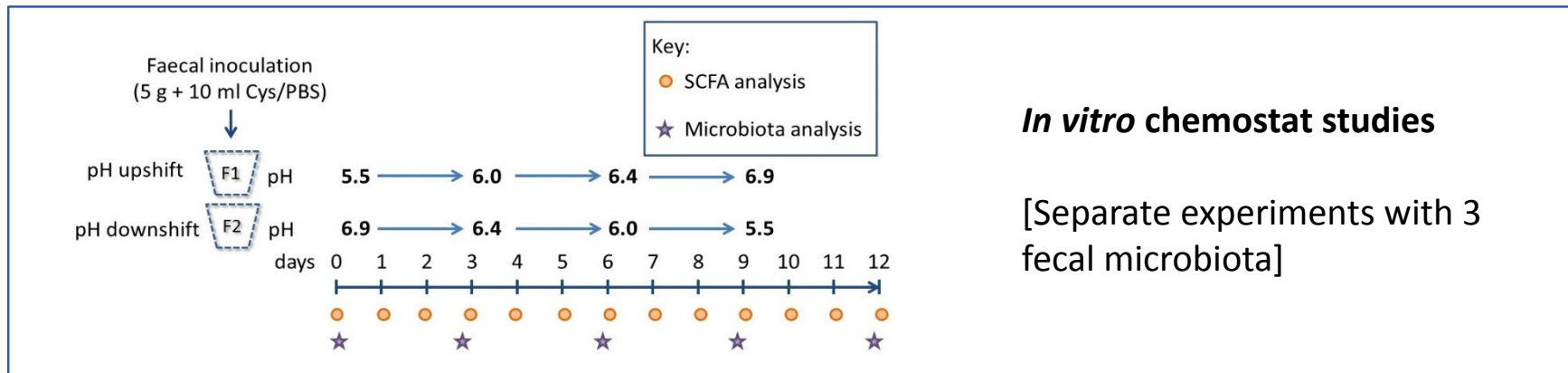
[Separate experiments with 3 fecal microbiota]

Microbiota composition (family level)



Modulation of the human gut microbiota by dietary fibres occurs at the species level

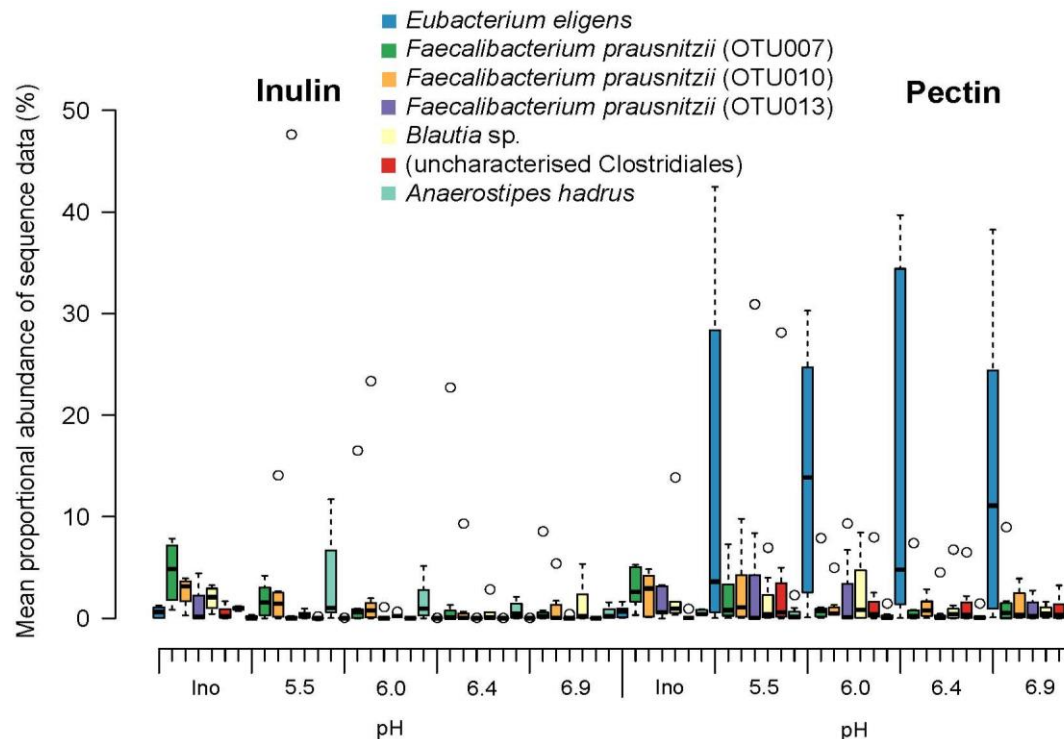
[Chung WSF *et al* BMC Biology, 2016]



In vitro chemostat studies

[Separate experiments with 3 fecal microbiota]

Firmicutes



Impact of dietary non-digestible carbohydrates *in vivo*

14 obese male volunteers with metabolic syndrome

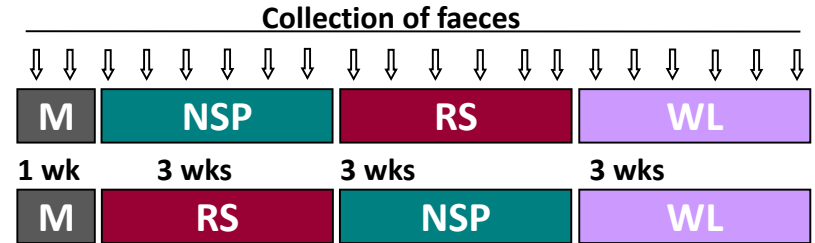
(mean age 54 years, mean BMI 39.4 kg/m²)

M: maintenance diet

NSP: high non-starch polysaccharide, low RS

RS: high resistant starch, low NSP

WL: high protein, moderate carbohydrate



Mean dietary intake [g/d]:

Diet	CHO	starch	RS	NSP	protein	fat
M	427	230	5	28	103	126
NSP	427	138	2	42	102	136
RS	434	275	26	13	109	127
WL	201	110	3	22	144	63

Weight maintenance

Weight loss

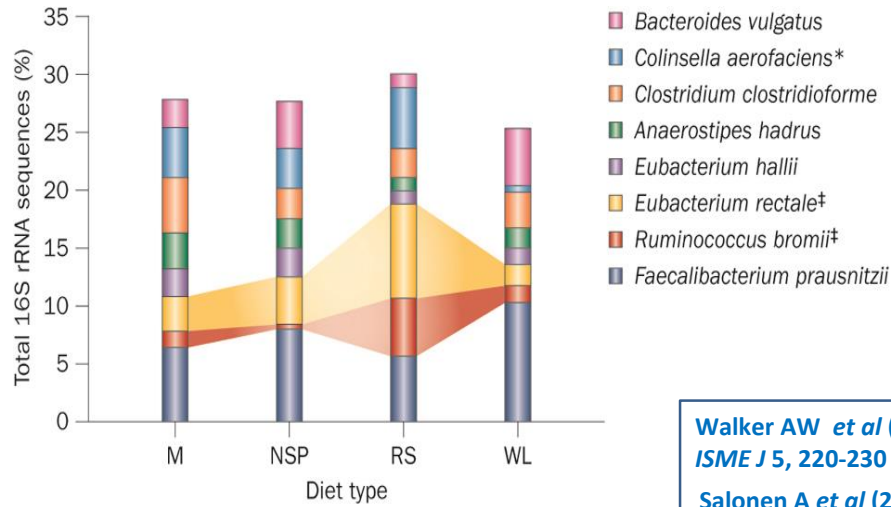
CHO: carbohydrate

added wheat bran

added type 3 resistant starch

★ Some but not all species are diet-responsive

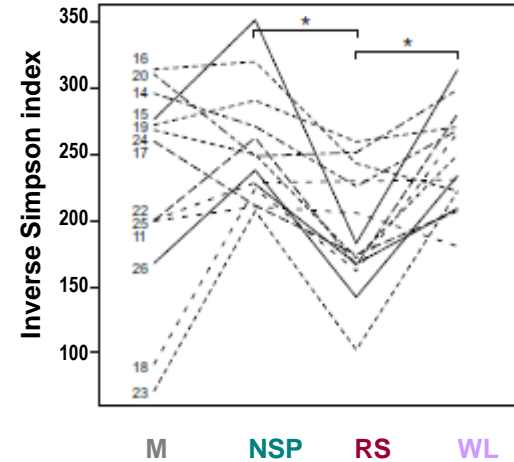
[16S rRNA gene sequences]



Walker AW *et al* (2011)
ISME J 5, 220-230

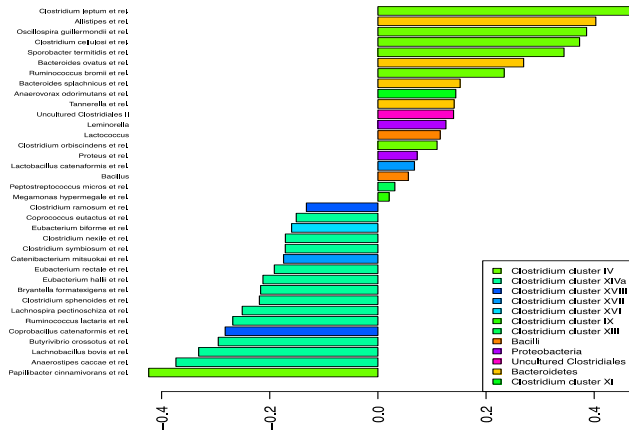
Salonen A *et al* (2014)
ISME J 8, 2218-2230

