

# ***The Role of the Microbiome in Health and Disease***

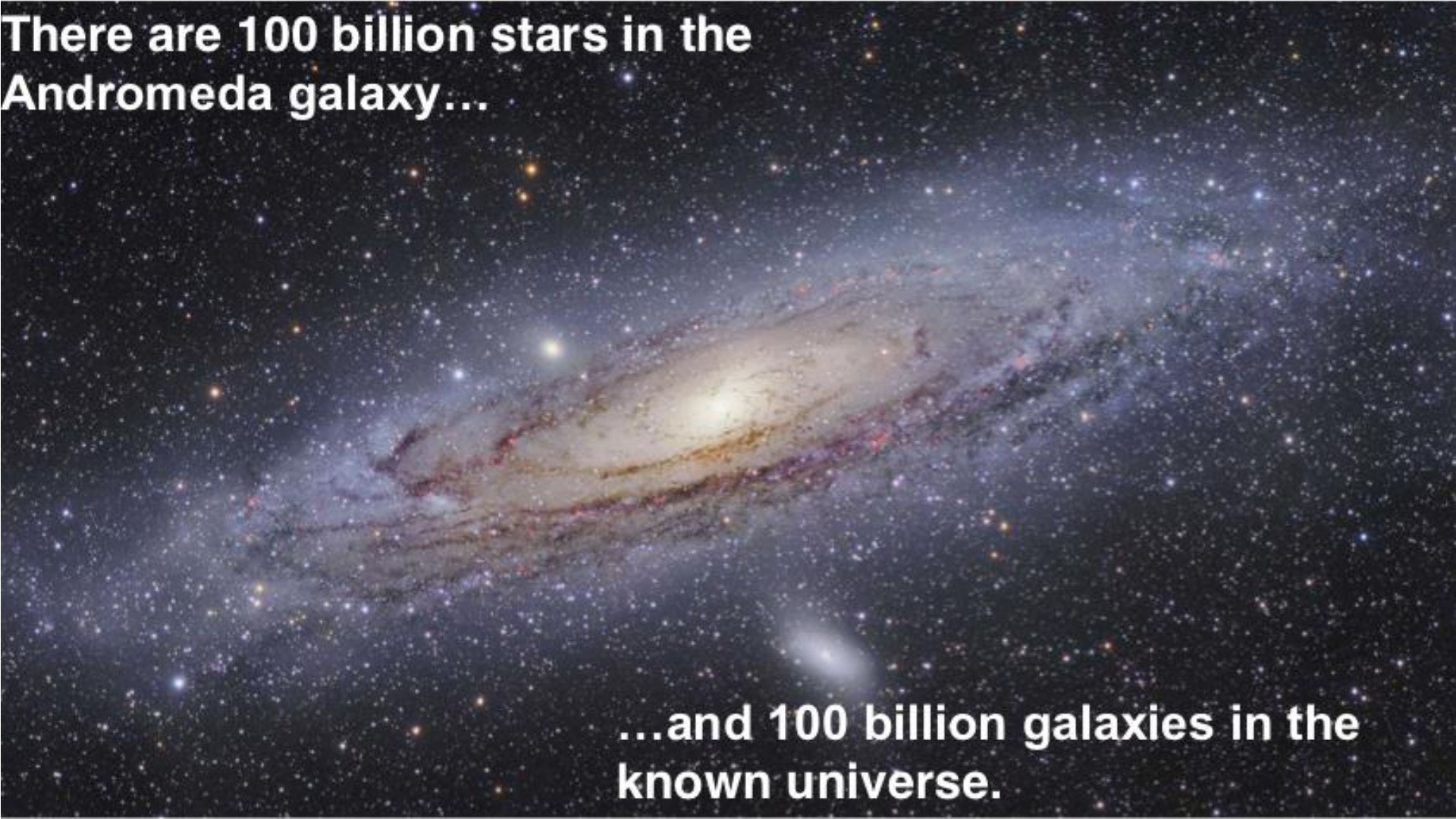
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*[research.mater.org.au](http://research.mater.org.au)*

**There are 100 billion stars in the  
Andromeda galaxy...**

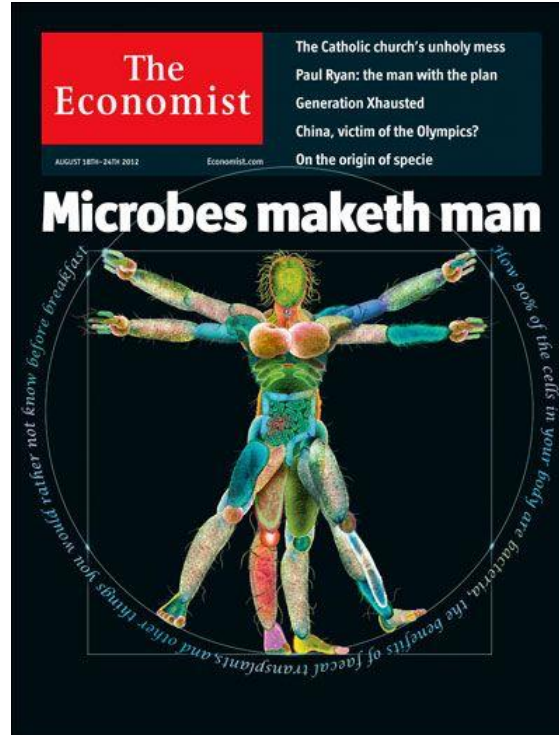
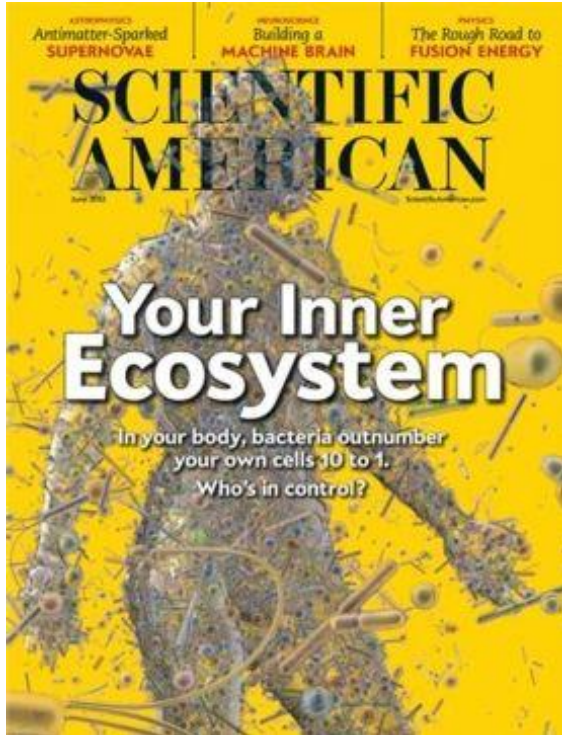


