Section One Slides

Botanicals, Microbiome, Biofilms, and Chronic Infections

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http://naimh.com
Notes and readings http://naimh.com/csch-biofilms

Seminar Overview

SECTION One

- The New Microbiology
- The Human Microbiome

SECtION TWO

- Infection
- Biofilms
- Berberine and related alkaloids
- Microbial defenses

SECTION THREE

- Host defenses
- Constituent synergy herbal therapeutics

SECTION FOUR

- Antifungal therapeutics

SECTION FIVE

- Biofilms in the gut
- Internal Biofilms

Themes for the weekend

- Germs are not the enemy, and attempts to eradicate them have led to serious unintended consequences, collectively and individually
- Biofilms are the natural base state of bacteria, archaea, and some fungi. Biofilms are not the enemy, and attempts to eradicate them may also produce unexpected and unintended adverse consequences.
- The microbiomes in the various regions of the body perform essential functions, and, if damaged, can allow increased pathogenic infections
- A **single** course of antibiotics ***can*** cause lasting damage to the microbiome. **Repeated** courses ***will*** cause lasting and irreversible damage

THE NEW MICROBIOLOGY

Key Points

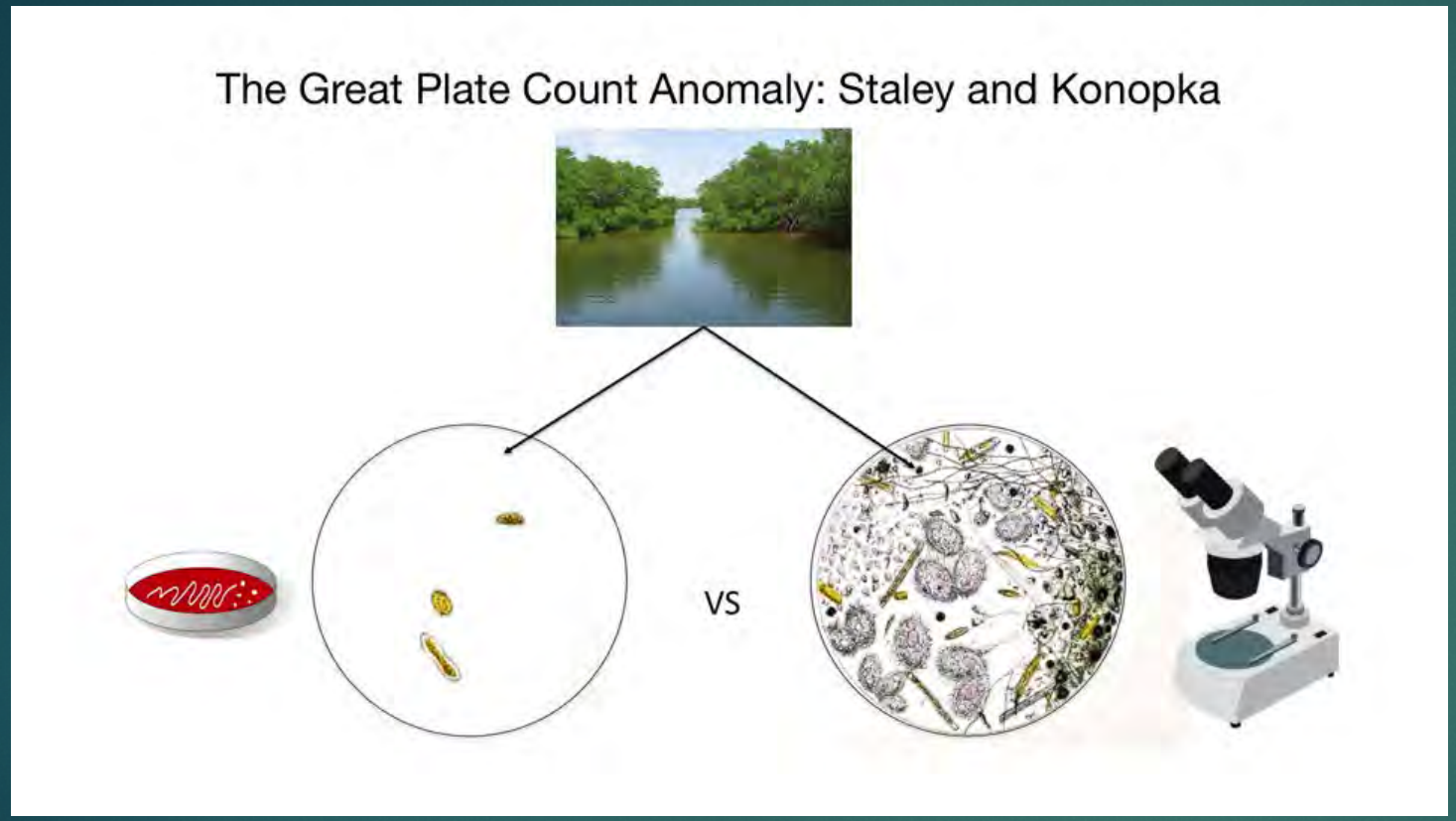
- Most bacteria cannot be cultured in a lab dish or visually identified by appearance.
- Identification of genetic material has led to a radical expansion and clarification of the phylogenetic tree.
- Microbes in an on the human body are now recognized as commensal and essential to human life
- Aggregates of microorganisms in the biofilm form are now recognized as the natural baseline state of bacterial and fungal life.

Terms

- **Microbe.** A microscopic living organism. Many be single or multi-celled. Includes bacteria, archaea, most protozoans, yeasts, and (depending on who you ask) viruses.
- **Microbiota** - the microorganisms of a particular site or anatomical zone
- **Microbiome** – the combined ***genetic material*** of the microorganisms of a particular site
- **Bacteriome** and **Archaeome** – Bacteria and Archaea
- **Virome** – The viruses in the human, or in one region of the organism
- **Fungome** – Yeasts and other fungi

New concepts in microbiology

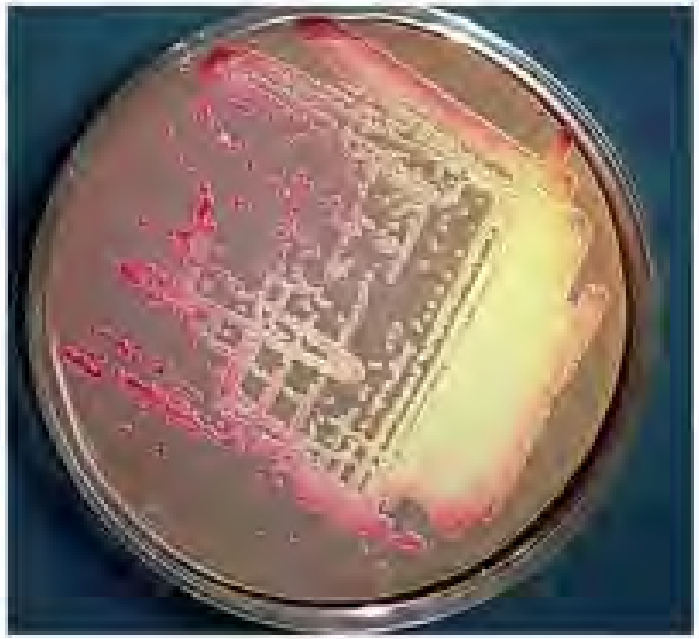
- Old Model. Freely mobile microbial invaders infect a sterile body. They can be cultured on lab plates. Antibiotics can kill and remove them.
- New methods of detection have increased not only the number of microorganisms we can recognize but have completely overthrown the previous models of detection, infection, and therapeutics.



- Typically of bacteria observed in lake water in a microscope, only 0.1 to 1% can be cultured in media.
- ***The great majority of microbes in the human gut cannot be cultured, including some of the dominant species***.
- We know a lot about *Escherichia coli* because it is easy to culture but It is a very minor part of the gut population, less than 0.1%.

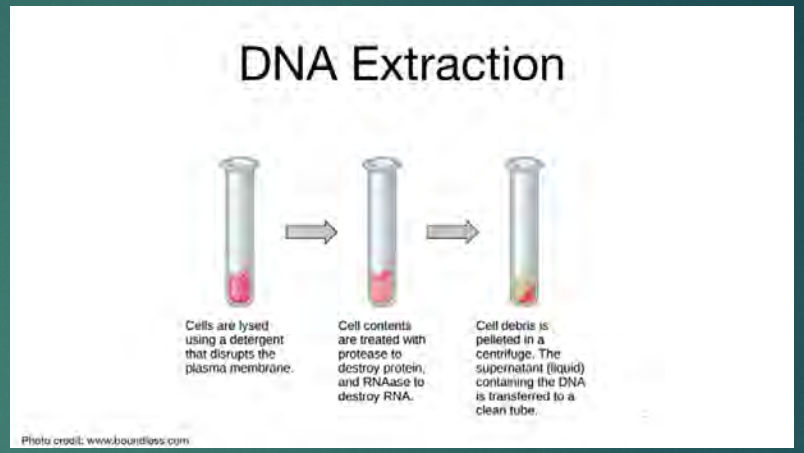
Easy to grow does not mean most important or abundant

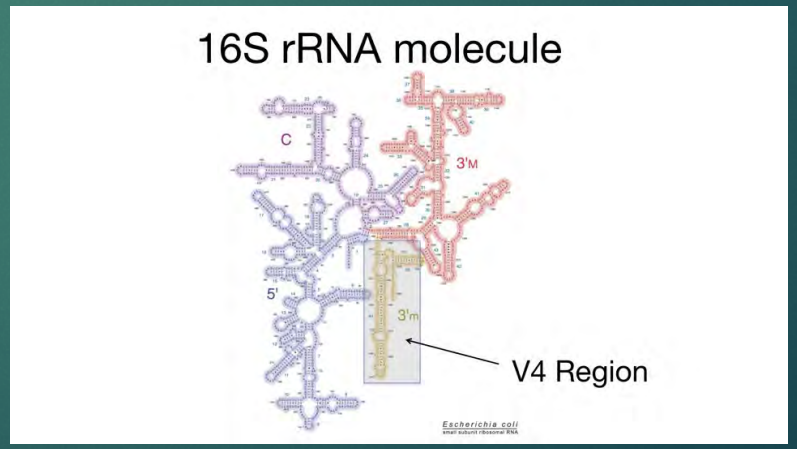
Tame ≠ Wild



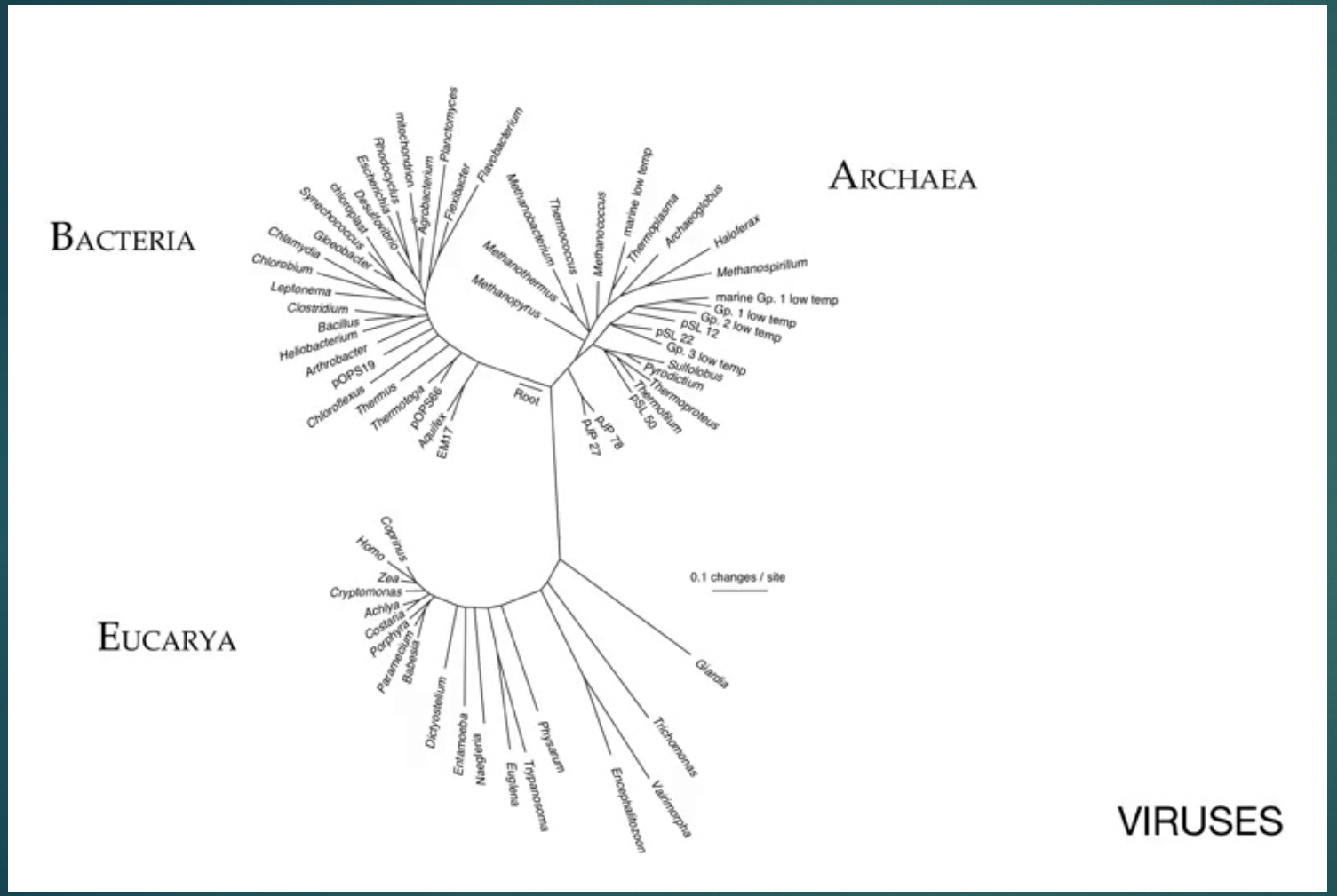
DNA sequencing to identify bacteria

- DNA Extraction
- Polymerase Chain Reaction
- Sequencing





Phylogenetic tree



Genetic sequencing has greatly expanded our knowledge of the phylogenetic tree with identification of the category of Archaea and many new orders and families of microbes

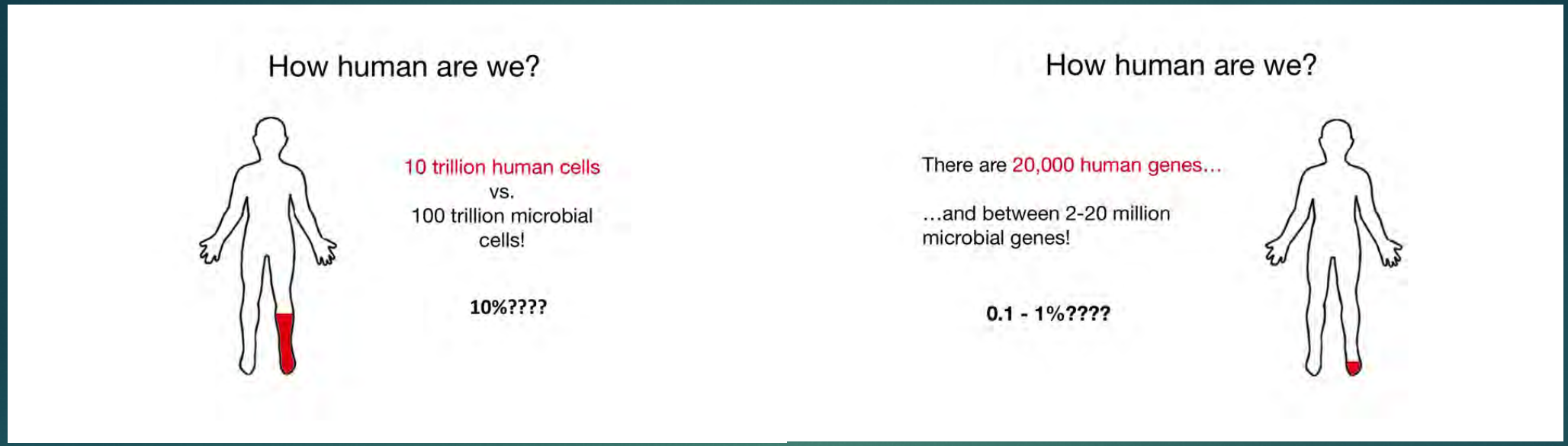
Prokaryotes are single-celled organisms whose genetic material is not enclosed in a nucleus. They include **bacteria** and **archaea**.

