Selective enrichment of key bacterial groups within the human colon in response to changes in diet

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Wellcome Trust Sanger Institute

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Rowett Institute of Nutrition and Health
University of Aberdeen

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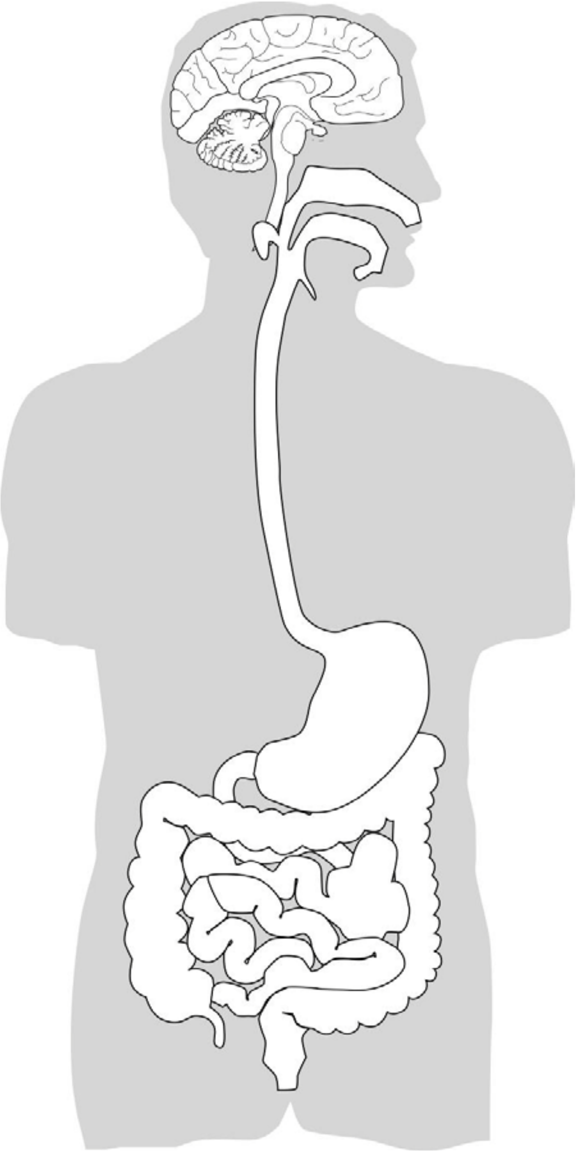
Diet and the human gut microbiota

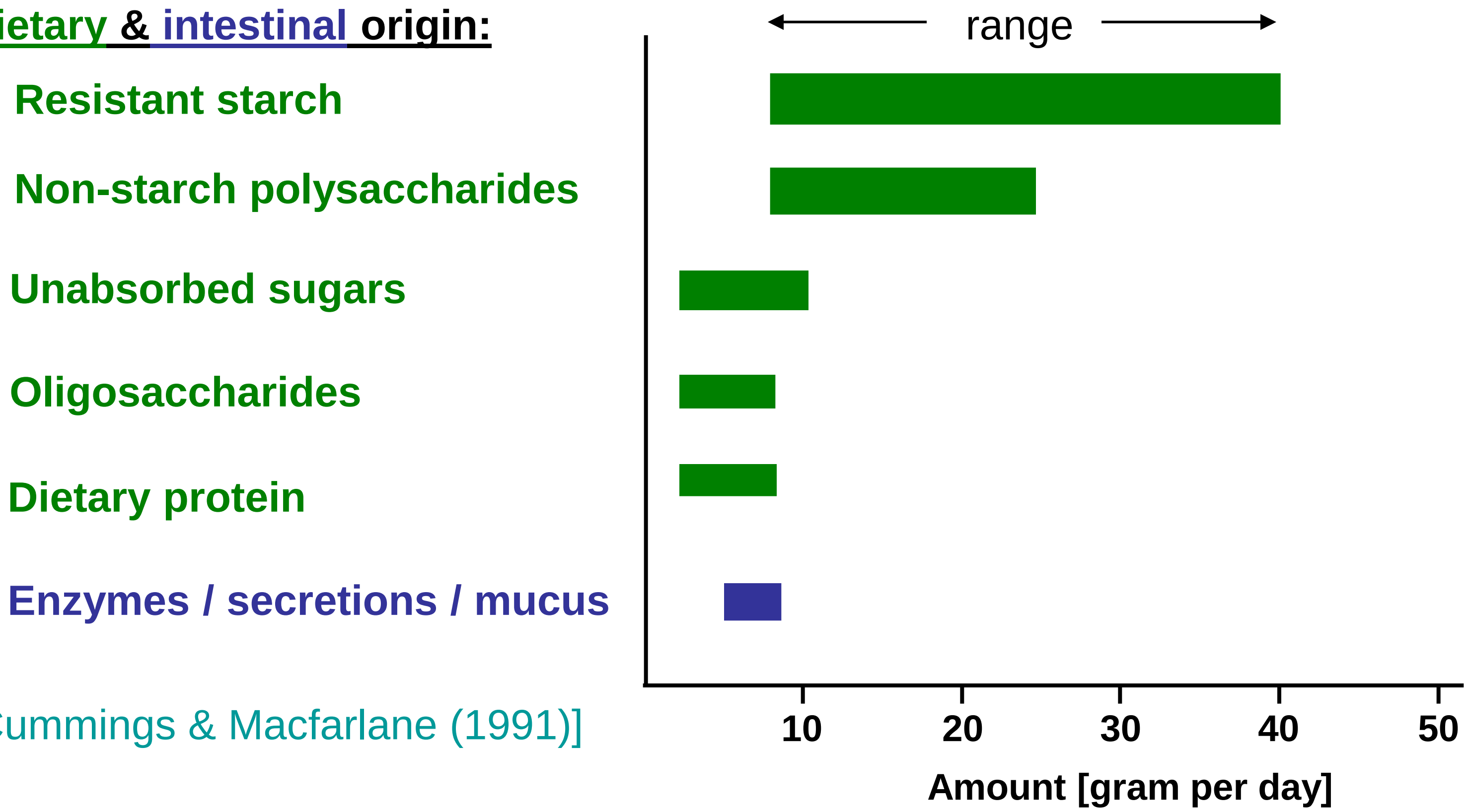
- A significant proportion of dietary compounds escape digestion in the small intestine.

- Non-digestible carbohydrates are the predominant growth substrates for gut bacteria.



Principal substrates available for utilization by intestinal microbes

Of dietary & intestinal origin:



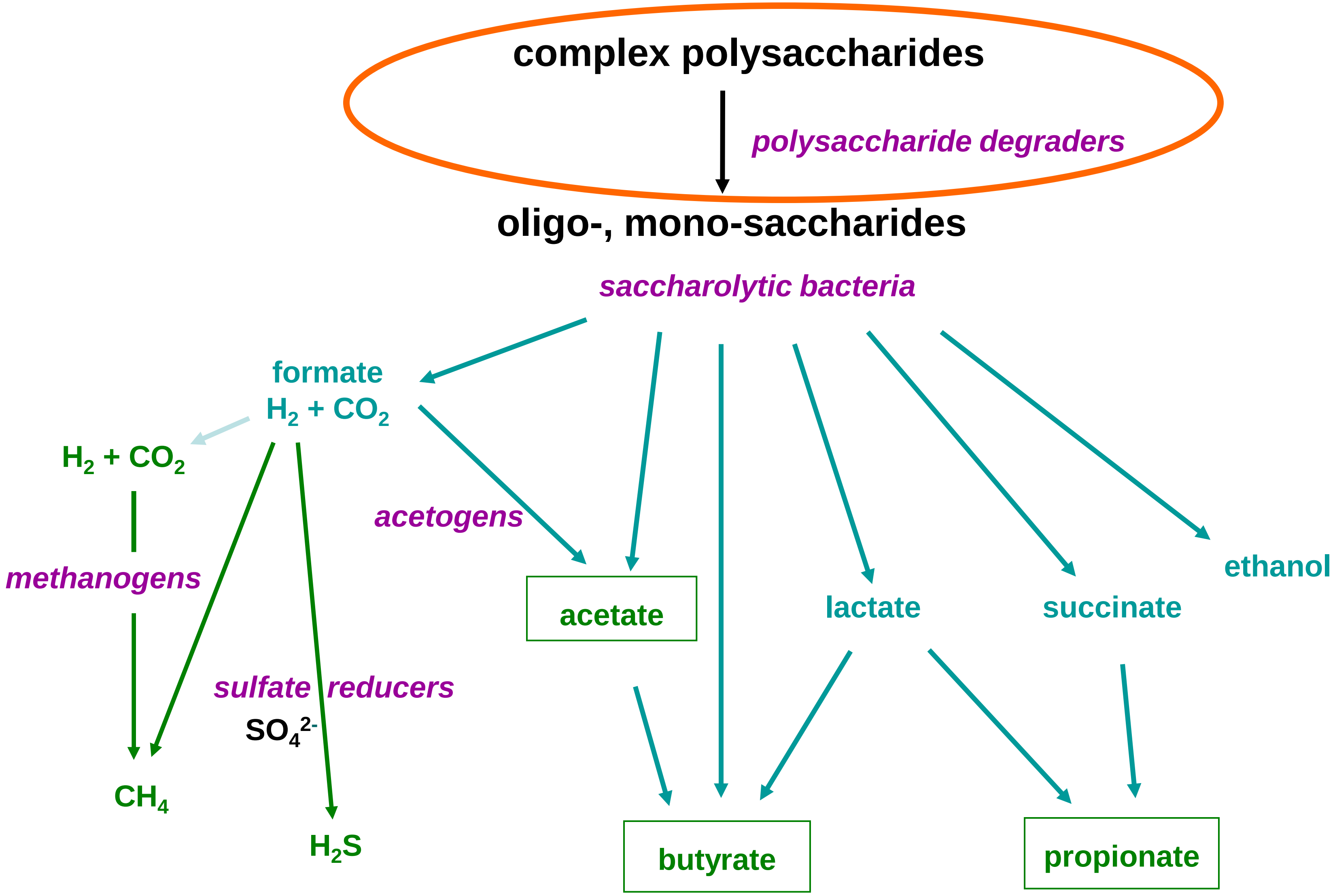
[from Cummings & Macfarlane (1991)]

