National Science Challenges

HIGH-VALUE NUTRITION

Ko Ngā Kai Whai Painga

Priority Research Programme
Foods for improving gut function and comfort

Are microbes the missing piece of the gut comfort puzzle?

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Host institution

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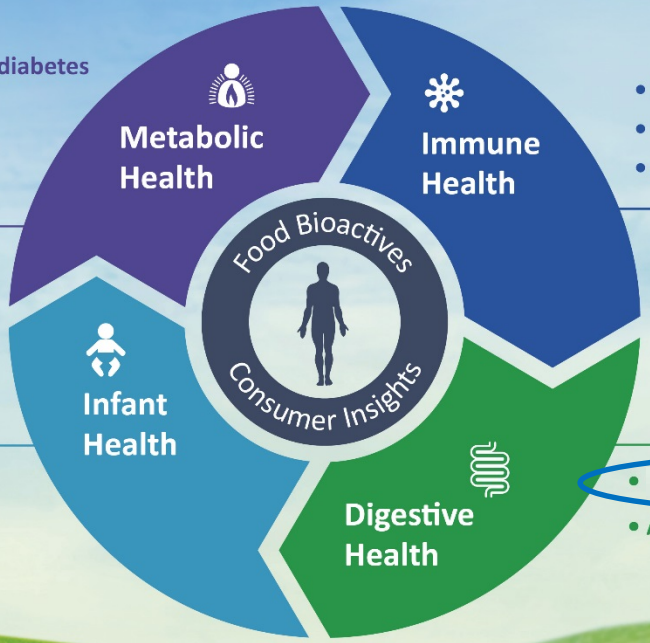
High-Value Nutrition National Science Challenge research themes and projects



- TOFI: Thin on the outside, fat inside: preventing diabetes
- Kiwifruit for glucose control
- Combined proteins for lean body mass
- Grass-fed beef for cholesterol control

- Building immune defence
- Natural milk for allergy management
- Greenshell™ mussels to manage inflamed joints

- Complementary feeding for immune protection
- Fibres for sustained energy release

- Foods for gut function and comfort
- A2 Milk for gut comfort

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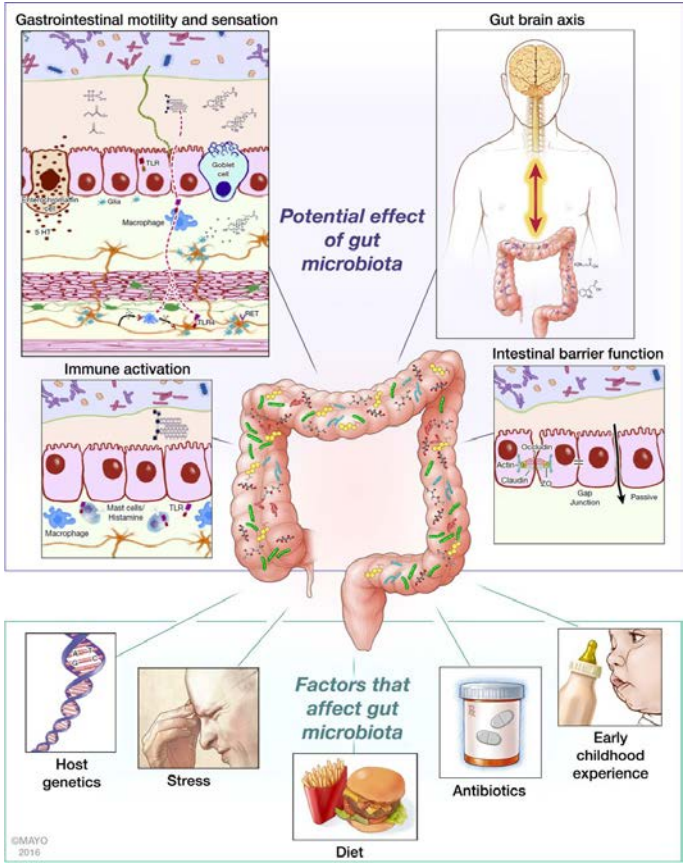
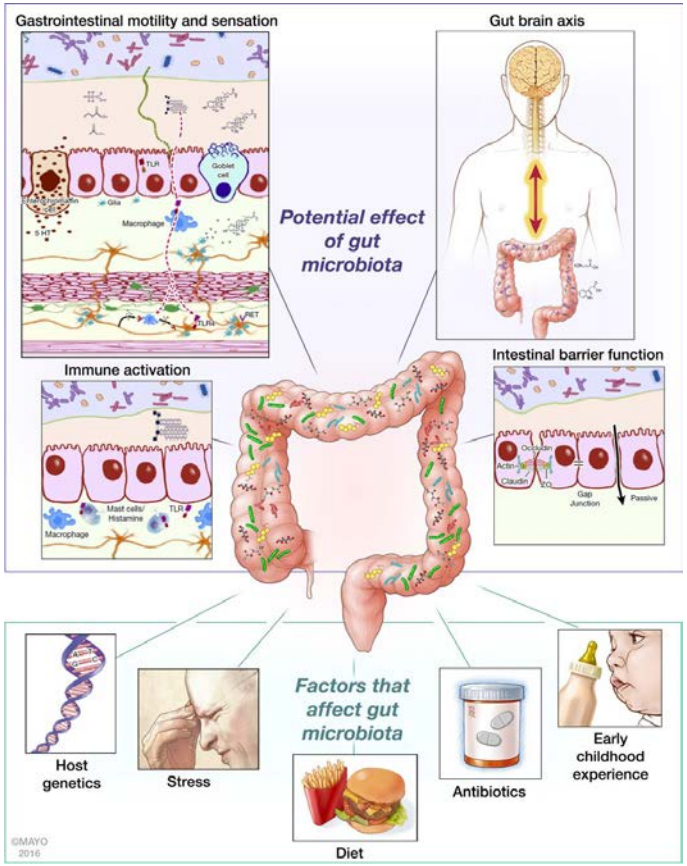
Irritable Bowel Syndrome is the ideal model for developing future foods with clinical evidence to support claims for healthy people