Eukaryotic cells contain a nucleus and multiple organelles, and are enclosed in a plasma membrane. They include fungi, protozoa, and all orders of plants and animals. This includes us.

Viruses are strands of genetic material which must infect a living cell in order to engage in the process of life and reproduction. They are arguably not living organisms (and thus cannot be killed).

- From 1980 to 2012, the category of Archaea has been identified.
- The number of known bacterial families has grown from 12 to more than 100. The great majority have never been cultured or identified in a microscope, and their existence cannot be detected through traditional methods.
- Only about 27 percent of the bacterial phyla have species that can be grown in the laboratory .
- Unculturable phyla, known as *candidate phyla*, make up 103 out of approximately 142 known phyla.

Virtually all animal life on earth is dependent on microbes for their survival as only bacteria and some archea possess the genes and enzymes necessary to synthesize vitamin B12, Involved in the metabolism of every cell in the human body.



- About ten times as many viruses as bacteria exist in an on the human body and its microbiota. Most viruses are bacteriophages which infect bacteria, not human cells. They are protective by preventing overgrowth.
- A beneficial fungome also exists in normal health, in and on most of the body.
- The interdependent community of bacteria, archaea, virus, and fungi has been called the "Third Arm of the Immune System" due to its barrier function, resistance to invasion, and secretion of antimicrobial compounds.
- It also fulfills essential nutritional and metabolic functions.

One set of authors recently estimated the ratio of bacteria to human cells 1:1, downgrading previous estimates of bacteria, and upgrading estimates of cells. The bacteria remain a significant and essential presence in the living organism.

Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016 Aug 19;14(8):e1002533.

Some surprising results

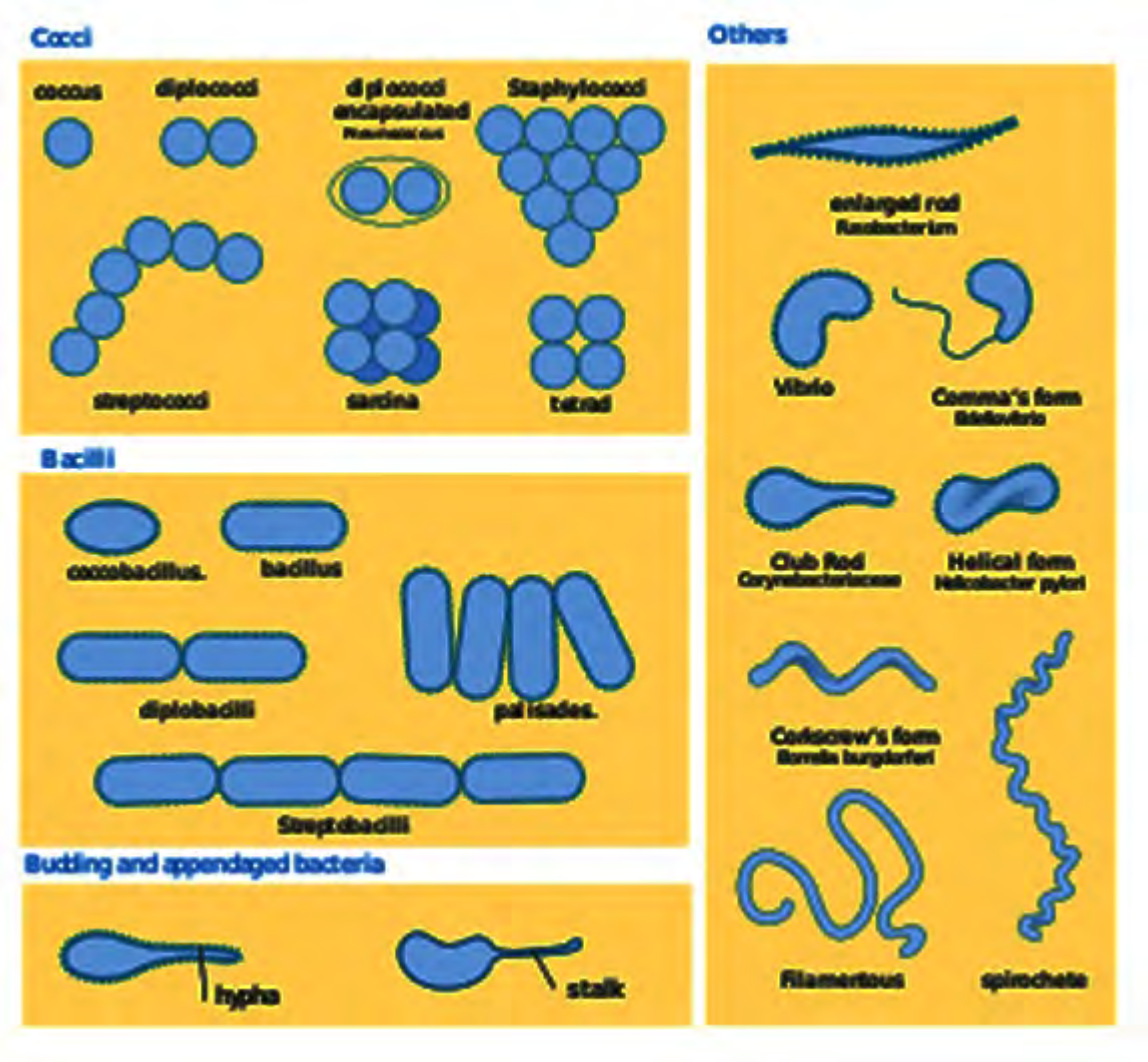
- Different regions and skin and body have their own unique microbiome.
- An estimated 1,000 different microbial species inhabit the human gut. No more 400 of these have been identified through genetic studies, and only a small minority have been cultured.
- More than 30 bacteria species have been found in the normal flora of the bladder which had never been detected on urine culture or microscopy.
- A microbiome has been discovered in the normal placenta. It is composed of bacteria similar to the oral microbiome with no similarity to the vaginal microbiome.

Bacteria

Among the first forms of life to appear on earth. They are present in virtually all the habitats on earth including soil, water, acidic hot springs, radioactive waste, and the deep portions of the earth.

They are about 1/10 the size of most eukaryotic cells.

They reside in and on all plants and animals and fulfill functions essential to life.

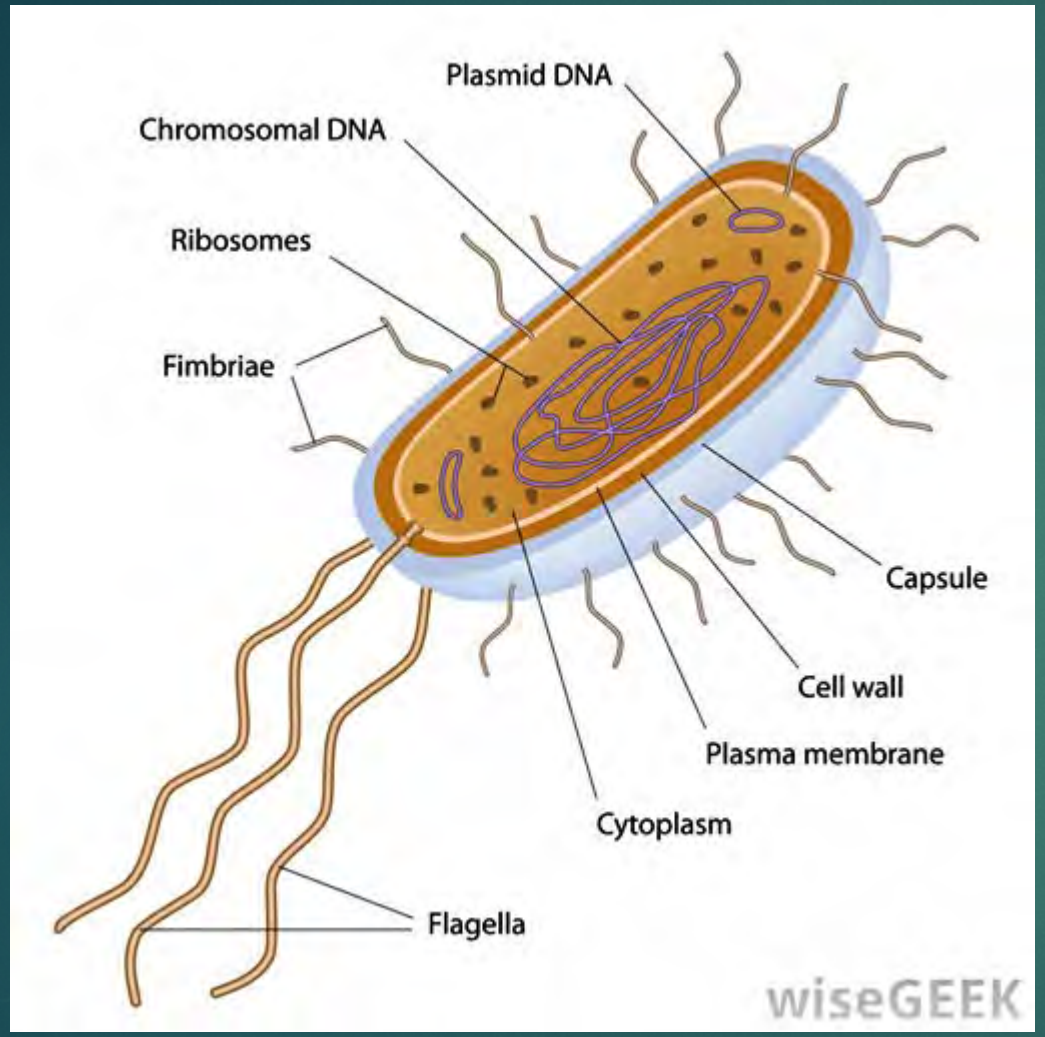


Bacteria have many forms, shapes, and habits of association.

They can reproduce very rapidly, some less than every ten minutes. A single bacterium can potentially produce millions of offspring in a few hours.

When the bacterial population reaches a certain threshold density, it converts to a biofilm form, and develops new emergent properties

Population growth is limited by available nutrients and competing populations

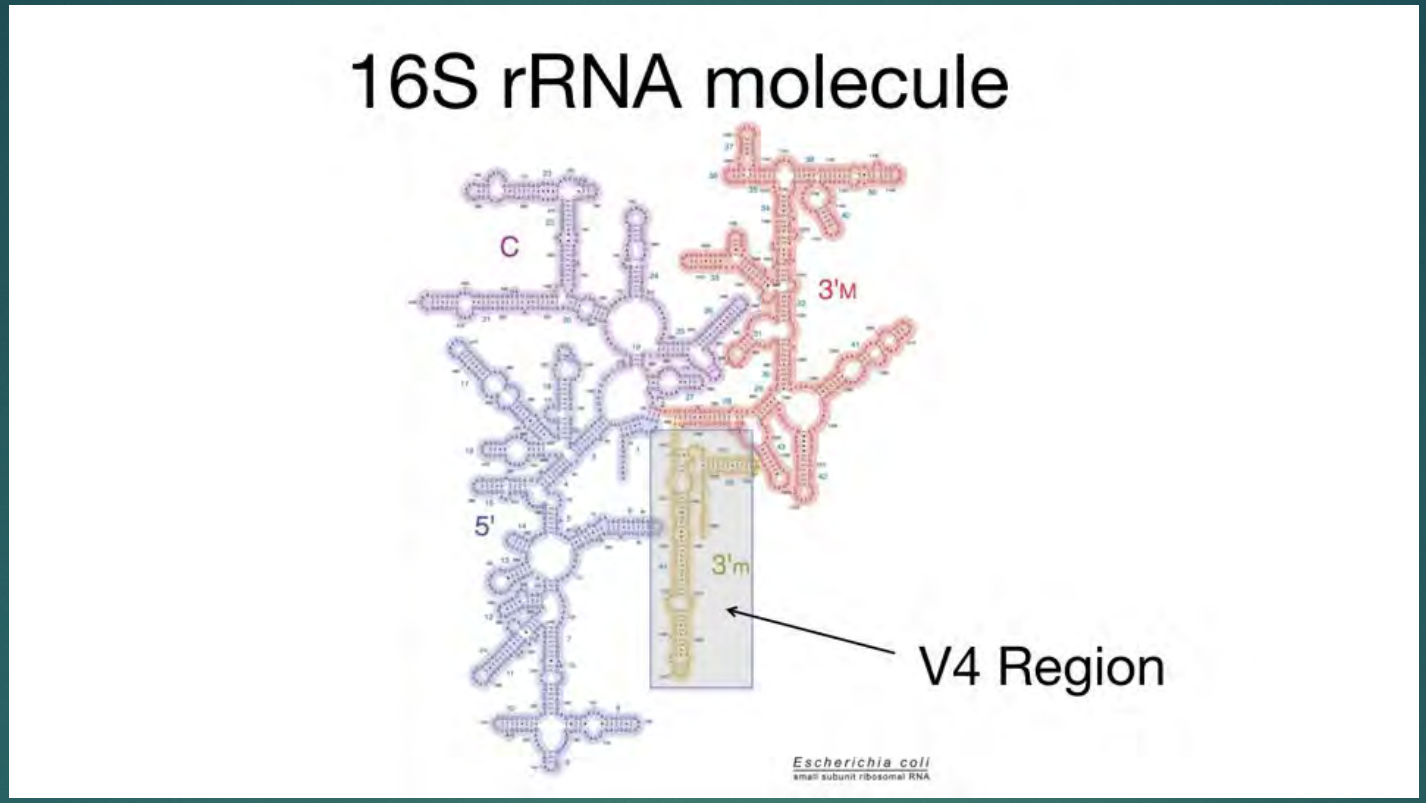


Bacterium

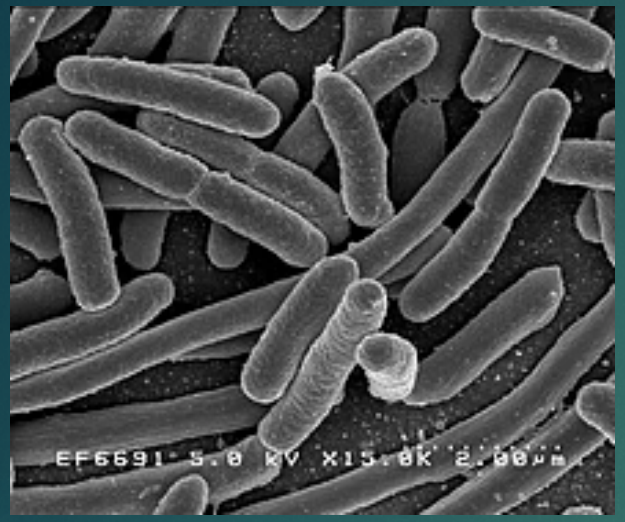
Note the genetic material unencapsulated in the cell cytoplasm.

This DNA is replicated when the bacterium divides during reproduction.