**...and 100 billion galaxies in the  
known universe.**

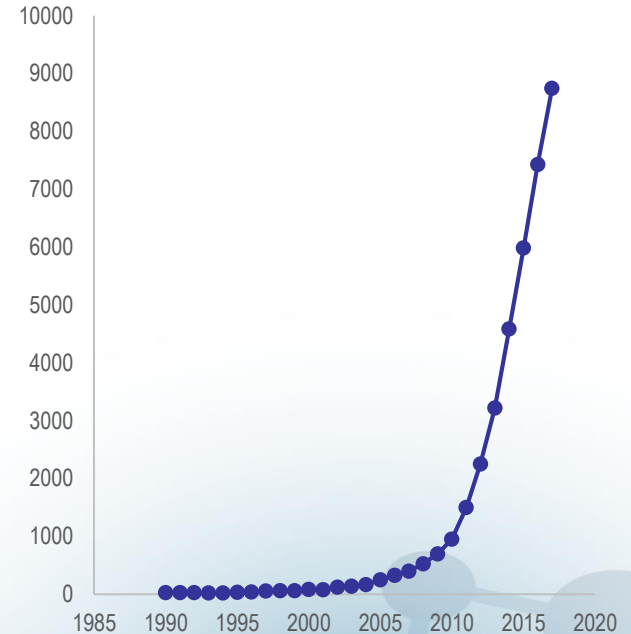
**But there are 100 million times as many bacteria on earth as there are stars in the universe**