Digestibilities for plant cell wall polysaccharides – 7 subjects (Slavin *et al* J. Nut 1981)

Pure cellulose (Solka Flok)	minimal
Cellulose (in normal diets)	69.7% (+/-10.7)
Hemicellulose	71.7% (+/- 5.4)

[Harry Flint - Rowett Institute of Nutrition and Health]

Microbial metabolism of dietary compounds



complex polysaccharides

polysaccharide degraders

oligo-, mono-saccharides

saccharolytic bacteria

formate

$H_2 + CO_2$

$H_2 + CO_2$

acetogens

methanogens

acetate

lactate

succinate

ethanol

sulfate reducers

SO_4^{2-}

CH_4

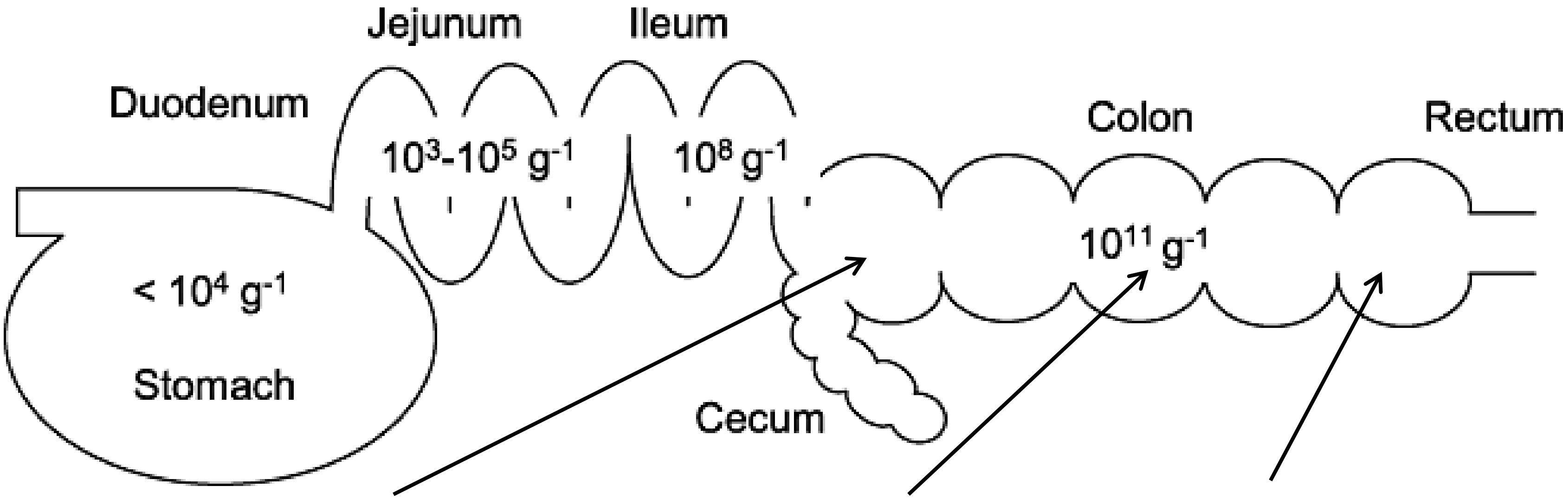
H_2S

butyrate

propionate

[Harry Flint - Rowett Institute of Nutrition and Health]

Microbial metabolism of dietary compounds



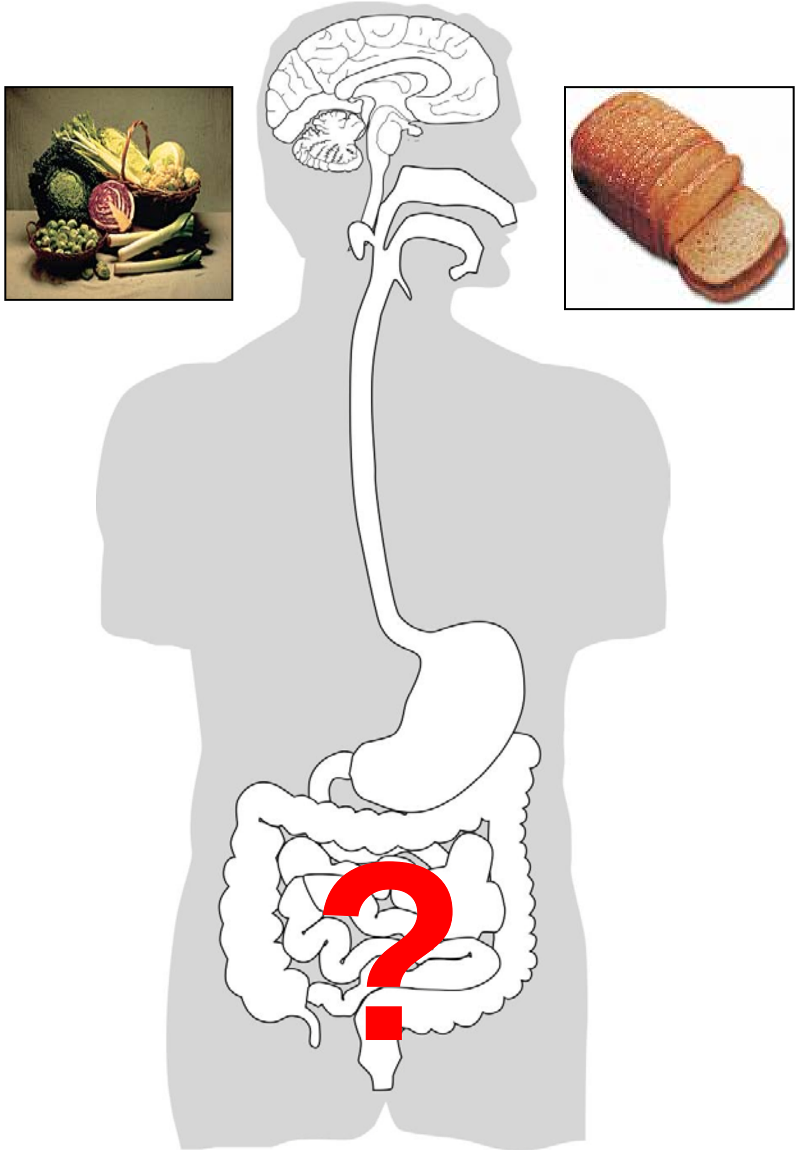
Proximal Colon	Transverse Colon	Distal Colon
Active fermentation	Depletion of substrates	Reduced carbohydrate fermentation
High bacterial growth rates	Reduction in bacterial activity	Increase in protein fermentation
Total SCFA around 127mM	Total SCFA around 117mM	Total SCFA around 90mM
pH 5.5-5.9	pH around 6.2	pH 6.5-6.9

- Regional differences along the length of the GI tract drive the development of distinct microbial communities with differing fermentative activities.

Figure adapted from Leser & Mølbak (2009) and Cummings & Macfarlane (1991)

Diet and the human gut microbiota

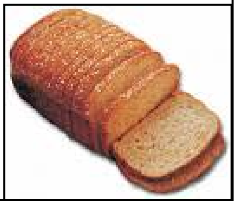
- A significant proportion of dietary compounds escape digestion in the small intestine.
- Non-digestible carbohydrates are the predominant growth substrates for gut bacteria.
- The ND carbohydrate content of the diet may have a considerable influence on human health.
- “Prebiotic” dietary supplements (e.g. inulin, FOS) have been extensively studied.
- Relatively little understood about the effect of the major dietary ND carbohydrates on microbial growth *in vivo*.