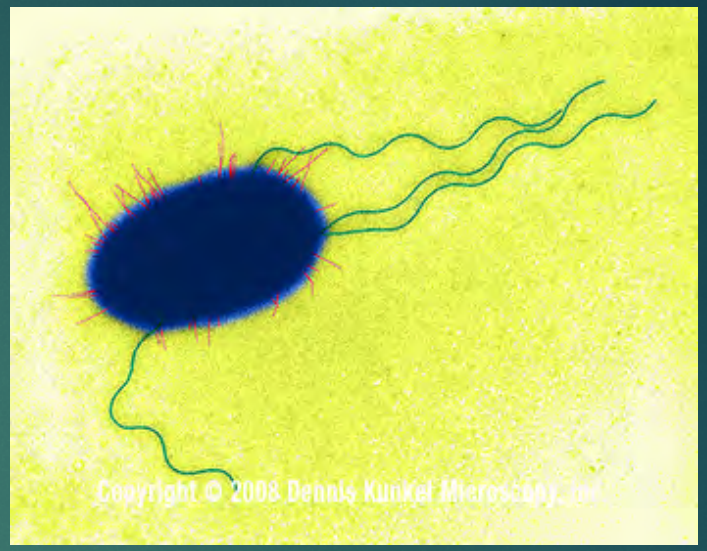
See also the small packets of **plasmid** DNA. These snippets of DNA can be shared and exchanged with other bacterial, archaeal, or some fungal species.

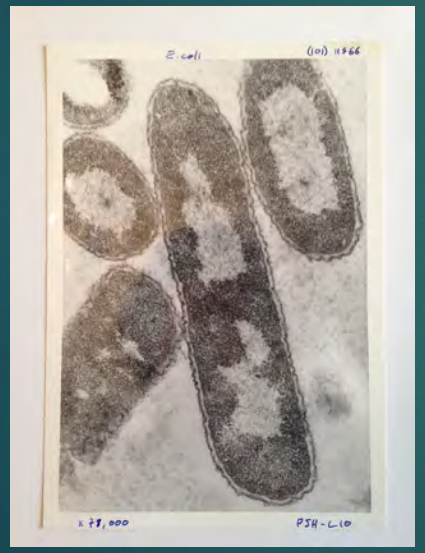


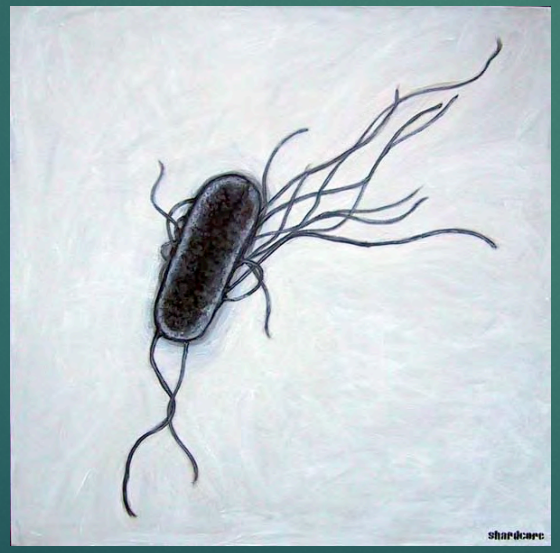
Large portions of bacterial and archaeal genetic material is common to all species likewise related metabolic functions. In this image of bacterial RNA, the red and blue portions are common to all bacteria. Thus **broad-spectrum** antimicrobial drugs and constituents are possible, which damage common core genetic material, proteins, or metabolic functions.

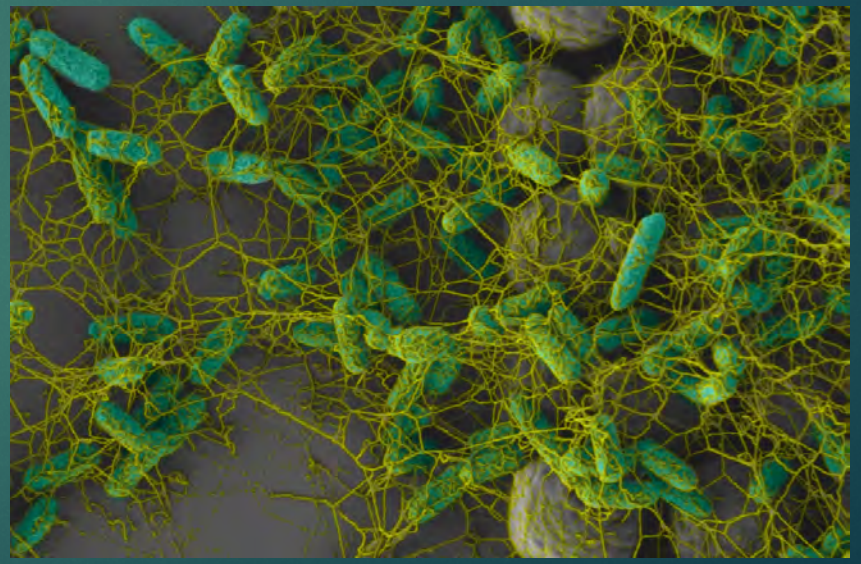


E. coli bacterium with fimbrae, flagellae, and a biofilm colony



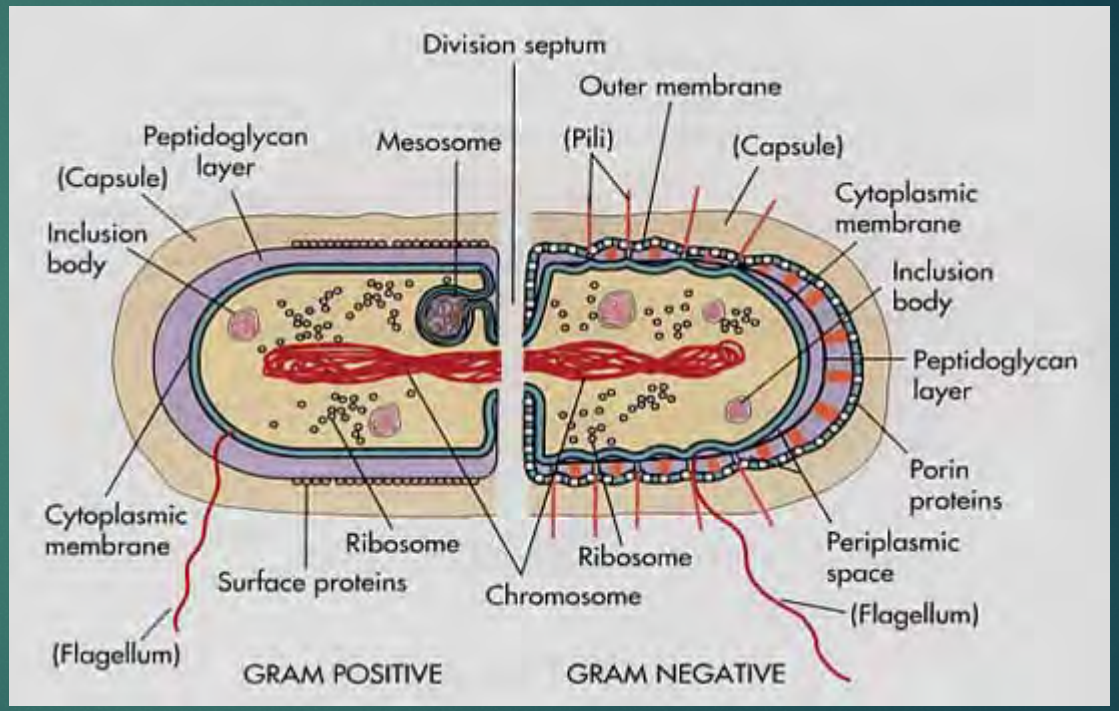




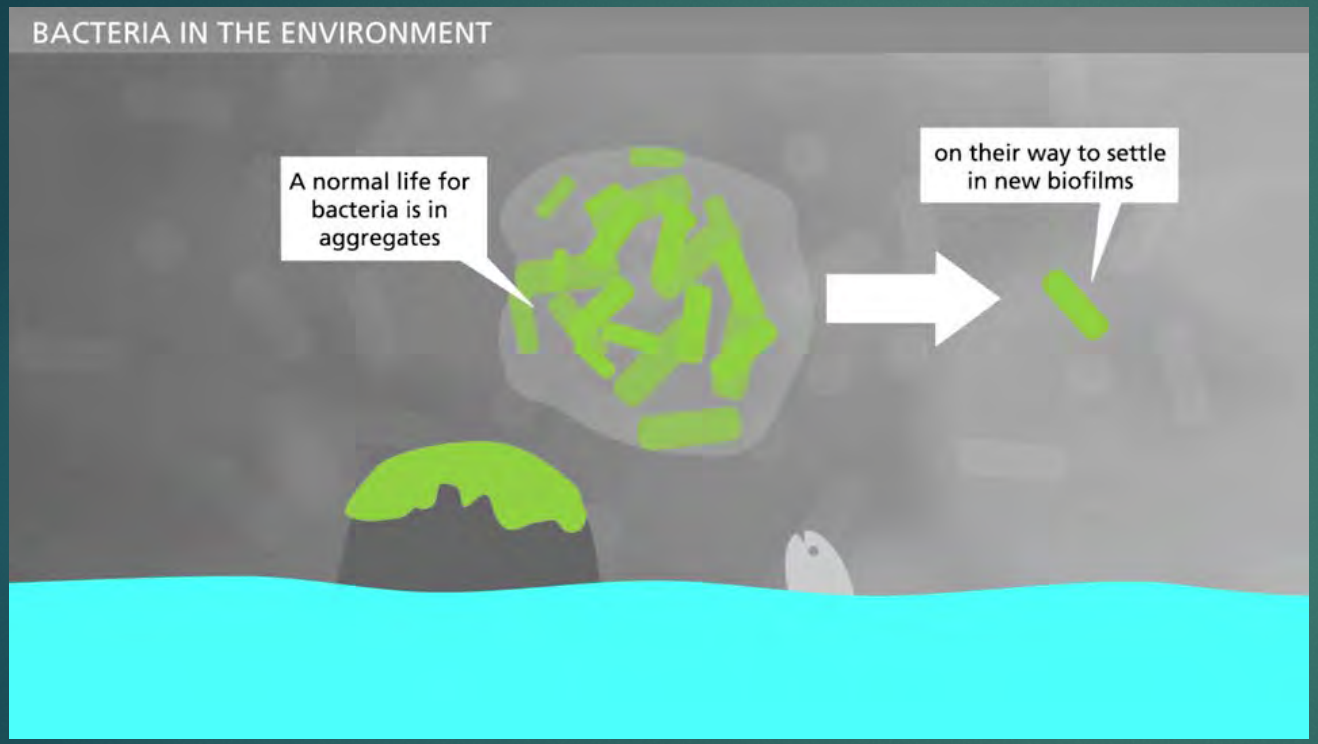


Gram-positive vs Gram-negative

- Refers to the type of stain (Gram) that the bacterium cell wall will take up.
- These two categories have different types of structures in the cell wall.
- Antimicrobial drugs or plant constituents may be effective against one type or another, or both.



Bacteria live in a biofilm state

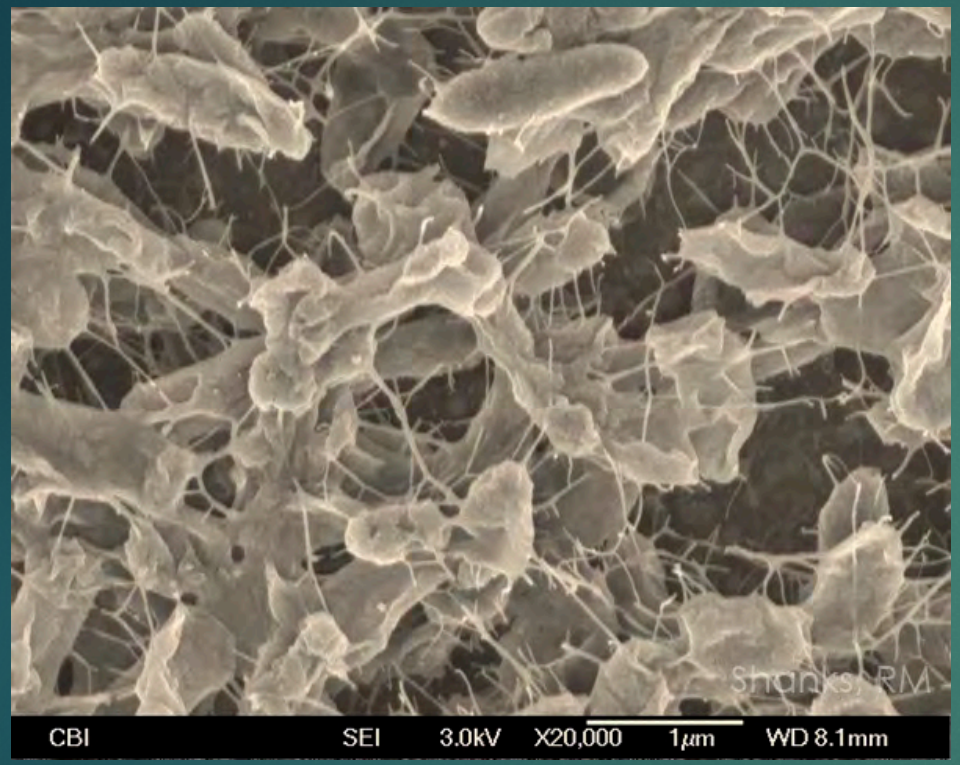


Planktonic form.
Free moving

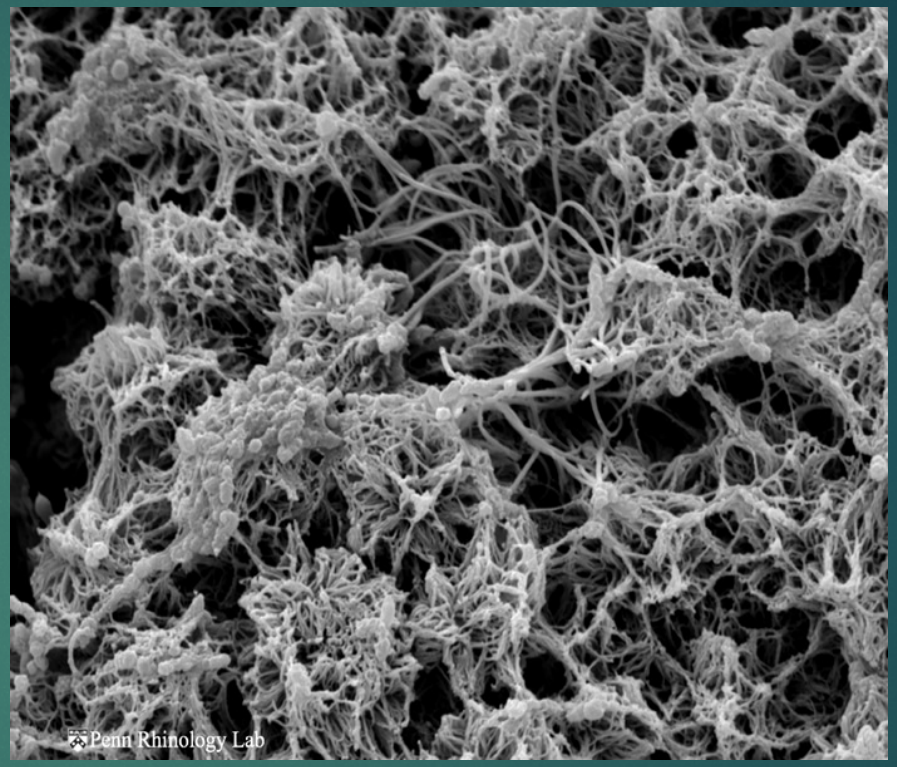
Biofilm form.
Non-mobile, linked in a matrix

The biofilm form of bacteria is resistant to both antibiotic therapy and the immune system