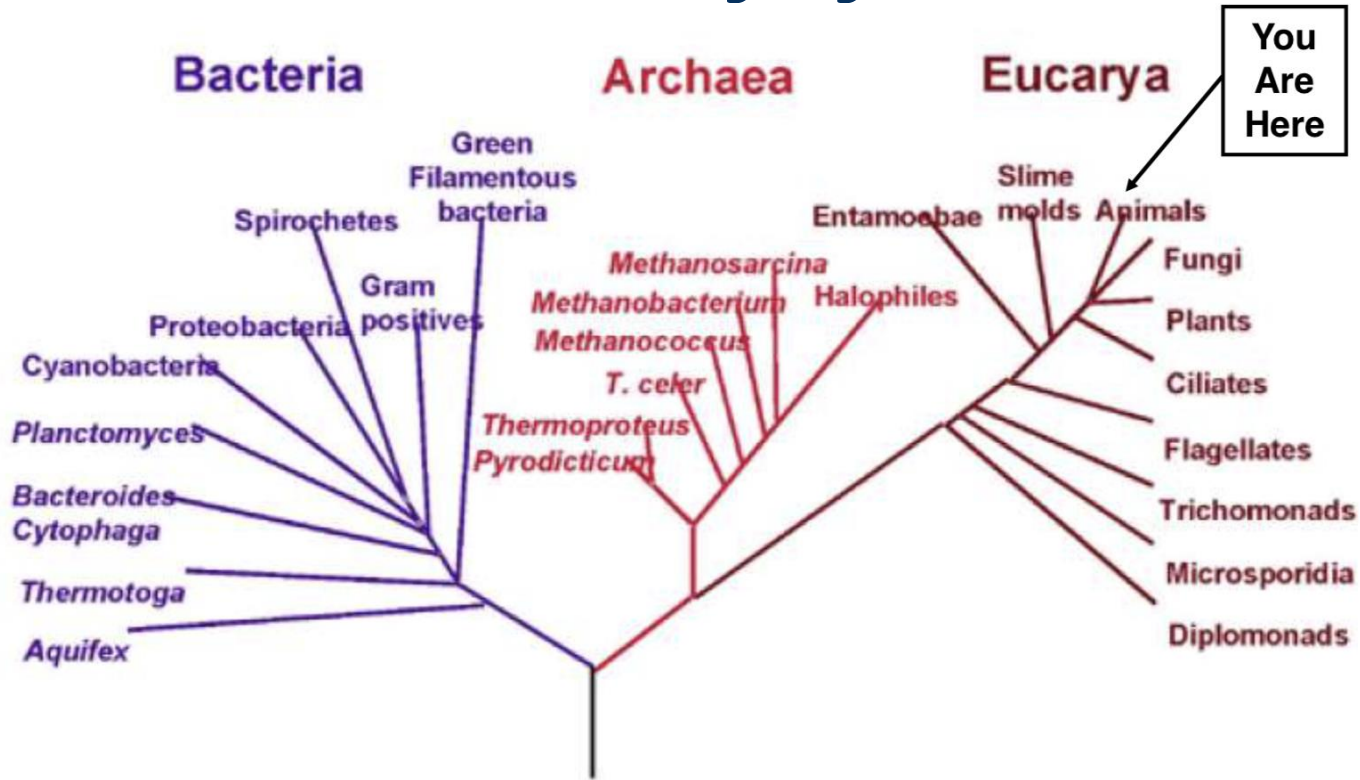
# The microbiome is hot



Pub Med References



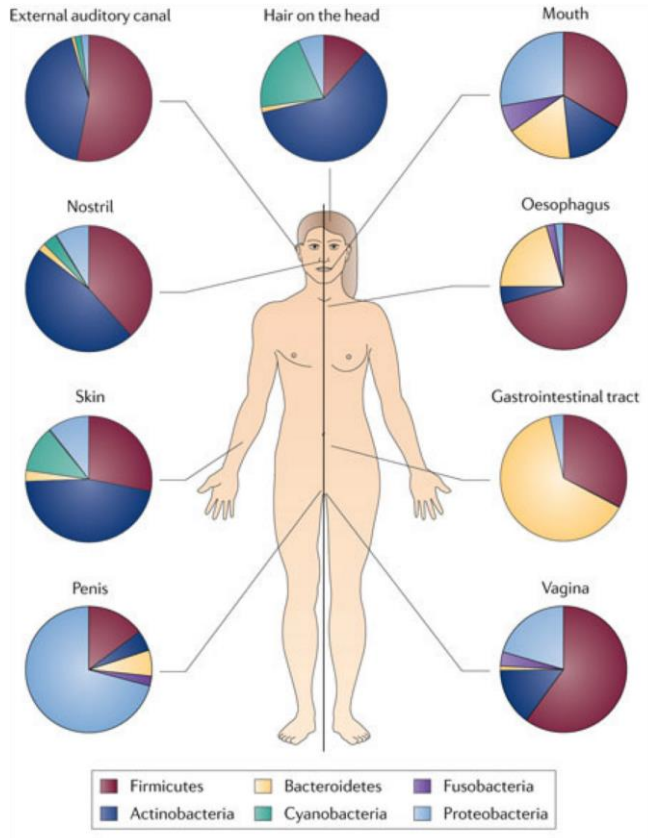
# The taxonomic tree of life



Tree of Life Derived from 16S rRNA Sequences

Source: Carl Woese, et al

# What is the human microbiome

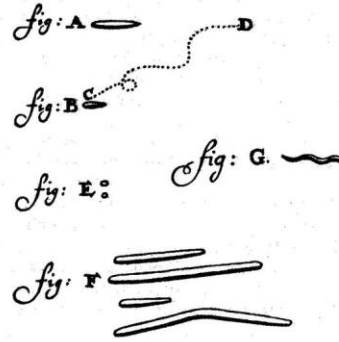


- **Microbial community colonising humans - over 10 trillion organisms**
  - Majority of microbiome mass localised within the gut ~ 2 kg
- **By DNA content we are >90% microbe and <10% *H. sapiens* = human holobiont**
- **Complex interactions with the epithelium, metabolism, immune system, hormonal axes, and nervous system**
- **A dynamic relationship to human health**

# Studying the human microbiome

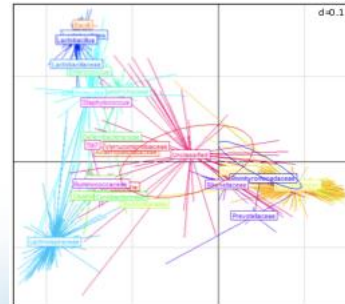
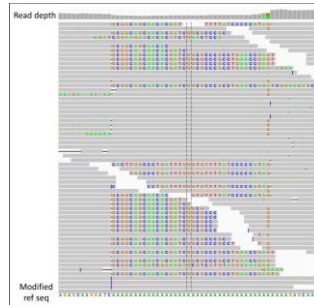
- *Historically – viewed through the lens of culture dependent methods and microscopic observation*
- *Primarily in context of disease and identification of pathogens*
- *Successful approach for diagnosis and treatment*

PLATE XXIV



# *Culture independent investigation*

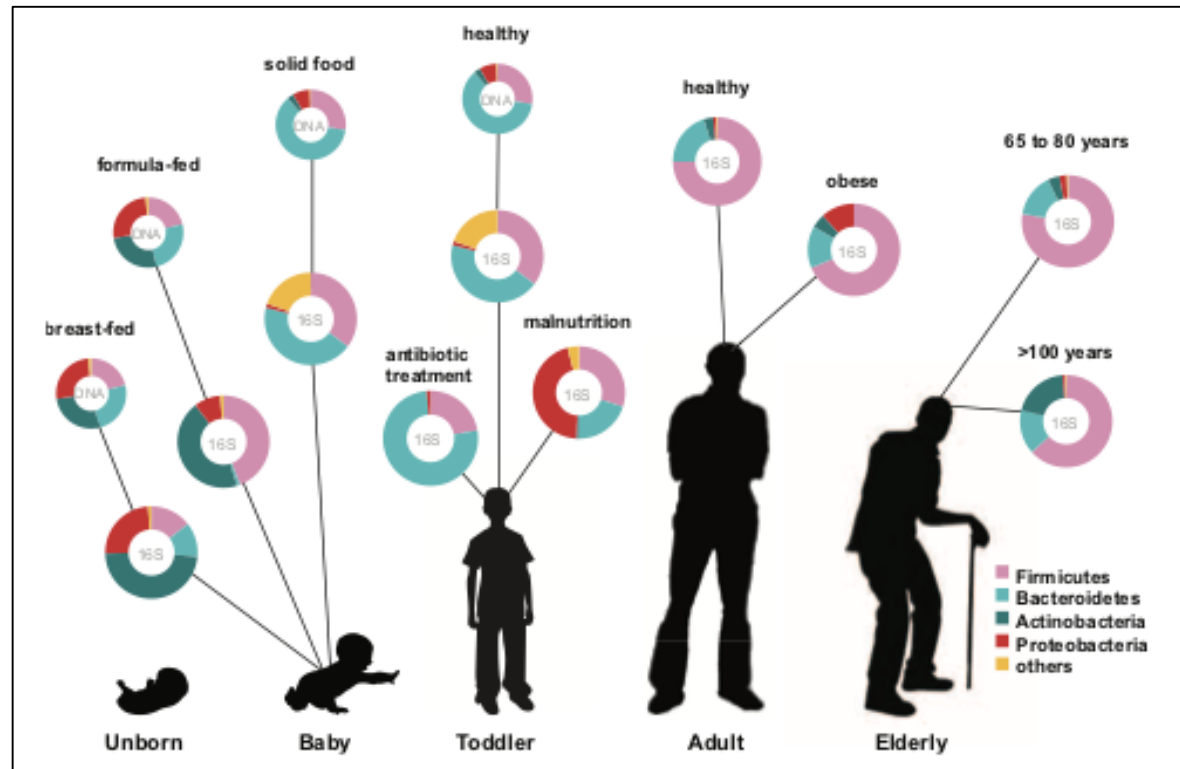
- *Advent of next generation sequencing and bioinformatics has revolutionised the field*
  - *16S rRNA – allows selective identification of bacterial species*
  - *Metagenomic approaches provide detailed views of microbial genomes*
  - *New sequence based approaches for studying the virome and mycobiome*





# A dynamic microbiome

- *The microbiome is a dynamic community*
- *Significant impact of environmental exposures*
- *Significant changes with age*



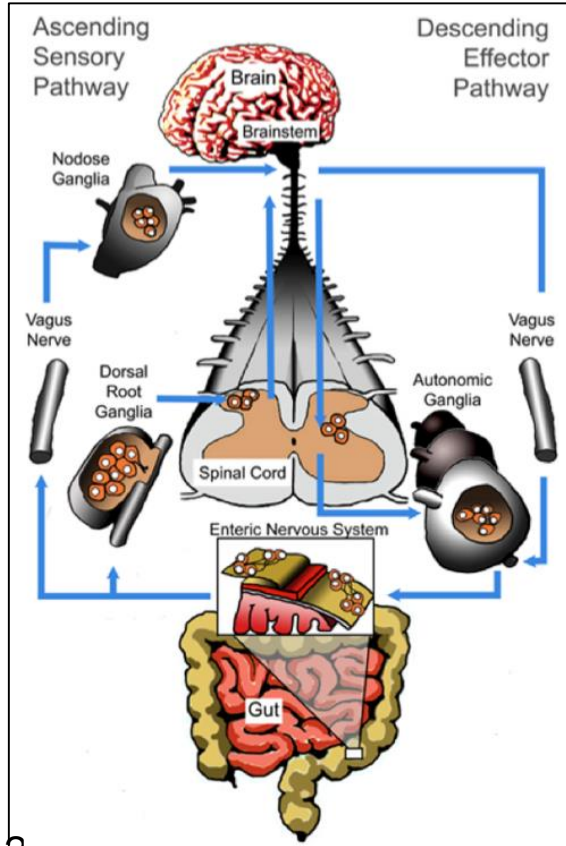
# *Microbiome associations with disease*