Microbiome and gut comfort

- Gut microbiota modulates mechanisms underlying gut comfort;
  - Motility
  - Immune system
  - Barrier function
  - Gut-brain axis
- Food is the obvious choice to fine-tune the microbiome for health conscious consumers

Bhattarai *et al* (2017). *Am J Physiol Gastrointest Liver Physiol.* 312:G52-G62



Microbiome and gut comfort

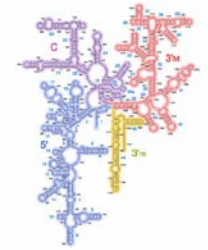
- Altered microbiota associated with perturbed gut function, including irritable bowel syndrome (IBS)
- Changes differ between studies, e.g. Firmicutes, *Faecalibacterium*, *Blautia*, bifidobacteria, methanogens, *Prevotella*[1-4]
- For food to be effective, we need to know where we are coming from and where we are going

Bhattarai *et al* (2017). *Am J Physiol Gastrointest Liver Physiol.* 312:G52-G62

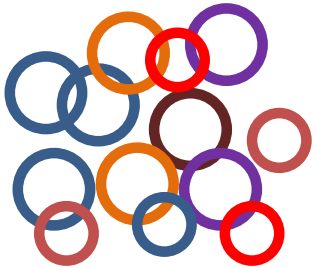
1. Jeffery *et al* (2012); 2. Tap *et al* (2017); 3. Labus *et al* (2017); 4. Malinen *et al* (2005)

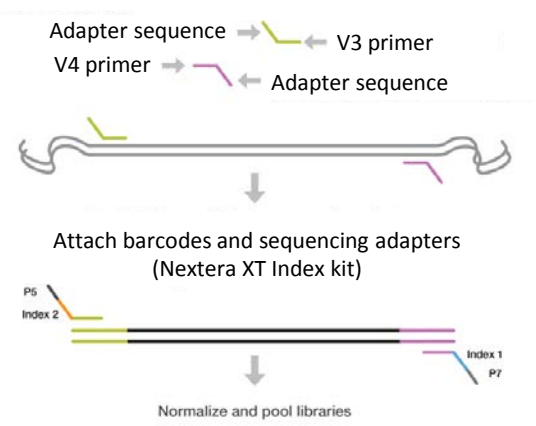
Which bacteria are present?