★ Lower diversity with RS than WB NSP



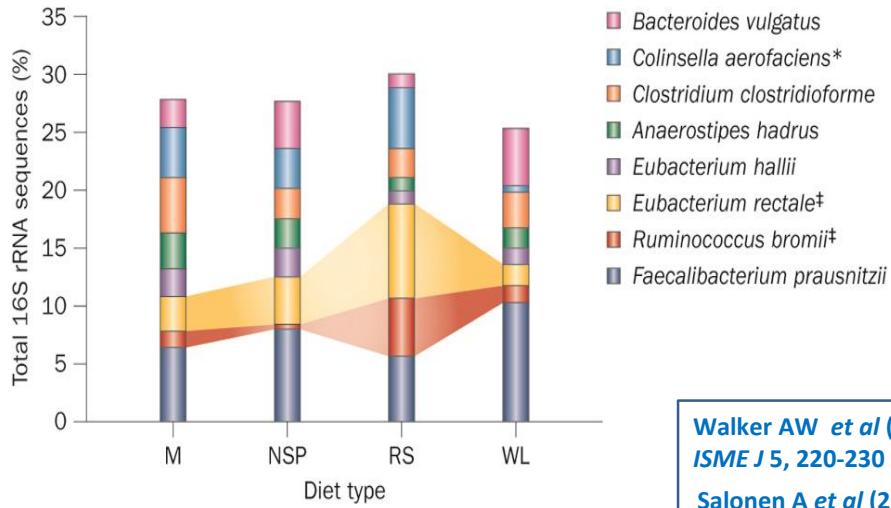
[Hitchip]

Increase with wheat bran NSP ← R - N → Increase with RS



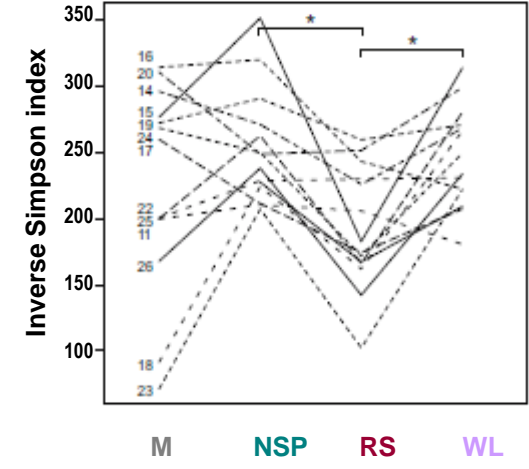
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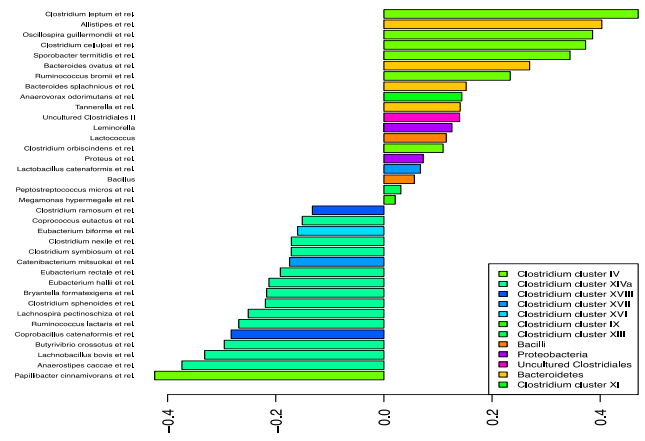
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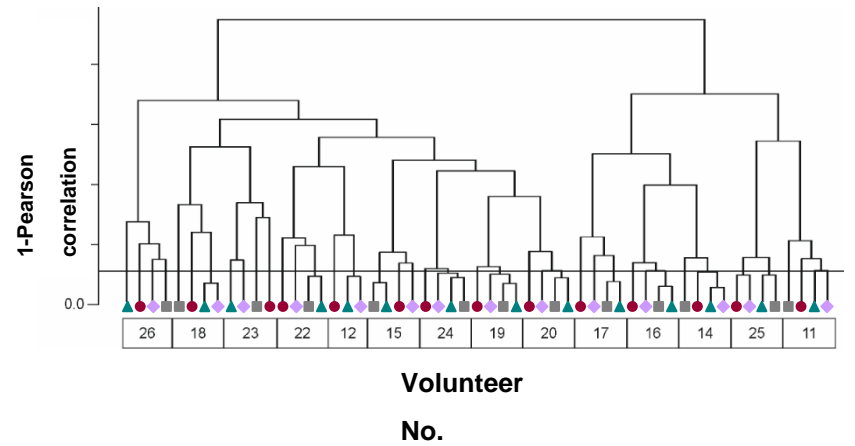


[Hitchip]

Increase with wheat bran NSP ← R - N → Increase with RS



★ Individual variation still dominates



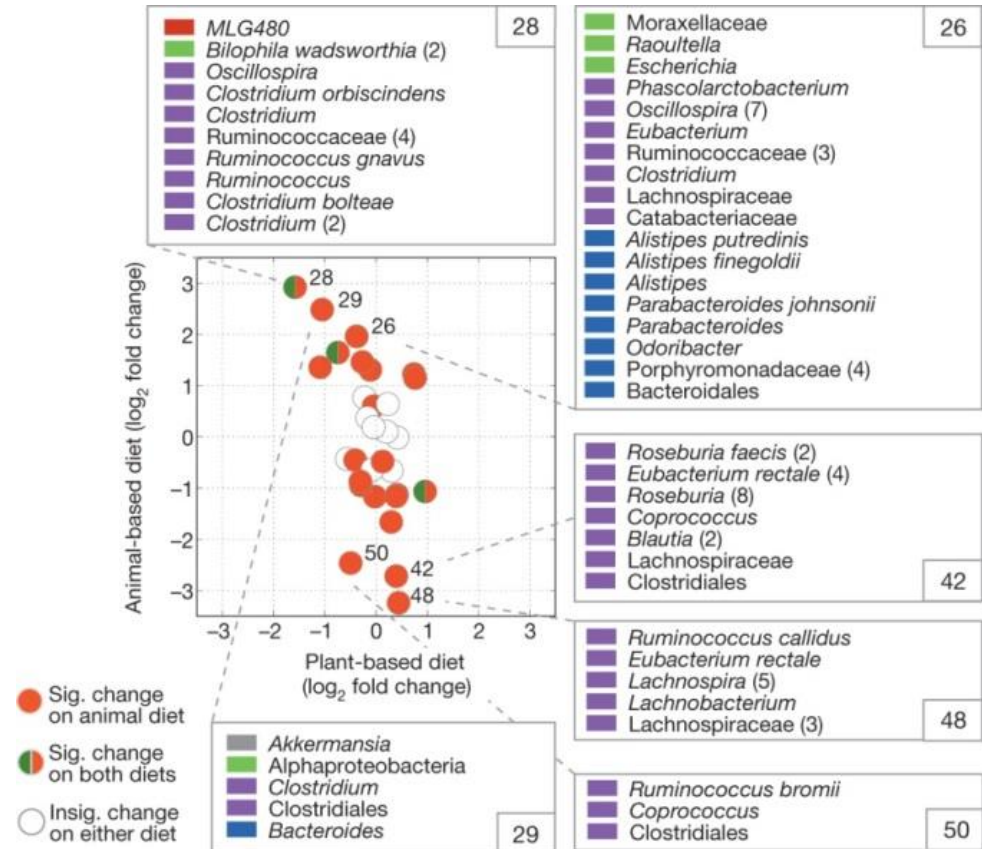
Very extreme dietary changes can lead to wide-ranging shifts in the gut bacterial community in humans

'animal based' – 69.5 % (!) calcs from fat, 30% from protein, <1% (!) from carbs/fibre

'plant based' - 32.5% from fat, 16.5% from protein, 50% from carbs/fibre

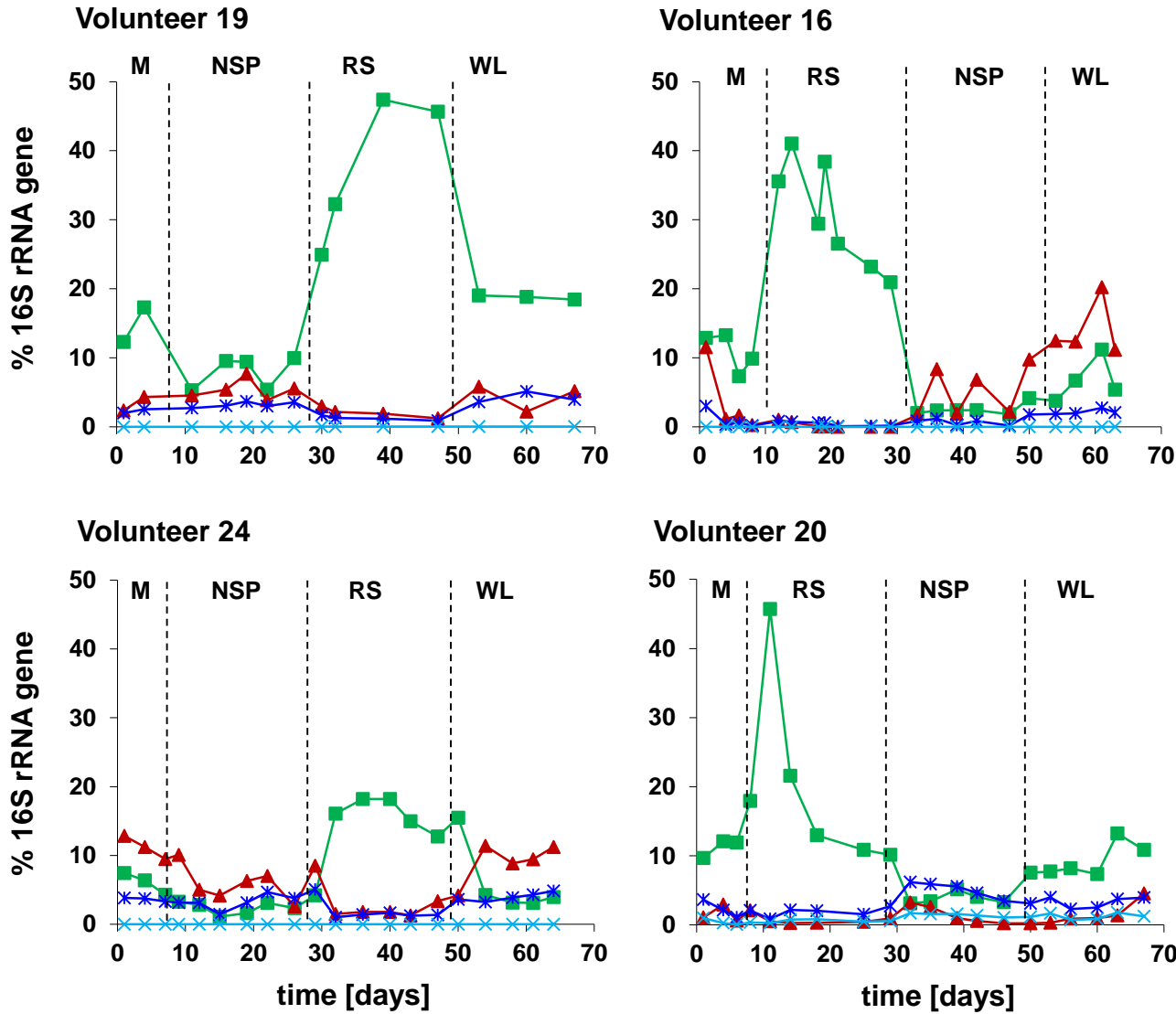
[David LA *et al Nature* 2014]