Impact of dietary non–digestible carbohydrates

High NSP, low starch | **High resistant starch, low NSP**

High	Phenolics	Low
Distal colon	Fermentation	Proximal colon
Fibrolytic	Bacteria	Amylolytic



Human volunteer trial – 14 overweight/ obese males

M	NSP	Starch	Weight loss	**(n =7)**
M	Starch	NSP	Weight loss	**(n =7)**
1 week	3 weeks	3 weeks	3 weeks	

M = weight maintenance, mixed diet (55% energy from carbohydrates)

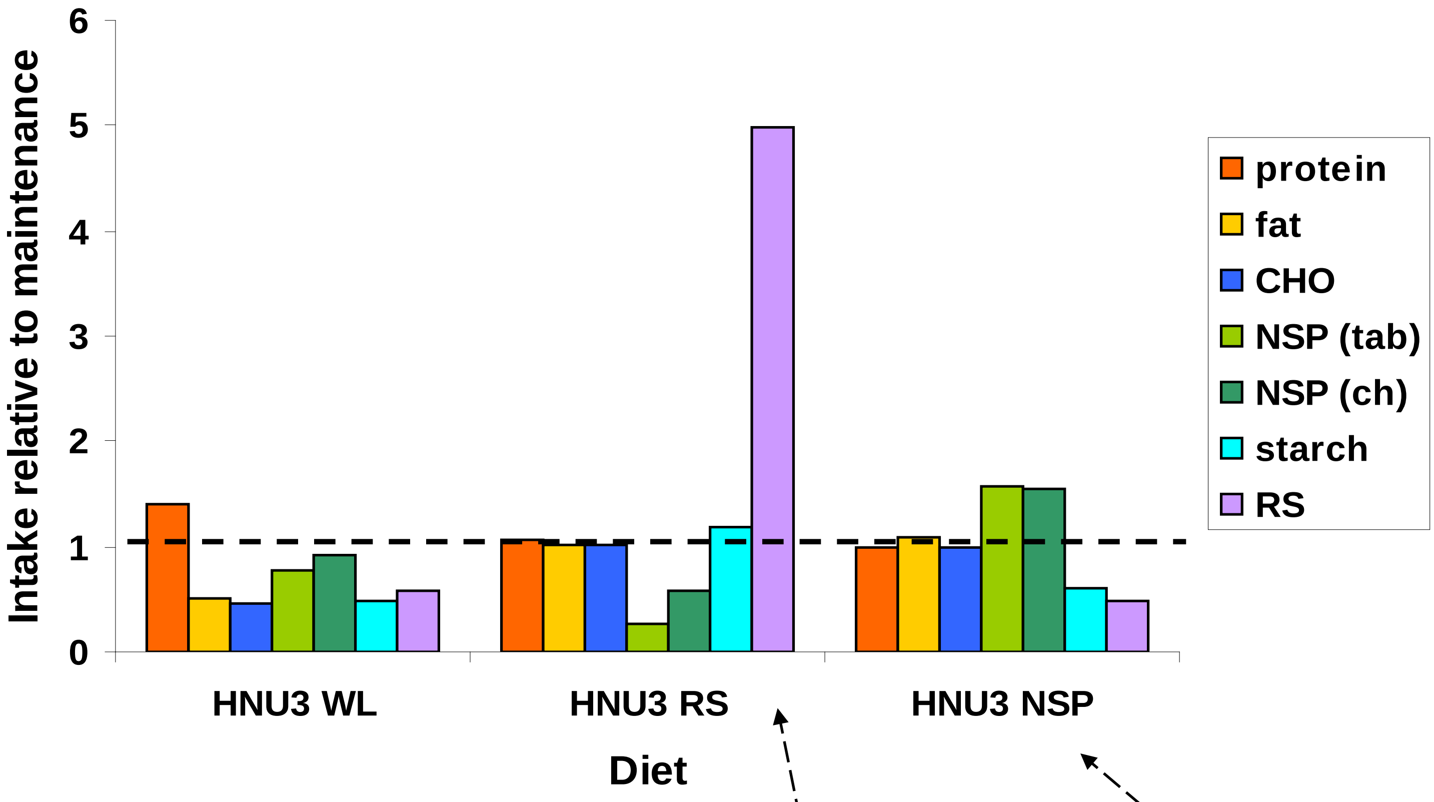
NSP = high non-starch polysaccharides (added bran), minimal starch

Starch = Added resistant starch (Type III), reduced NSP

Weight loss = reduced calorie intake. Increased % protein.

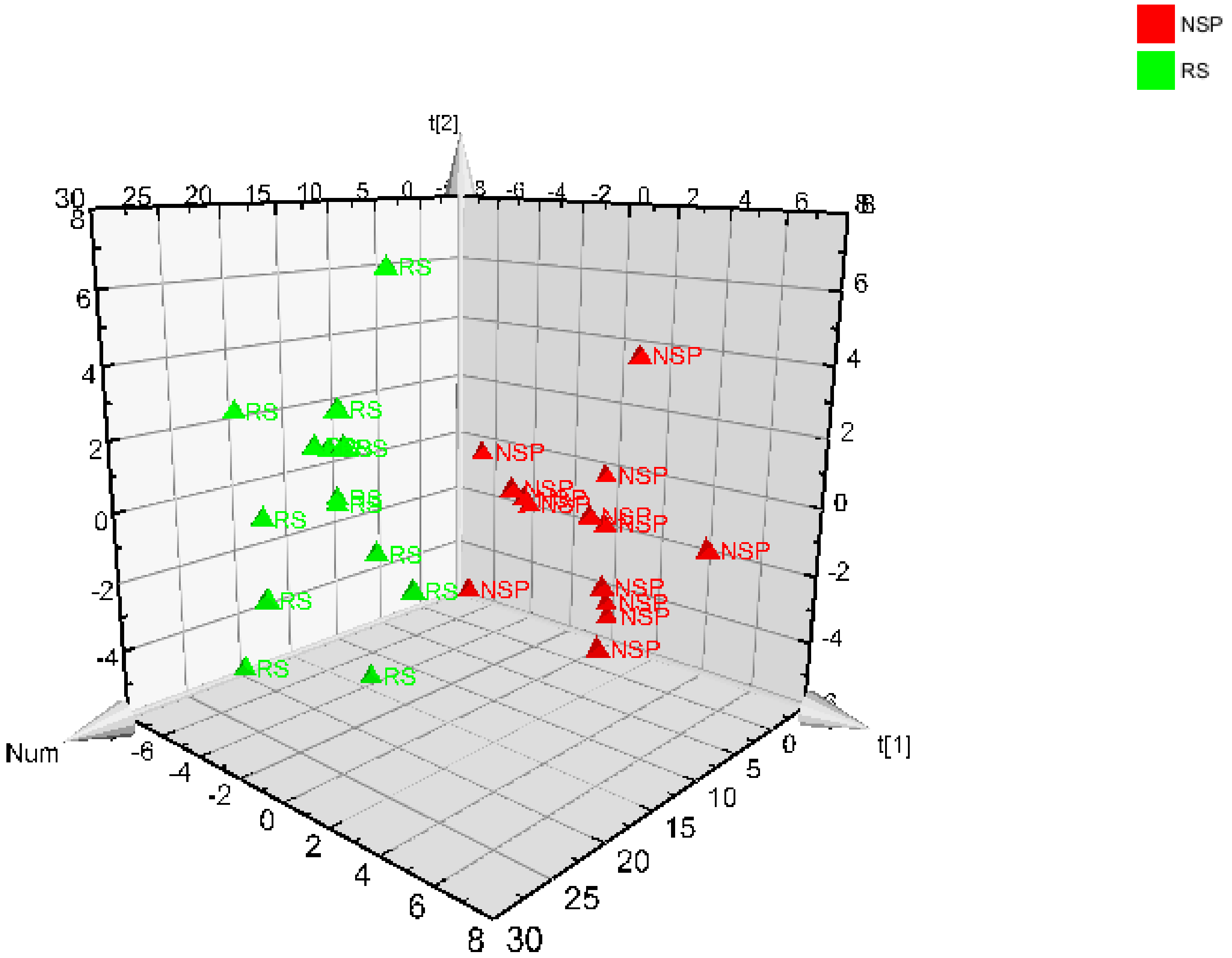
[Walker AW *et al* ISME Journal (2011)]

Diet compositions



Easier to boost resistant starch (Type III) than NSP (bran)

Principal Component Analysis - effect of non-starch (NSP) and resistant starch (RS) diets on fecal metabolites