PCR amplify regions of bacterial 16S rRNA gene

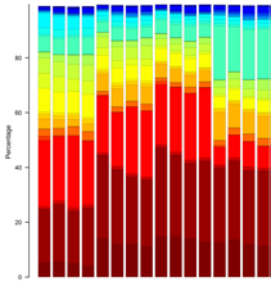


Extract metagenomic DNA from faecal sample





Community taxonomic information



- To date, most IBS microbiome studies have been based on 16S rRNA gene amplicon sequencing
- Relatively easy to do
- **Only provides taxonomy** (who is there?)

Our whole systems approach to High-Value Nutrition science

Our biology
Organ networks
Cellular networks
Molecular networks
Genetic interaction

Our environment
Where we live
Cultural backgrounds
Social networks
Food choices

Our research focuses on understanding biological processes as complex integrated systems.
Nutrition to keep us healthy and well requires an holistic approach.

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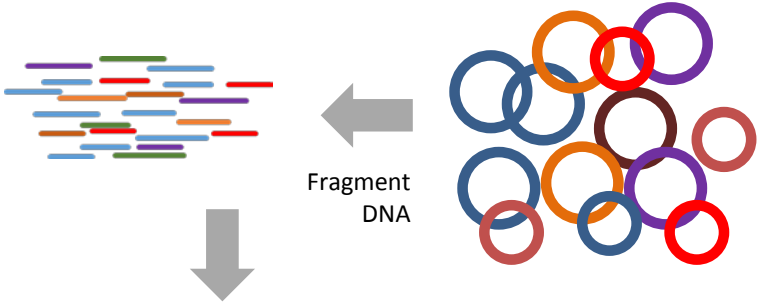
What are the bacteria doing?

- We want to know what the bacteria are capable of doing
- Sequencing all DNA in the community (the **metagenome**) provides this insight

Shotgun metagenomic sequencing

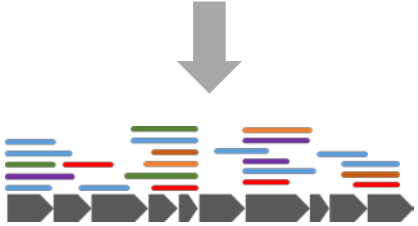
Extract metagenomic DNA from faecal sample



Fragment DNA

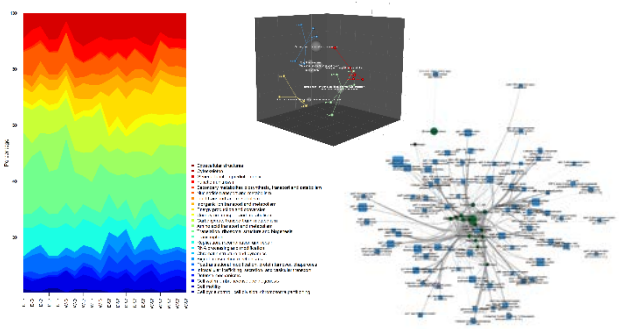
Attach barcodes and sequencing adapters
Nextera XT Index kit/Rubicon ThruPLEX kit