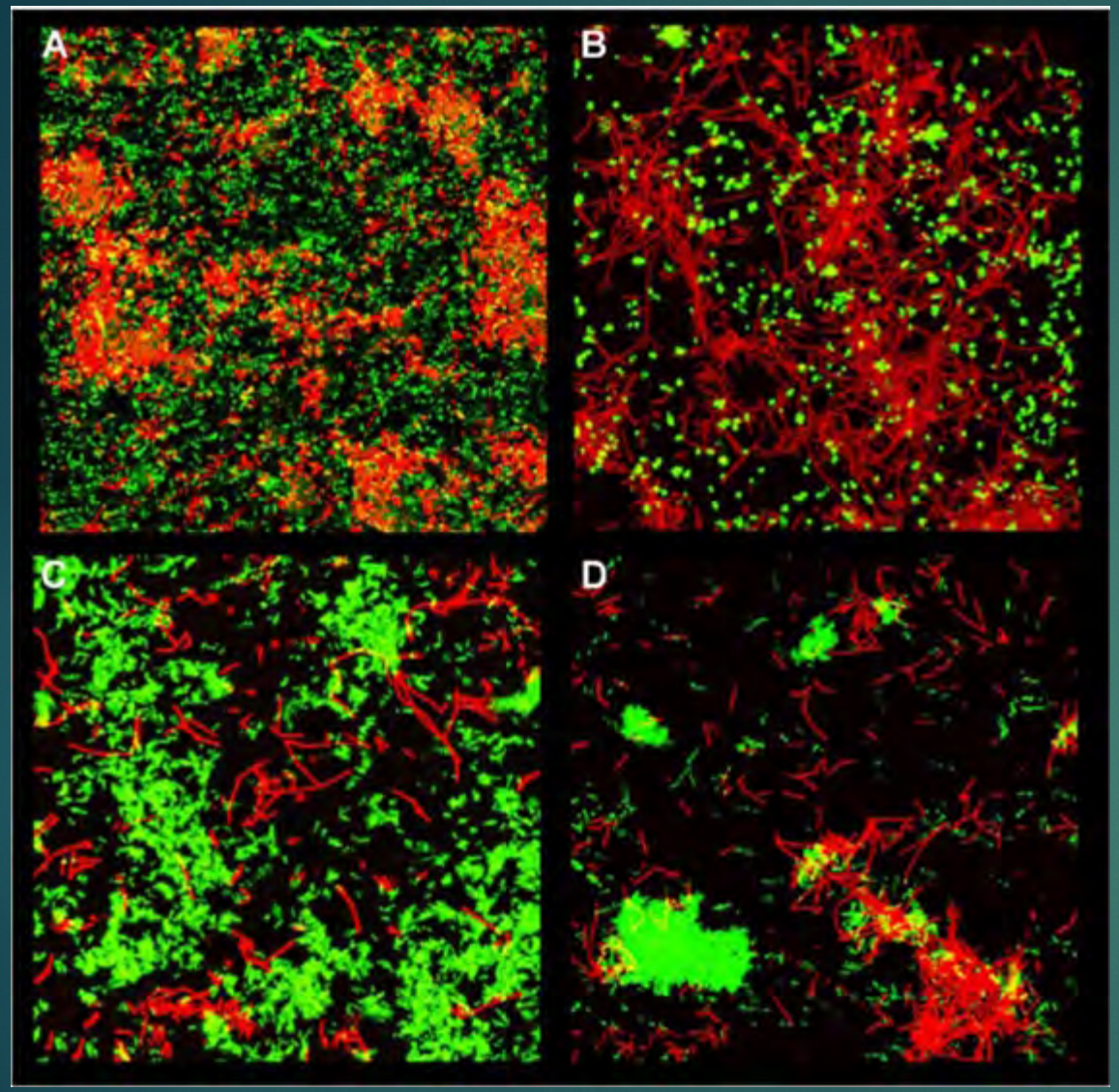
Most bacteria in an on the human body exist in biofilm form. Most are ***beneficial*** commensal bacteria and provide barrier, immune, and metabolic functions, and damage to the biofilm can promote disease



MRSA biofilm



Pseudomonas biofilm

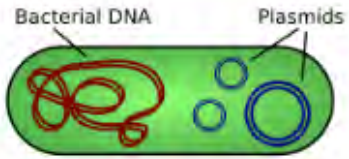


In the natural world, including the body, **multispecies biofilms** are most common, where the biofilm offers some advantage to both species.

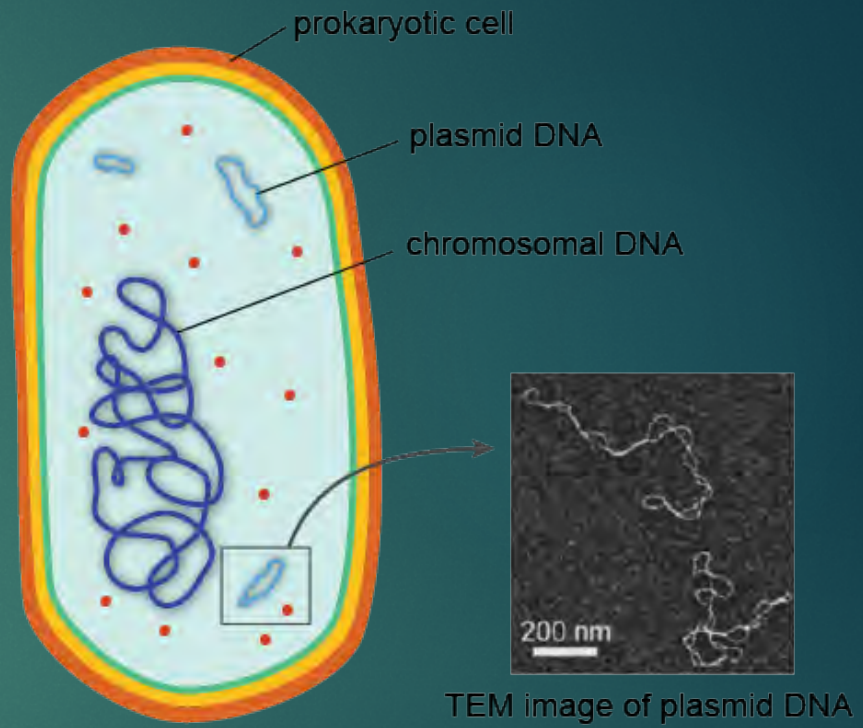
Mixed organisms might include bacteria, archaea, or fungi/yeast.

In these pictures, *Bacillus subtilis* (cherry red) Is mixed with 2 different types of *Staphylococus* (top) or 2 of *E. coli* (bottom)

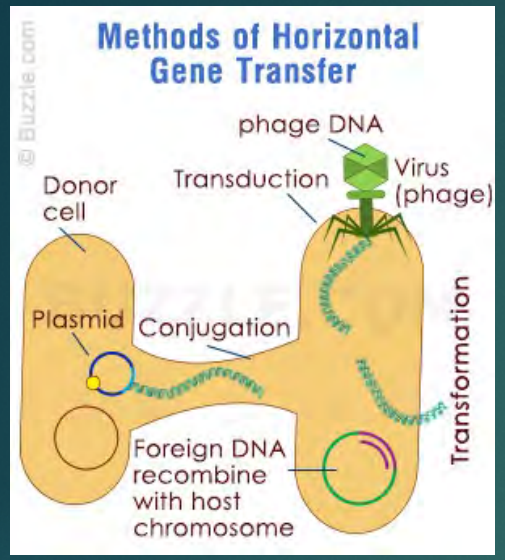
Plasmid

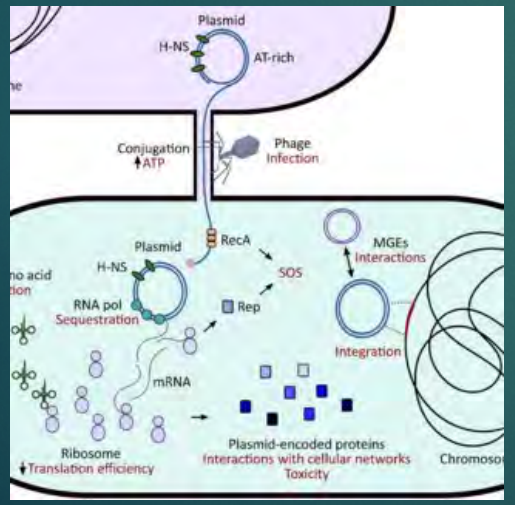


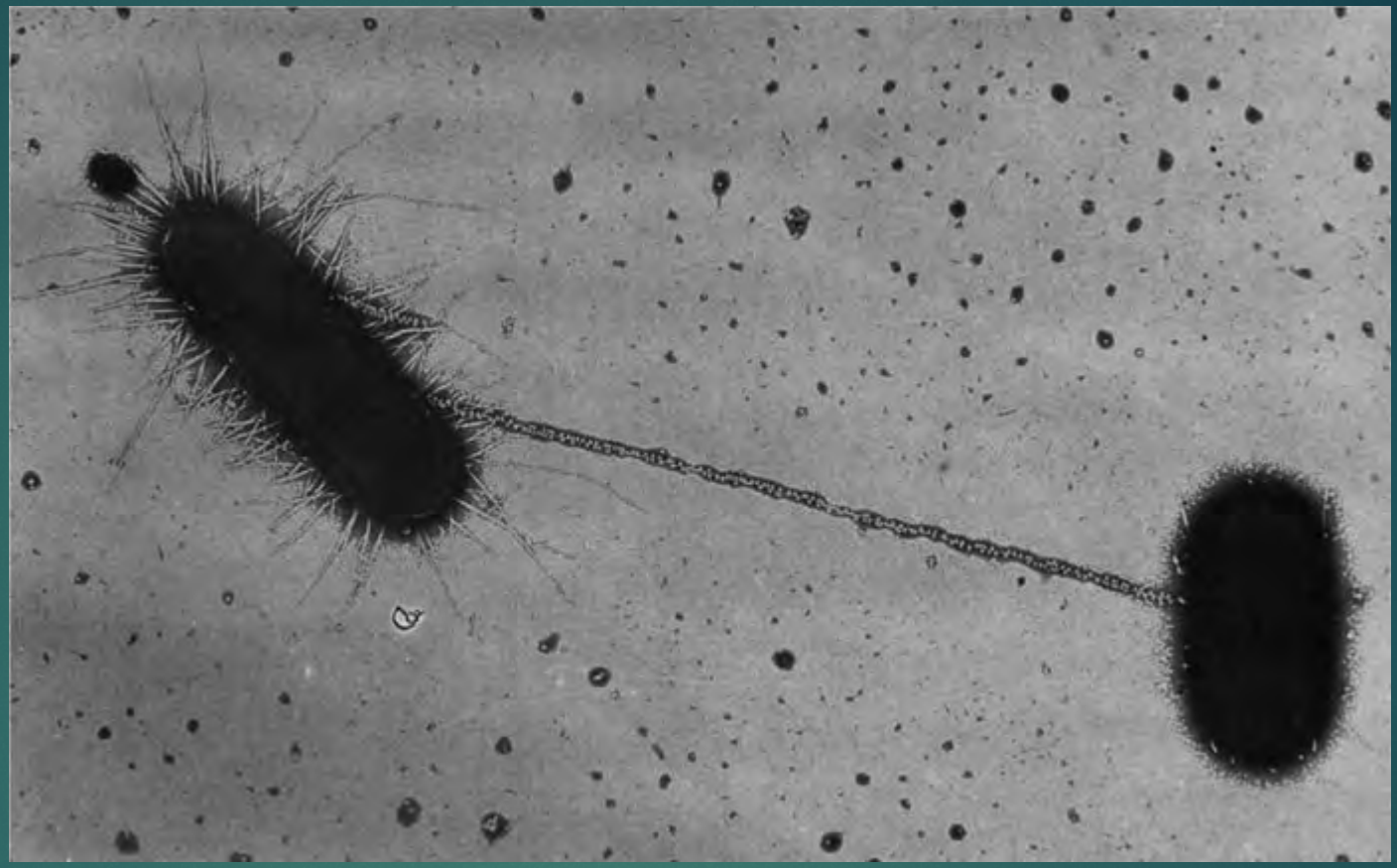
A plasmid is a small DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently. They are most commonly found as small circular, double-stranded DNA molecules in bacteria; however, plasmids are sometimes present in archaea and eukaryotic organisms. Wikipedia



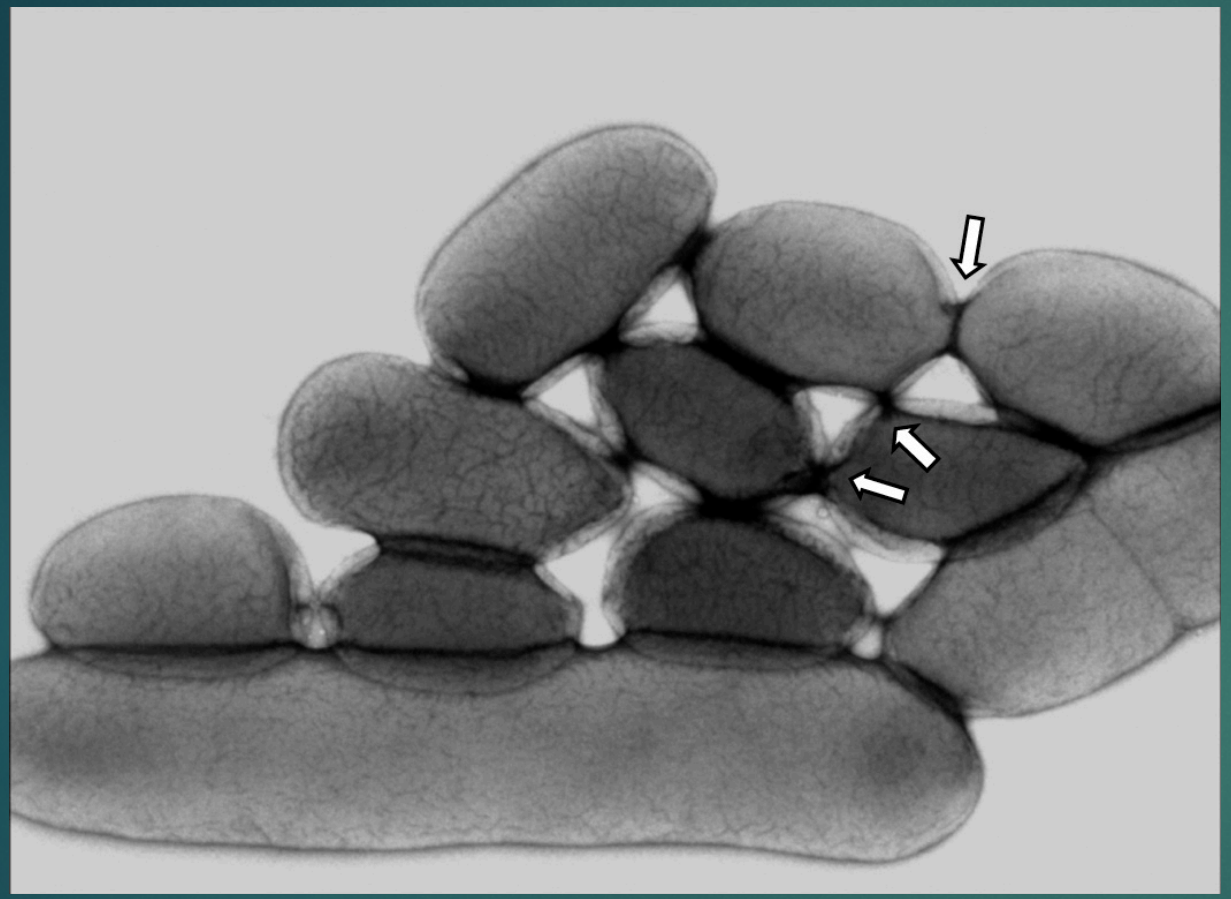
TEM image of plasmid DNA







Antibiotic resistance or other genes promoting survival genes can be passed between bacteria and other organisms via plasmid exchange.

