THE HUMAN MICROBIOME: OUR "HIDDEN" ORGAN

PRESENTED BY

Associate Professor Sabrina D. Dyall, PhD

Department of Biosciences

Faculty of Science

UNIVERSITY OF MAURITIUS

The Medical Update Group

ELT2, University of Mauritius

Wed 26th April 2017

OUTLINE

A. Overview of the human microbiota

B. Gut microbiota in health and disease

C. Research on gut microbiota at UoM

D. Applications

“*E pluribus unum*.”

Out of many, one: the motto of the United States of America

We, humans, are one with microbes.

A. OVERVIEW OF THE HUMAN MICROBIOTA

- Most microorganisms are benign
  - Few contribute to health, and fewer pose direct threats to health
- Human microbiota/biome = sum total of all microorganisms that live in or on the body
- The "normal" microbiota have developed a symbiotic relationship with the human host.

OVERVIEW OF THE HUMAN MICROBIOTA

- The human microbiota consists of around 1000 species comprising:
  - Bacteria,
  - Archaea,
  - Microbial Eukarya,

  and their associated viruses

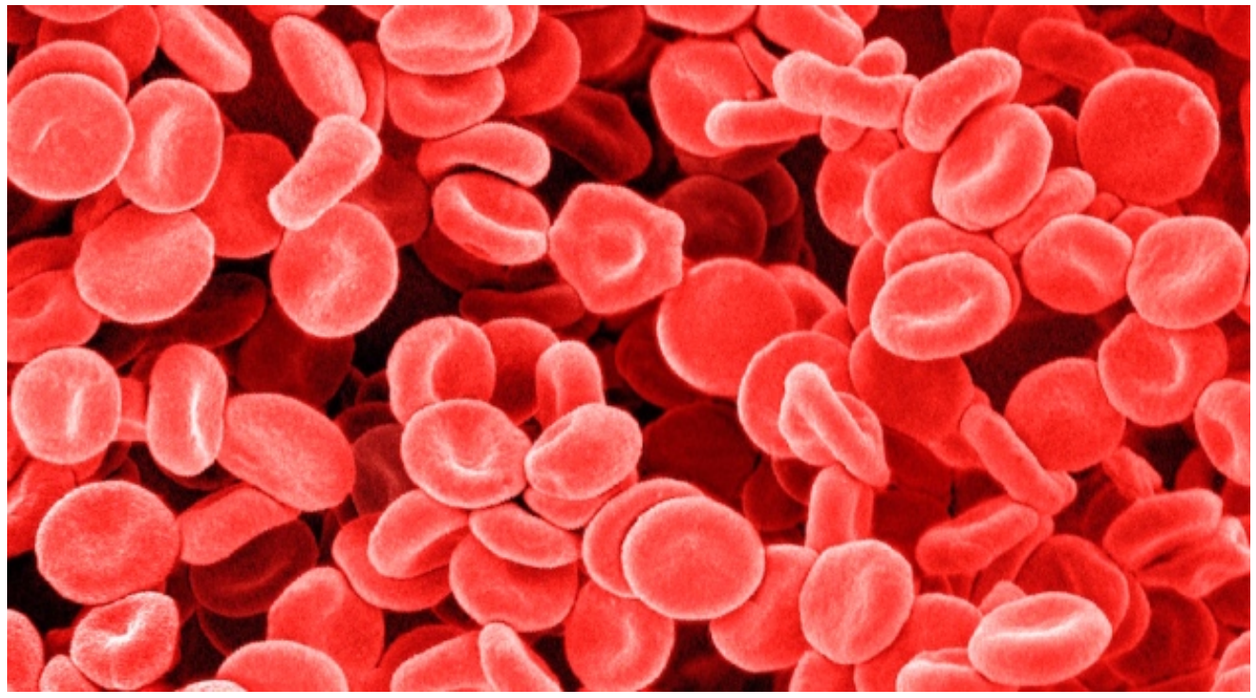
Are we human?

Scientists bust myth that our bodies have more bacteria than human cells

Decades-old assumption about microbiota revisited.

Alison Abbott

08 January 2016



SUSUMU NISHINAGA/SCIENCE PHOTO LIBRARY

Red blood cells dominate our total cell count by number (though not by mass).

"A reference man (one who is 70 kilograms, 20–30 years old and 1.7 metres tall) contains on average about 30 trillion human cells and 39 trillion bacteria, say Ron Milo and Ron Sender at the Weizmann Institute of Science in Rehovot, Israel, and Shai Fuchs at the Hospital for Sick Children in Toronto, Canada."

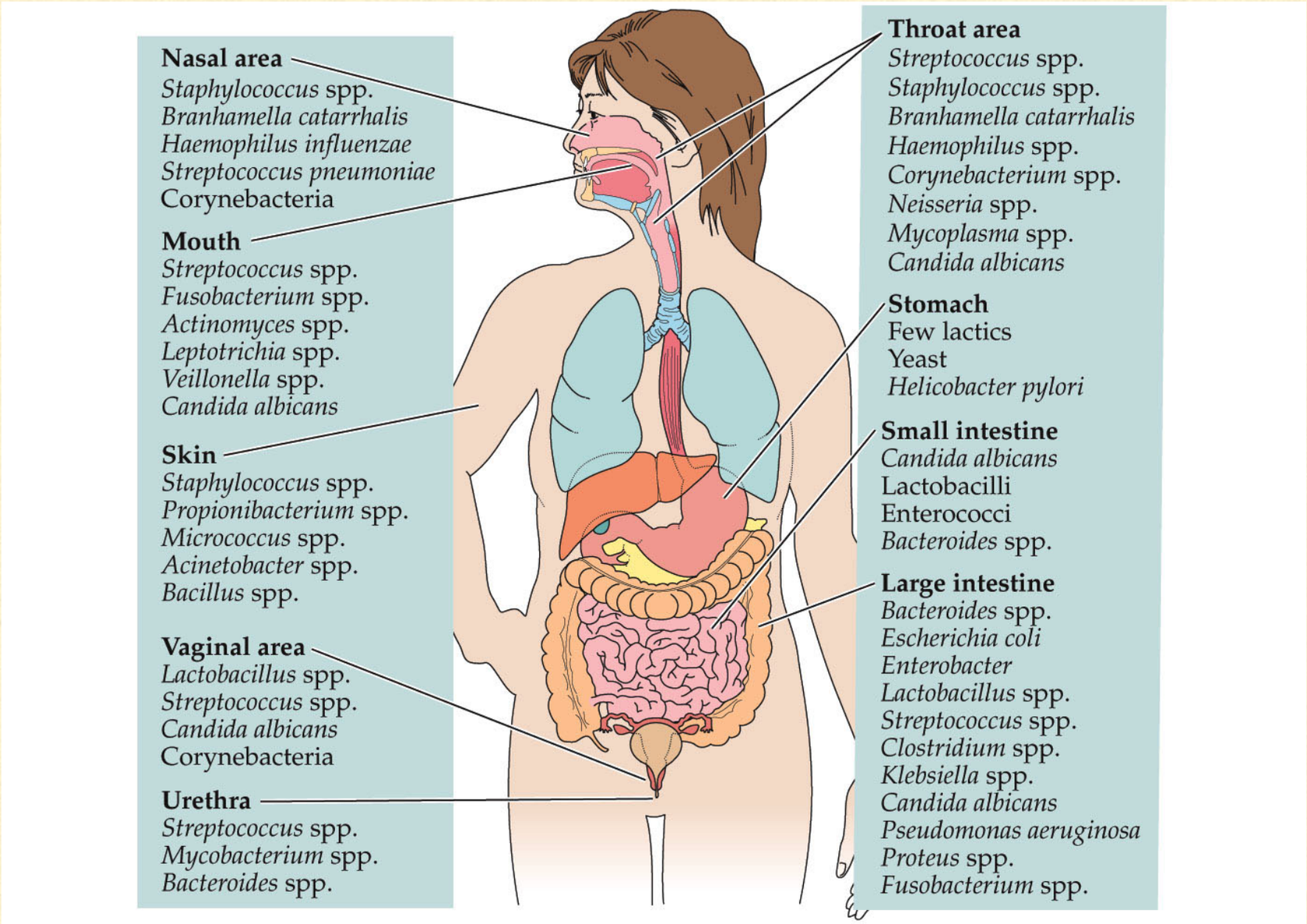
Are we human?