Normalise and pool libraries
Illumina HiSeq paired-end sequencing



Align DNA sequences against reference genomes using MG-RAST/IMG

Community taxonomic and functional gene abundance information

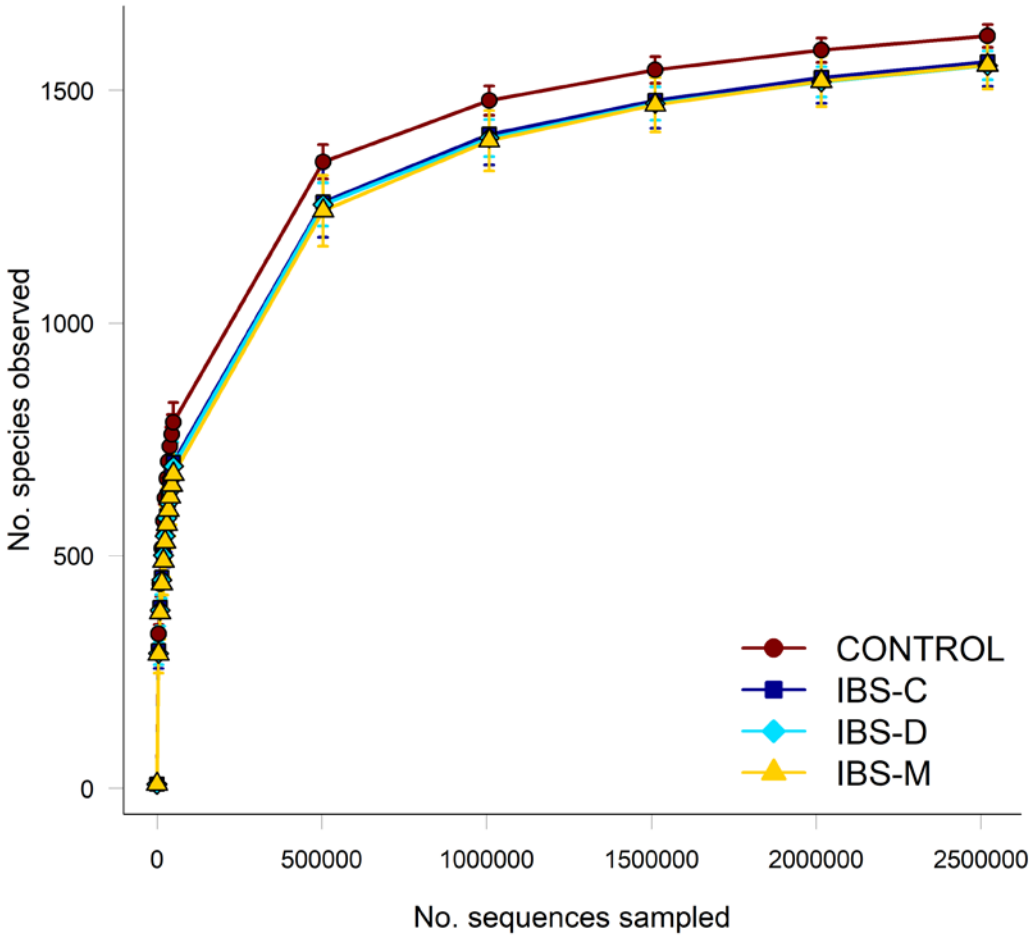


Metagenomic sequencing gives insight into what the community is doing

Metagenomic sequencing of COMFORT cohort faecal microbiome

- Faecal microbiome analysed by shotgun metagenome sequencing using Illumina NextSeq 500 paired-end 2x150 bp (APC/Teagasc)
- 112 samples sequenced
  - 41 case-controls
  - 9 IBS constipation (IBS-C)
  - 22 IBS diarrhoea (IBS-D)
  - 10 IBS mixed (IBS-M)
  - 16 functional constipation (FC)
  - 5 functional diarrhoea (FD)
  - 9 not determined

What did we find?