Rise in bile acids with high fat + lack of fibre considered key factors in 'animal-based' diet



Responses occur rapidly and tend to be species-specific

Cluster IV *Ruminococcus* species - qPCR



R. bromii

R. albus

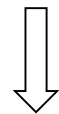
+ *R. bicirculans*

R. flavefaciens

+ *R. champanellensis*

+ *R. callidus*

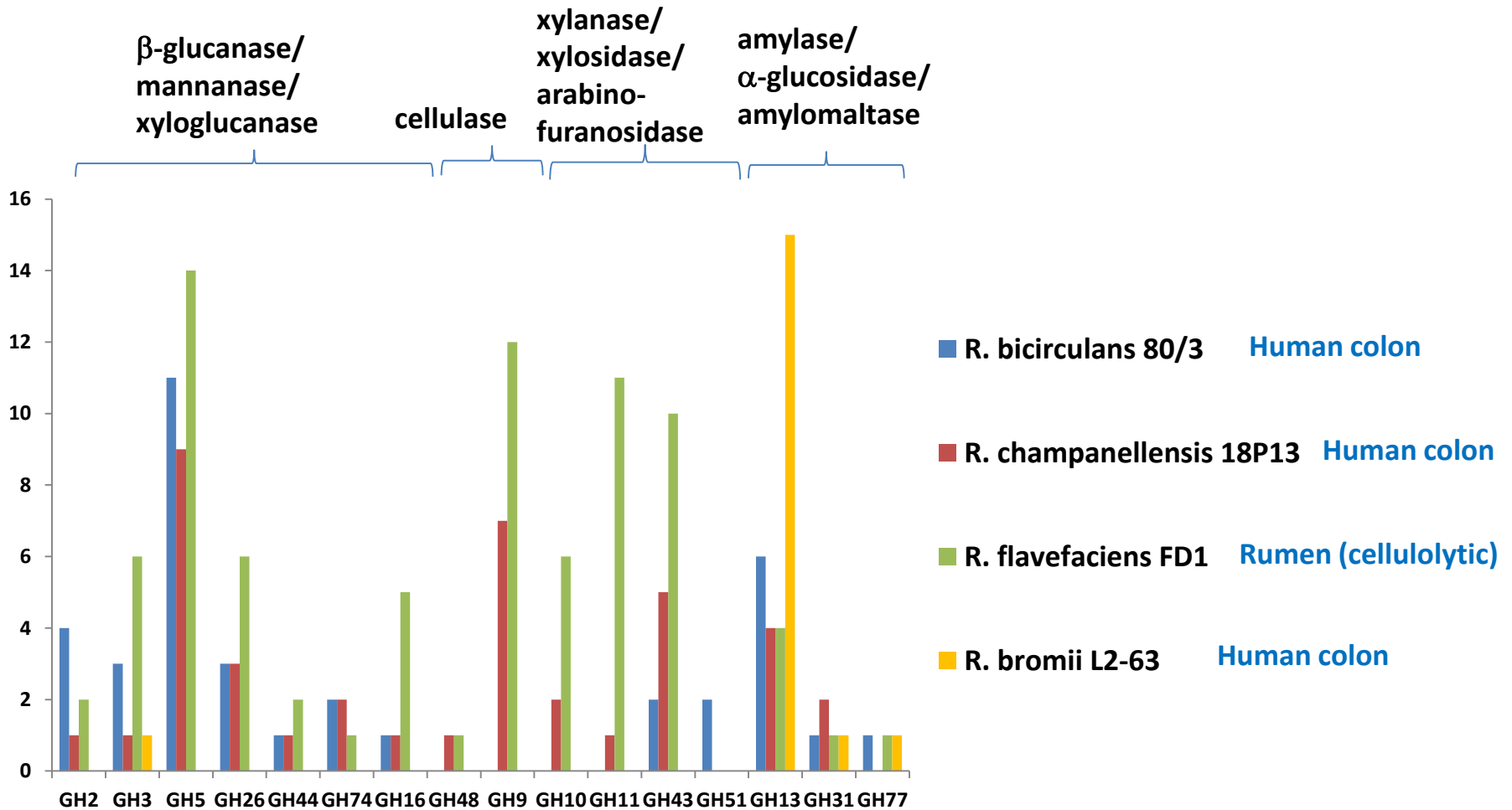
R. champanellensis



Response on
RS is due to
R. bromii

Nutritional specialization among *Ruminococcus* spp.

- selected glycoside hydrolase families encoded by genomes

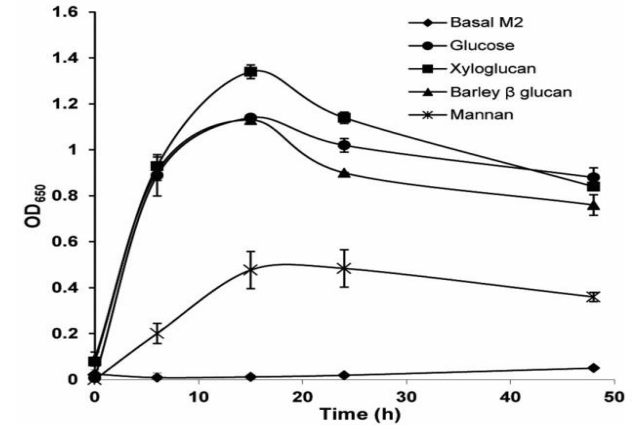


“*Ruminococcus bicirculans*”

Able to utilize xyloglucan, beta-glucan for growth, but not cellulolytic

Complete genome of a new Firmicutes species belonging to the dominant human colonic microbiota (*Ruminococcus bicirculans*) reveals two chromosomes and a selective capacity to utilize plant glucans.

Wegmann U *et al* (2014) *Environ Microbiol* 16:2879-2890

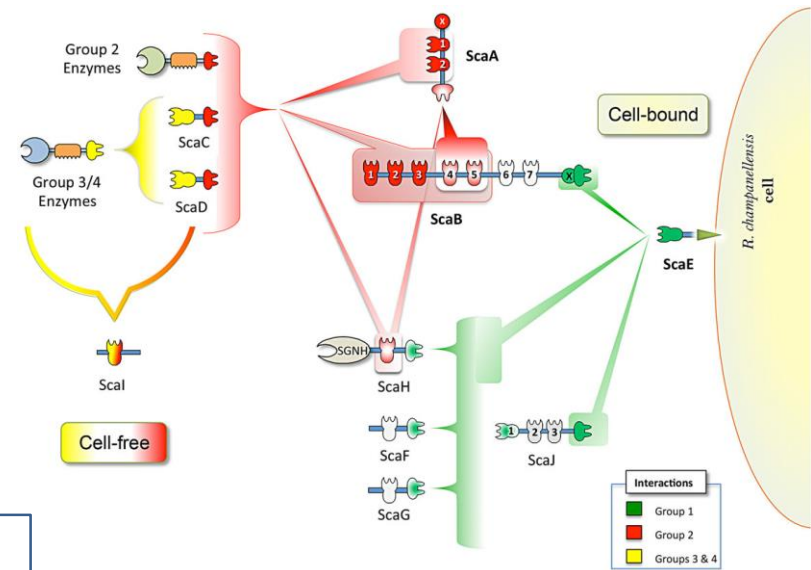


Ruminococcus champanellensis

The only human colonic anaerobe able to degrade filter paper (crystalline) cellulose

Ruminococcal cellulosome systems from rumen to human.

Ben David *et al* (2015) *Environ Microbiol* 17:3407-3426

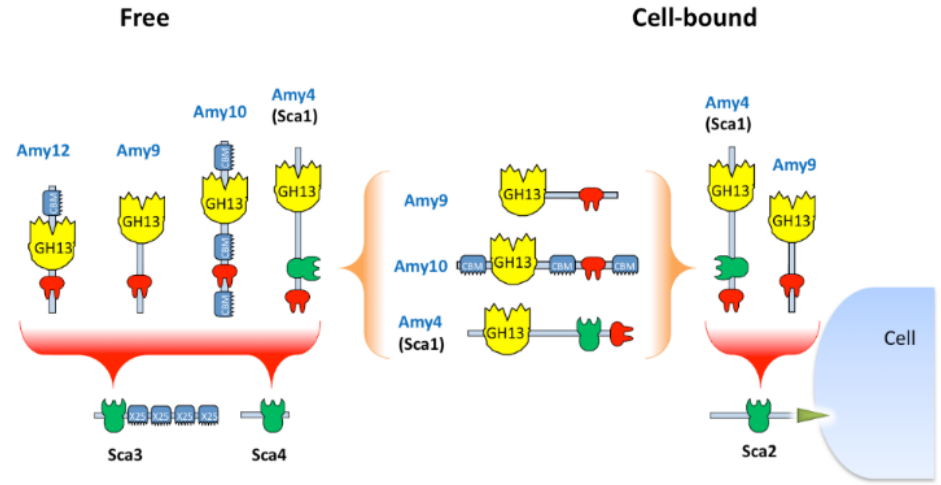


Ruminococcus bromii

Specialist starch-degrader

Exceptional starch-degrading activity in the human colonic anaerobe *Ruminococcus bromii* coincides with unique organization of its extracellular enzymes into 'amyloosomes'.