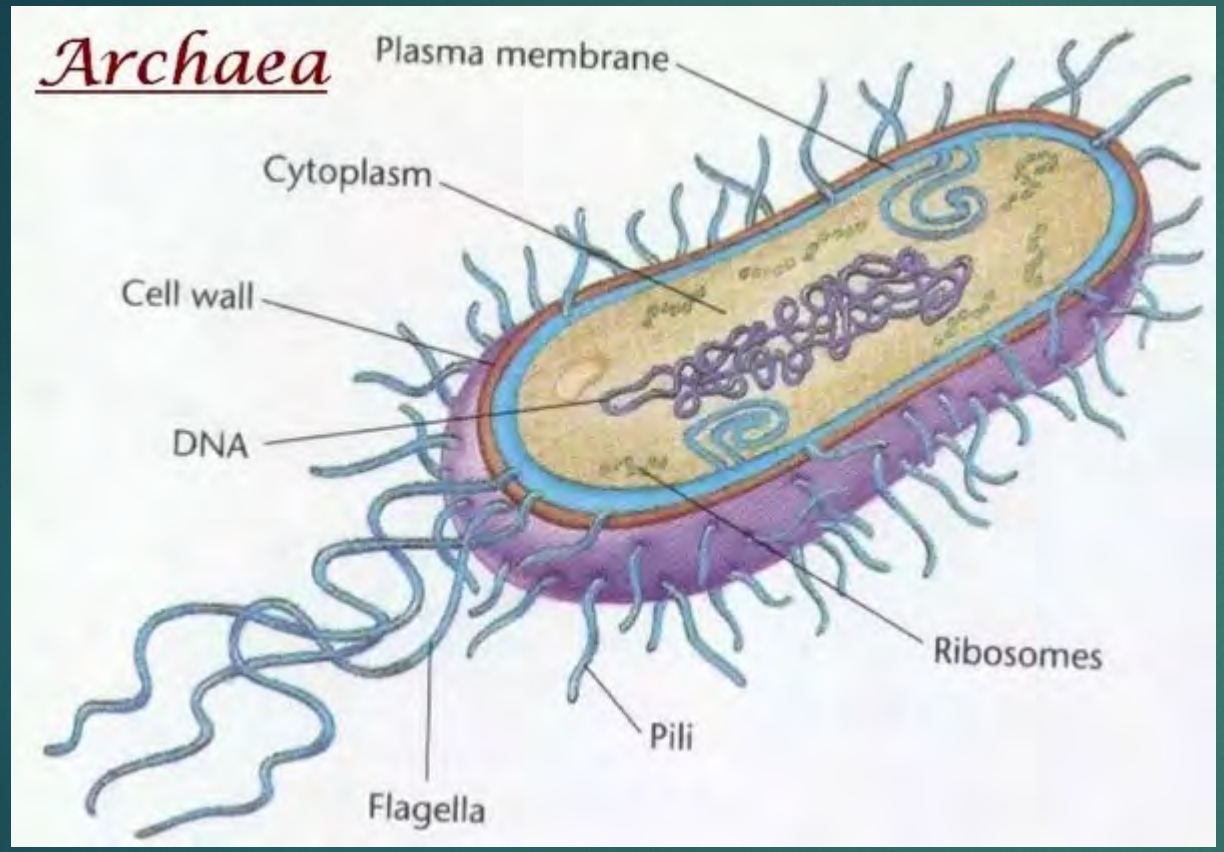


Plasmid exchange can occur rapidly and extensively throughout a biofilm.

In this example, almost all the bacteria are conjugated and exchanging genetic and other materials with each other.

For instance “resistance genes” to an antimicrobial constituent or drug, or other genes which allow survival, can be shared and passed throughout the biofilm until all surviving members possess them.

Archaea



Archaea are indistinguishable from bacteria in structure or appearance.

Their metabolism and functions are radically different, and they can live and thrive in extreme environments

Formerly classified as "archaebacterial" They were distinguished from bacteria by studies of their metabolic byproducts

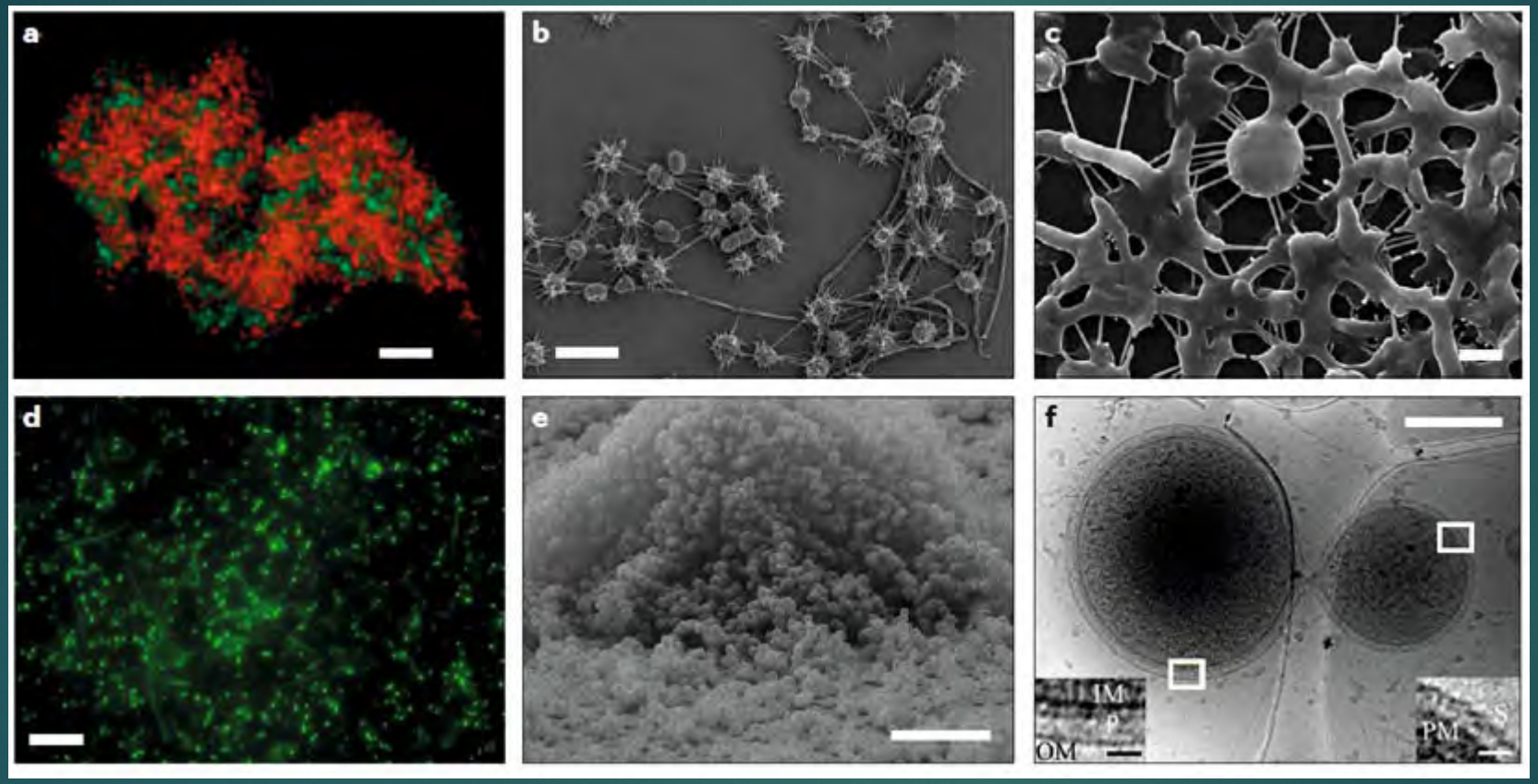
Some are normal residents of the human microbiome and physiologically significant.

Some types of Archaea

- **Methanogens** produce methane in anaerobic environments, such as swamps, or the human gut. Methanogens in the human gut are associated with chronic constipation, but whether the cause, or the result of the dense anaerobic environment is not clear. A common test for SIBO assesses methane secretions.
- **Halophiles** are found in environments with a high salt count, typically 5x or more the salinity of the ocean. They have altered structures and functions in order to eliminate or tolerate salt.
- **Thermoacidophiles** can thrive in hot and acidic environments

Methane-producing archaea such as these inhabit human tissues.

Archaea also form biofilms

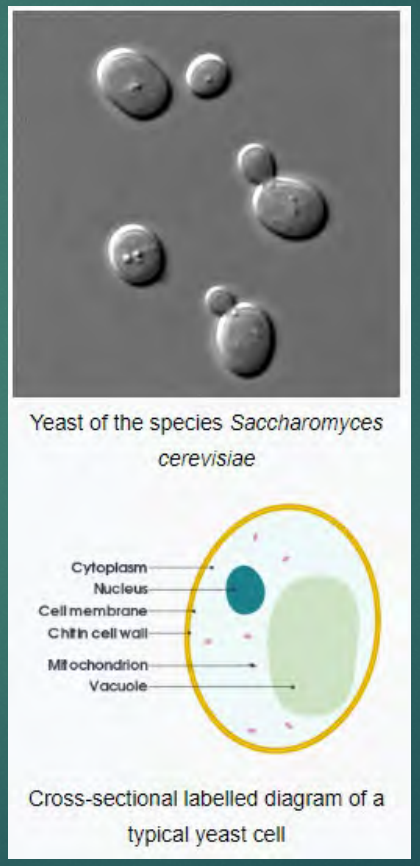


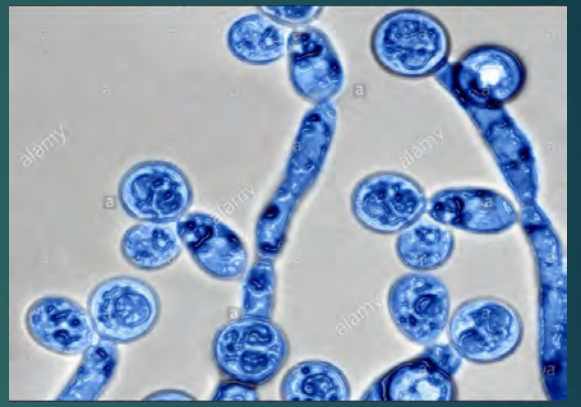
The Fungal Kingdom

EUKARYOTES SEPARATE FROM PLANT AND ANIMAL KINGDOMS

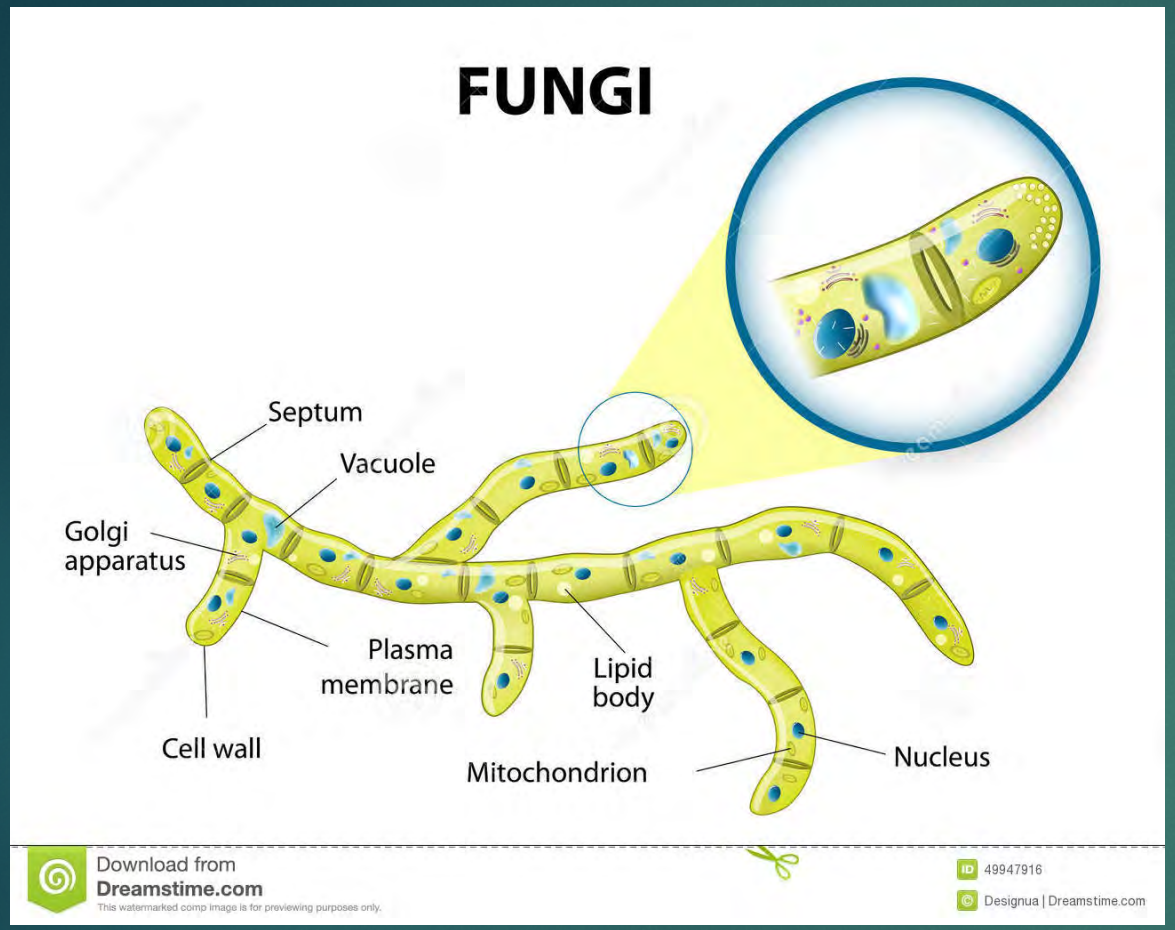
POSSESSING NO CHLOROPLASTS AND A CHITENOUS EXOSKELETON

- Include yeasts, molds, and mushrooms
- **Yeasts** are single celled organisms, but descended from multicellular organisms, and which may form strings of connected cells with the properties of multicellular organisms
- A mold grows in the form of multicellular filaments.
- A dimorphic fungus can exist in either mold or yeast form.