- *Alterations in microbiome composition have been associated with many human diseases and states of health*
  - *Metabolic disease – diabetes, heart disease*
  - *Cancer – bowel cancer, melanoma, hepatocellular carcinoma, lung cancer*
  - *Neurologic/psychiatric – Parkinson's, anxiety/depression, Autism*
  - *Inflammatory diseases – rheumatological, pulmonary, genitourinary, neurologic, autoimmune disease*
  - *Gastrointestinal disease – irritable bowel syndrome, Coeliac disease, inflammatory bowel disease*
- *Focus on the gut microbiome*

# *Metabolic outputs of the microbiome*

- *Gut microbiota utilise insoluble fibre, mucus, and non-absorbed carbohydrates, protein and fat as energy sources*
- *Metabolic outputs vary as a function of the microbial composition and diet*
- *Produce a variety of secondary metabolites*
- *Metabolise drugs*

# Gut-Brain Axis



- *Bi-directional signalling between the CNS and the gut microbiome*
- *CNS → Gut*
  - *Motility, secretion, intestinal permeability*
- *Gut → CNS*
  - *Modulation of neurotransmitter release by the gut and direct microbial production*
  - *Modulation of endocrine and immune signalling*

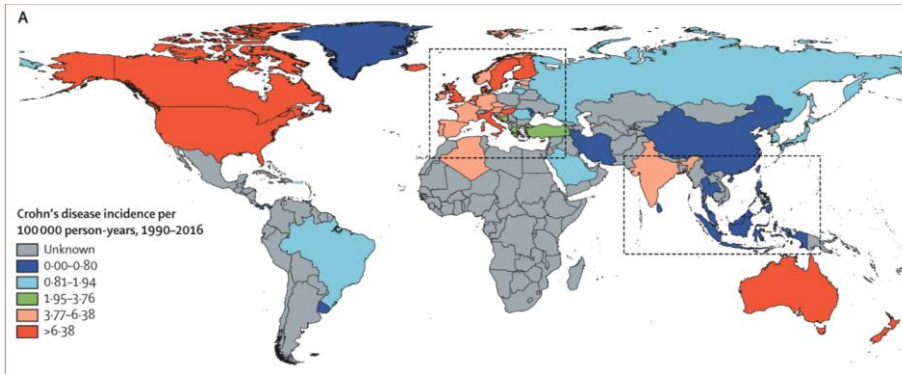
# ***Microbiome in rodents***

- ***Natural behaviours of mice (e.g BALB/c – impaired sociality and exaggerated caution)***
  - *Altered in the germ free state*
  - *Can be manipulated through microbiome transfer from different strains*
- ***Antibiotic treatment can cause behavioural changes and changes in stress hormone levels***
- ***Probiotic studies in animals can reduce anxiety/depression***
  - *Lactobacillus rhamnosus directly stimulates vagus nerve*
- ***Gut derived serotonin production influenced by the microbiome composition***

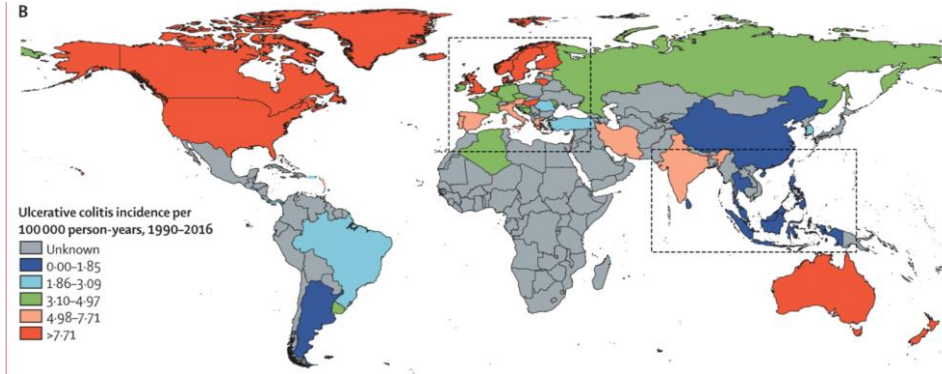
# Microbiome - Autism Spectrum Disorder

- **ASD is increasing in incidence (as high as 1 in 45)**
  - Higher incidence in developed world
  - GI symptoms are prominent in ASD individuals
- **In animal models manipulation of the microbiome can result in Autism like behaviours**
- **In human studies the microbiome signature of ASD is distinct**
  - Decreased Bacteroidetes/Firmicutes ratio
  - Decreased SCFA production, and carbohydrate fermentation
  - Confounded by diet
  - Small trial with oral vancomycin and minocycline showed some efficacy signal

# Worldwide burden of IBD



*Crohn's disease incidence*



*Ulcerative colitis incidence*

- *Worldwide prevalence ~400/100,000 (~30M patients)*
- *Incidence in Australia 29.3/100,000 → Approximately 85,000 patients diagnosed with IBD*
- *Current treatment paradigms consist primarily of immunosuppressive therapy including potent biologic therapy*