Ze X *et al* (2015) *MBio* 6 (5) e01058-15.

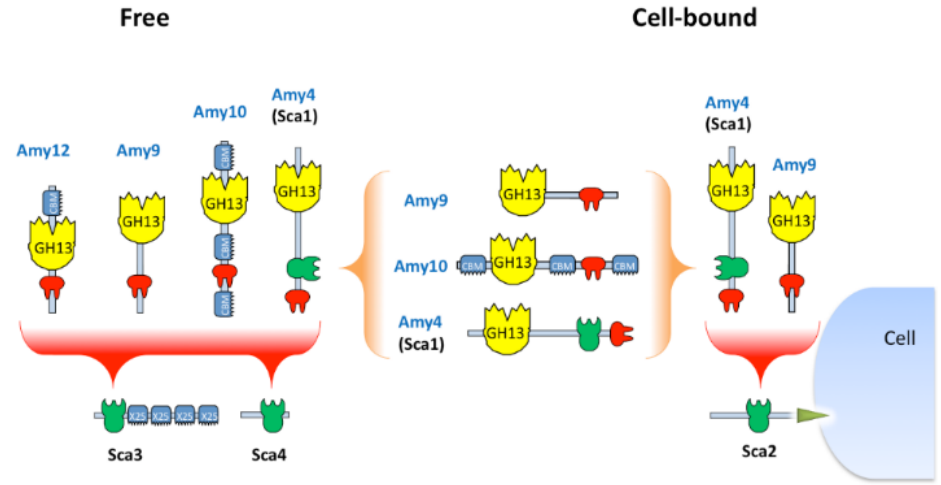


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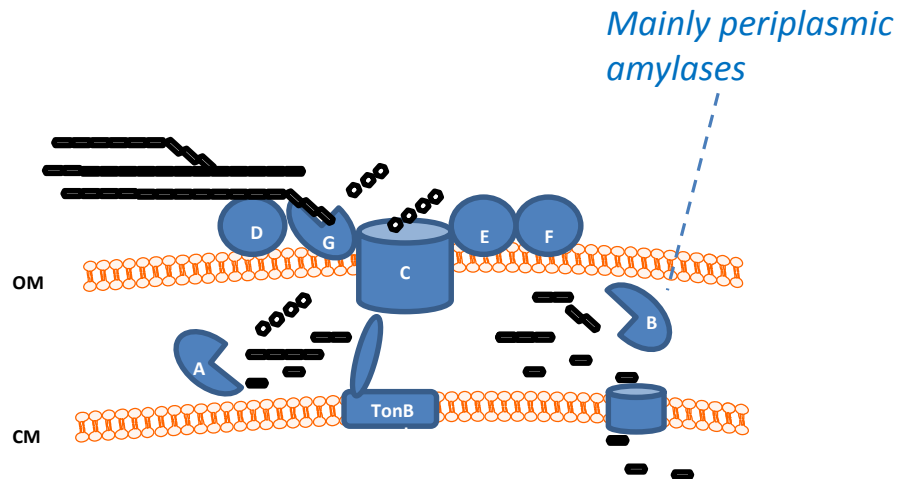
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Ze X *et al* (2015) *MBio* 6 (5) e01058-15.



Contrast - *Bacteroides thetaiotaomicron* starch utilization (*sus*) system (Salyers)

- soluble starches

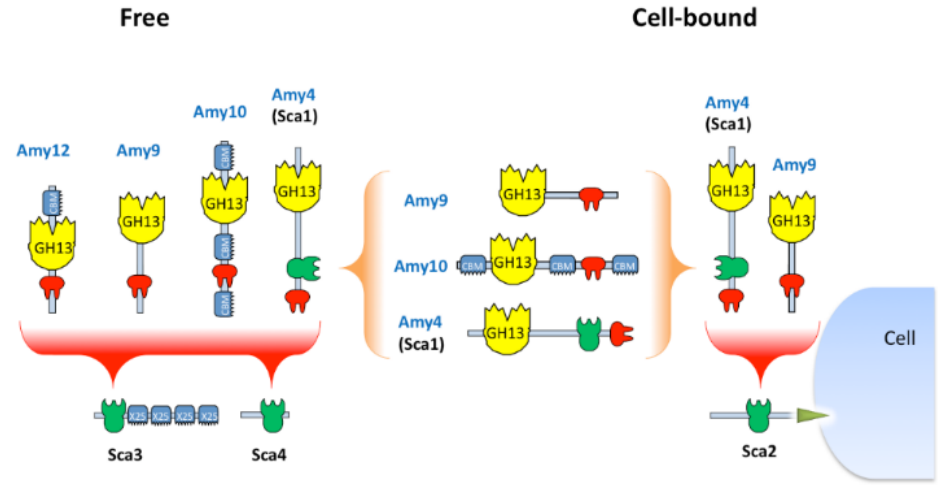


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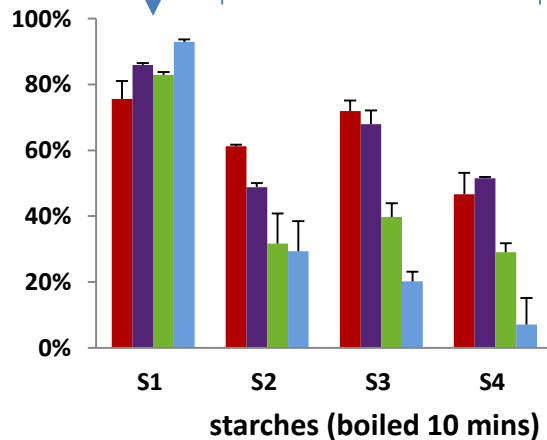


Degradation of corn starches by four amyolytic human gut bacteria

[Ze X *et al* 2012 *ISME J*]

Digestible starch (S1)

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



Ruminococcus bromii
(Firmicutes)

Bifidobacterium adolescentis
(Actinobacteria)

Eubacterium rectale
(Firmicutes)

Bacteroides thetaiotaomicron
(Bacteroidetes)

Gram-positive

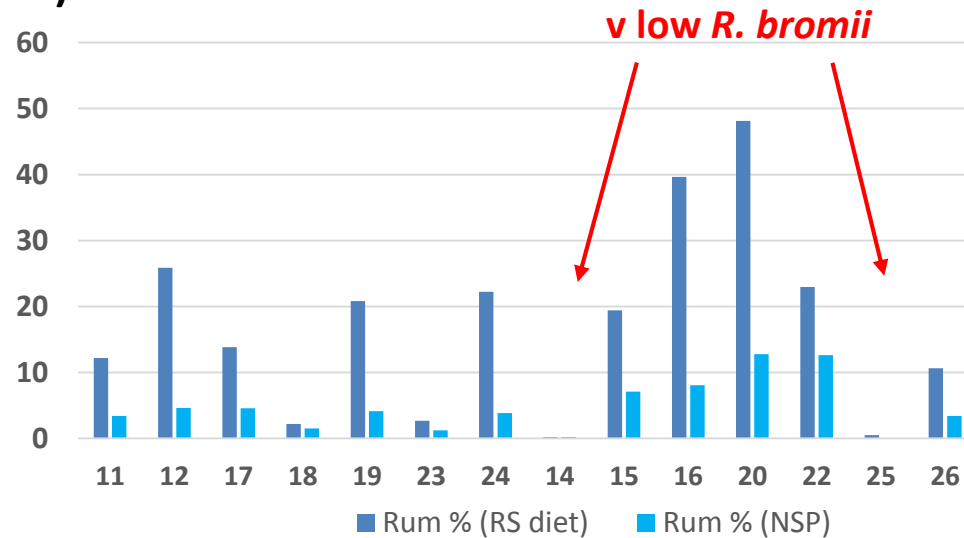
Gram-negative

R. bromii as a 'keystone' species in degradation of resistant starch

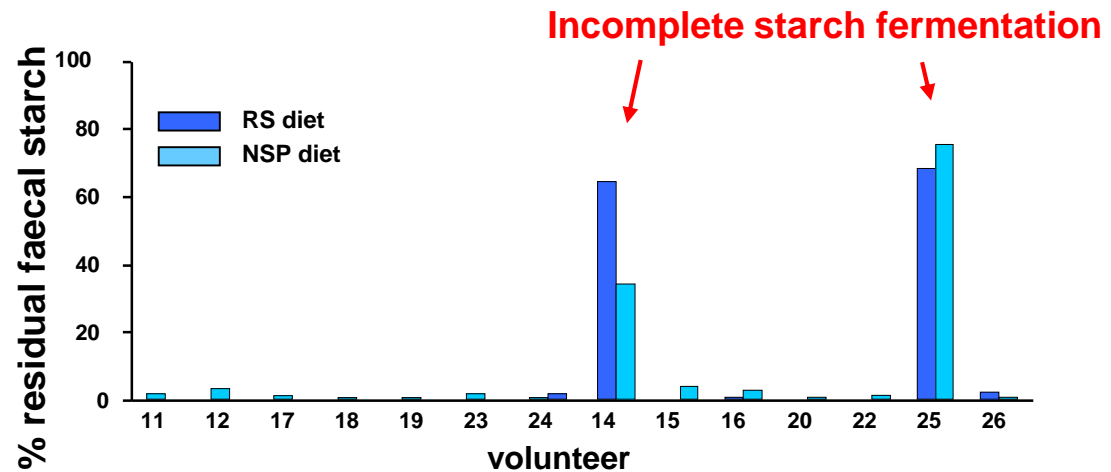
[Walker AW *et al.* ISME J 2011
Ze X *et al.* ISME J 2012]

qPCR - % *Ruminococcus* (cl IV)