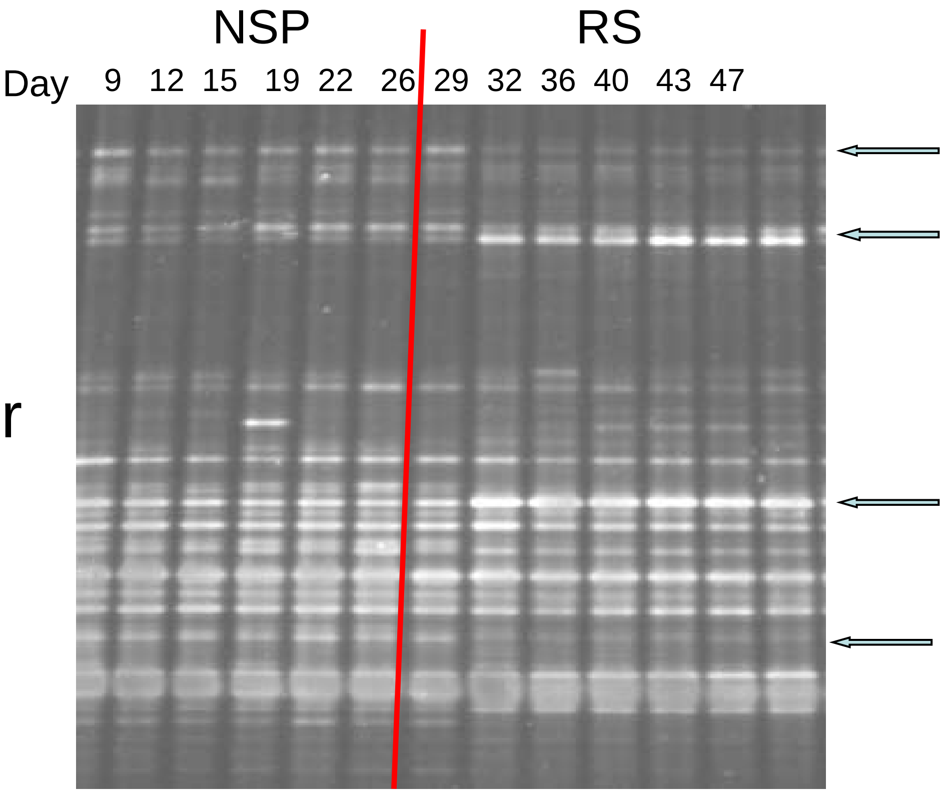


[Wendy Russell - Rowett Institute of Nutrition and Health – in prep]

Microbiota response – experimental design

1. 16S rRNA gene DGGE analysis – time series (14 subjects, all time points)

Volunteer 24



2. 16S rRNA gene sequencing analysis on final sample of each dietary period (n=6)

3. qPCR analysis on selected bacterial groups, plus methanogens (14 subjects – all time points)

16S rRNA gene sequencing – sample clustering

6 overweight male volunteers

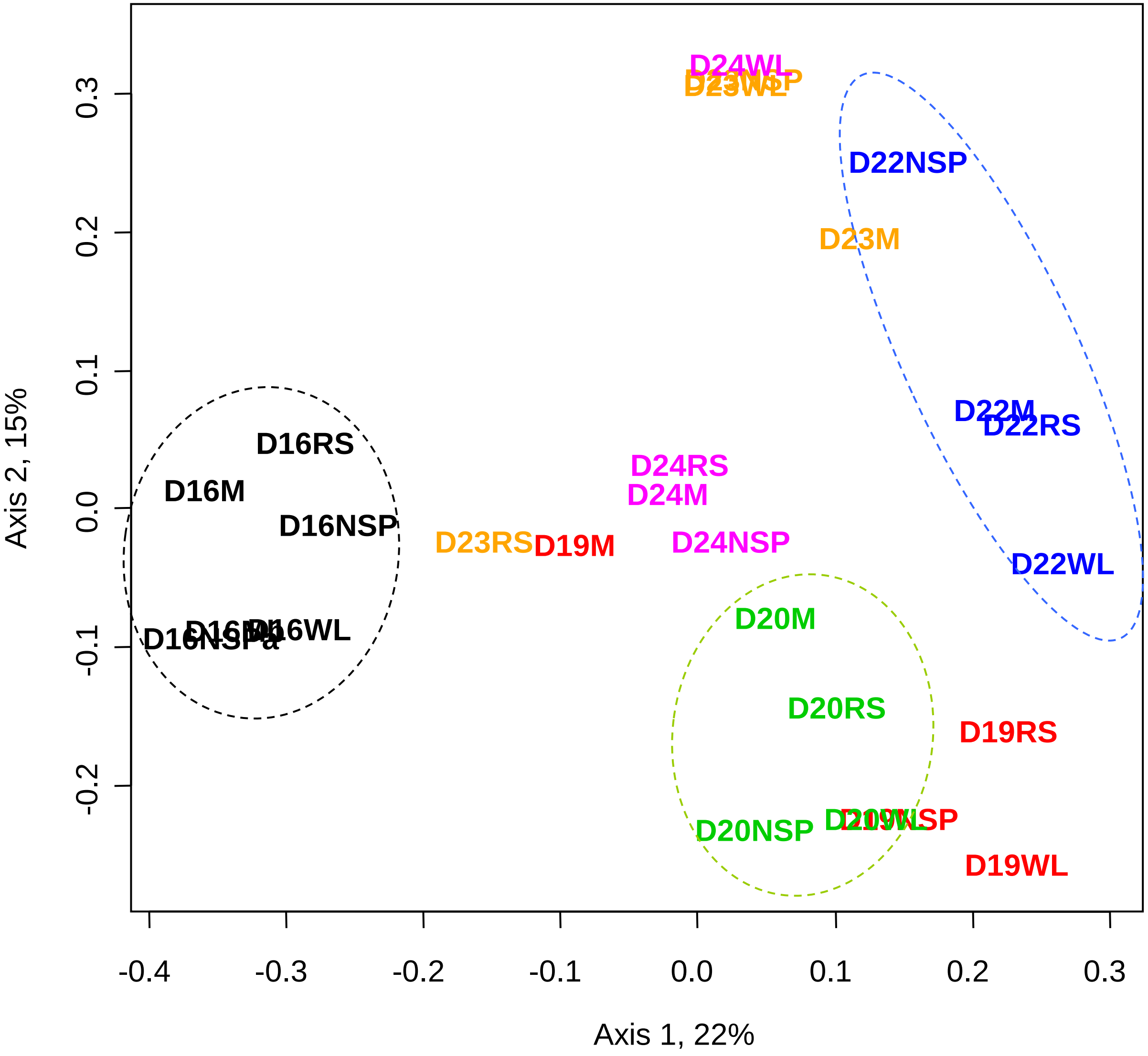
Four diets:-

M = maintenance

RS = resistant starch

NSP = non-starch polysaccharide

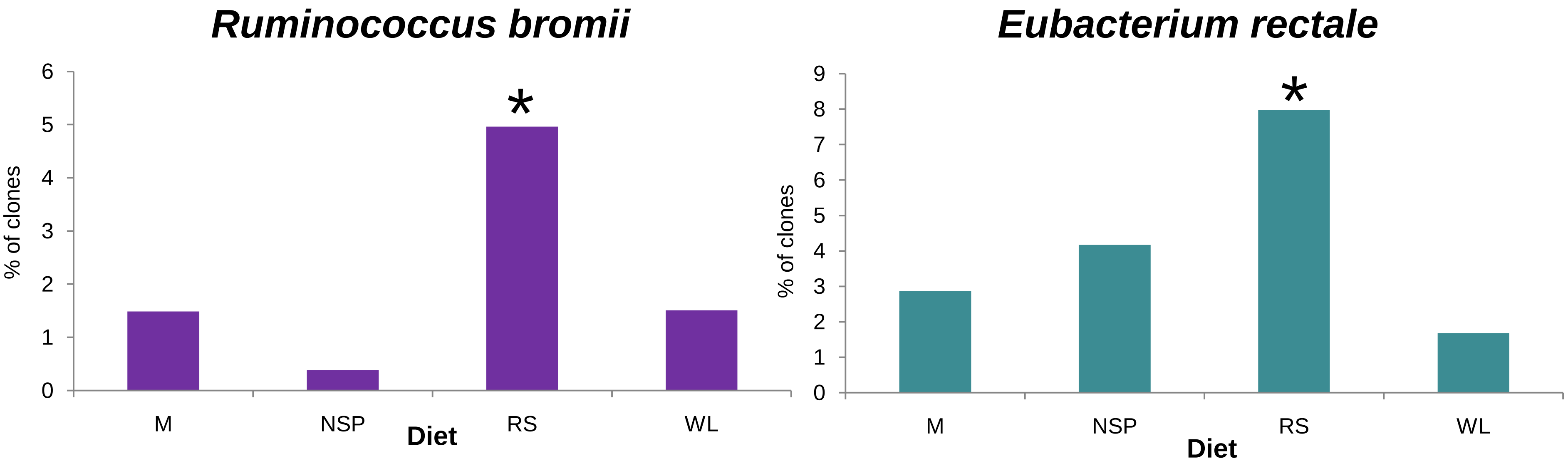
WL = weight loss



[Grietje Holtrop– BioSS]