# The current model of IBD pathogenesis

## Multiple hits:

- *Genetics*
- *Dietary factors*
- *Environmental factors*
- *Microbial changes*



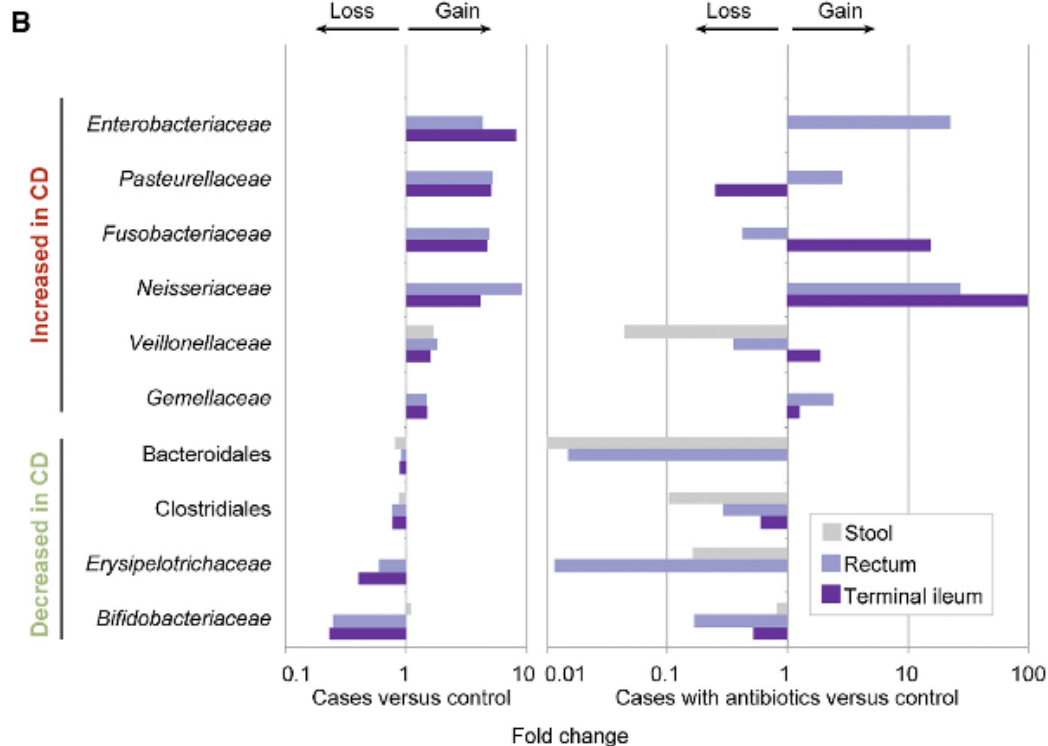
*Immune dysregulation*



Your body is  
mostly microbes



# Microbial “dysbiosis” associated with IBD



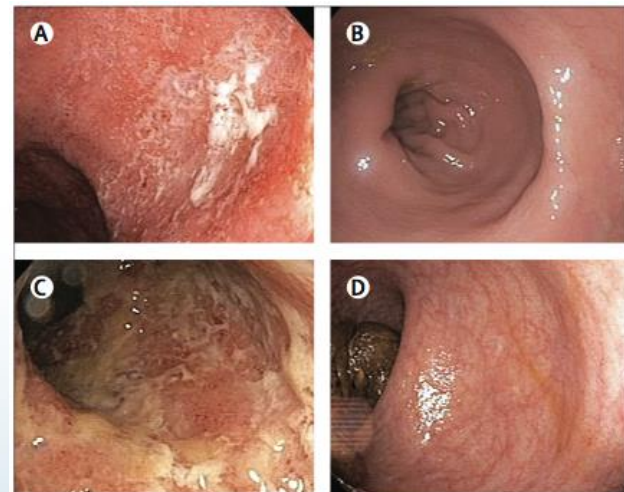
*Multiple studies show increased Firmicute abundance and decreased Bacteroidites*

*Decreased microbial diversity in patients with IBD*

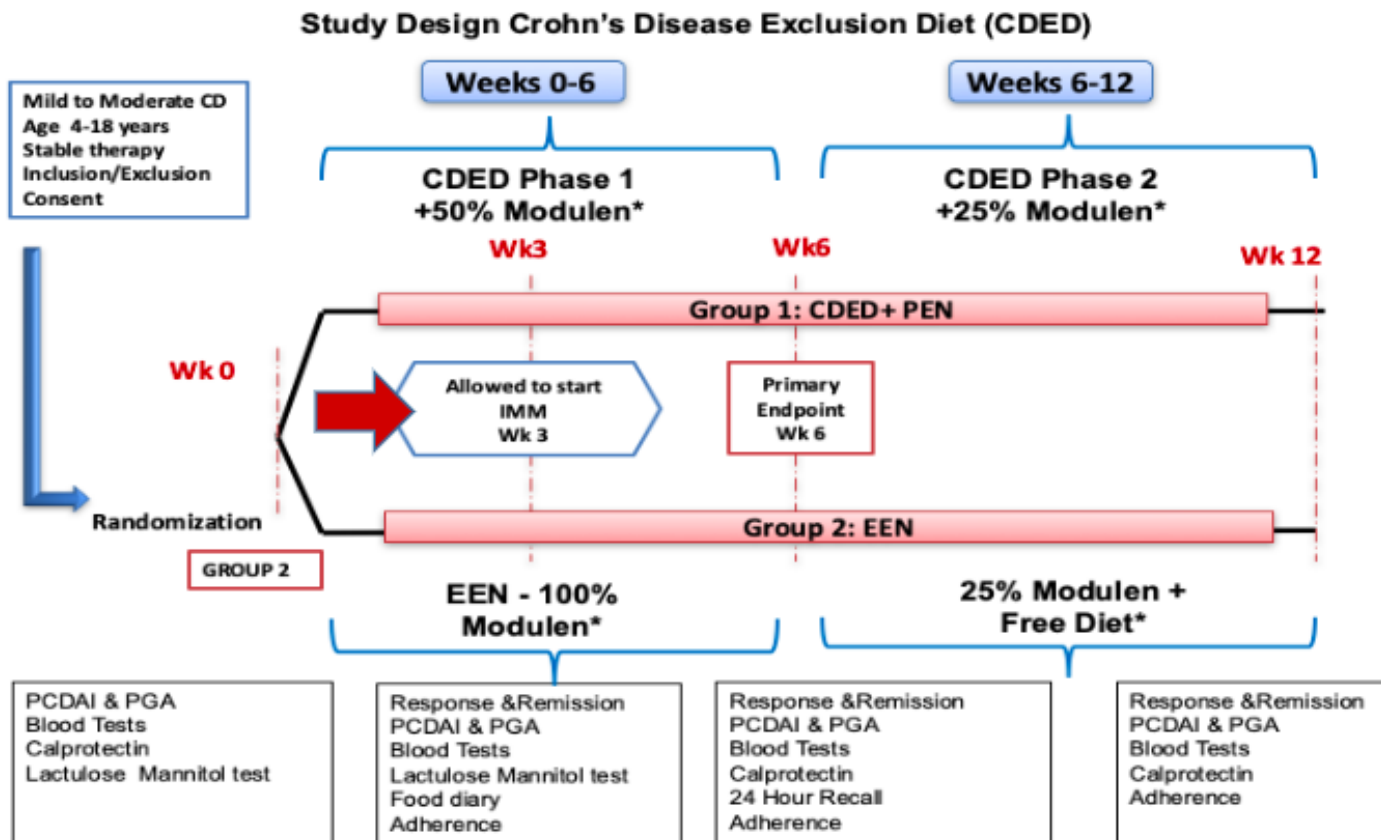
# Manipulation of microbiome in IBD

- *Antibiotics are effective in luminal and perianal Crohn's disease*
- *Faecal microbiota transplantation(FMT) is effective at inducing remission in ulcerative colitis*
- *"Intensive" regimen = 5 enemas per week x 8 weeks*