16S rRNA gene copies

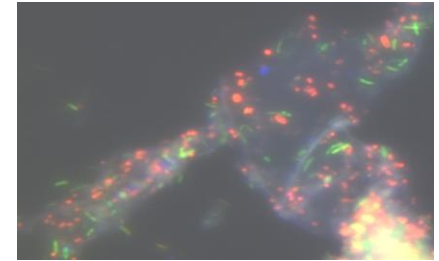


Residual starch in faeces

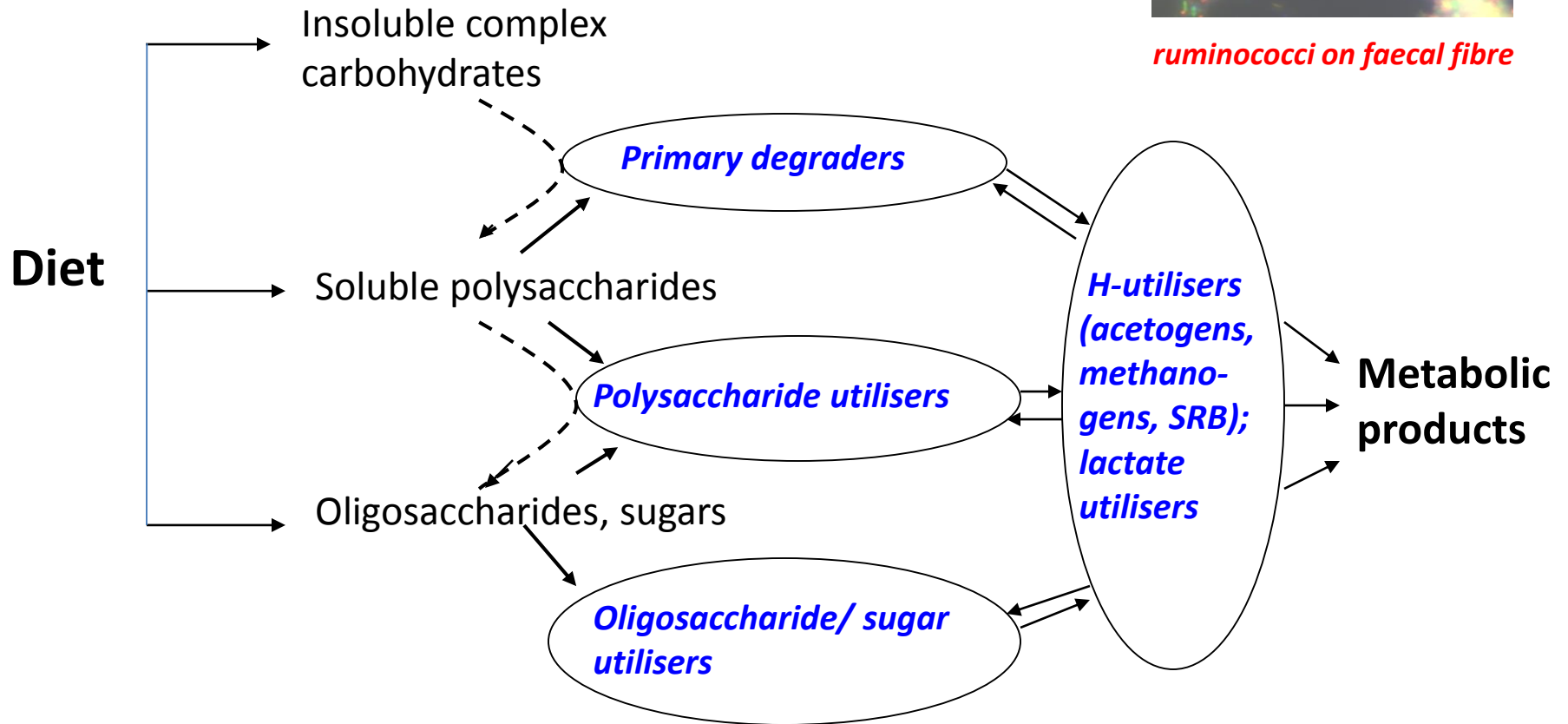


Microbial ecology of carbohydrate utilization in the gut: functional groups

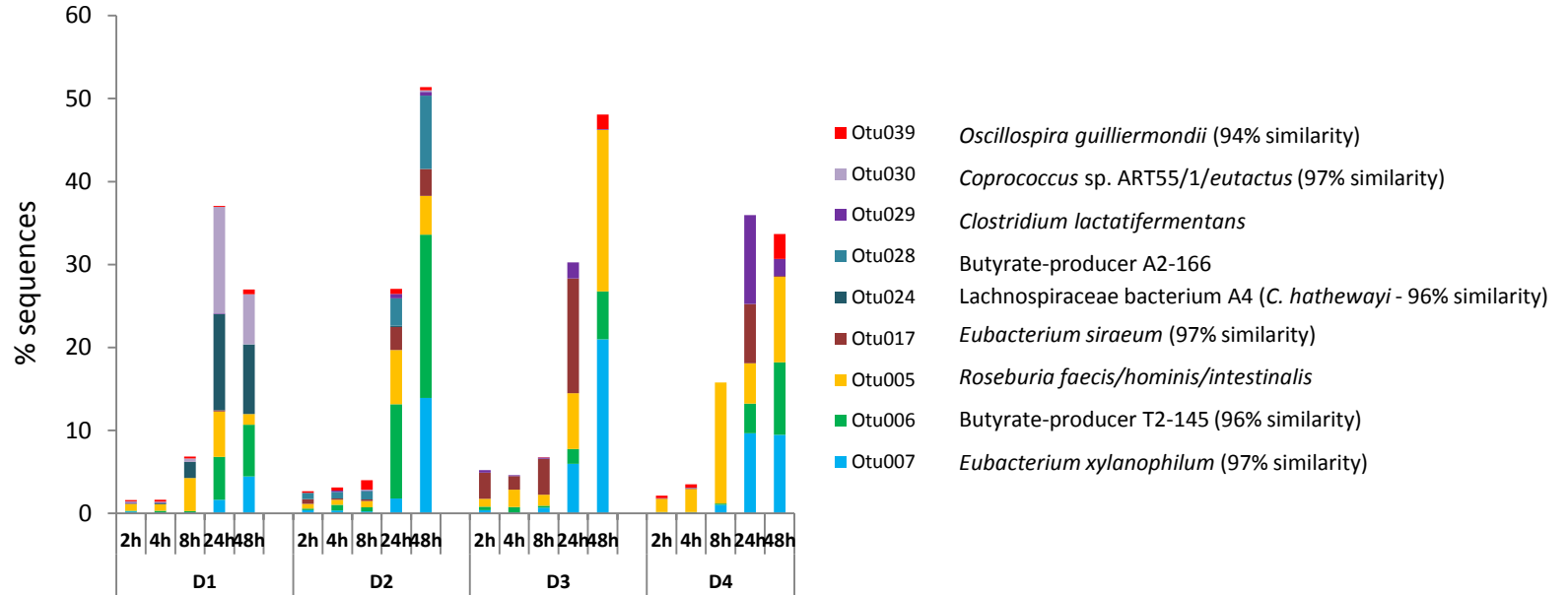
(Flint HJ *et al* Env Micro 2007)



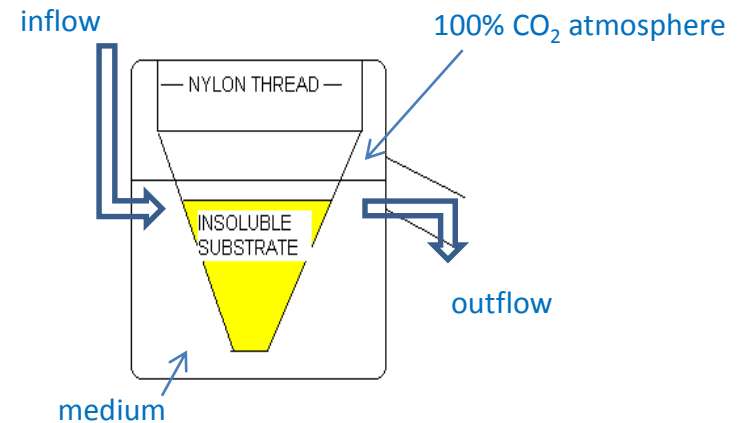
ruminococci on faecal fibre



Enrichment of Firmicutes bacteria on wheat bran *in vitro**



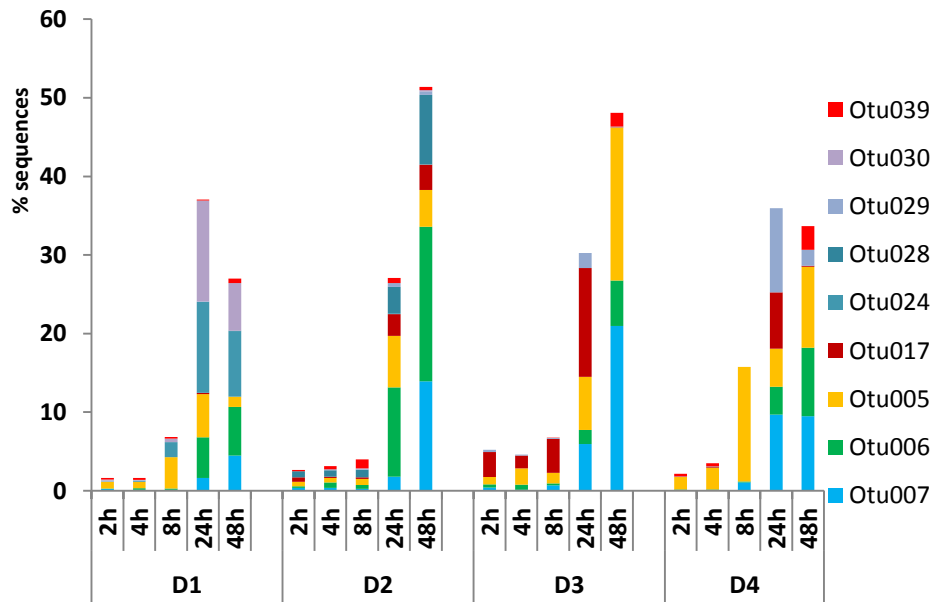
pH- controlled, anaerobic *in vitro* fermentor system -