16S rRNA gene sequencing - Compositional analysis

- Analysis of individual phylotypes reveals significant differences:-



Ruminococcus bromii

Eubacterium rectale

Mean results across 6 volunteers and each dietary regime

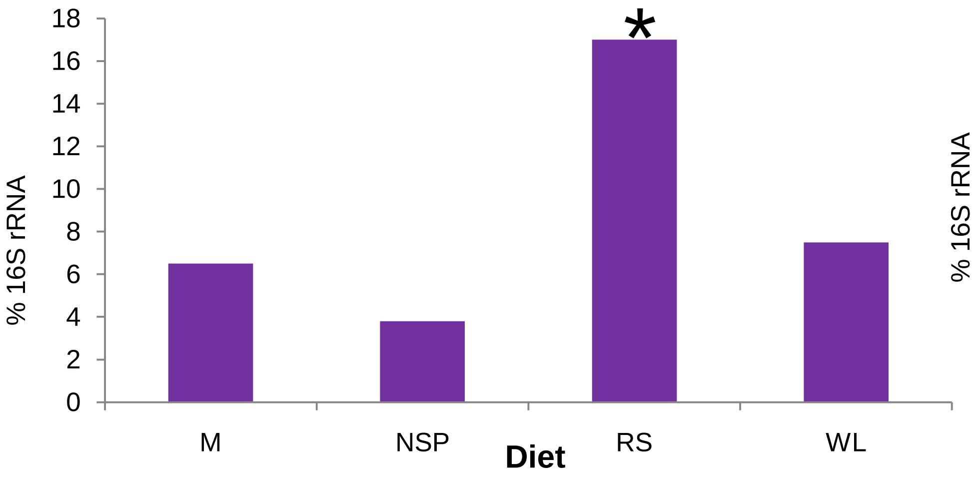
- Proportional abundance of *Ruminococcus bromii* (*Ruminococcaceae*) + *Eubacterium rectale* (*Lachnospiraceae*) increased on RS diet

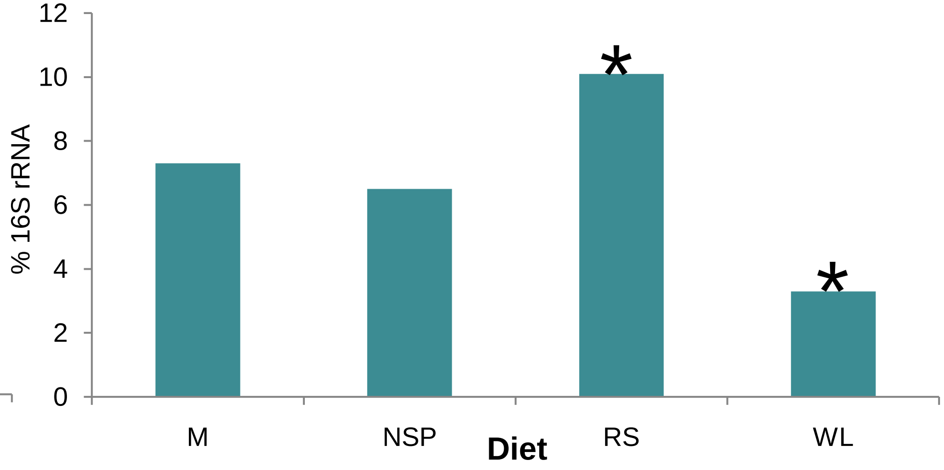
- *Collinsella aerofaciens* proportion reduced on WL diet

- Used qPCR to monitor selected bacterial groups across all donors and all samples

qPCR results

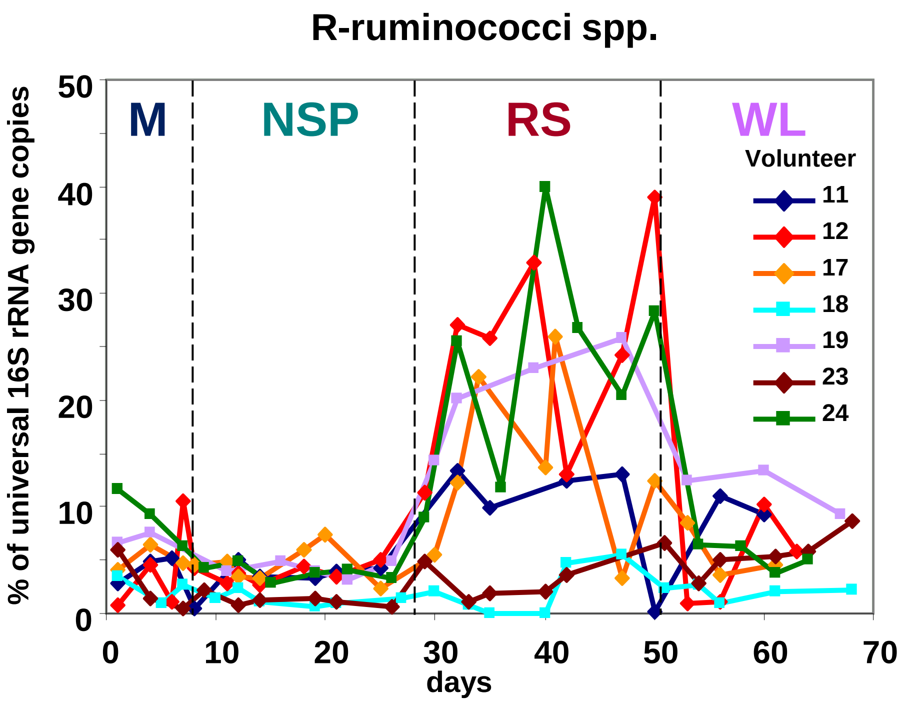
Mean results across all 14 volunteers and each dietary regime

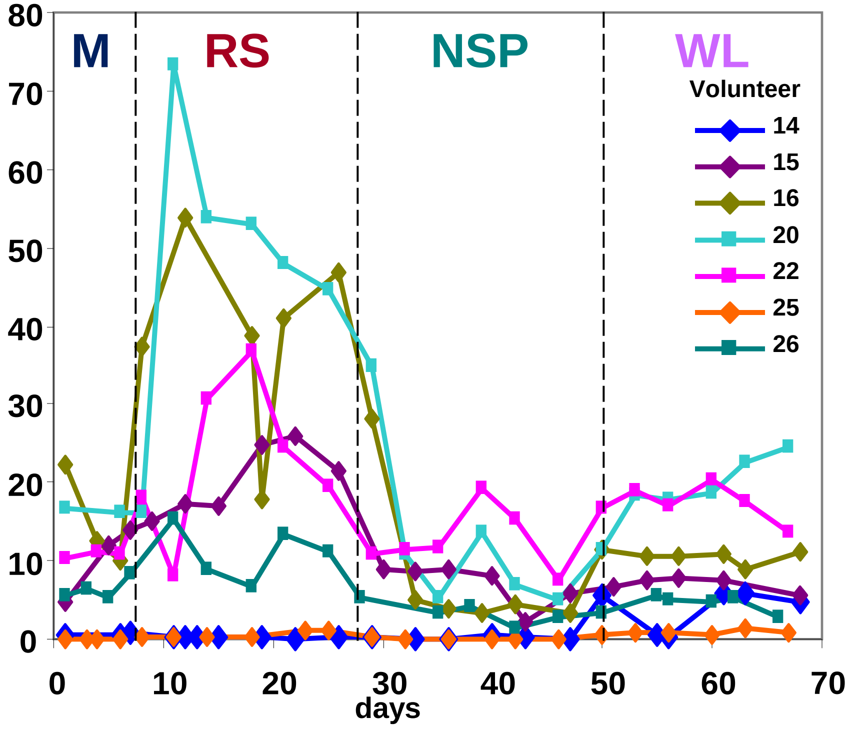

R-ruminococci


E. rectale/Roseburia

- R-ruminococci increased on RS diet
- *E. rectale/Roseburia* spp. increased on RS and decreased on WL diet

qPCR results



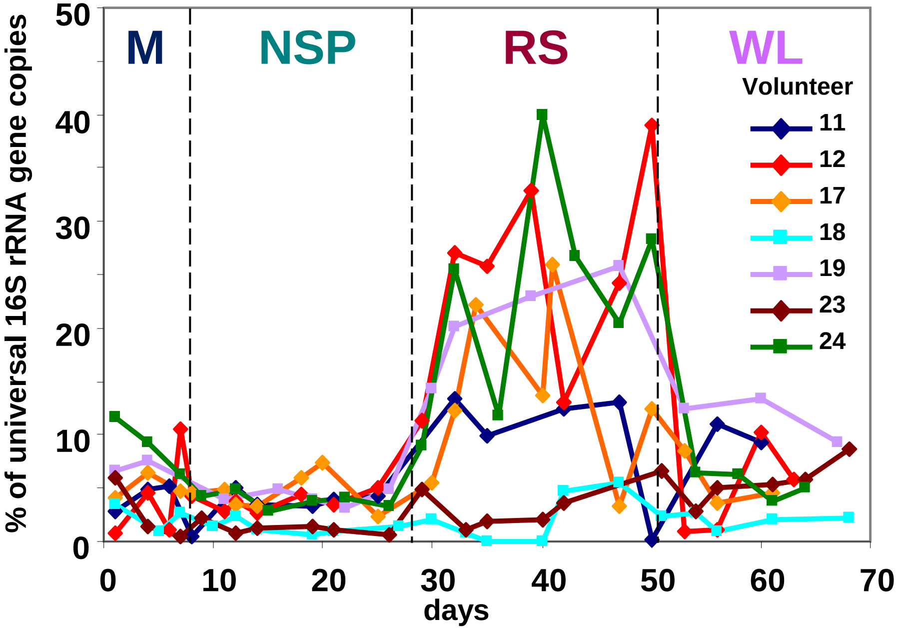


- Rapid responses to RS diet in most individuals
- ‘Bloom’ of ruminococci (related to *R. bromii*) on resistant starch diet
- Marked inter-individual variation in responses