- An estimated 4 X 10^{14} microbes live in or on an adult human.
- Estimated mass of one microbial cell = 6.65 X 10^{-13} g.
- Total microbial mass = 266 g
- Percentage of the body mass of a reference 70 kg human adult that consists of bacterial mass = 0.38%

Are we really human?

- A human genome contains 20,000 - 25,000 genes that code for proteins.
- The human microbiota associated with one individual collectively contains at least 3,000,000 genes that code for proteins.
- Humans are metaorganisms.

Most areas of the body in contact with the outside environment harbour resident microbiota.



***Microbial Life 2e*, Figure 26.1**

Sterile sites

- Tissues and organs: Heart and circulatory system, Liver, Kidneys and bladder, Lungs, Brain and spinal cord, Muscles, Bones, Ovaries/Testes, Glands (pancreas, salivary, thyroid), Sinuses, Middle and inner ear, Internal eye

- Fluids within organs and tissues: Blood, Urine in kidneys, bladder, Cerebrospinal fluid, Saliva prior to entering oral cavity, Semen prior to entering the urethra, Amniotic fluid surrounding the embryo and foetus (?)

- Microorganisms in these sites = infection

Colonization through the ages

- Mammals develop *in utero* in a sterile (?) environment
- Colonization begins as neonates are exposed to microorganisms in the birth process.
- Skin surfaces are readily colonized by many species.
- The mucous membranes of the oral cavity and gastrointestinal tract acquire microorganisms through feeding and exposure to the mother's body.
- Along with other environmental sources, such contact initiates colonization of the skin, oral cavity, upper respiratory tract and gastrointestinal tract.

Factors affecting microbiota

- Diet
- Mode of delivery of neonate
- Infection
- State of the immune system
- Xenobiotics (inc. antibiotics)
- Host genetics
- Sex, age
- Host behavior (diurnal rhythm, activity, lifestyle etc)

Why do they like us so much?

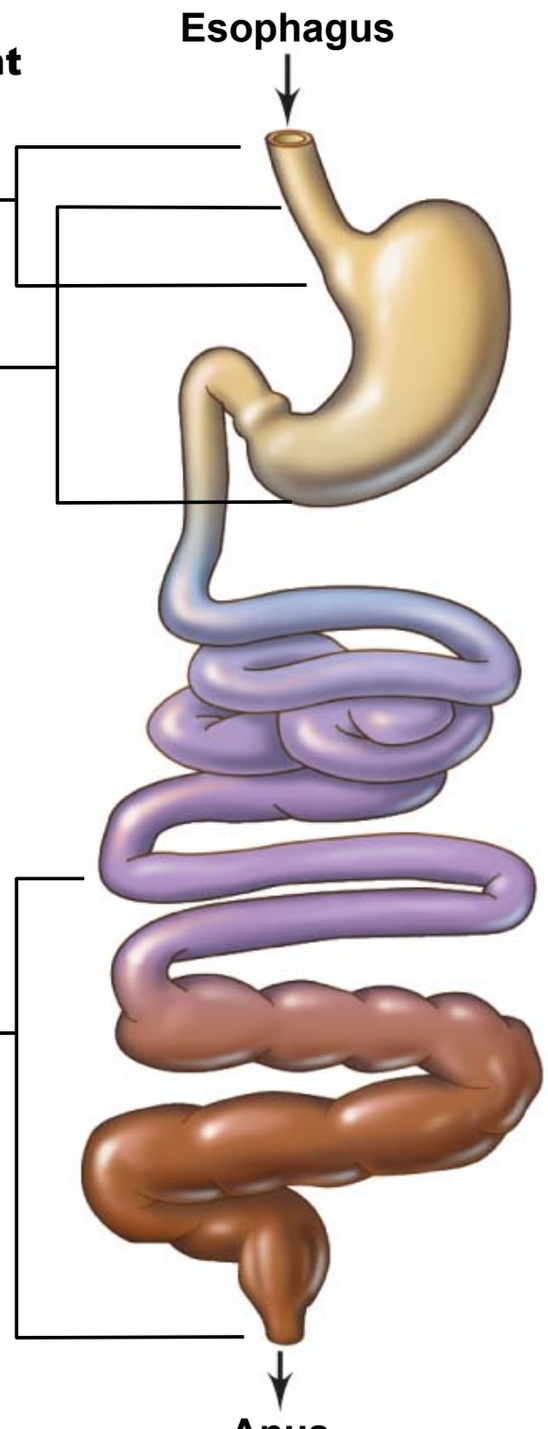
- Rich in organic nutrients and growth factors
- Controlled pH
- Stable osmotic pressure
- Small temperature range
- Mesophilic

  Body locations vary greatly chemically and physically and offer selective environments for certain microorganisms. Hence, each location has a unique microbial bias.

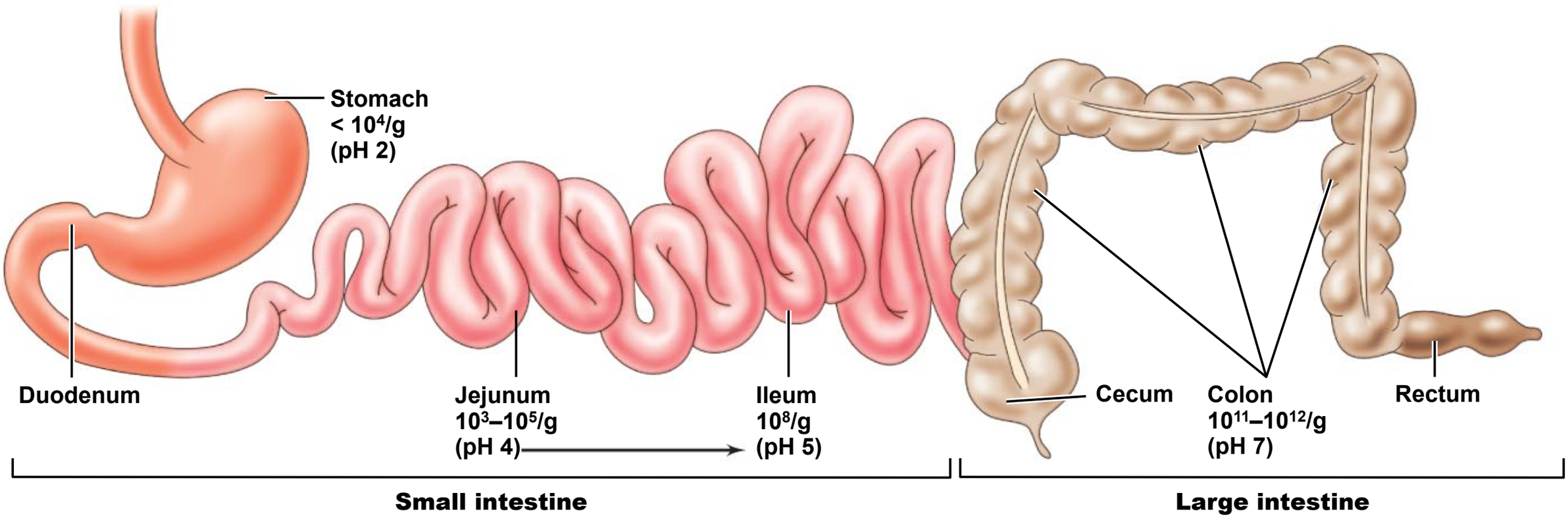
MICROBIOTA OF THE GASTRO-INTESTINAL TRACT

- Surface area = 400 square meters (inc. stomach, small intestine, and large intestine)
- Responsible for digestion of food, absorption of nutrients, and production of nutrients by the indigenous microbiota
- Contains 10^{13} to 10^{14} microbial cells

Major bacteria present		Organ	Major physiological processes
Prevotella *Streptococcus* *Veillonella*		Esophagus	
Helicobacter *Proteobacteria* *Bacteroidetes* *Actinobacteria* *Fusobacteria*		Stomach	Secretion of acid (HCl) Digestion of macromolecules **pH 2**
Enterococci Lactobacilli	Duodenum	Small intestine	Continued digestion Absorption of monosaccharides, amino acids, fatty acids, water **pH 4–5**
Bacteroides *Bifidobacterium* *Clostridium*	Jejunum		
Enterobacteria *Enterococcus* *Escherichia* *Eubacterium* *Klebsiella* *Lactobacillus* *Methanobrevibacter* (*Archaea*) *Peptococcus* *Peptostreptococcus* *Proteus* *Ruminococcus* *Staphylococcus* *Streptococcus*	Ileum		
	Colon	Large intestine	Absorption of bile acids, vitamin B_{12} **pH 7**