Candida albicans is dimorphic and can exist in single-cell or filamentous hyphal forms


FUNGI
Septum
Vacuole
Golgi apparatus
Plasma membrane
Lipid body
Cell wall
Mitochondrion
Nucleus
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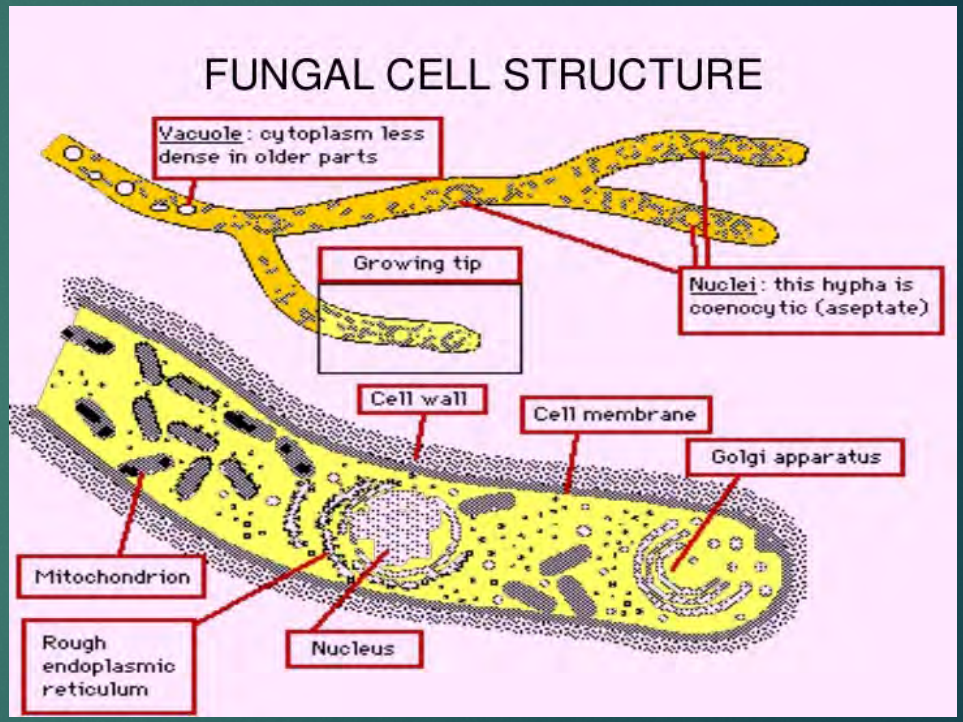
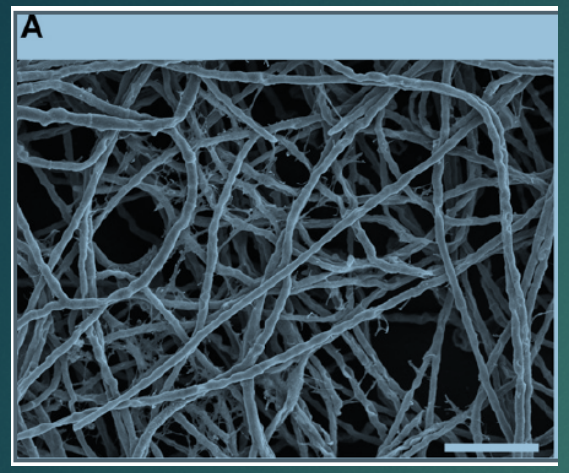
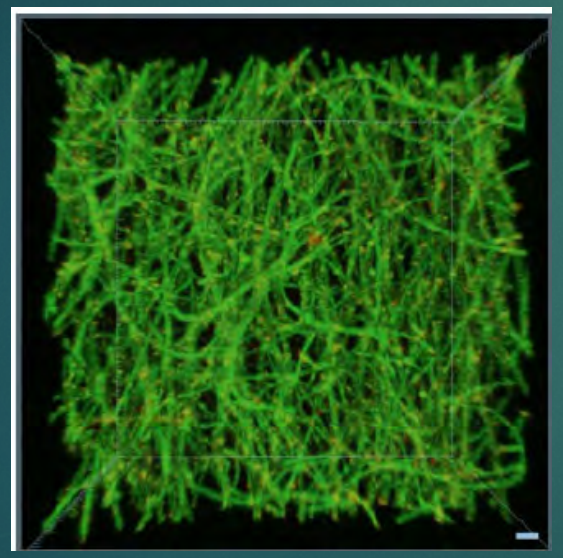
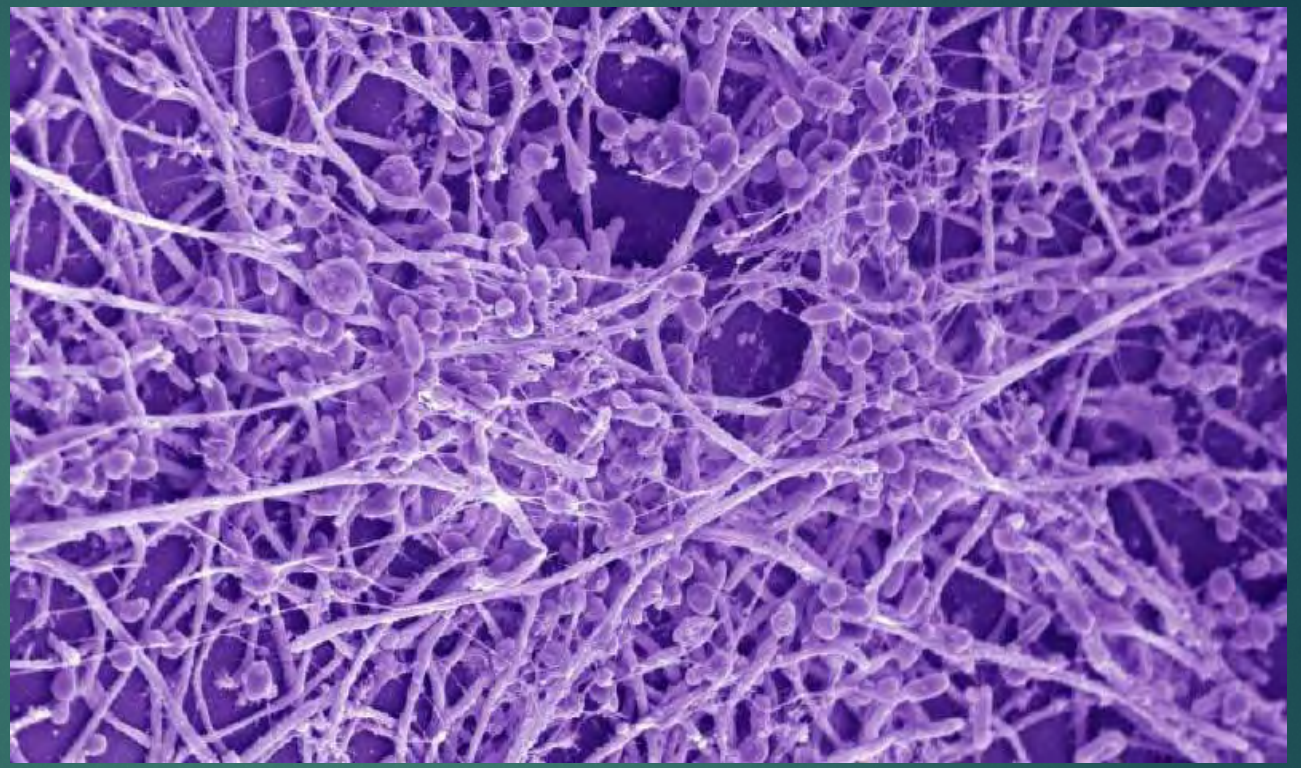

FUNGAL CELL STRUCTURE
Vacuole: cytoplasm less dense in older parts
Growing tip
Nuclei: this hypha is coenocytic (aseptate)
Cell wall
Cell membrane
Golgi apparatus
Mitochondrion
Rough endoplasmic reticulum
Nucleus



Aspergillus biofilm

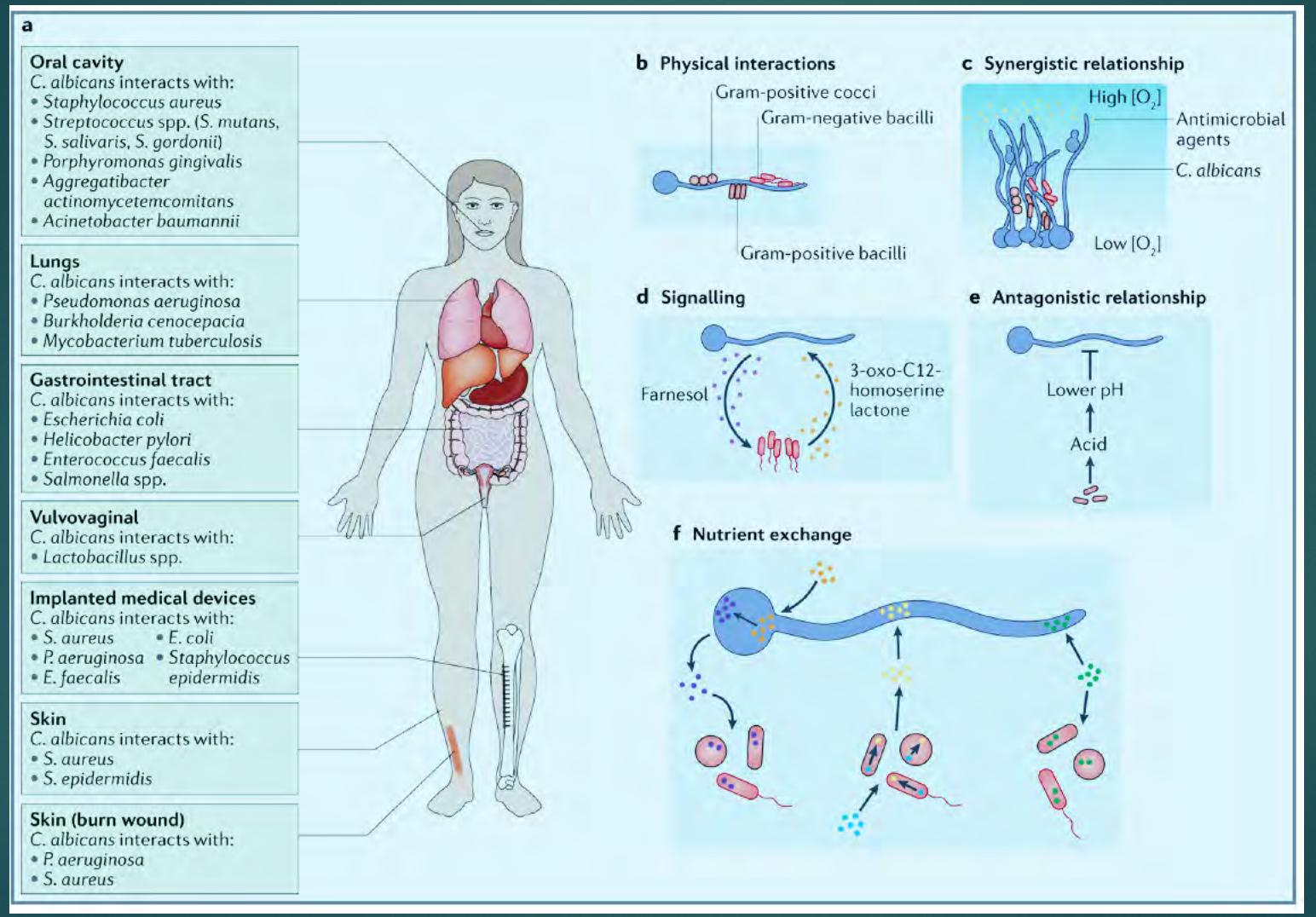


Candida biofilm



Candida in round and filamentous states

Candida multispecies biofilms



Clinical Preview

For disinfection of biofilm-based infections, include broad spectrum antiseptics which possess both antibacterial *and* antifungal properties, or add antifungal “specialists” to a formula. Always include botanicals with effects against both microbial types

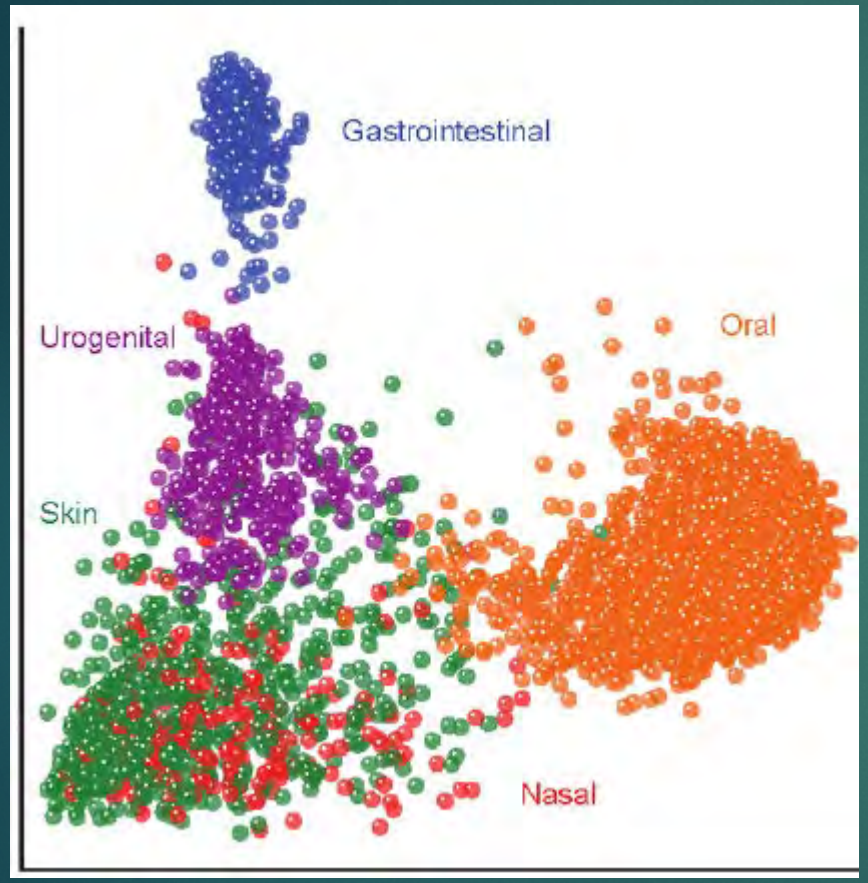
THE HUMAN MICROBIOME

Human Microbiota

The community of microbes residing in and on the human body, including

- Bacteria
- Archaea
- Viruses
- Microkaryotes (fungi, protozoa, other)

Human microbiome



- " . . . healthy individuals differ remarkably in the microbes that occupy habitats such as the gut, skin, and vagina."
- " . . . diversity and abundance of each habitat's signature microbes vary widely among healthy subjects."
- Strong niche specialization occurs both within and among individuals

Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012 Jun 13;486(7402):207-14.