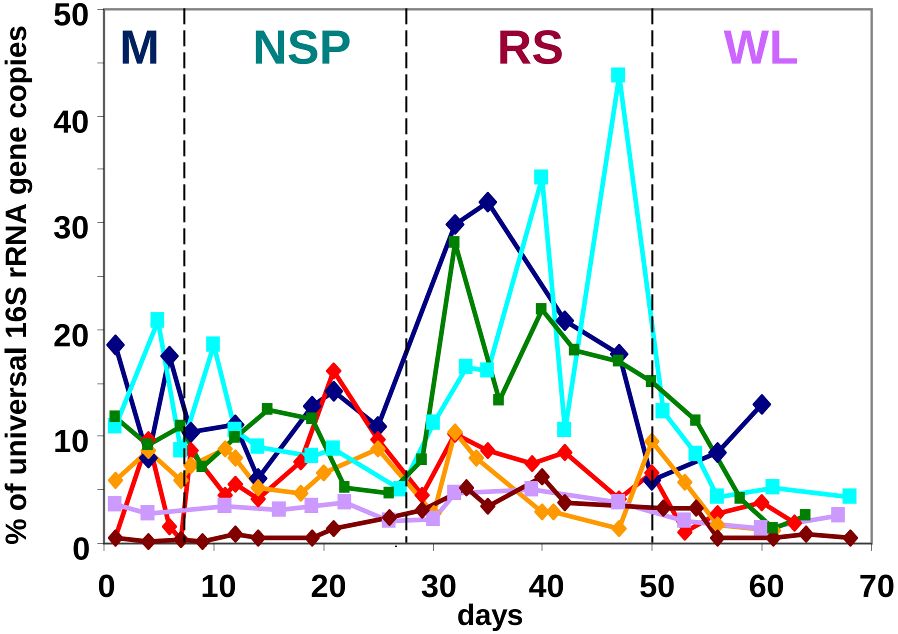
[Petra Louis - Rowett Institute of Nutrition and Health]

qPCR results

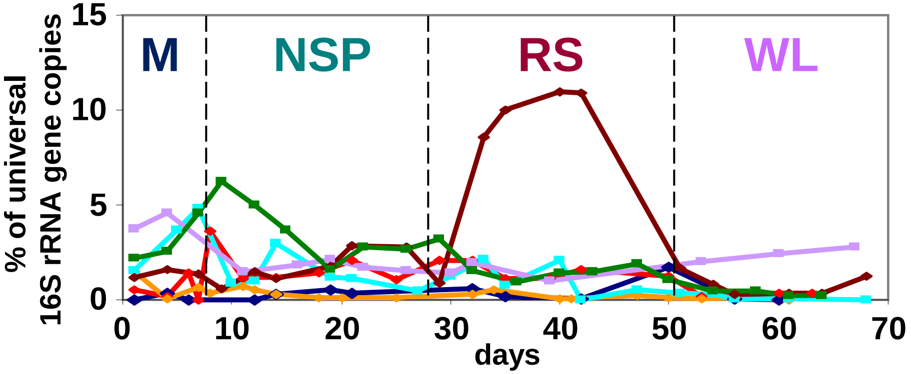
R-ruminococci spp.



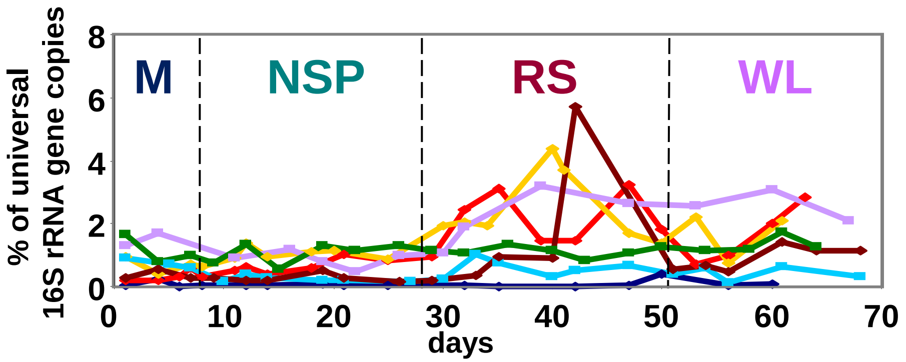
***E. rectale/Roseburia* spp.**



***Bifidobacterium* spp.**

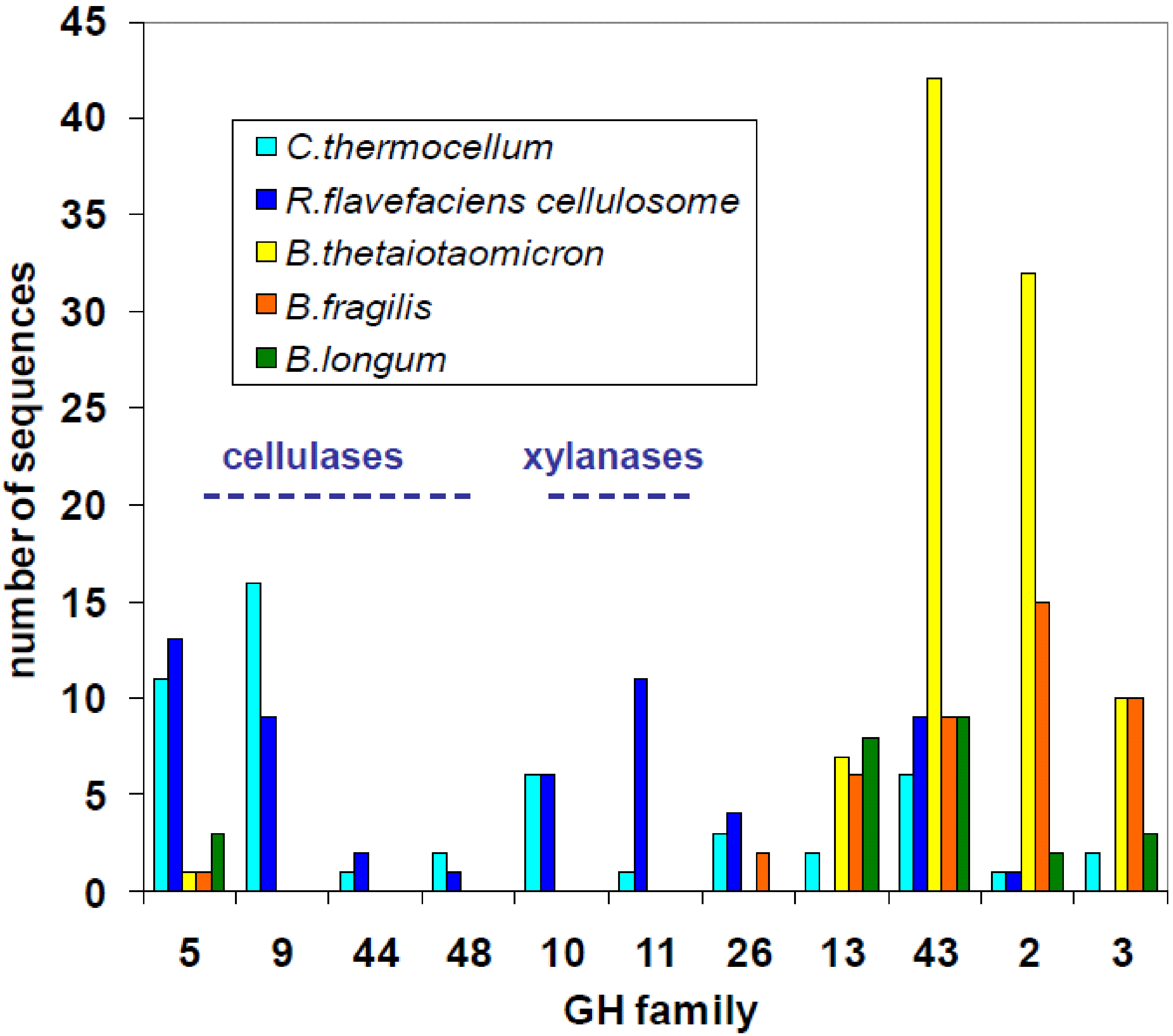


***Oscillibacter* spp.**



[Petra Louis - Rowett Institute of Nutrition and Health]

R-ruminococci

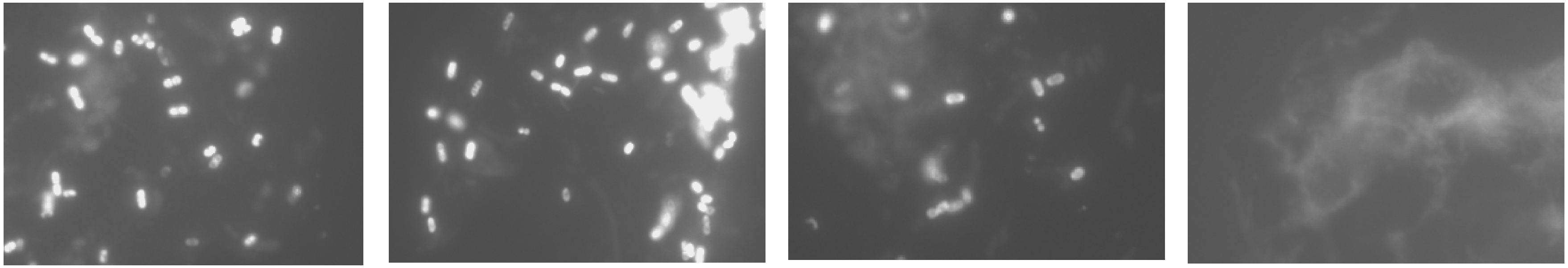


Distribution of glycoside hydrolase families in the genomes of five polysaccharide-utilizing bacteria