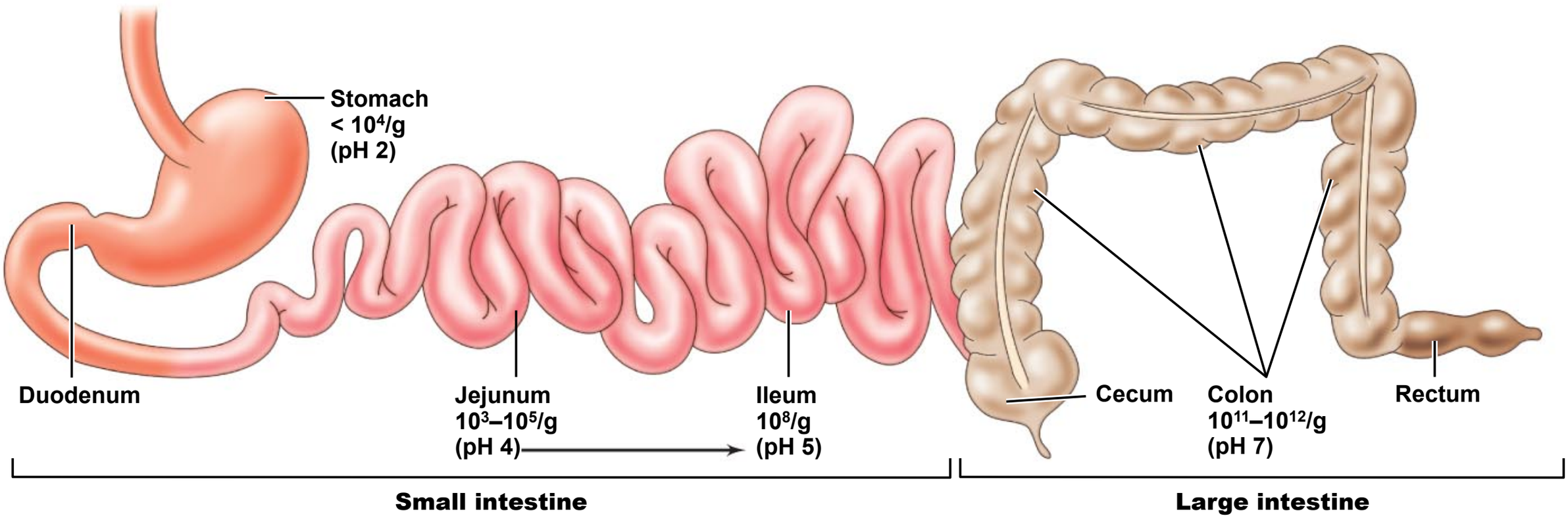
Microbial distribution along the GI tract



The stomach

- The acidity of the stomach and the duodenum of the small intestine (~pH 2) prevents many organisms from colonizing the GI tract (10,000 cells/g)
- Several different bacterial taxa populate the stomach.
- Each person has a unique population but have in common several species of gram positive bacteria and *Proteobacteria*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria*.
- Home to *Helicobacter pylori*, which colonizes the stomach of many individuals. Causes ulcer in susceptible individuals.

Microbial distribution along the GI tract

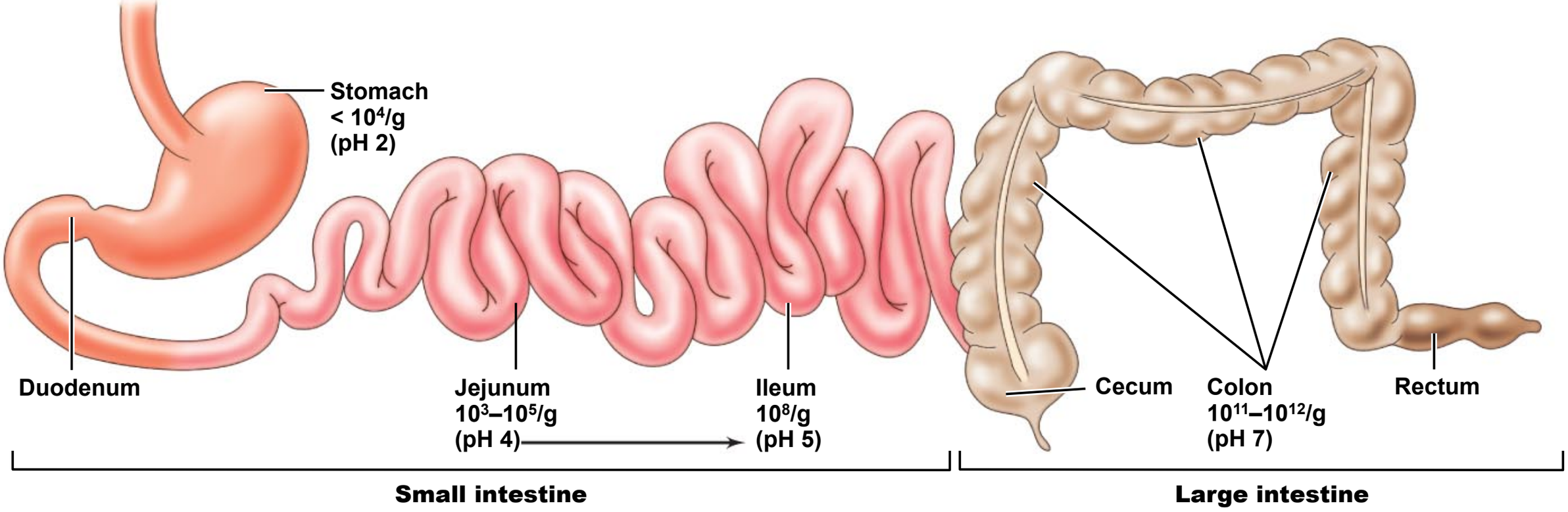


The small intestine

- Duodenum: fairly acidic, normal microbiota resembles that of the stomach.
- From duodenum to ileum, as pH gradually increases, bacterial numbers increase.
- In the lower ileum: 100,000 to 10,000,000 cells per gram of intestinal contents.
- Proceeding down the small intestine, the environment becomes gradually more anoxic.