Likewise, each body site contains a distinct microbiome

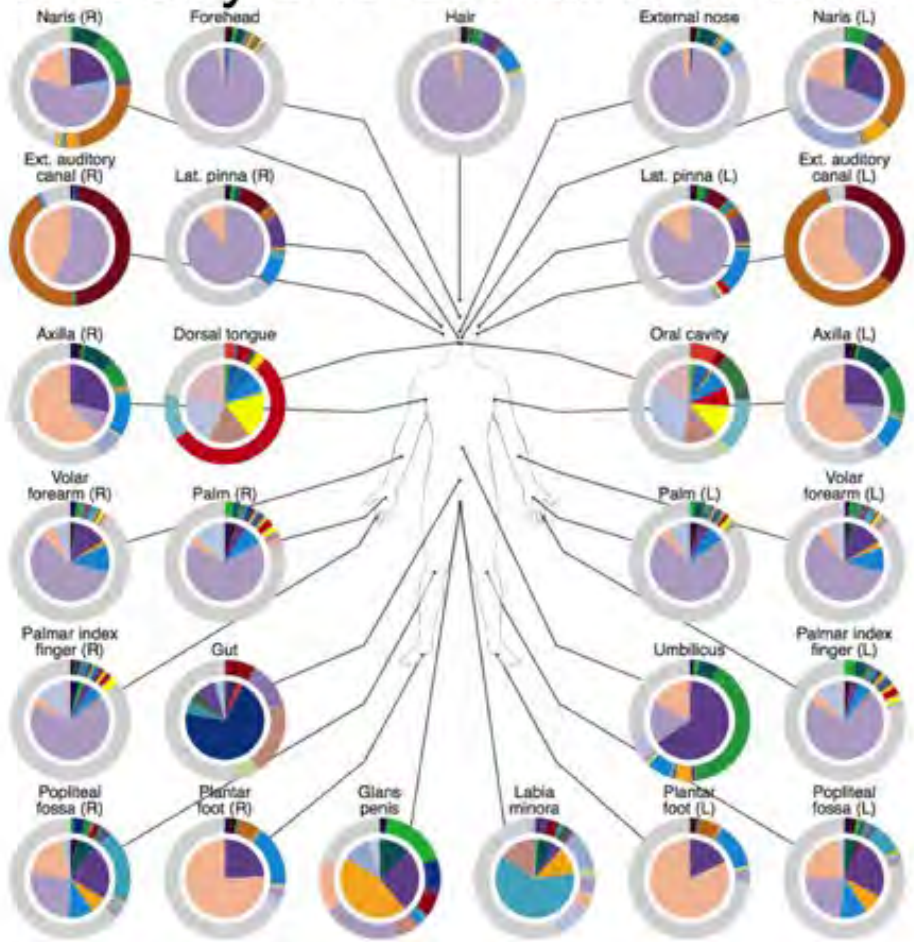
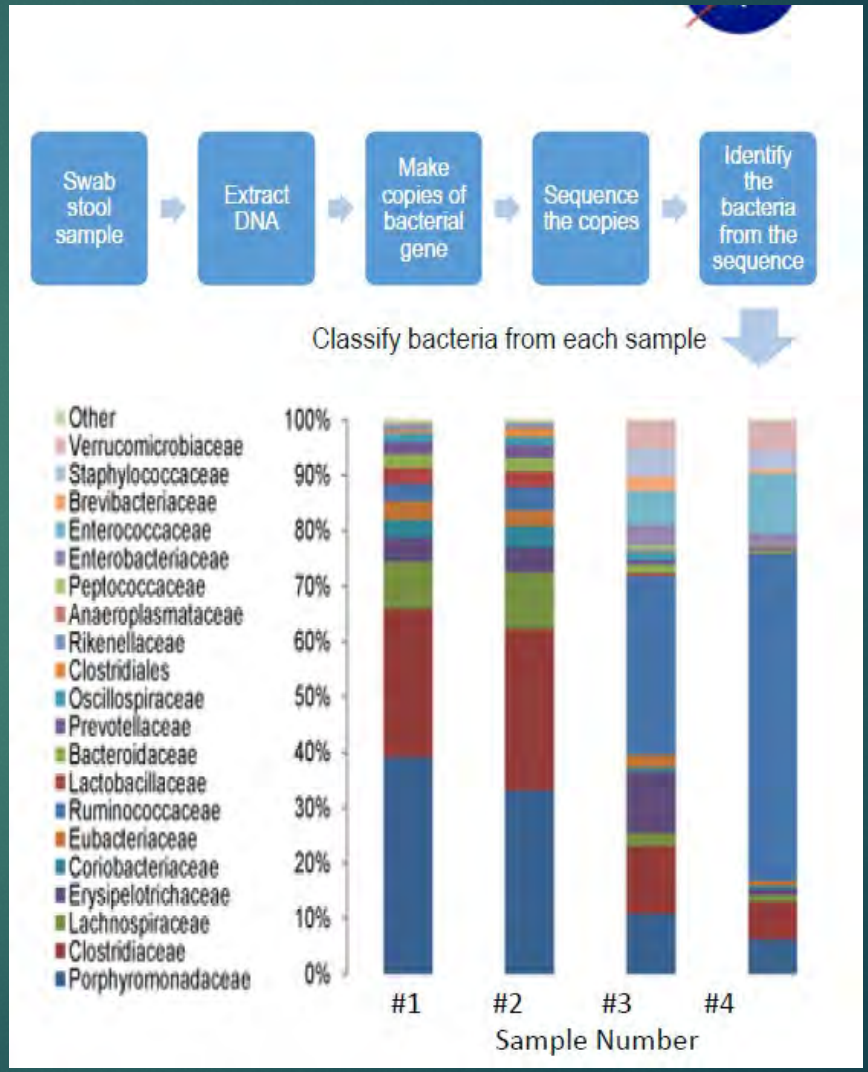
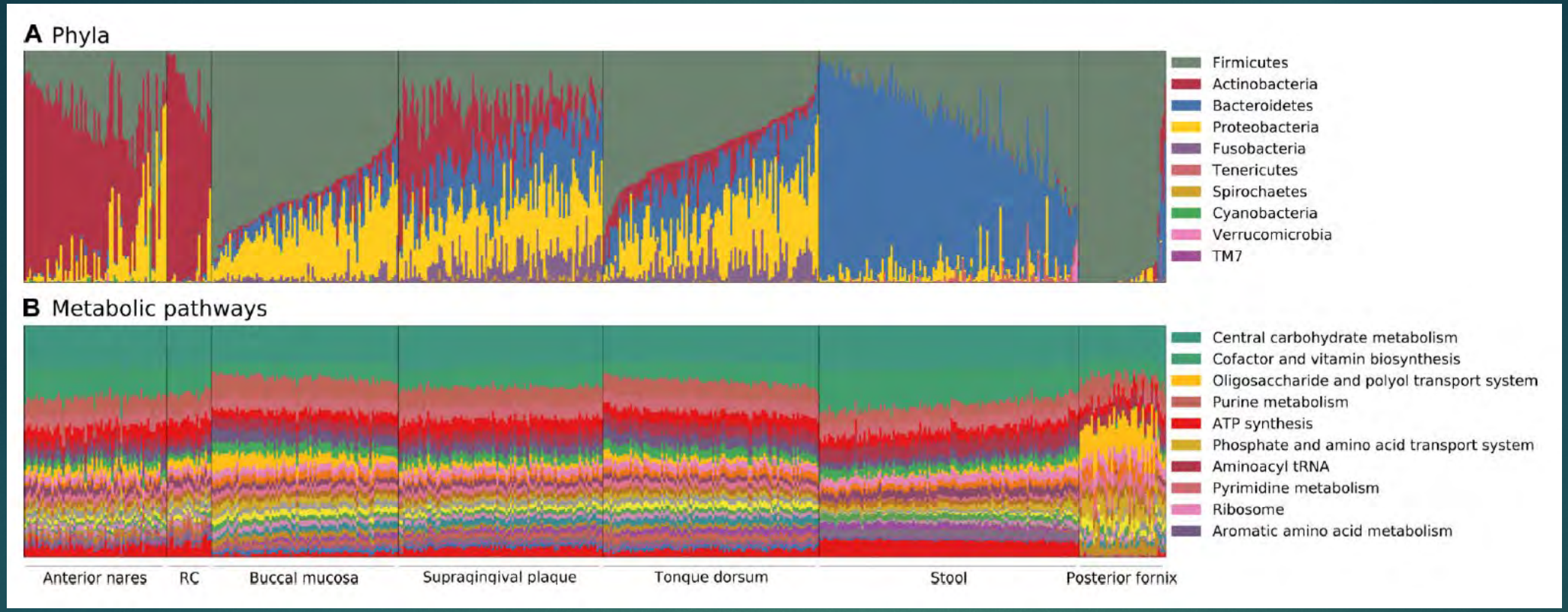


Figure adapted from Costello et. al (2009) *Supplementary materials*

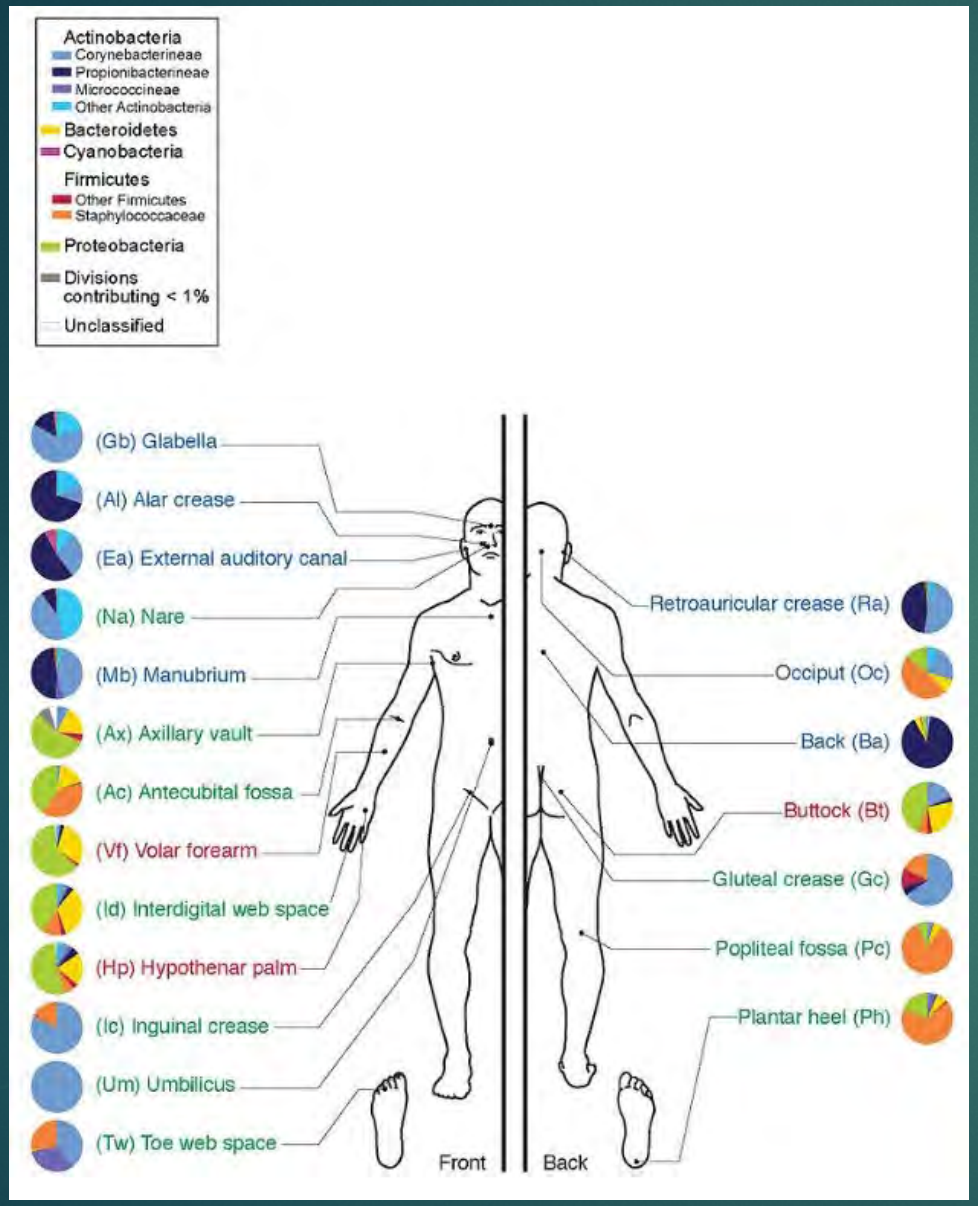
Diversity in the human gastrointestinal microbiota





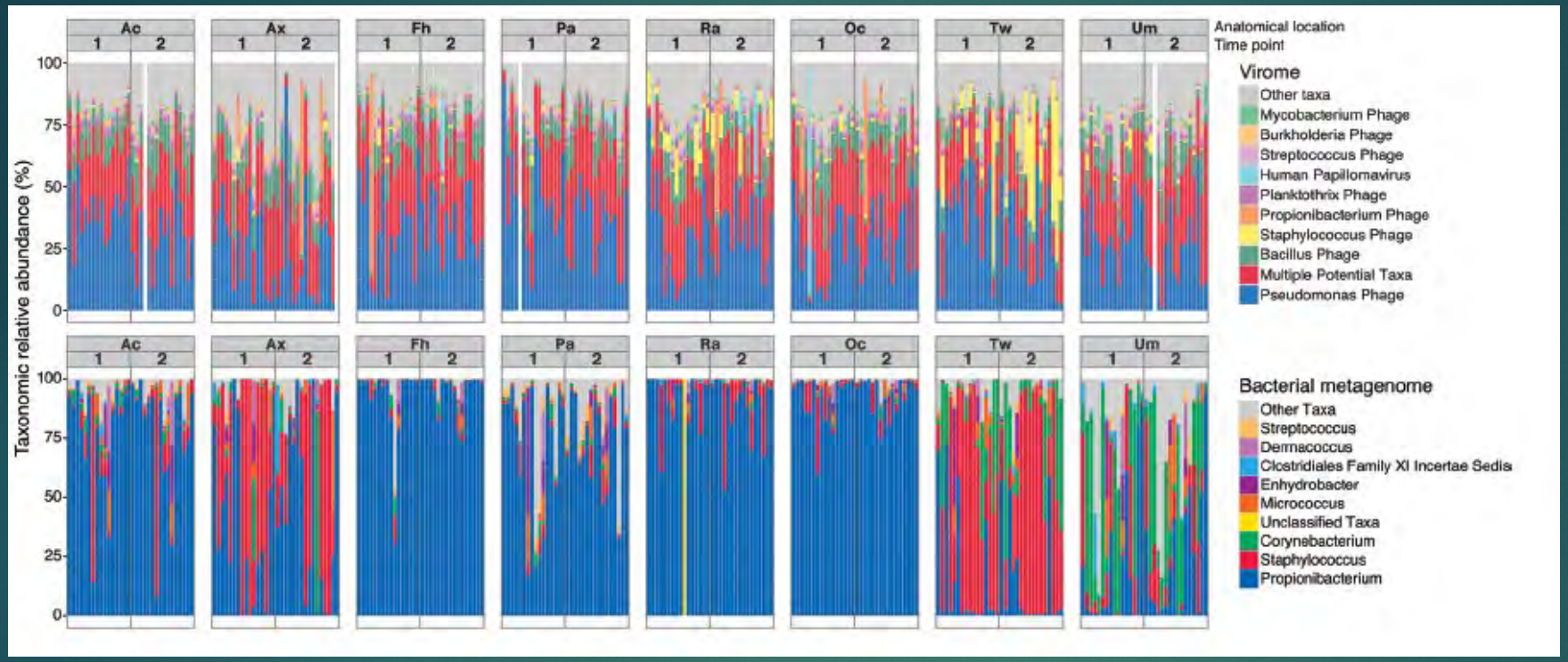
Top series shows dominant bacterial types in each of the 7 regions.
Bottom series shows functional bacterial metabolic pathways in each region.
Diverse microbiota may produce similar metabolic output

Skin Microbiome



- The skin microbiome varies widely between different areas.
- The microbiomes of different areas are more similar between individuals than with other areas in the same individual.

Skin Virome and Bacteriome



Hannigan GD, Meisel JS, Tyldsley AS, Zheng Q, Hodkinson BP, SanMiguel AJ, Minot S, Bushman FD, Grice EA. The human skin double-stranded DNA virome: topographical and temporal diversity, genetic enrichment, and dynamic associations with the host microbiome. MBio. 2015 Oct 20;6(5):e01578-15.

Urinary Microbiome

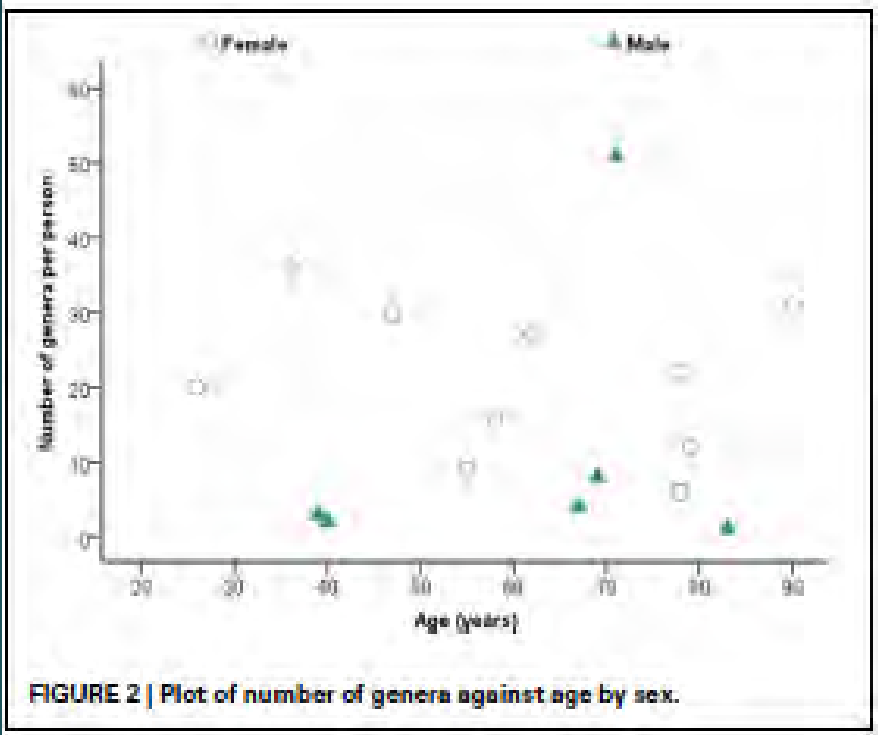


FIGURE 2 | Plot of number of genera against age by sex.