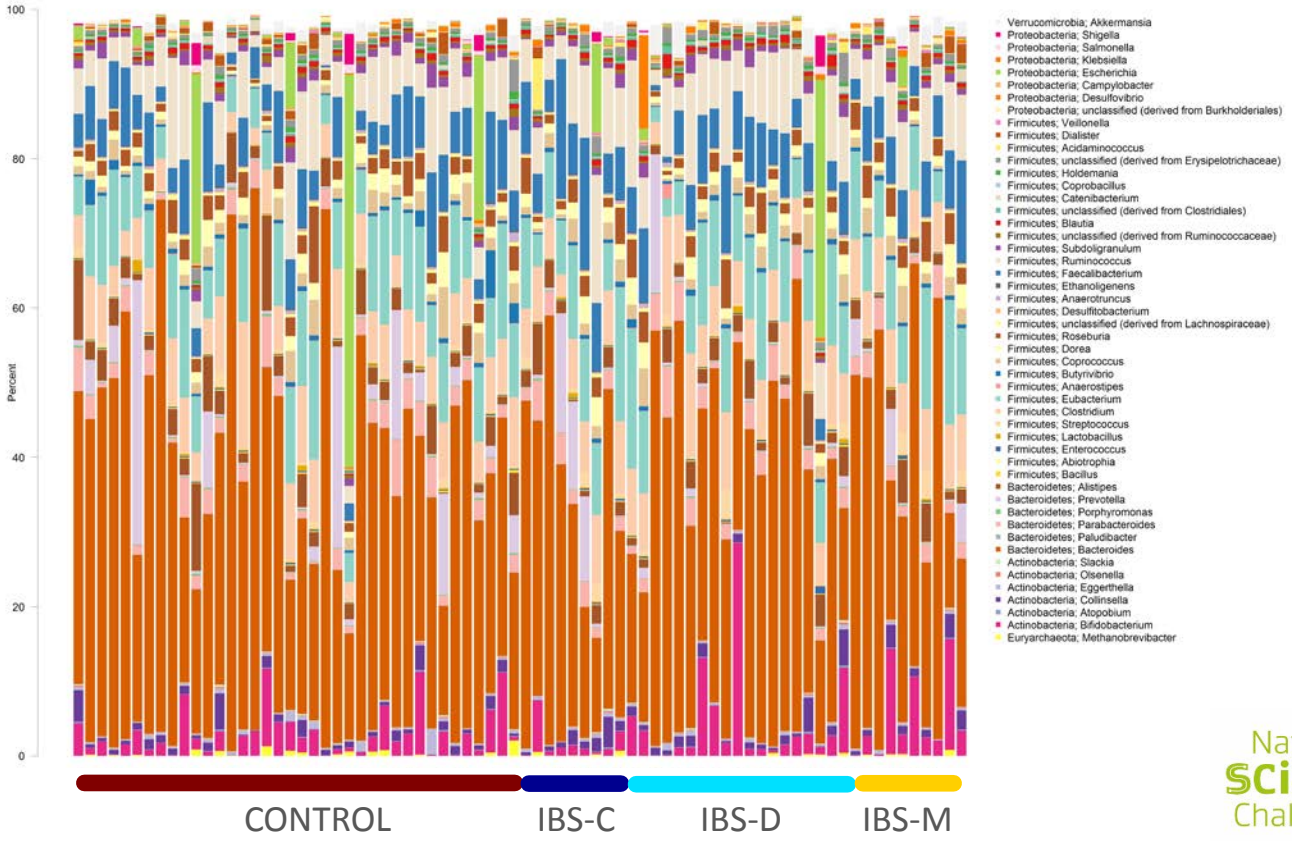


Alpha diversity
(how many different types)

- IBS groups have lower alpha diversity than controls (P=0.05)