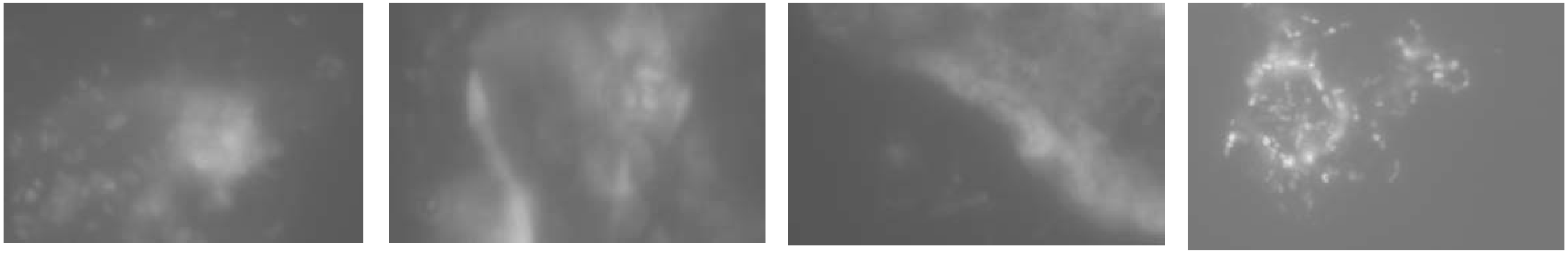
[Flint HJ *et al* Nat Rev Microbiol (2008)]

R-ruminococci

Bran | Cabbage | Carrot | Corn Starch



Ruminococcus flavefaciens-like



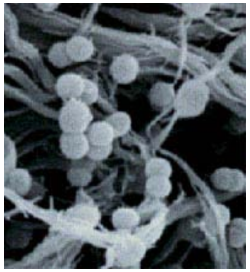
Ruminococcus bromii-like

RESEARCH ARTICLE

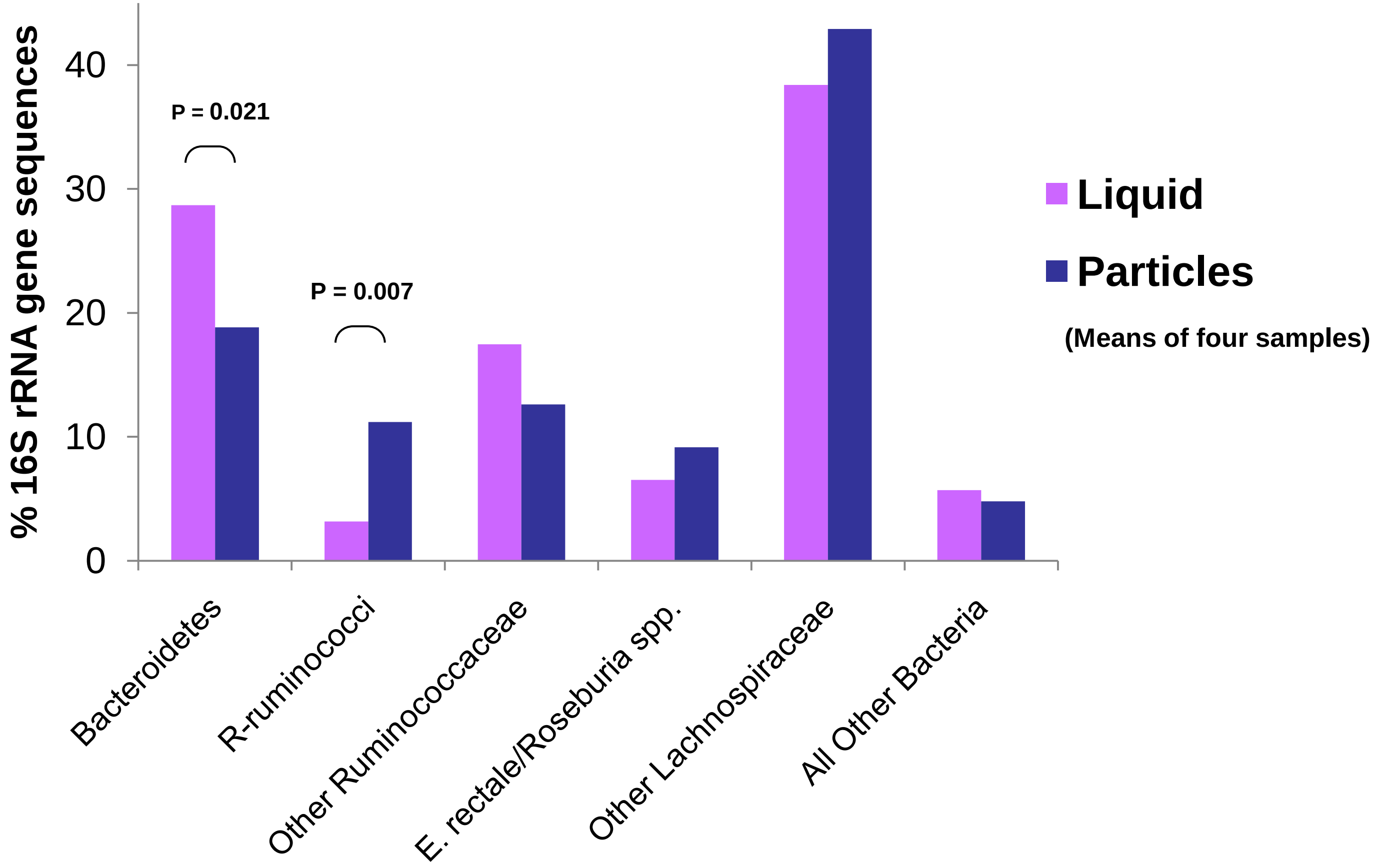
Phylotypes related to *Ruminococcus bromii* are abundant in the large bowel of humans and increase in response to a diet high in resistant starch

Guy C.J. Abell, Caroline M. Cooke, Corinna N. Bennett, Michael A. Conlon & Alexandra L. McOrist

R. albus



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

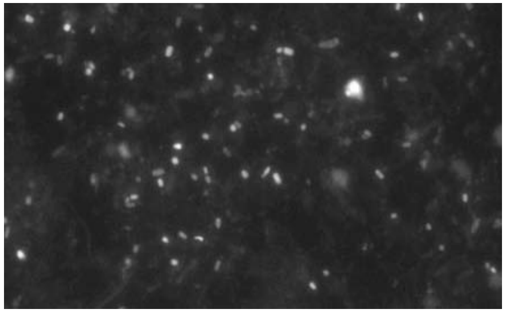


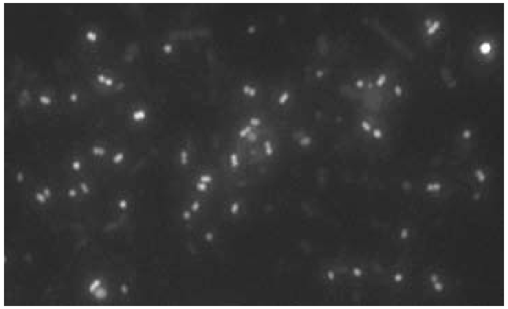
- **R-ruminococci are preferentially associated with fiber particles in stool samples.**
- **Bacteroidetes partition more into the liquid phase**

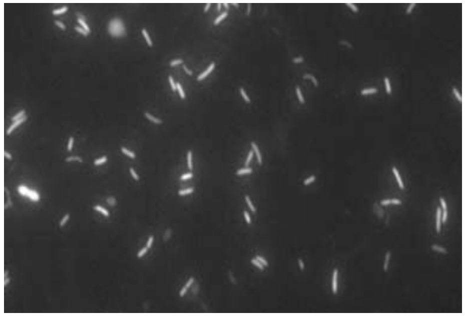
[Walker AW *et al* Env Microbiol (2008)]