	Faecal microbiota transplantation (n=41)	Placebo (n=40)	Risk ratio (95% CI)	p value
<b>Primary outcome</b>				
Steroid-free clinical remission and endoscopic remission or response*	11 (27%)	3 (8%)	3.6 (1.1-11.9)	0.021
<b>Secondary outcomes</b>				
Steroid-free clinical remission†	18 (44%)	8 (20%)	2.2 (1.1-4.5)	0.021
Steroid-free clinical response‡	22 (54%)	9 (23%)	2.4 (1.3-4.5)	0.004
Steroid-free endoscopic remission§	5 (12%)	3 (8%)	1.6 (0.4-6.4)	0.48
Steroid-free endoscopic response¶	13 (32%)	4 (10%)	3.2 (1.1-8.9)	0.016



# Effect of diet on Microbiome in IBD



\*Modulen will be given ORALLY

# The Crohn's Disease Exclusion Diet...

## Phase 1

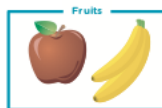
### Mandatory foods



**Protein-rich foods**  
Unlimited intake of fresh **chicken breast** minimum of 150-200g/day  
2 eggs/day



**Carbohydrate-rich foods**  
2 fresh potatoes/day peeled, cooked and cooled before consumption



**Fruits**  
2 bananas/day  
1 apple/day peeled

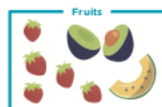
### Allowed foods



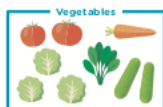
**Protein-rich foods**  
One portion once a week of fresh lean fish replacing the chicken



**Carbohydrate-rich foods**  
White rice unlimited  
Rice noodles without preservatives - one portion per day  
Rice flour for baking



**Fruits**  
1 avocado/day (not more than 1/2 avocado per meal)  
5 ripe strawberries/day  
1 slice of melon/day



**Vegetables**  
2 tomatoes/day (or 6 cherry tomatoes). Additional tomatoes may be used to prepare home made tomato sauce  
2 cucumbers/day peeled  
1 carrot/day  
Fresh spinach (1 cup uncooked leaves/day)  
3 lettuce leaves once daily

Foods that are not on the list of mandatory or allowed foods are disallowed

### Disallowed foods



**Protein-rich foods**  
Meats and fish processed, precooked or smoked  
Seafood  
Red meat, pork, turkey, other parts of the chicken  
Soy products  
Dairy products  
Ice-cream  
Non-dairy milk beverages soy milk, rice milk, almonds milk



**Carbohydrate-rich foods**  
Wheat product (breakfast cereals, breads and baked goods of any kind, yeast for baking)  
Gluten free products not listed above  
Soy products  
Legumes (lentils, peas, chickpeas and beans)  
Corn  
Frozen potatoes  
All other flours



**Fruits**  
Dried fruits  
All other fruits



**Vegetables**  
Frozen vegetables  
Kale  
Leeks  
Asparagus  
Artichoke  
All other vegetables not mentioned as allowed

## Phase 2

### Mandatory foods



**Protein-rich foods**  
Unlimited intake of fresh **chicken breast** minimum of 150-200g/day  
2 eggs/day



**Carbohydrate-rich foods**  
2 fresh potatoes/day peeled, cooked and cooled before consumption



**Fruits**  
2 bananas/day  
1 apple/day peeled

### Allowed foods



**Protein-rich foods**  
One portion once a week of fresh lean fish replacing the chicken  
One can of tuna once a week packed in olive or canola oil  
Red meat is not recommended. Please avoid. If really desired it should be limited to fresh unprocessed beef lean steak, up to 200g per week (only once a week)



**Carbohydrate-rich foods**  
White rice (unlimited)  
Rice noodles without preservatives - one portion/day  
Rice flour for baking  
½ sweet potato/day  
Whole grain bread 1 slice/day  
Lentils, peas, chickpeas or beans ½ cup of dry, uncooked/day  
Quinoa (unlimited)  
½ cup of oatmeal (allowing for a portion of oatmeal or cookies 1-2/day)



**Fruits**  
1 avocado/day (not more than 1/2 avocado per meal)  
5 ripe strawberries/day  
1 slice of melon/day  
Pear, peach or kiwi/day can be added in week 7-9  
10 blueberries or ½ cup can replace the allowed strawberries  
From week 10, all fruits (except for those in the disallowed list) can be introduced in small quantities. For example ½ cup cubes of mango, pineapple OR slices of orange



**Vegetables**  
2 tomatoes/day or 6 cherry tomatoes. Additional may be used for home made tomato sauce  
2 cucumbers/day (peeled)  
1 carrot/day  
Fresh spinach (1 cup uncooked leaves/day)  
3 lettuce leaves once daily  
Zucchini, fresh mushrooms, broccoli or cauliflower (but not all at the same time)  
From week 10, all vegetables (except for those in the disallowed list) can be produced. For example ½ sweet red pepper, cabbage and beetroots

Foods that are not on the list of mandatory or allowed foods are disallowed

### Disallowed foods



**Protein-rich foods**  
Meats and fish processed, precooked or smoked  
Seafood  
Red meat, pork, turkey, other parts of the chicken  
Soy products  
Dairy products  
Ice-cream  
Non-dairy milk beverages soy milk, rice milk, almonds milk



**Carbohydrate-rich foods**  
Wheat product (breakfast cereals, breads and baked goods of any kind, yeast for baking)  
Gluten free products not listed above  
Soy products  
Corn (allowed from week 10)  
Frozen potatoes  
All other flours



**Fruits**  
Dried fruits  
All other fruits



**Vegetables**  
Frozen vegetables  
Kale  
Leeks  
Asparagus  
Artichoke

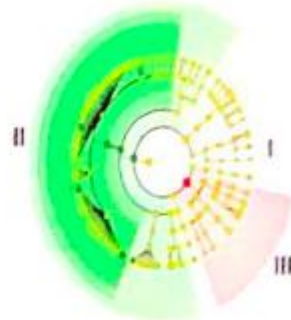
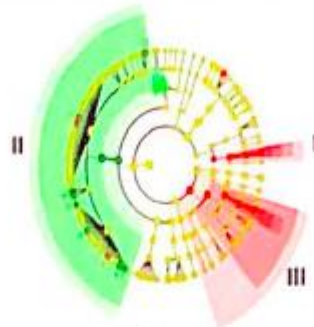
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change in community composition from baseline

decrease

week 0 to week 6

week 0 to week 12

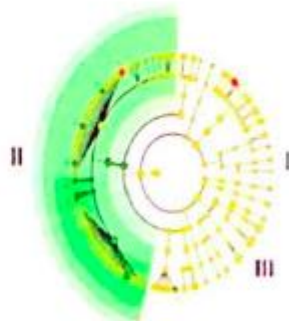
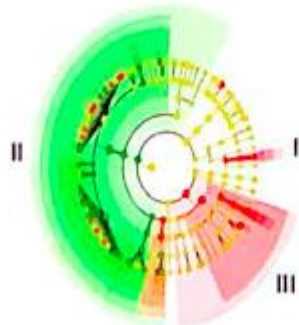