Microbiome composition

Genus level

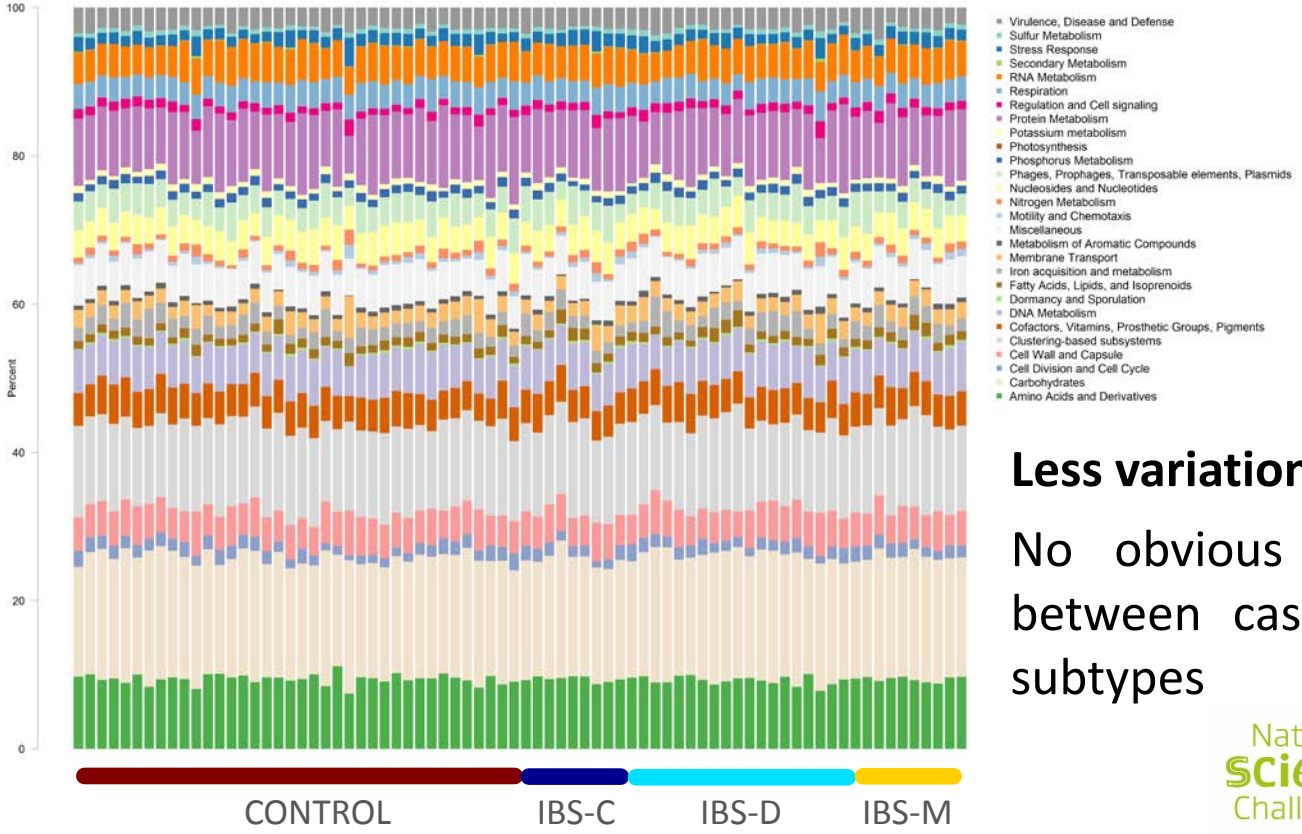


Highly variable

No obvious division immediately apparent between case-controls and IBS subtypes

Gene functions

Level 1



Less variation between subjects

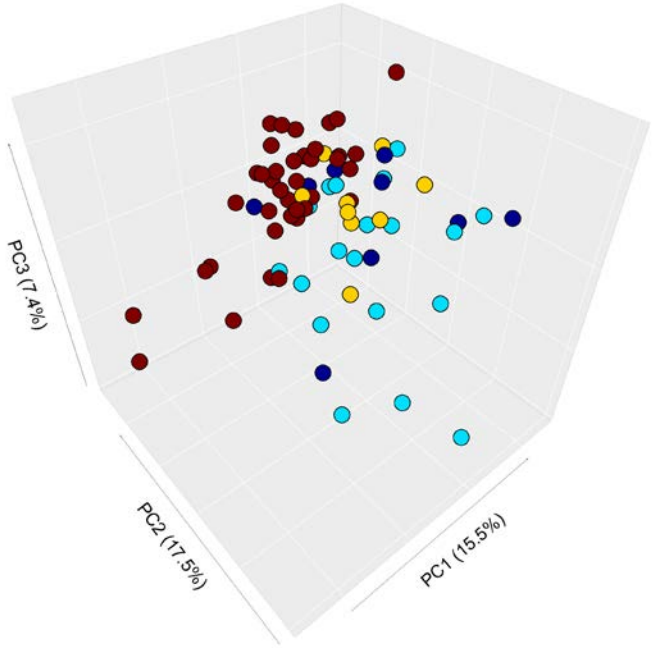
No obvious division apparent between case-controls and IBS subtypes

Are there differences in microbiome composition and function?

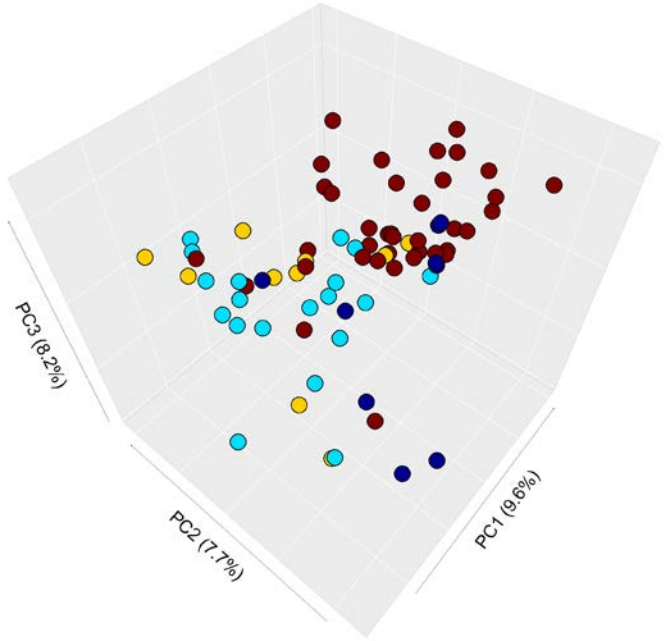
- Standard statistical methods show few differences between case-controls and IBS subtypes
- Machine learning methods (e.g. PLS-DA, SVM, random forest) could help to separate groups