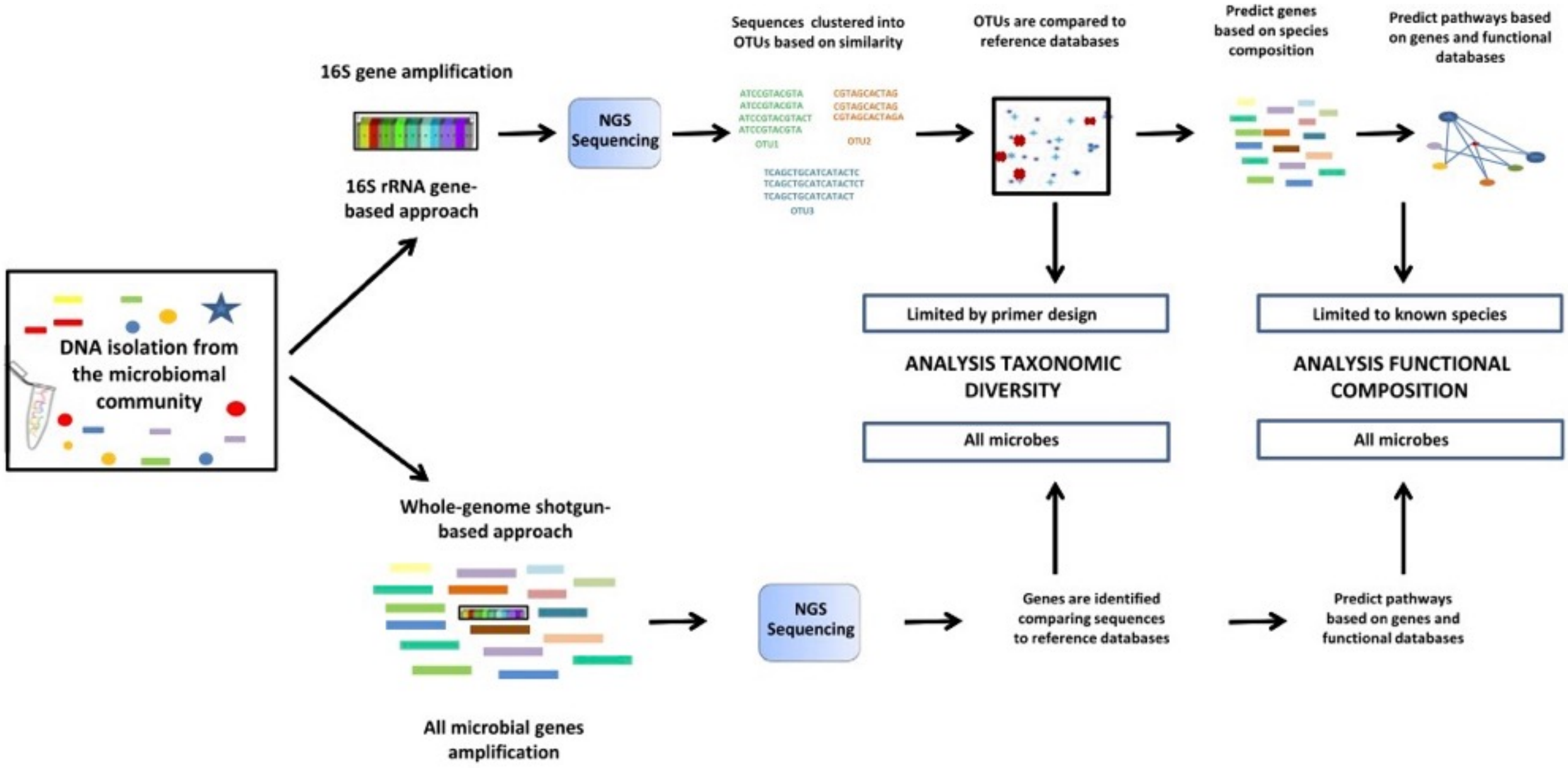
The large intestine

- In the colon, prokaryotes are present in enormous numbers.
- Colon = *in vivo* fermentation vessel where microorganisms use nutrients derived from food

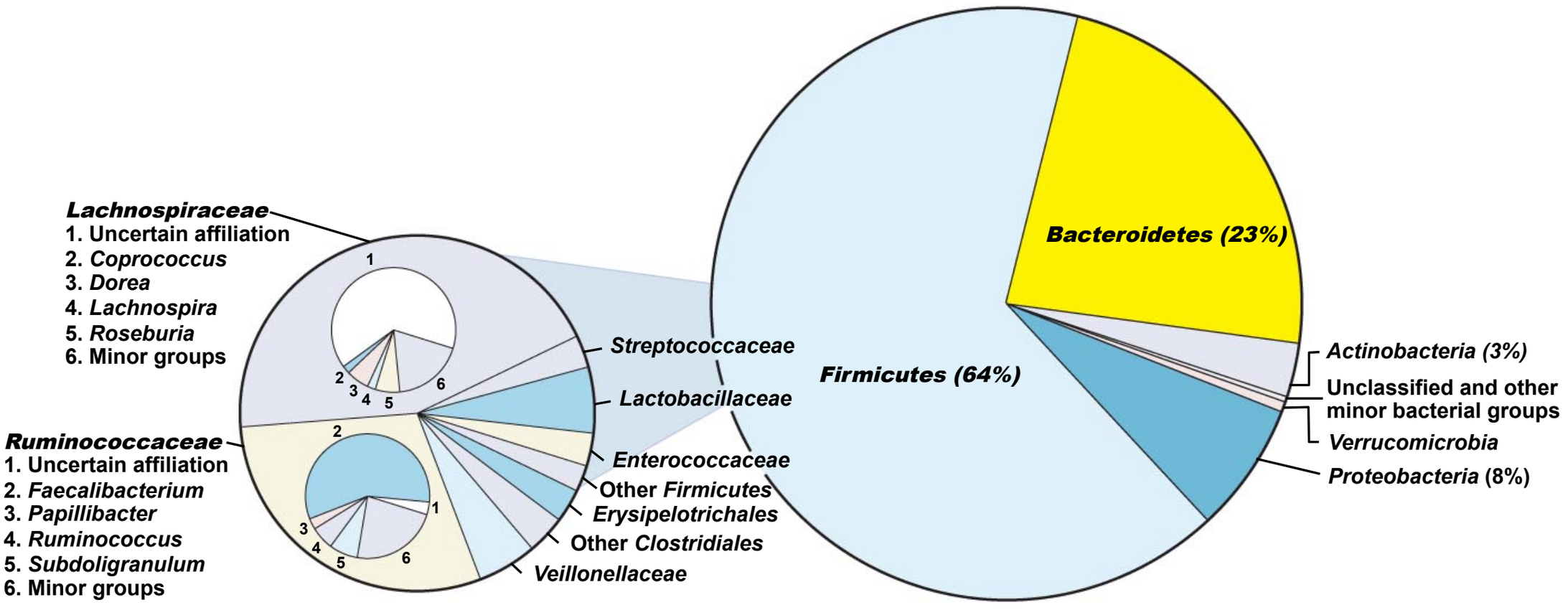


The colon

- Facultative aerobes = 10 million per gram of contents. Facultative aerobes, , e.g. *Escherichia* coli, consume any remaining oxygen, making the large intestine strictly anoxic.
- Obligate anaerobes = 10 billion (10^{10}) to 1 trillion (10^{12}) per gram of distal gut and faecal contents.
- Gram-positive bacteria and *Bacteriodetes* species account for >90% of all Bacteria
- Archaeal methanogen *Methanobrevibacter smithii* may also be present in significant numbers.


16S gene amplification
16S rRNA gene-based approach
NGS Sequencing
Sequences clustered into OTUs based on similarity
ATCCGTACGTA
ATCCGTACGTA
ATCCGTACGTACT
ATCCGTACGTA
OTU1
CGTAGCACTAG
CGTAGCACTAG
CGTAGCACTAGA
OTU2
TCAGCTGCATCATACTC
TCAGCTGCATCATACTCT
TCAGCTGCATCATACT
OTU3
OTUs are compared to reference databases
Predict genes based on species composition
Predict pathways based on genes and functional databases
DNA isolation from the microbiomal community
Whole-genome shotgun-based approach
All microbial genes amplification
NGS Sequencing
Genes are identified comparing sequences to reference databases
Predict pathways based on genes and functional databases
Limited by primer design
ANALYSIS TAXONOMIC DIVERSITY
All microbes
Limited to known species
ANALYSIS FUNCTIONAL COMPOSITION
All microbes

Microbial composition of the human colon inferred from 16S RNA gene sequences



B. GUT MICROBIOTA IN HEALTH AND DISEASE

Why "hidden organ"?

- Collectively, human microbiota contribute 150-fold more genes to the human metaorganism than the human host does = Metabolic diversity.

- The microbial genes encode diverse functions and pathways that provide metabolites and signaling molecules <u>essential</u> for host survival and development.

Functions of the gut microbiota

Nutritional benefits:

- Food breakdown
- Digestion of polysaccharides
- Production of essential vitamins: Vitamin K and B12
- Regulation of fat storage
- Production of organic acids (SCFA)
- Steroid metabolism
- Bile salt metabolism

Functions of the gut microbiota

Host development: Influences on -

- Angiogenesis
- Cell proliferation
- Bone density
- Vascularization
- Endocrine function
- Neurological signalling

Functions of the gut microbiota

<u>Barrier, chemical and microbial defense</u>:

- The microbiota provides a physical barrier to protect its host from pathogens through
  - plugging up binding sites
  - consuming nutrients to starve the invader
  - producing antimicrobial substances
  - modulating oxygen and pH

Functions of the gut microbiota

The host immune system:

- Microbiota is essential in the development of the intestinal mucosa and immune system of the host.
- Germ-free animals (mice) have abnormal numbers of several immune cell types, defects in lymphoid structures, deformed spleens and lymph nodes and general perturbations in cytokine levels.
- Immune modulation functions of the microbiota are primarily involved in promoting the maturation of immune cells.

Functions of the gut microbiota

Interactions with xenobiotics:

- Implication in absorption, distribution, metabolism and excretion of therapeutic drugs and other xenobiotics.
- Diversity of enzymes that can directly metabolize drugs and hence influence their effect on the host.
- On the other hand, the drugs may also exert a causal effect on microbiota structure.