### CDED

- I. Actinobacteria:
- II. Clostridia:
- III. Proteobacteria:

- I. decrease
- II. increase
- III. decrease

- I. minor rebound
- II. increase (expanded)
- III. decrease (sustained)



### EEN

- I. Actinobacteria:
- II. Clostridia:
- III. Proteobacteria:

- I. decrease
- II. increase
- III. decrease

- I. minor rebound
- II. increase (contracted)
- III. major rebound

## CDED associated with:

- Increase in beneficial Clostridia family
- Decreased in harmful Proteobacteria

- This change in microbiome composition is associated with clinical response

# ***Microbiome as a pharma target***

- ***Historically the market has been dominated by nutraceuticals with a paucity of clinical trials***
- ***Evidence of FMT in *C. difficile* colitis is established, and IBD is growing with significant interest***
  - *Openbiome – Finch, currently conducting Phase 2 trials in *C. difficile**
  - *Janssen Microbiome Institute – targeting IBD, T2D*
  - *Seres Therapeutics – Spore based formulation in Ph 2 trials for UC and CDI*
  - *Rebiotix acquired by Ferring Pharmaceuticals – CDI*
  - *Nextbiotix – Bacterial treatment for IBD (*F. prau*)*
- ***FDA/EMA regulatory pathways***



# Summary

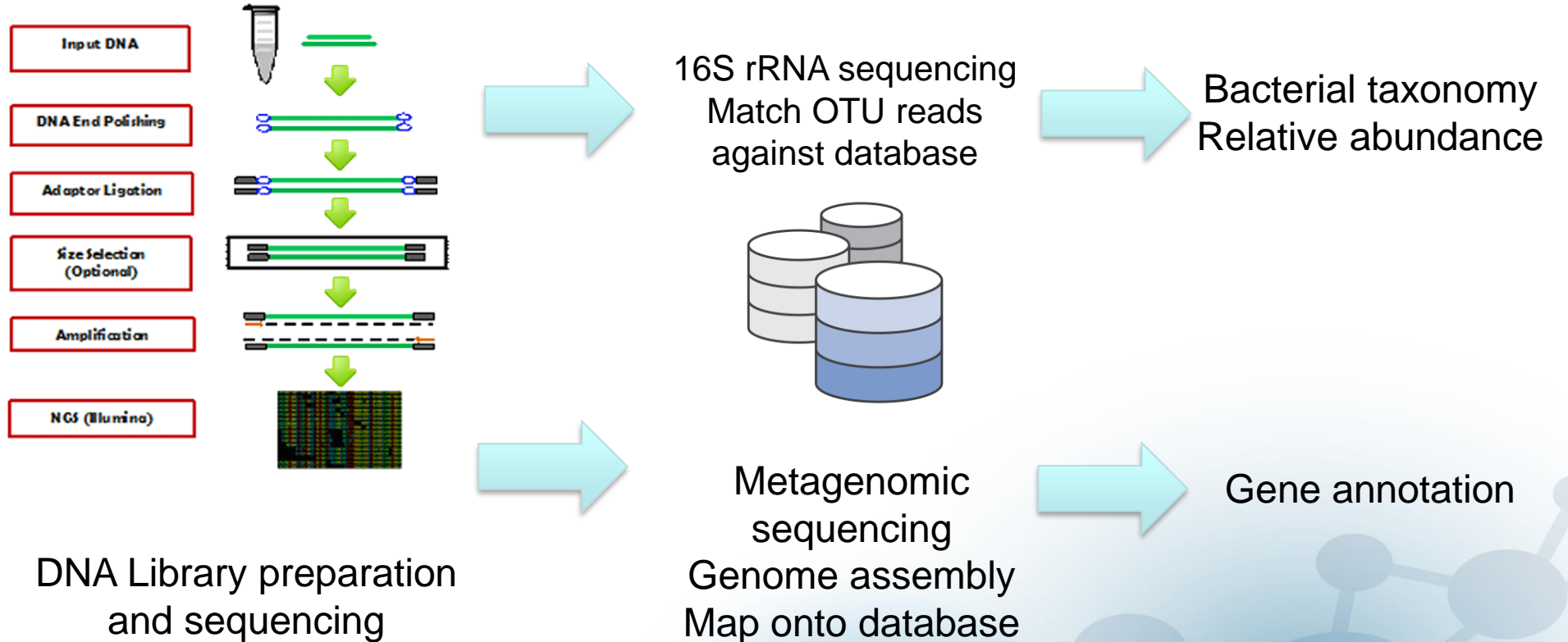
- *New techniques in microbiome research have led to unprecedented detail of human microbiome composition*
- *Multiple associations between microbiome alterations and disease states*
- *Functional aspects of these changes remain relatively unexplored and are somewhat limited by existing databases*
- *Evidence in gastrointestinal disease that targeting the microbiome can treat disease such as IBD*