FISH analysis of liquid and particulate fractions

Liquid fraction



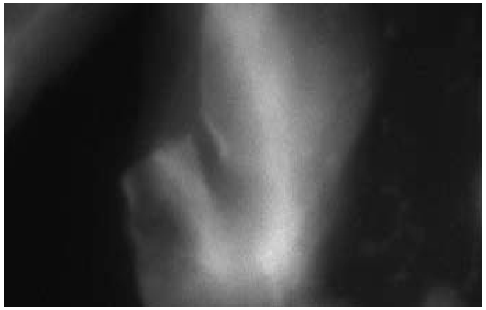


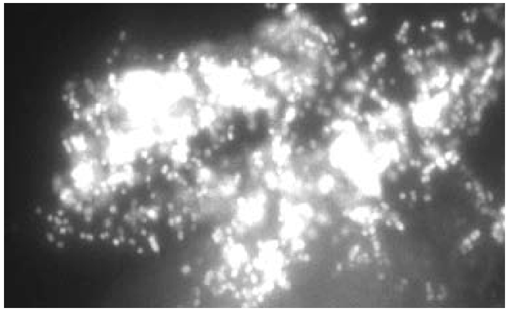


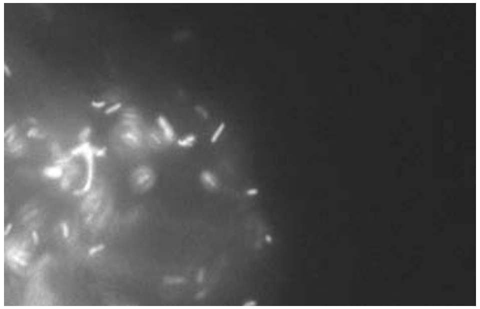
Bacteroides/Prevotella

R-ruminococci

Roseburia/E.rectale

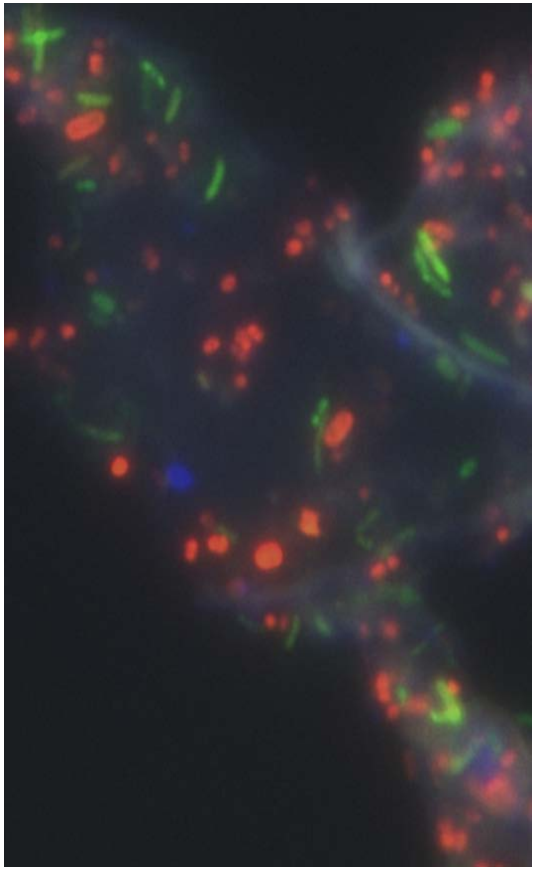


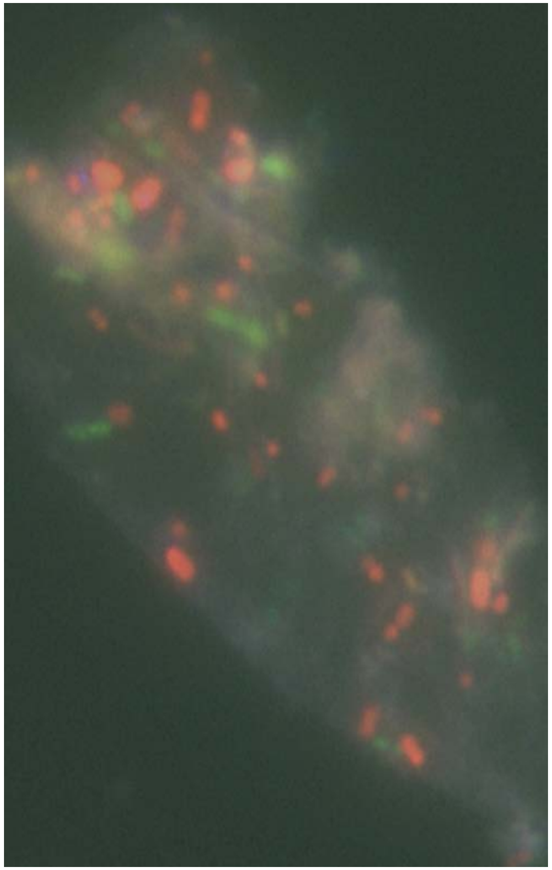


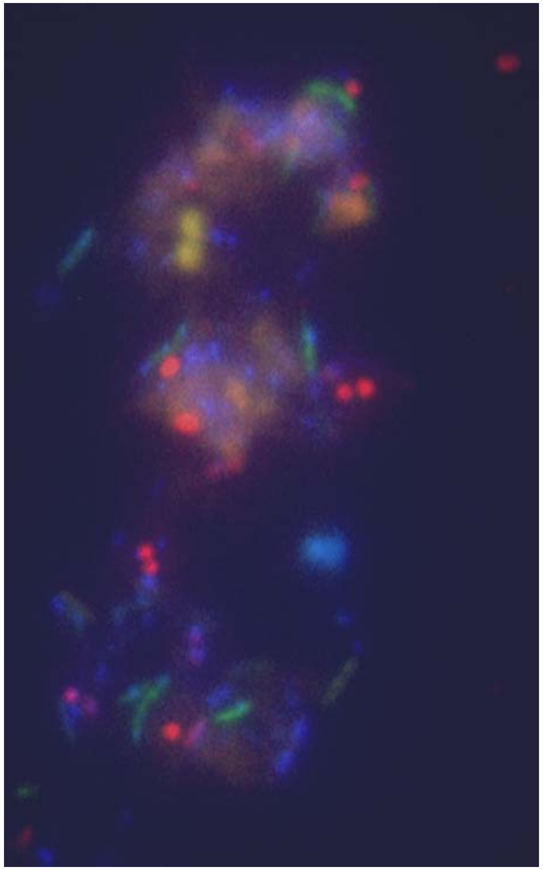


Particulate fraction

Multi-probe FISH analysis of particulate fraction


Donor A


Donor B


Donor C

Red = R-ruminococci Green = *Lachnospiraceae* Blue = DAPI

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

RS diet responses in 14 volunteers

Stimulation on RS diet relative to NSP diet

Volunteer	R-ruminococci	*E. rec/Roseburia*	*Bifidobacterium*
11	+	+	-
12	++	-	-
17	+	-	-
18	+	+	-
19	++	-	-
23	+	+	++
24	++	+	-
14	-	+	+
15	+	-	-
16	+++	-	-
20	+++	-	-
22	+	-	+++
25	-	+	-
26	+	+	-
	acetate, ethanol fibre degrader?	butyrate	lactate, acetate immune modulation?

< 40% resistant starch fermented

<2-fold

>2-fold

>4-fold

>8-fold

- **Likely to have different consequences for host health**

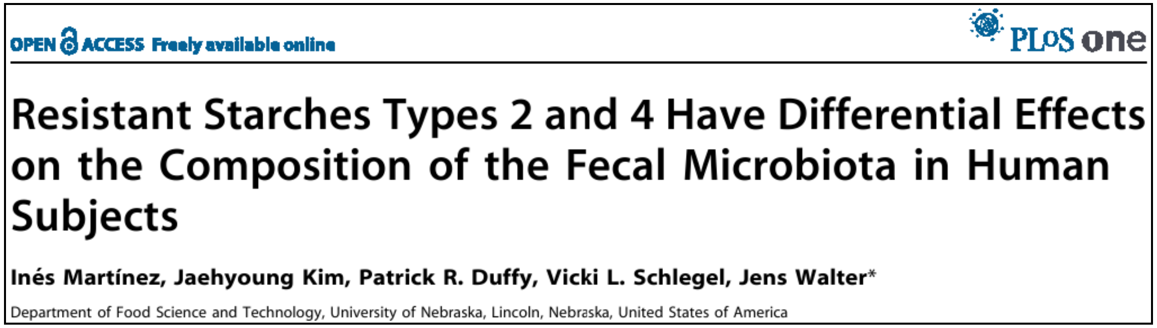
[Walker AW *et al* ISME Journal (2011)]

Summary

- Specific bacterial groups/species respond strongly to dietary change, but there is inter-individual variation in the groups that respond.

- Ruminococci may be important for resistant starch degradation.

- Other dietary substrates will likely drive different microbiota responses:



OPEN ACCESS Freely available online PLoS one

Resistant Starches Types 2 and 4 Have Differential Effects on the Composition of the Fecal Microbiota in Human Subjects

Inés Martínez, Jaehyoung Kim, Patrick R. Duffy, Vicki L. Schlegel, Jens Walter*

Department of Food Science and Technology, University of Nebraska, Lincoln, Nebraska, United States of America

- Implications for human health?
  - Does this affect energy harvest from the diet?
  - Does this impact delivery of SCFA (e.g. butyrate) to the distal colon?

- Implications for therapeutic dietary intervention:
  - Even if rational prebiotics/functional foods are designed the microbiota response may depend on the individual.

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