Partial least squares discriminant analysis (PLS-DA)

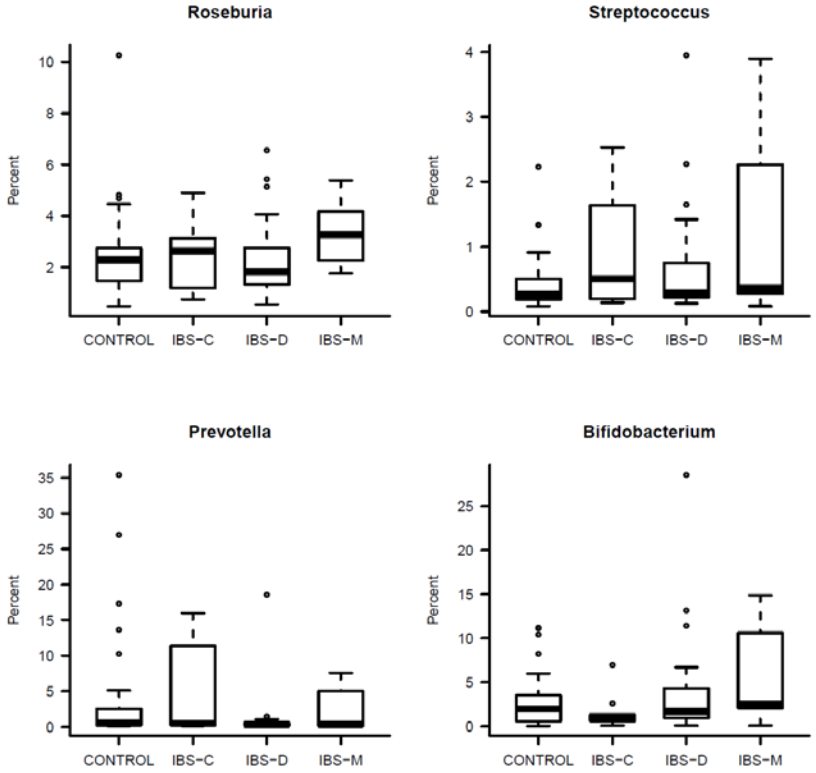
Taxonomy



Gene function



- IBS-C
- IBS-D
- IBS-M
- CONTROL

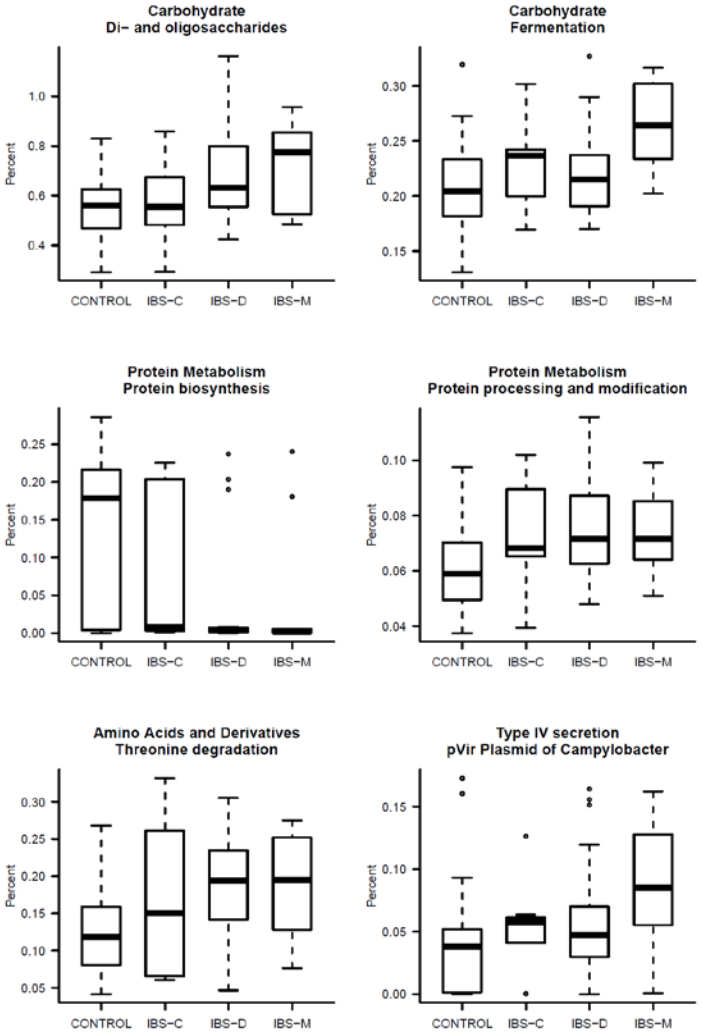


What are the taxa and gene functions that lead to separation in PLS-DA models?

Genera include *Roseburia*[1], *Streptococcus*, *Prevotella*[1,2], *Bifidobacterium*[3]

- Complex carbohydrate fermenters.
- Some generally thought of as “good” bacteria, but appear to be higher in IBS groups

1. Rigsbee *et al* (2012) Am J Gastrol 107:1740-71
2. Jeffery *et al* (2012) Gut 61:997-1006
3. Tap *et al (*2017) Gastroenterology 152:111-123



What are the taxa and gene functions that lead to separation in PLS-DA models?

- Functions include those related to carbohydrate and protein metabolism
- Aligns with taxonomy data

How robust is the separation between groups?

Support vector machines (SVM)

Taxonomy

predict	CONT	IBS
CONT	**35**	7
IBS	3	**31**

Gene function

predict	CONT	IBS
CONT	**38**	3
IBS	0	**35**

How robust is the separation between groups?

Support vector machines (SVM)

Taxonomy

predict	CONT	IBS-C	IBS-D	IBS-M
CONT	**38**	7	10	9
IBS-C	0	**2**	0	0
IBS-D	0	0	**9**	0
IBS-M	0	0	0	**1**

Gene function

predict	CONT	IBS-C	IBS-D	IBS-M
CONT	**38**	7	6	8
IBS-C	0	**2**	0	0
IBS-D	0	0	**13**	0
IBS-M	0	0	0	**2**

Improved predictive power with gene function

How robust is the separation between groups?

Support vector machines (SVM)