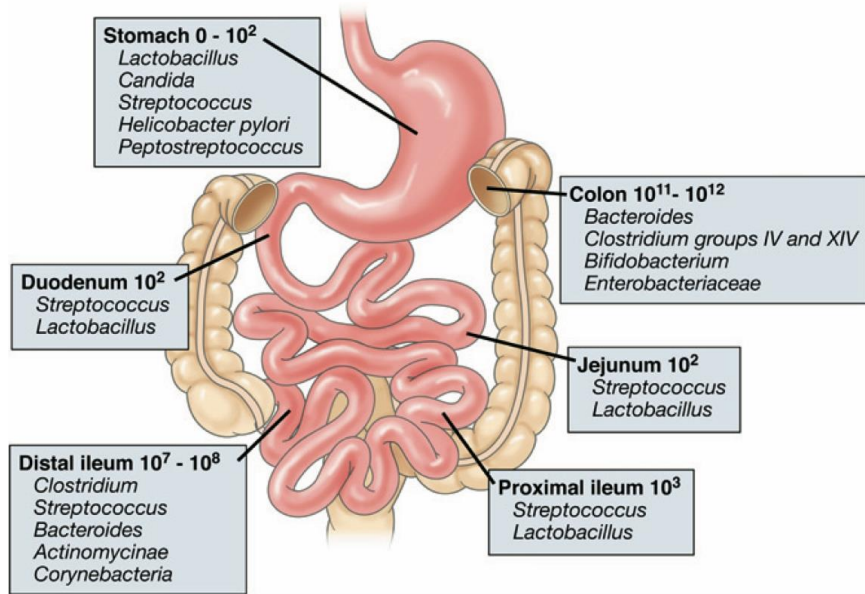




# Coupling sequence with big data



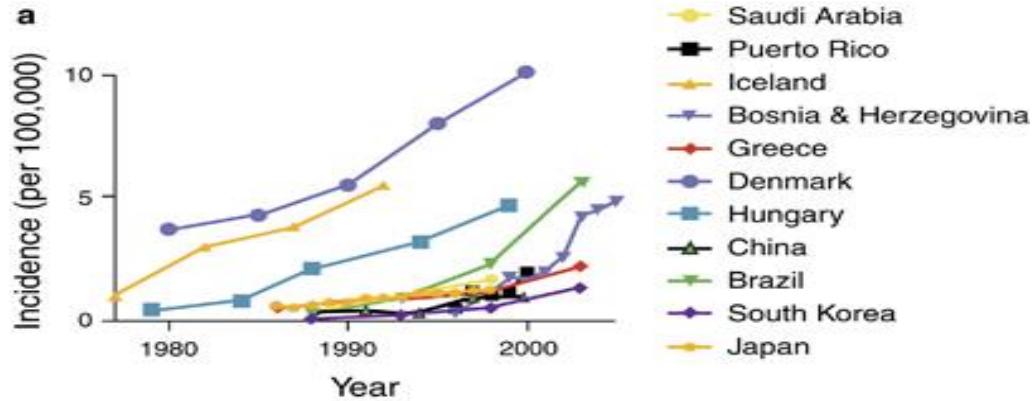
# A word about the gut microbiome



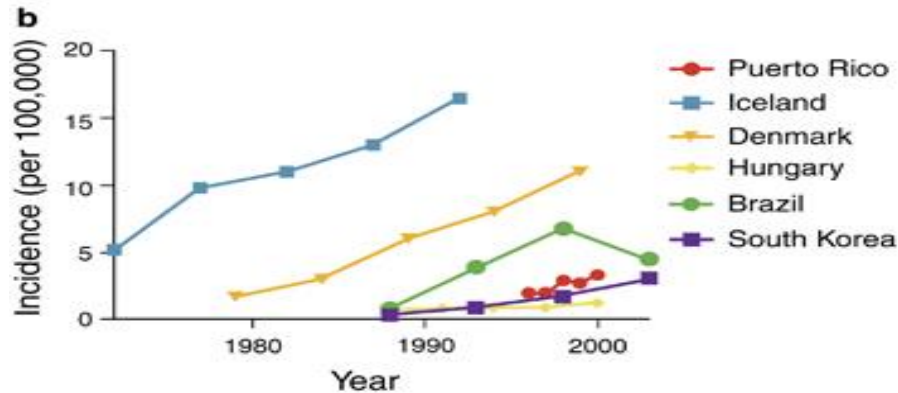
- *Oral cavity contains multiple distinct communities*
- *Upper GI tract characterized by limited diversity adapted to harsh environment (acidic, bile, digestive enzymes)*
- *The lower intestine is oxygen poor, nutrient rich, and neutral pH*

# Increasing incidence of IBD over time

Crohn's Disease

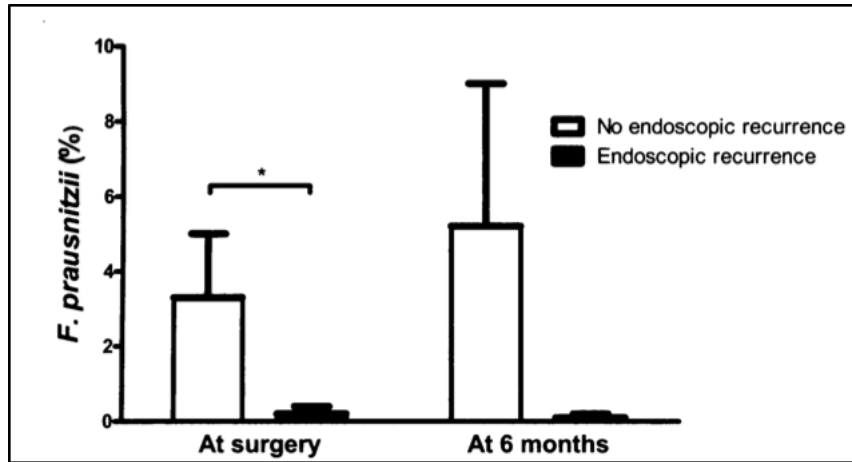


Ulcerative Colitis

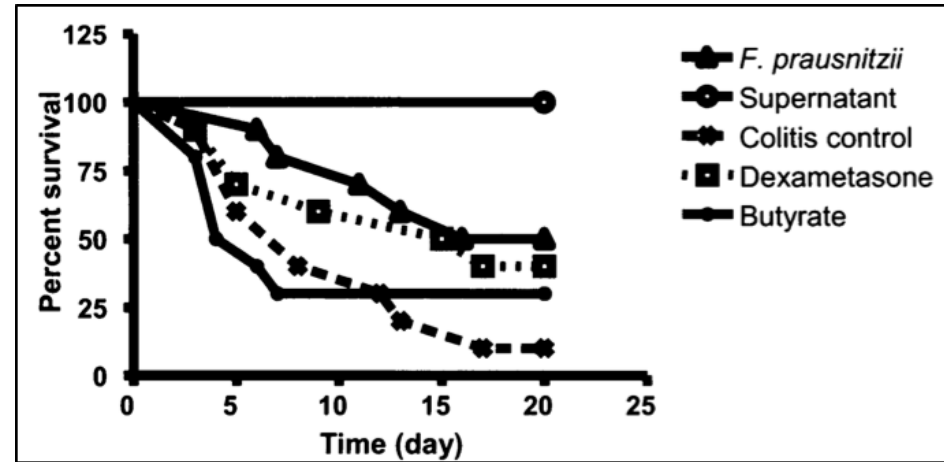


# Role of individual bacteria in IBD

- **Protective effect of bacteria - *F. prausnitzii***
  - Produces the MAM peptide – which is NF- $\kappa$ B suppressive



*F. prau* abundance post-operatively



Protective effect in a mouse model