Dysbiosis

- Imbalances in the composition and function of the gut microbes. Associated with many diseases:
  - inflammatory bowel disease, and other GI-associated diseases
  - rheumatoid arthritis
  - Type I and II diabetes
  - autoimmune diseases
  - obesity and metabolic syndrome
  - allergies
  - cancer
  - neurodegenerative diseases
  - CVD

Dysbiosis of microbiota and disease

- Cause or Effect?
- Does dysbiosis cause the disease or does the disease cause the symbiosis?
- Evidence is emerging to suggest that gut microbiota has a causal role in disease prevention.

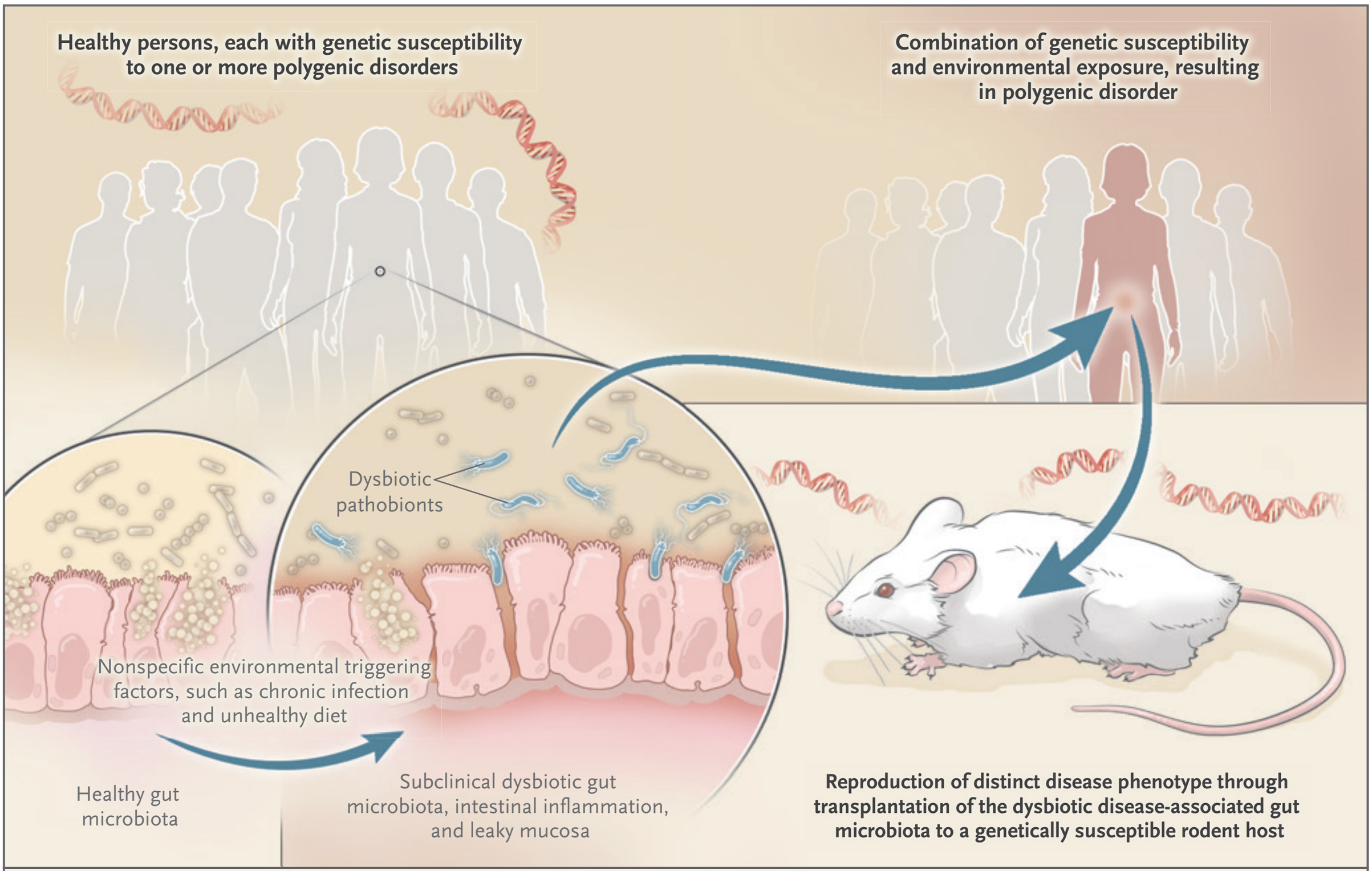


Figure 3. **"Common Ground" Hypothesis of Pathogenesis Caused by Dysbiotic Gut Microbiota.**

C. RESEARCH ON GUT MICROBIOTA AT UOM

Investigators:

- Dr Meera D. Manraj
- Assoc Prof. Prity Pugo-Gunsam

Personnel at SSR Resource Centre:

- Ms Annick Hébé

Technical Staff at Faculty of Science:

- Mrs Nathalie Sem Fa
- Mrs Sarojini Jankee
- Mrs Solange Lee Kwai Yan

Funded by the Mauritius Research Council

"Effects of vegetarianism on the gut microbiota structure of Mauritian vegetarians and omnivores"

Aims:

- To assess the effect of a vegetarian diet on the composition of faecal microbiota of human vegetarians and omnivores;
- To investigate how rapidly a vegetarian diet affects the faecal microbiota of long-term omnivores;
- To analyze relationships between the composition of faecal microbiota and levels of biomarkers associated with metabolic diseases

The specific objectives of this project are to:

1. collect stool samples from human subjects before, during and after fasting periods;

2. measure levels of biomarkers indicative of metabolic disease

3. investigate the diet habits of the subjects

4. identify the microbes in the stool samples by molecular tools;

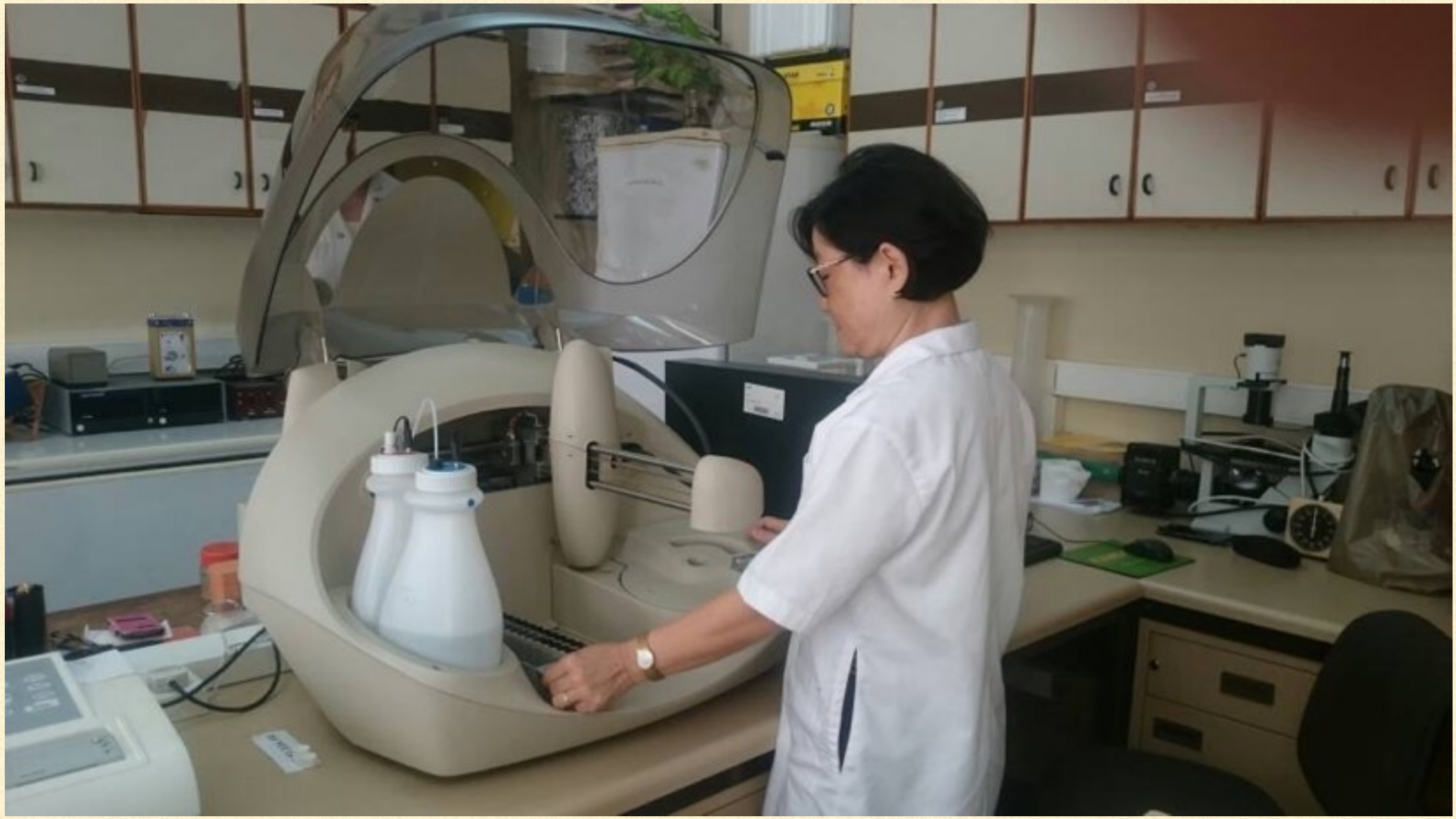
5. compare the levels of selected species of microorganisms between samples from subjects on vegan, lacto-vegetarian and omnivorous diets