Combined taxonomy and gene function

predict	CONT	IBS
CONT	**37**	2
IBS	1	**36**

predict	CONT	IBS-C	IBS-D	IBS-M
CONT	38	6	2	7
IBS-C	0	**3**	0	0
IBS-D	0	0	**17**	2
IBS-M	0	0	0	**1**

Predictive power further improved by combining taxonomy with gene function

Can we classify the undefined samples?

90040	90050	90055	90074	90075	90111	90121	90123	90126
CONT	CONT	IBS	CONT	IBS	IBS	CONT	IBS	IBS

Time will tell if these predictions are accurate

Conclusions

- Microbiome composition and function appear be different between controls and IBS subtypes, more pronounced with IBS-D
- Add to predictability of existing biomarkers?
- Carbohydrate fermentation appears to play a role
- Some ostensibly “good” bacterial increased in IBS
- What we need to know is “what they are doing”?

→ *Clinical and systems approach will de-risk developing new foods with validated gut health benefits that will be highly desirable and sought after by healthy consumers*

Research Team and collaborators

Programme Leader/Principal Investigator

- Nicole Roy, AgResearch, Riddet Institute

Principal Investigator, clinical

- Richard Gearry, University of Otago

Associate Investigators, biomarkers

- Metabolites: Karl Fraser, AgResearch
- Microbiota: Wayne Young, AgResearch
- Immune: Oliver Grasser, Malaghan Institute
- Proteins: Janine Cooney, Plant and Food Research

Collaborator

- Sequencing: Paul Cotter, APC/Teagasc

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