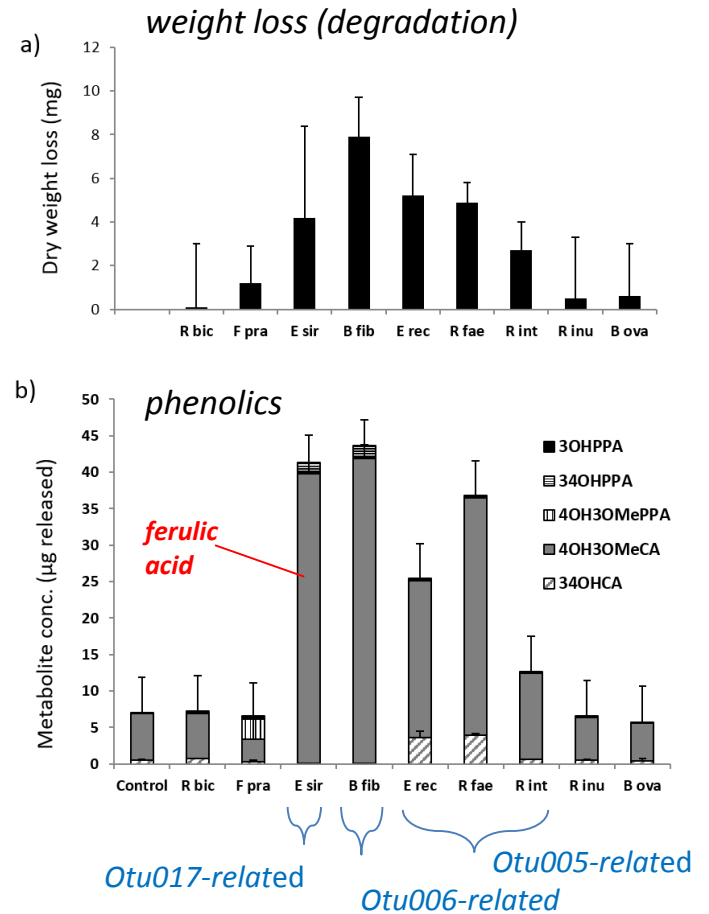
* Duncan SH *et al.* Environ Microbiol 2016 online

“Wheat bran promotes enrichment within the human colonic microbiota of butyrate-producing bacteria that release ferulic acid”

Duncan SH *et al* Environ Microbiol -2016 online



Action of isolated bacteria on wheat bran

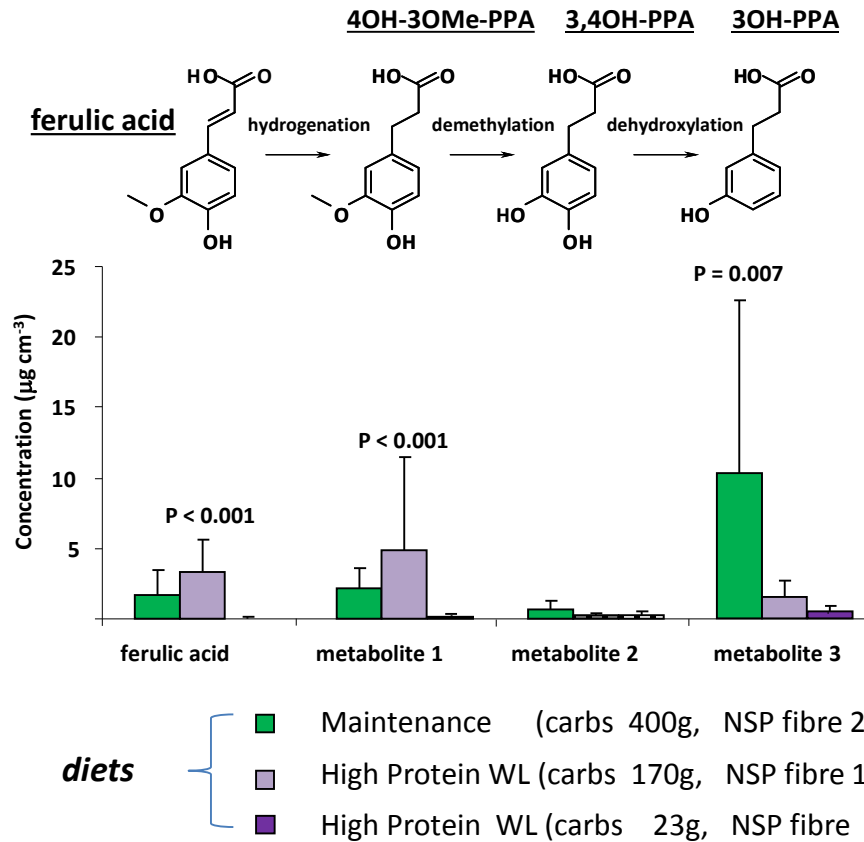


Impact of diet on plant-derived phenolic acids and microbial metabolites in human volunteers

[Russell WR *et al*
AJCN 2011]

Faecal concentrations

(means, 8 overweight male volunteers)