SSR Resource Centre, UoM:

- Health Check
- Diet Survey
- Collection of biological specimen

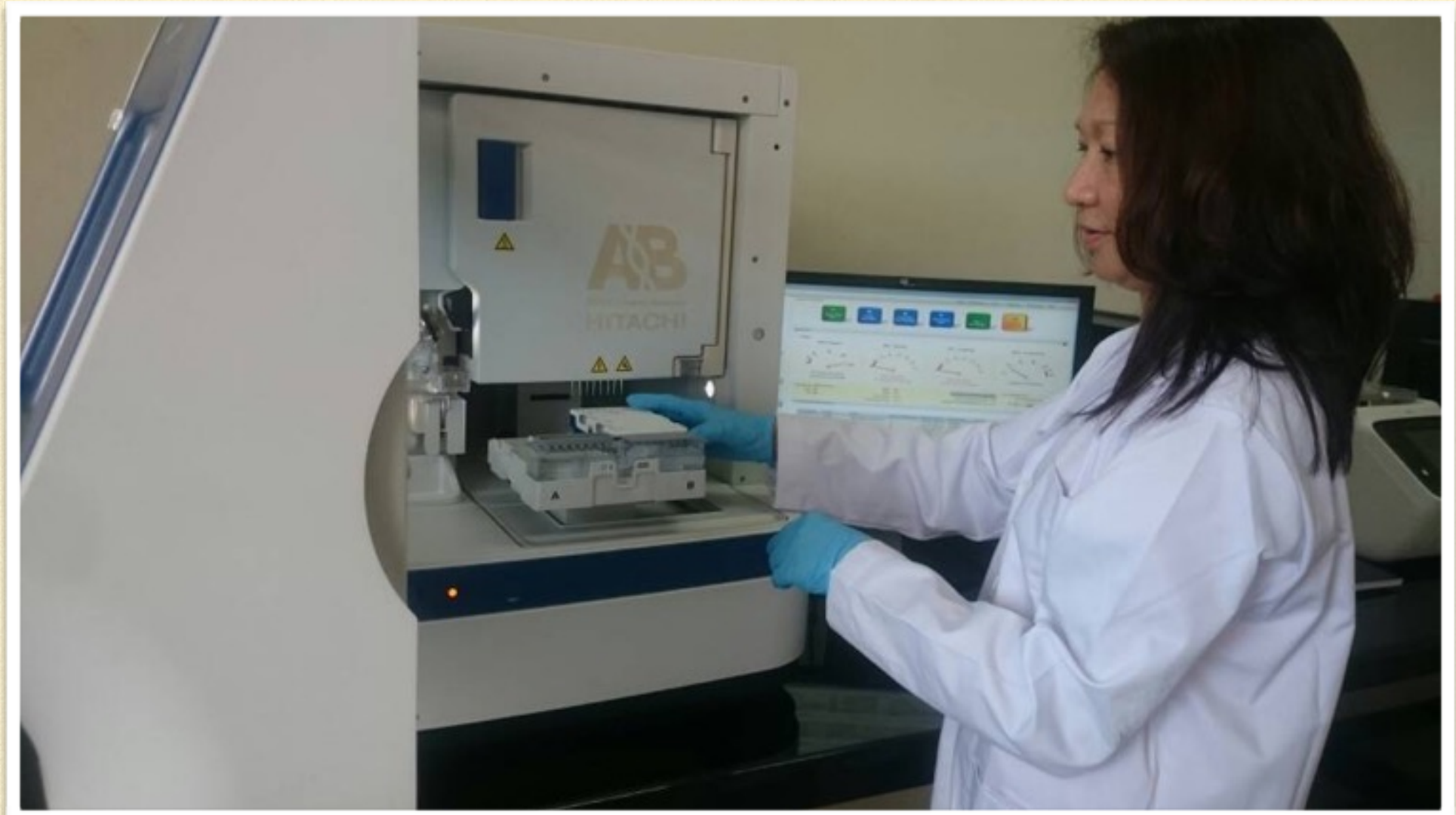
Faculty of Science, UoM:

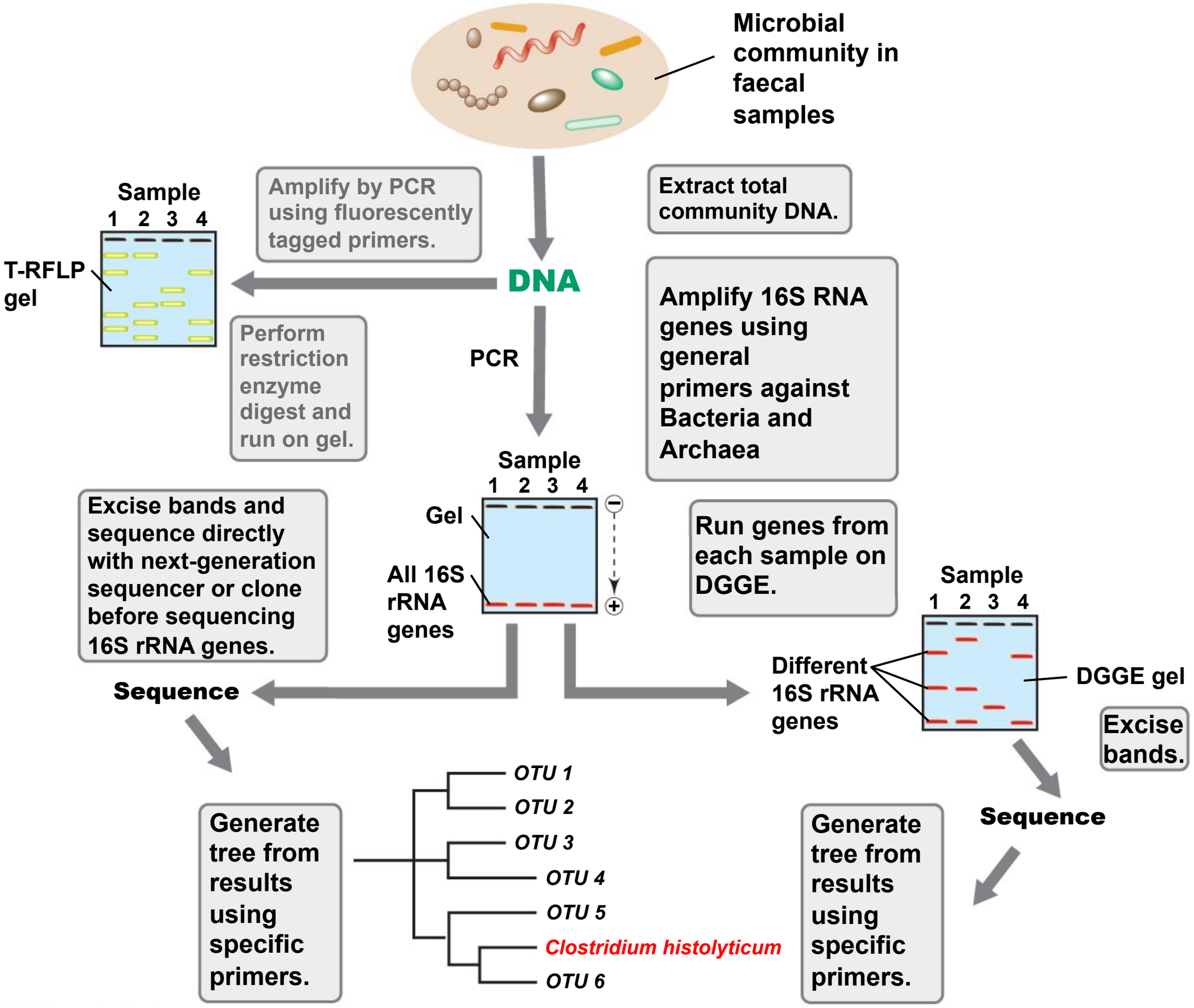
Dept of Medicine: Blood biochemistry; Urine tests



Faculty of Science, UoM:

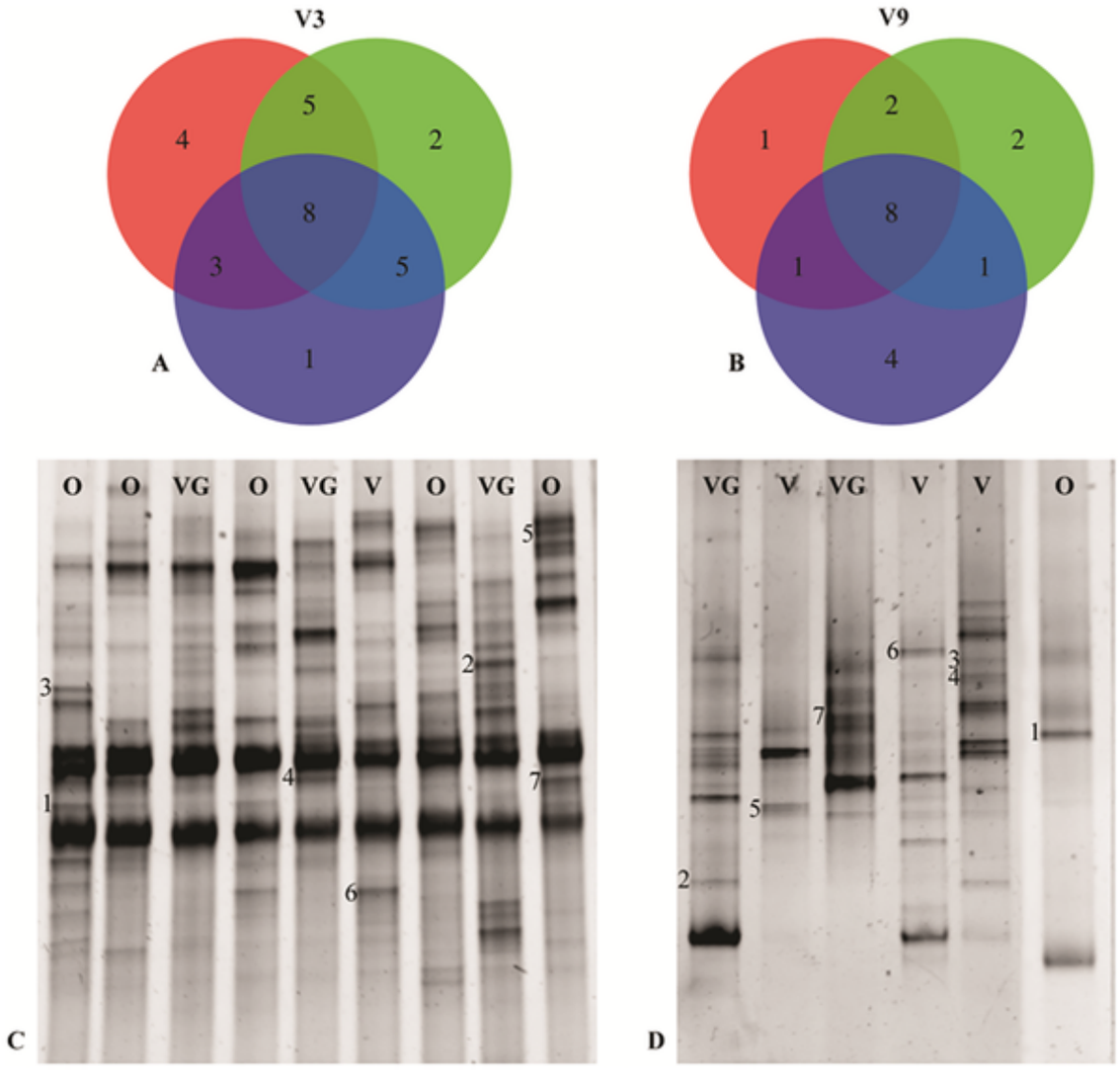
Dept of Biosciences: DNA extraction from stool; PCR amplification; Denaturing Gradient Gel Electrophoresis; DNA sequencing; Quantitative PCR




Microbial community in faecal samples
Extract total community DNA.
DNA
Amplify by PCR using fluorescently tagged primers.
Sample
1 2 3 4
T-RFLP gel
Perform restriction enzyme digest and run on gel.
PCR
Amplify 16S RNA genes using general primers against Bacteria and Archaea
Sample
1 2 3 4
Gel
All 16S rRNA genes
Run genes from each sample on DGGE.
Excise bands and sequence directly with next-generation sequencer or clone before sequencing 16S rRNA genes.
Sequence
Different 16S rRNA genes
Sample
1 2 3 4
DGGE gel
Excise bands.
Sequence
Generate tree from results using specific primers.
OTU 1
OTU 2
OTU 3
OTU 4
OTU 5
Clostridium histolyticum
OTU 6
Generate tree from results using specific primers.

An example of DGGE:

Fig 6. RNA-DGGE band distribution.



Ferrocino I, Di Cagno R, De Angelis M, Turroni S, Vannini L, et al. (2015) Fecal Microbiota in Healthy Subjects Following Omnivore, Vegetarian and Vegan Diets: Culturable Populations and rRNA DGGE Profiling. PLOS ONE 10(6): e0128669. https://doi.org/10.1371/journal.pone.0128669
http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0128669

"Effects of vegetarianism on the gut microbiota structure of Mauritian vegetarians and omnivores"

Major Expected Outcomes:

- Determination of the differential composition of gut microbiota of vegans, lacto-vegetarians and omnivores
- Estimation of the time taken for the microbiota to change composition in response to diet changes
- Correlation, if any, between gut microbiota composition and diets and risk factors

D. APPLICATIONS

Manipulation of the gut microbiota composition through:

- Probiotics
- Prebiotics
- Antimicrobials
- Faecal transplantation
- Microbiota products