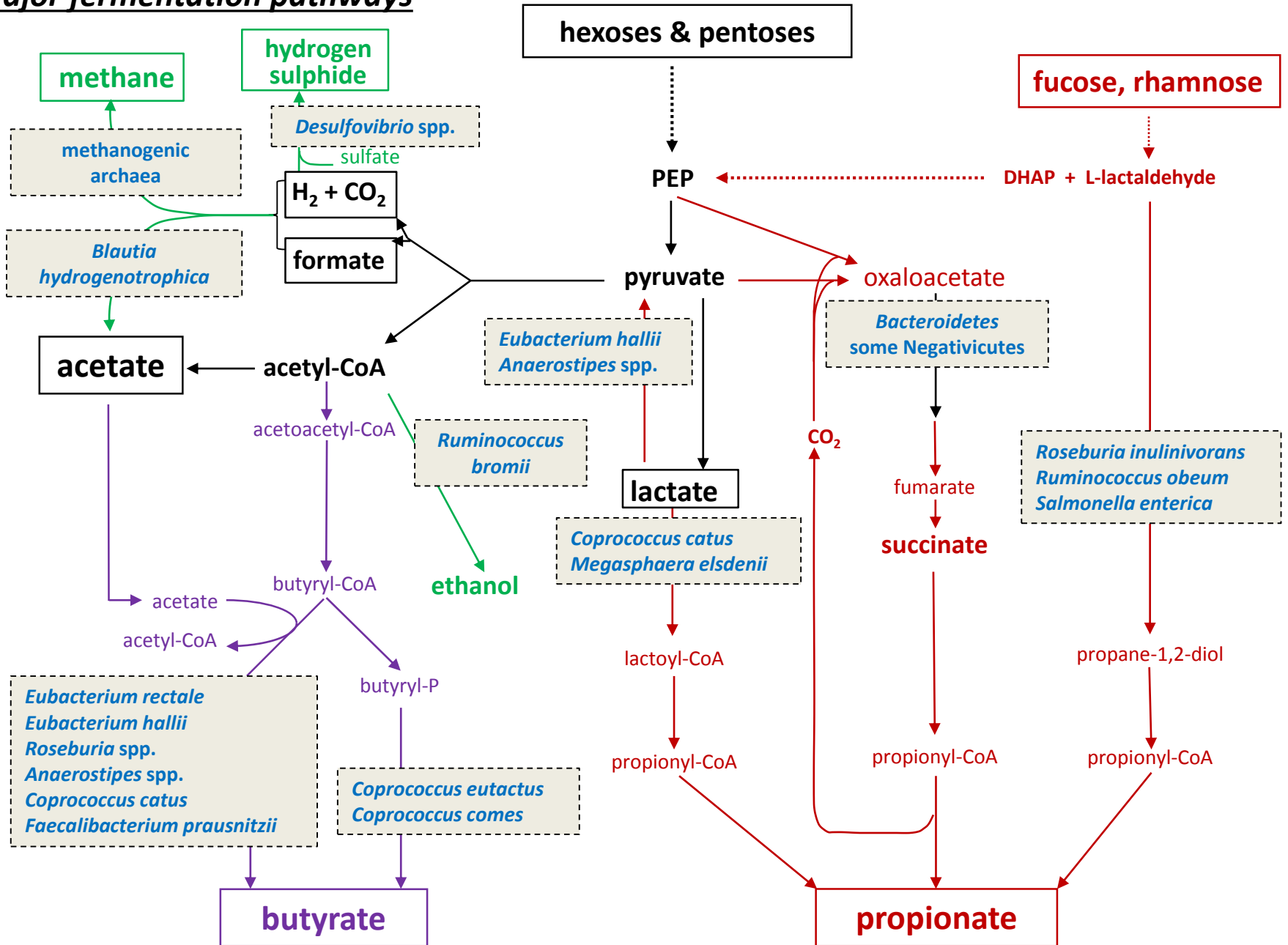


The gut microbiota, bacterial metabolites and colorectal cancer

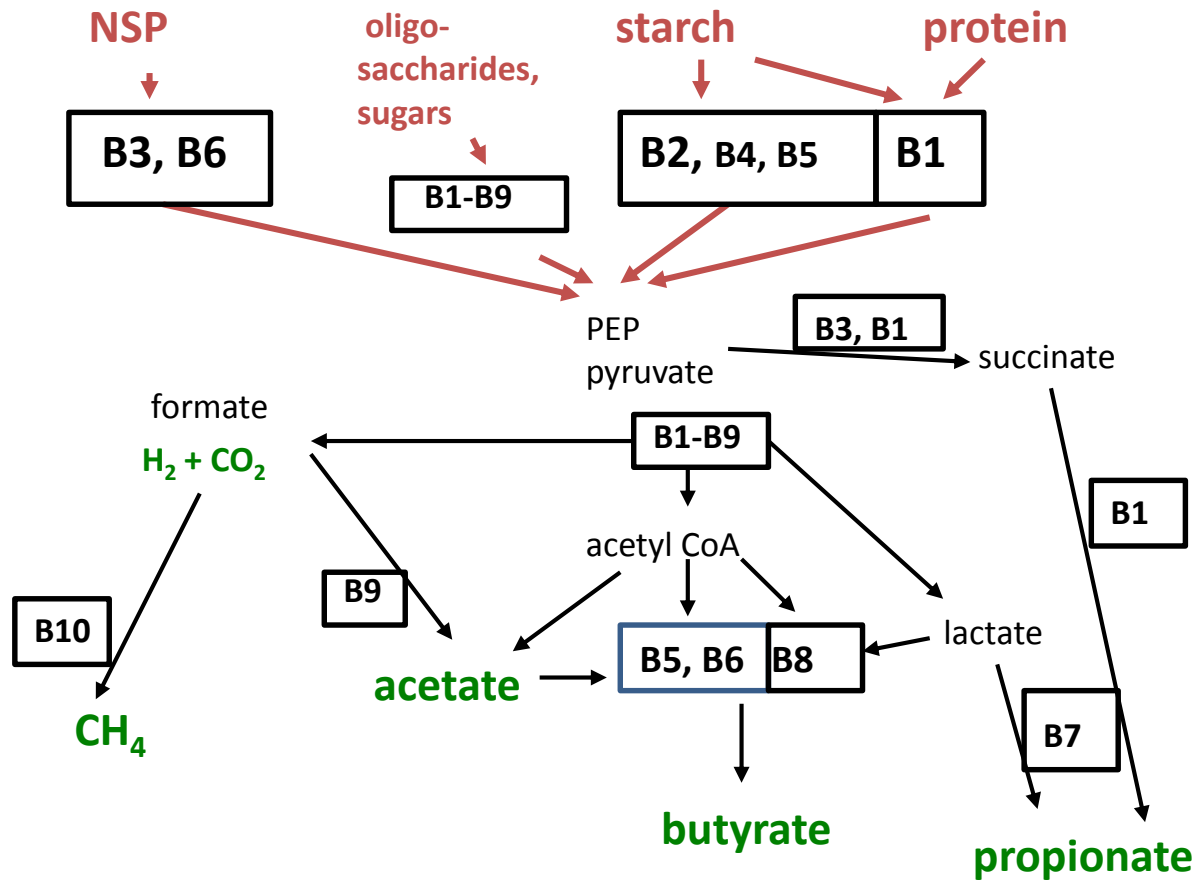
[Louis P, Hold GL & Flint HJ 2014 NRM]

Dietary and environmental compounds	Microbial products	Known effect on host
Non-digestible carbohydrates	SCFAs	<ul style="list-style-type: none"> • Microbiota modulation • Cellular differentiation; apoptosis • Inflammation
Phytochemicals	Phenolic acids; isothiocyanates	<ul style="list-style-type: none"> • Xenobiotic detoxification • Microbiota modulation • Cellular differentiation; apoptosis • Inflammation
Protein	NOCs; ammonia	<ul style="list-style-type: none"> • ROS production; genotoxicity
	Polyamines	<ul style="list-style-type: none"> • Inflammation • ROS production; genotoxicity
	Hydrogen sulphide	<ul style="list-style-type: none"> • Inflammation • ROS production; genotoxicity
Fat → Bile acids	Taurine	<ul style="list-style-type: none"> • Microbiota modulation
	Secondary bile acids	<ul style="list-style-type: none"> • Microbiota modulation • Cellular differentiation; apoptosis • ROS production; genotoxicity
Xenobiotics	Carcinogens	<ul style="list-style-type: none"> • ROS production; genotoxicity
Ethanol	Acetaldehyde	<ul style="list-style-type: none"> • ROS production; genotoxicity

Major fermentation pathways



Kettle H, Louis P, Duncan SH, Holtrop G, Flint HJ (2015) "Modelling the emergent dynamics of communities of human colonic microbiota: response to pH and peptide"
Environ Microbiol 15: 1615-1630

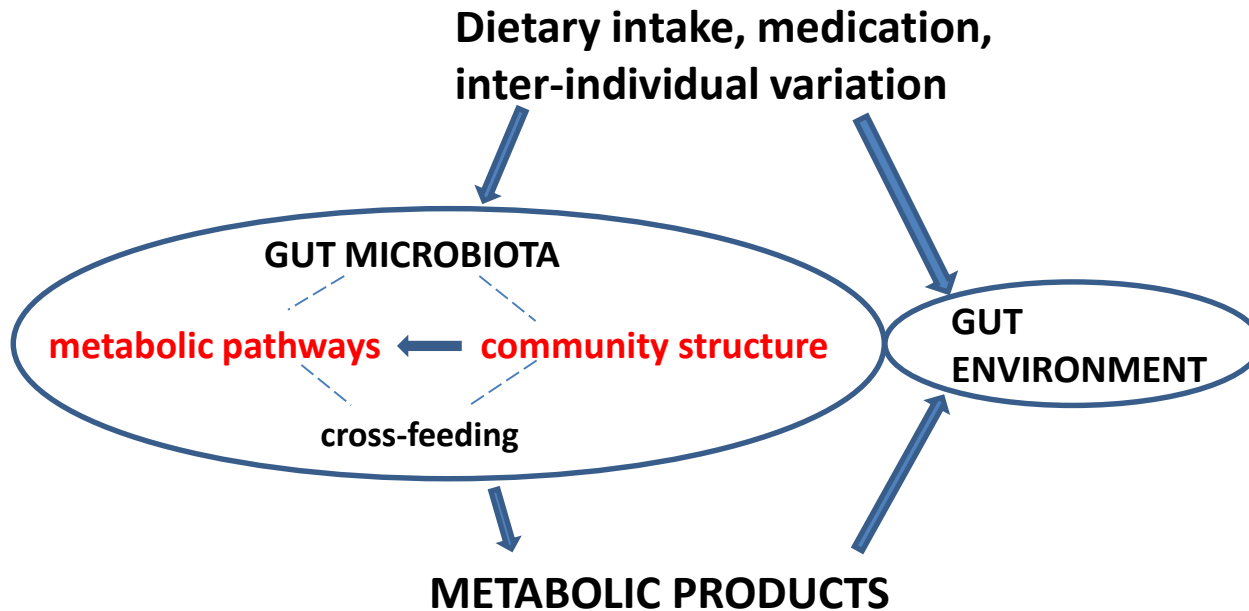


- Bacterial functional groups**
- B1 = Bacteroidetes
 - B2 = Firmicutes (eg. *R. bromii*)
 - B3 = Firmicutes (eg. *E. eligens*)
 - B4 = Actinobacteria
 - B5 = *Roseburia* group
 - B6 = *F. prausnitzii*
 - B7 = Negativutes
 - B8 = *E. hallii*, *Anaerostipes*
 - B9 = acetogens
 - B10 = methanogens

Changes in butyrate-producing bacteria within faecal microbiota in disease states/ extreme diets –

- ↓ *F. prausnitzii* in ileal Crohn's patients Sokol H *et al PNAS* (2008)
- ↓ *F. prausnitzii* in frail elderly Claesson *et al Nature* (2012)
- ↓ *Roseburia*, *E. rectale*, *F. prausnitzii* in type 2 diabetics Qin J *et al Nature* (2012)
- ↓ Butyrate- producers in type 1 diabetics de Goffau MC *et al Diabetes* (2013)
- ↓ *Roseburia*, butyrate-producers in CR cancer Wang T *et al ISME J* (2012)
- ↓ *Roseburia* in constipation-type-IBS Chassard C *et al Alim Ph Th* (2012)
- ↓ *Roseburia* in hepatic encephalopathy (mucosal flora) Bajaj JS *et al Am J Clin Nutr* (2012)
- ↑ Butyrate-producers with improved insulin sensitivity Vrieze A *et al Gastroenterol* (2012)
- ↓ *Roseburia*, *E. rectale* with low carb WL diets Duncan SH *et al AEM* (2007)
- ↓ *Roseburia*, *Faecalibacterium*, *Coprococcus* in LGC individuals (increased metabolic syndrome) Le Chatelier *et al Nature* (2013)

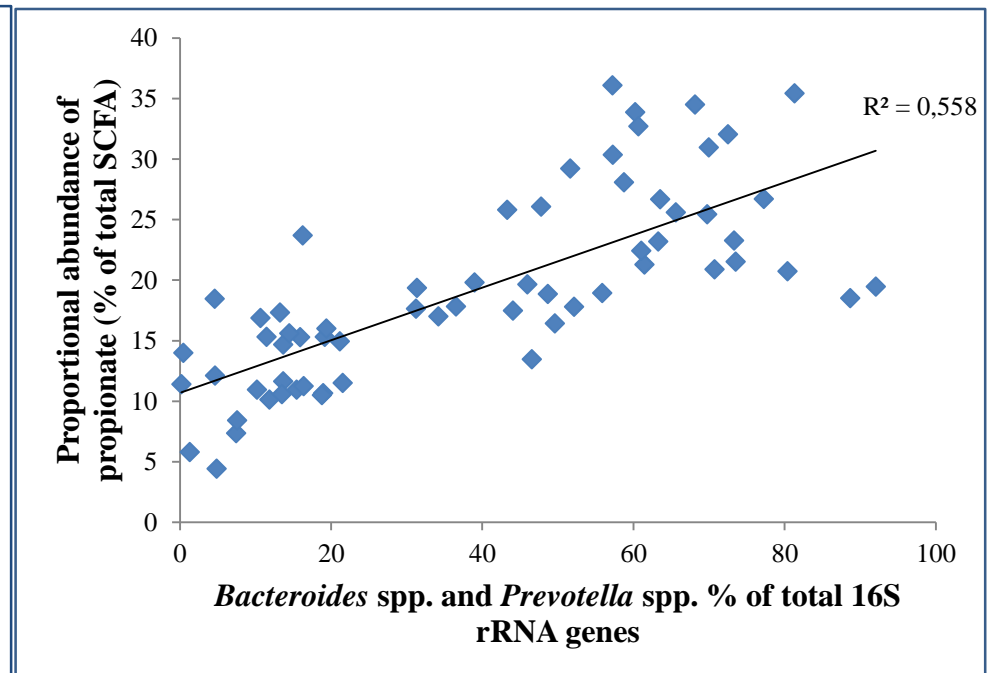
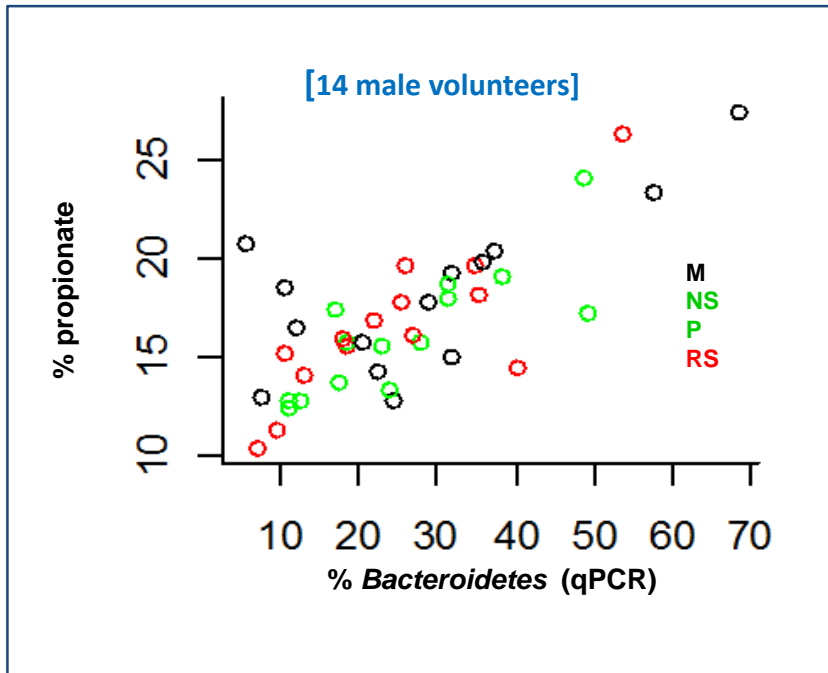
How do changes in gut microbiota composition impact on metabolite production?



Relative abundance of Bacteroidetes correlates with % propionate

- in faecal samples

- and in fermentor studies



Salonen A *et al.* *ISME J* (2014)

Chung WSF *et al.* *BMC Biology* (2016)

- Total faecal SCFA , especially butyrate, decrease with lower carbohydrate intake

Obese male subjects (UK)

1. *Duncan SH et al AEM 2007*

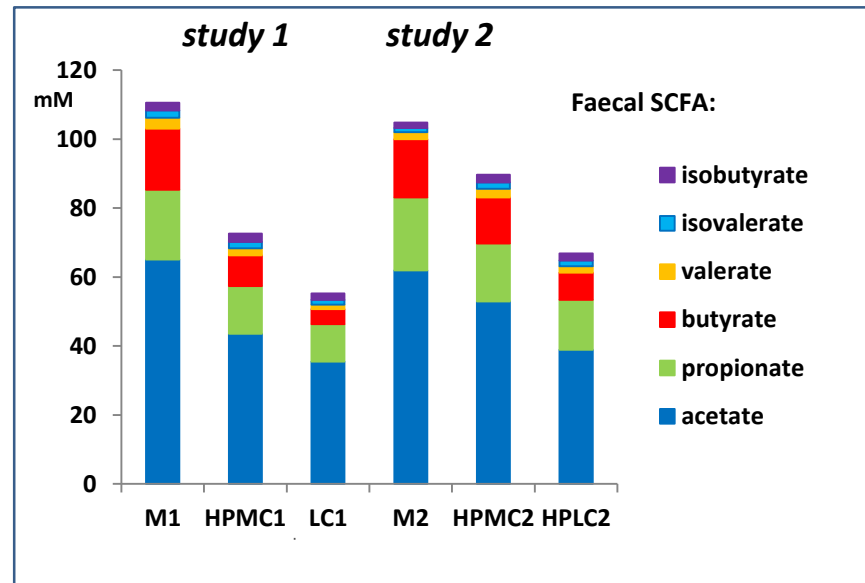
2. *Russell WR et al Am J Cl Nutr 2011*

Diets:

M – maintenance (50-52% cal as CHO)

HPMC – Moderate carbohydrate (35%)

HPLC – Low carbohydrate (4-5%)



- Total faecal SCFA, including butyrate, can be very high in populations that have high intakes of plant-derived foodstuffs

Ou J et al Am J Clin Nutr 2013

Native Africans -

Ace: Prop: But

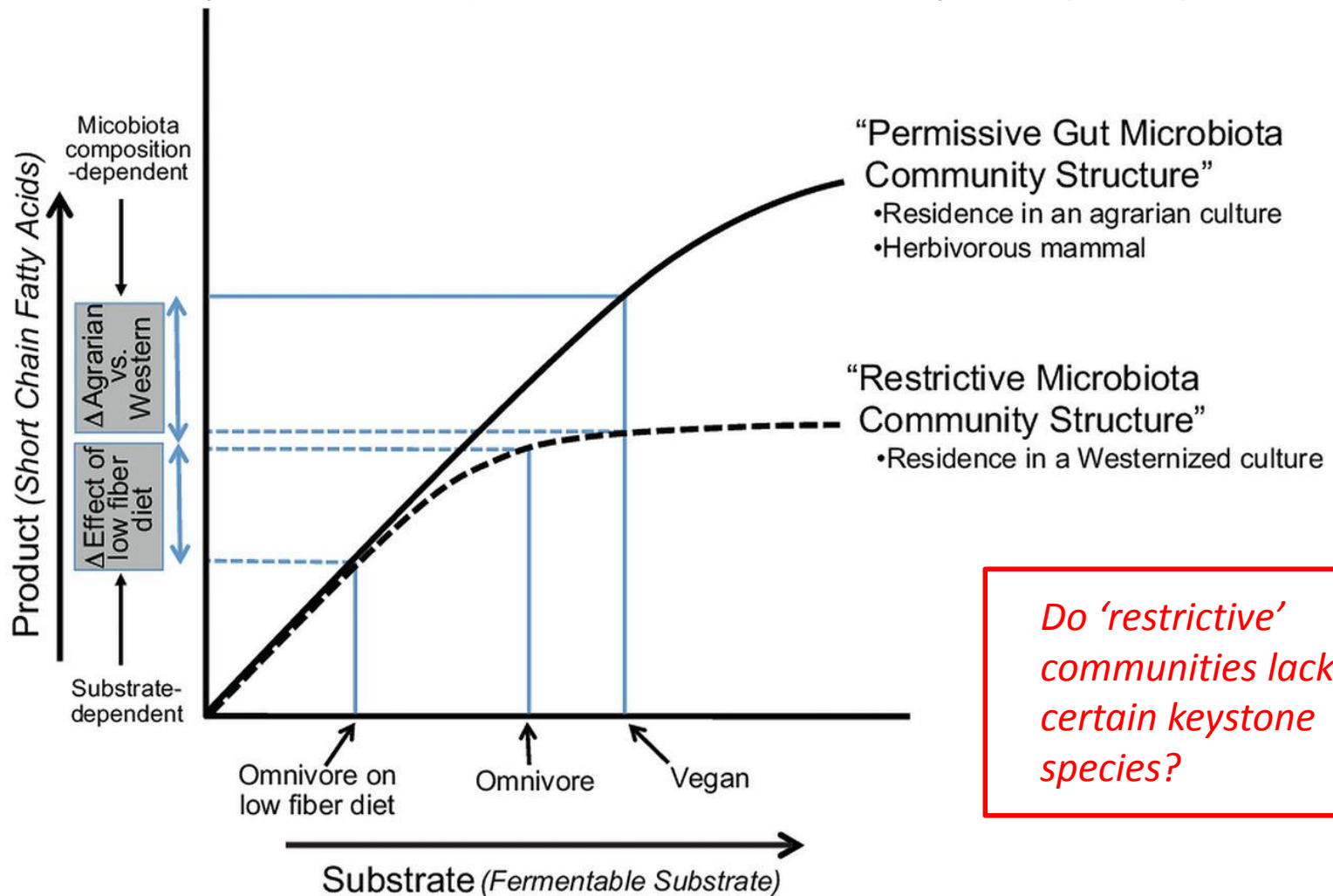
72: 27: 17 mM (total > 100 mM)

African Americans -

Ace: Prop: But

27: 7: 8 mM (total < 50 mM)

Model describing the relationship between substrate abundance and gut microbiota product formation and its dependence on gut microbiota composition using the consumption of fermentable carbohydrates and the production of short chain fatty acids (SCFAs) as an example.



Gary D Wu et al. Gut 2016;65:63-72



GENERAL CONCLUSIONS

- ★ Changes in diet composition change the representation of certain 'diet-responsive' species within the microbiota
- ★ This reflects the fact that many species (especially Firmicutes?) are highly specialised in their substrate utilization
- ★ 'Some are more equal than others' (Ze X *et al* Gut Microbes 2013) (after George Orwell) - certain 'keystone species' play primary roles in degradation of recalcitrant substrates
- ★ Changes in microbiota composition (due to diet or individual variation) impact on metabolite formation – especially loss of keystone species

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