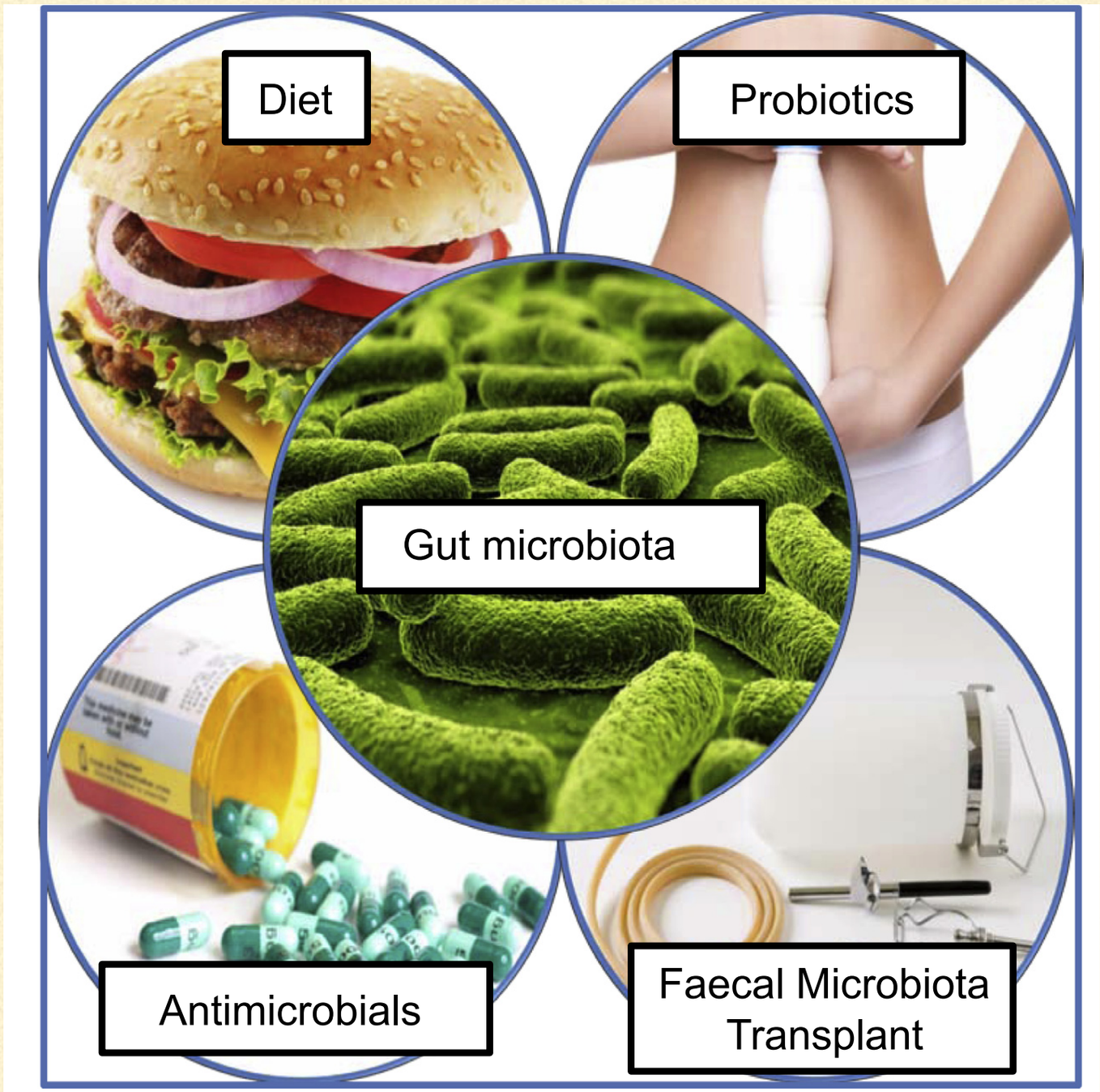


Fig. 1. Potential strategies for manipulation of the gut microbiota.

Probiotics

- Live bacteria taken orally e.g. through yoghurt, kefir
- Design personalized biotic capsules
- Q: Can they survive the journey down to the gut, through the harsh environment of the stomach?

Prebiotics

- Principles: To promote the growth of specific microbes
- Sugars and foodstuff taken to alter microbiota by providing substrates to microorganisms

Antimicrobials

- Could be used to modulate microbiota composition
- Problems:
  - collateral damage
  - induction of antibiotic resistance

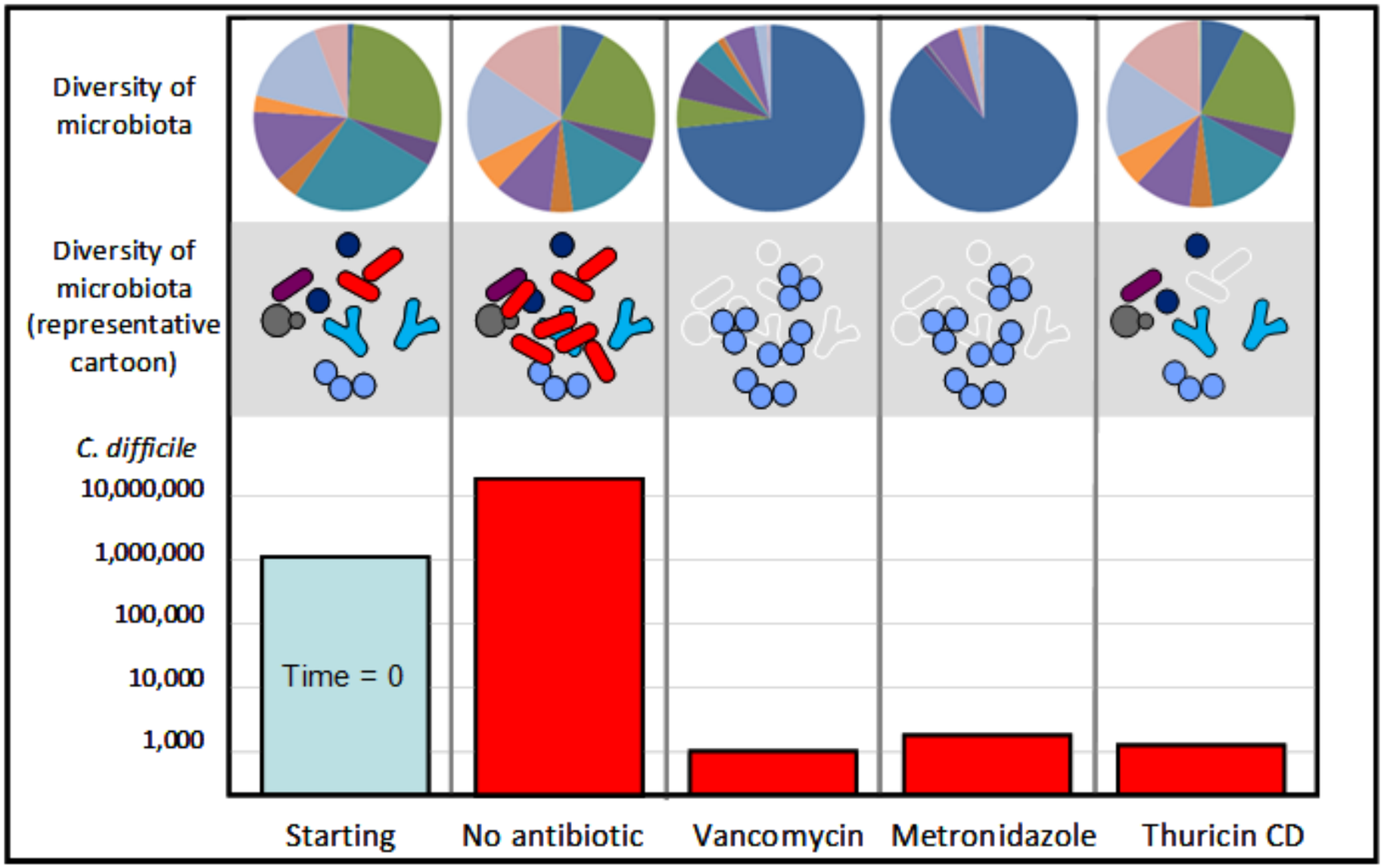


Figure 1. Impact of antimicrobials (all at 90 μM) after 24 hrs on the gut microbiota as revealed by high throughput sequencing-based analysis of a model colon containing faecal samples spiked with *C. difficile*. Top: different colors denoting different families of bacteria (blue indicates the *Enterobacteriaceae*); middle: cartoon representing the diversity of the microbiota (red rods indicate *C. difficile*); bottom: thuricin CD is as effective at eliminating *C. difficile* as vancomycin and metronidazole.

Faecal transplantation

- Used in *Clostridium difficile* infections (CDI)
- 2013: Patient with persistent CDI was administered a faecal microbiota transplant from an individual with similar diet from the same environment. Patient recovered from chronic infection.
- Issues to be considered:
  - may need to deplete microbiota with antibiotics
  - regulations regarding transplantation of human biological material

Microbiota products

- The human microbiome produces a large number of metabolites, some of which could have important potential applications.
- Examples:
  - MRSA-active antibiotics have been recently found in the microbiome (Chu *et al.* 2016).
  - a bacterial polysaccharide from *Bacteriodes fragilis* affects T-cell populations and could be used to modulate immune responses.
- Challenge: identification of the large number and diversity of microbial metabolites in the human microbiome

PERSPECTIVES

- The major challenge in microbiota research is no longer data generation but data analyses and interpretation. Informatics need to catch up with biological data generation.
- Personalized microbiota genomics could be integrated with personalized genomics to give a holistic picture of the human metaorganism.
- Longitudinal studies are essential to better gauge the crosstalk between microbial and human cells.
- Distinction between cause and effect.
- The not-so-hidden organ has much in store for us to discover and manipulate.

ACKNOWLEDGMENTS

- The Medical Update Group
- The Department of Medicine, Faculty of Science, University of Mauritius
- The Mauritius Pharmacy (Seegobin) Ltd