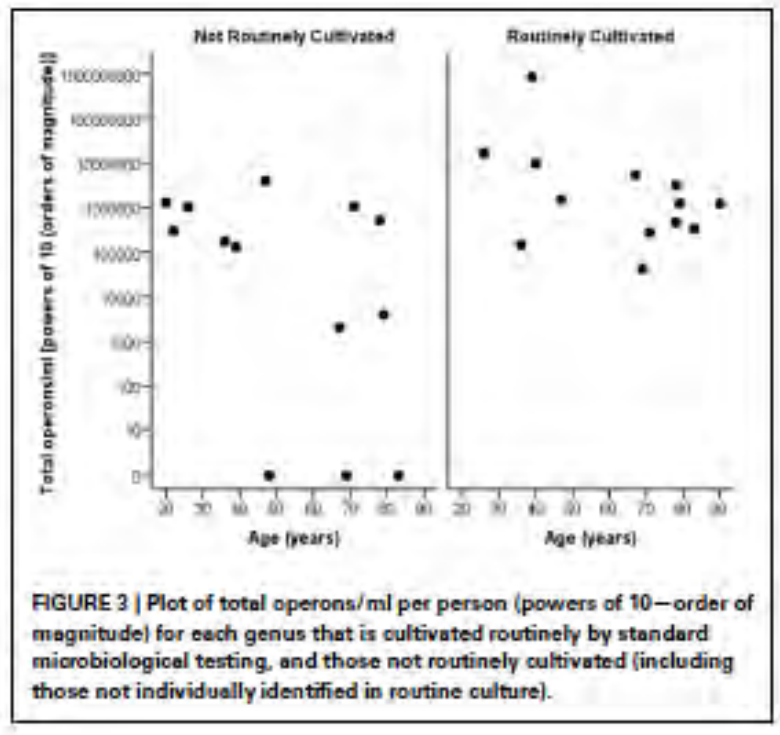
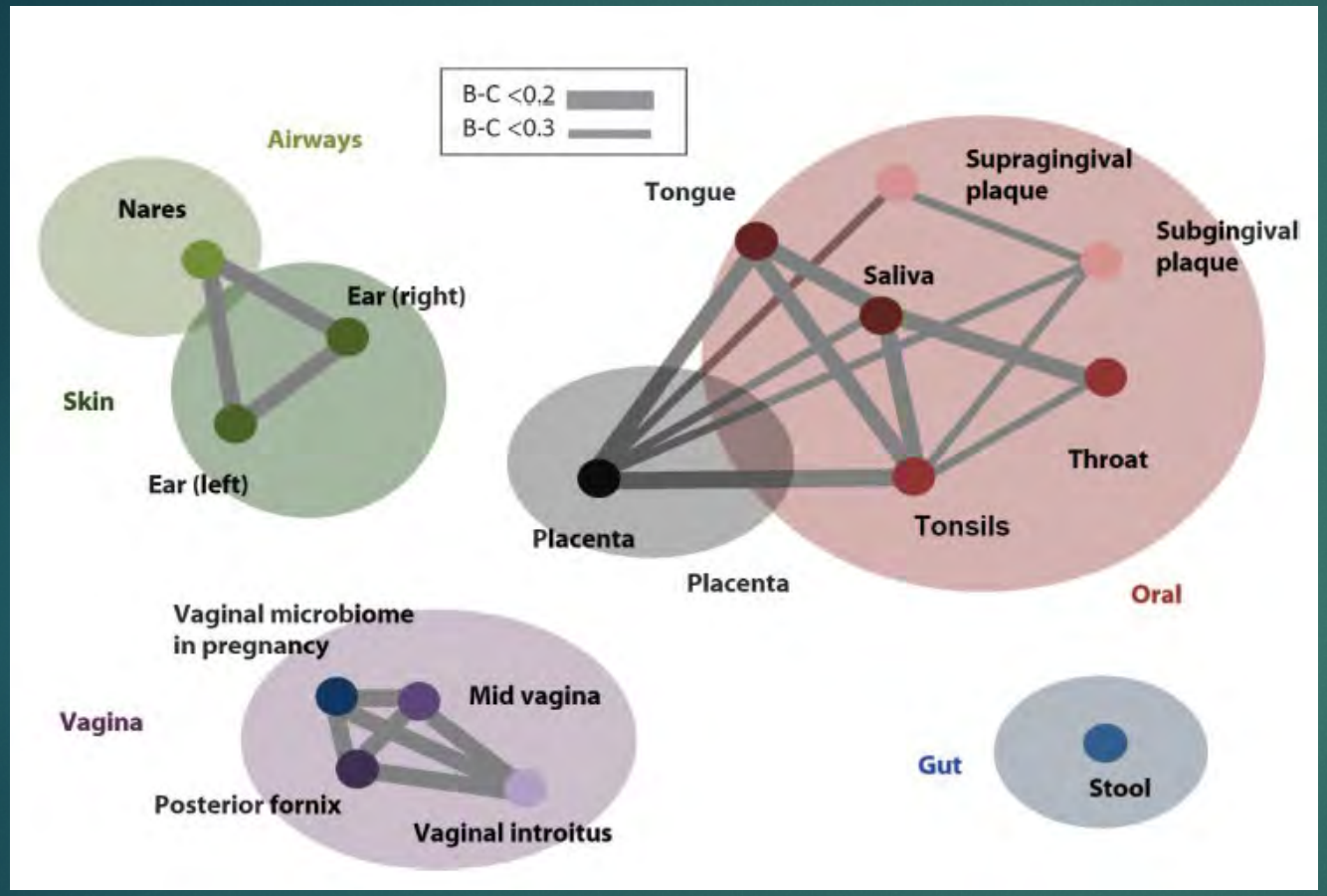


FIGURE 3 | Plot of total operons/ml per person (powers of 10—order of magnitude) for each genus that is cultivated routinely by standard microbiological testing, and those not routinely cultivated (including those not individually identified in routine culture).

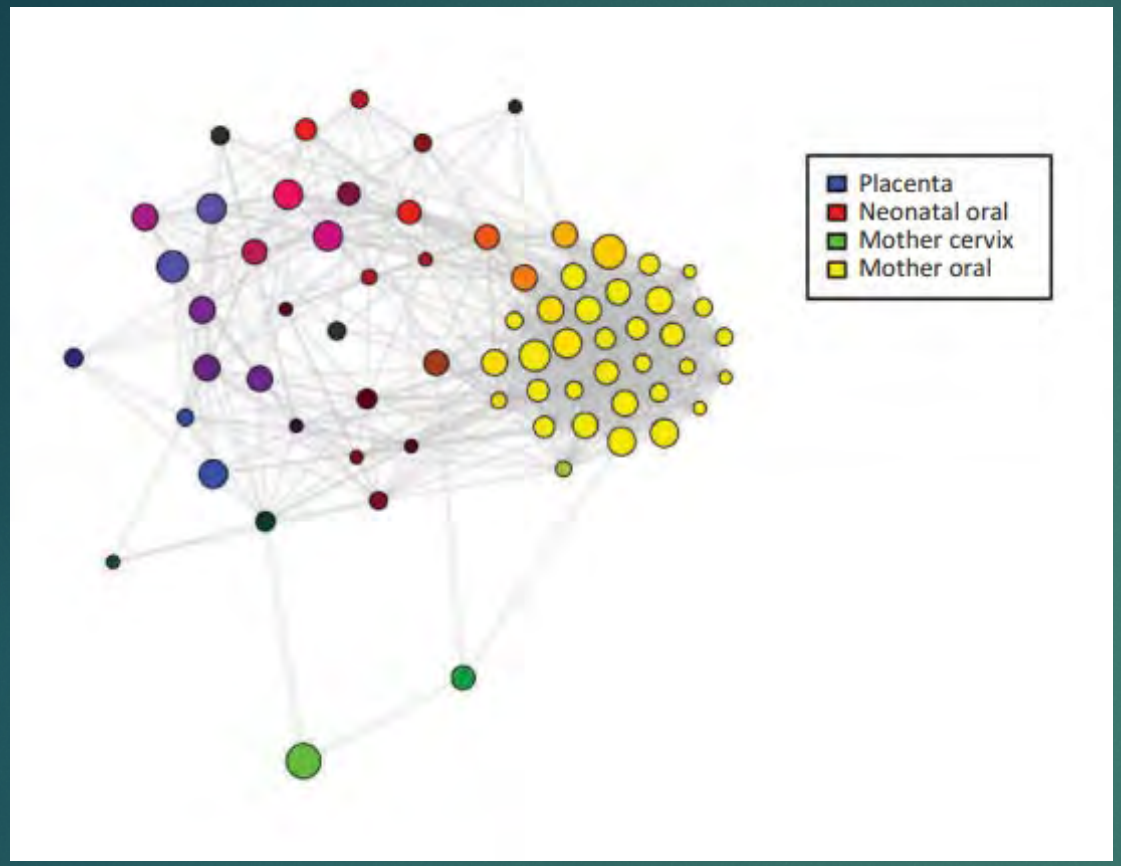
- Analysis by genetics rather than by culturing nearly doubles the known genera inhabiting the healthy male or female bladder.
- Female have much high numbers of bacterial genera.

Placental microbiome



- Taxonomic comparisons between regions of the body
- The thicker the connecting line, the greater the similarity of the taxonomic profile.
- Study has been criticized for possible contamination of samples with oral bacteria
- Implications for the theory of the sterile body

Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med. 2014 May 21;6(237):237ra65.

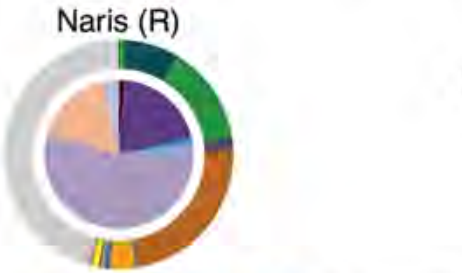


The oral microbiome of the neonate (swabbed at birth) (RED) has a close resemblance to the maternal oral (YELLOW) and placental (BLUE) microbiomes and little resemblance to the cervical microbiome (GREEN)

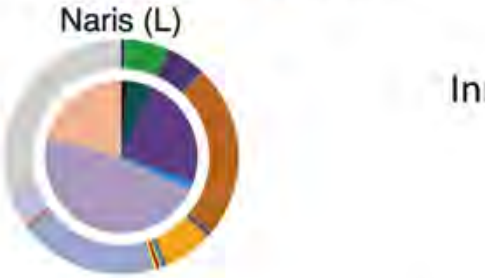
Tuominen H, Collado MC, Rautava J, Syrjänen S, Rautava S. Composition and maternal origin of the neonatal oral cavity microbiota. J Oral Microbiol. 2019 Sep 5;11(1):1663084.

Implications for antibiotic use during pregnancy, a known risk. Also oral health

Respiratory Microbiome - Nose



Staphylococcus



Despite the prescence of *Staphylococcus aureus*, infections don't occur

Innocuous bacteria keep infectious bacteria at bay

Figures adapted from Costello et. al (2009) *Supplementary materials*

In many conditions, establishment of colonies of pathogenic bacteria more likely to be due to ***disruption of the biofilms*** of the normal microbiome than to simple invasion and infection.

How much do we know?

- “The field is still young, and we have only a limited knowledge of the confounding factors, the natural variation in the healthy population (and also variation over time), the impact of low-abundance species that are difficult to detect . . . .”
- We indeed cannot be confident about the significance of the many observed differences between healthy and diseased individuals.
- The enthusiasm generated by early discoveries of correlations between elements of the microbiome and disease must be tempered by the sobering fact that correlation does not equate with causality, and the potential confounding factors across populations are many.
- See monthly ***Microbiome*** and ***Biofilms and Microbiomes*** open access journals

Blaser M, Bork P, Fraser C, Knight R, Wang J. The microbiome explored: recent insights and future challenges. Nat Rev Microbiol. 2013 Mar;11(3):213-7.

What we do know

- A healthy microbiota, in all regions of the body, depends on a broad ecological **diversity of species**.
- Local and gut microbiota produce systemic effects and in some instances these are critical to health.
- Once species diversity is lost, it does not recover spontaneously
- Minor species, by number, may play critical roles in systemic health
- Minor species can be extincted through antibiotic therapy.

Questions about infection in the era of the microbiome

- Could host conditions and microbial ecology and community structure be more important than the nature of the infectious agent?.
- Does a microbial-killing strategy resolve the infection?
- Can a microbial-killing strategy further damage microbial ecology?
- What factors can restore a damaged ecology?

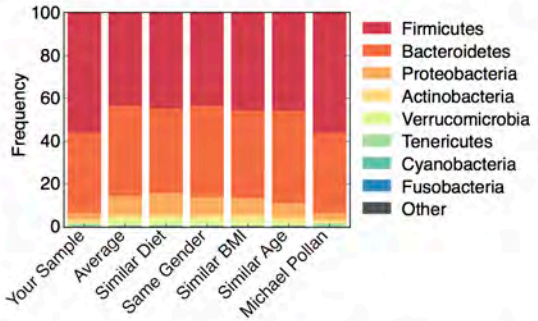
We should use great caution in viewing biofilms as “the enemy”
In the same manner that we have viewed microbes as “the enemy.”

MICROBIOME OF THE GUT

YOUR AMERICAN GUT SAMPLE

MICHAEL POLLAN

What's in your American Gut sample?



Your most abundant microbes:

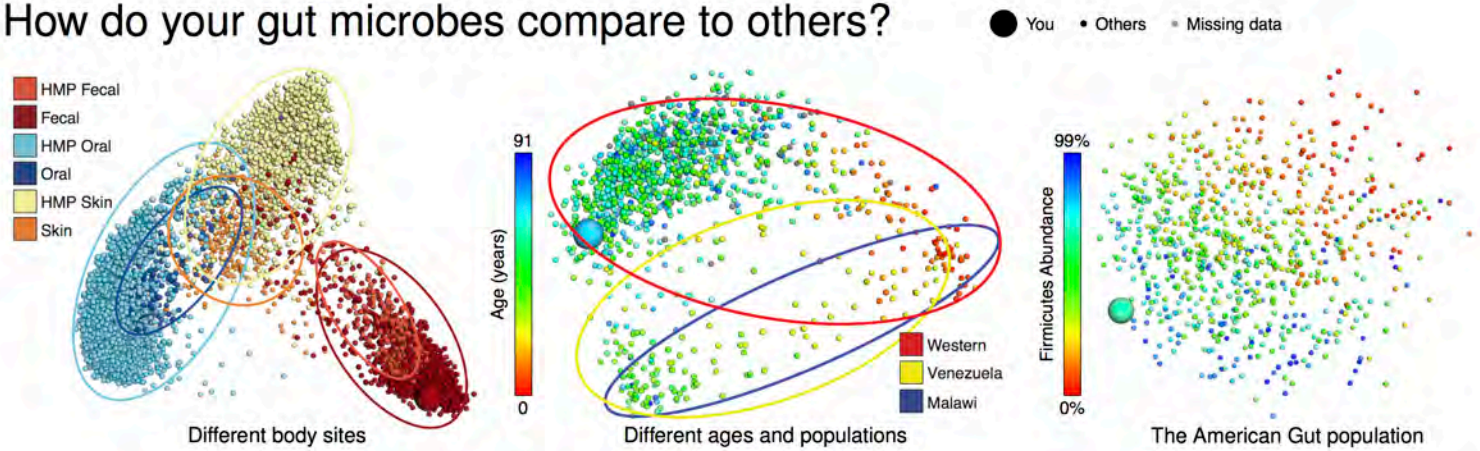
Taxonomy	Sample
Genus *Prevotella*	24.9%
Family Ruminococcaceae	13.4%
Family Lachnospiraceae	10.1%
Genus *Bacteroides*	10.0%

Your most enriched microbes:

Taxonomy	Sample	Population	Fold
Genus *Clostridium*	2.5%	0.3%	7x
Genus *Finegoldia*	0.7%	0.0%	17x
Genus *Prevotella*	24.9%	2.6%	9x
Genus *Collinsella*	0.9%	0.1%	8x

This sample included the follow rare taxa: Genus *Varibaculum*, Genus *Neisseria*, Genus *Campylobacter*, Order ML615J-28

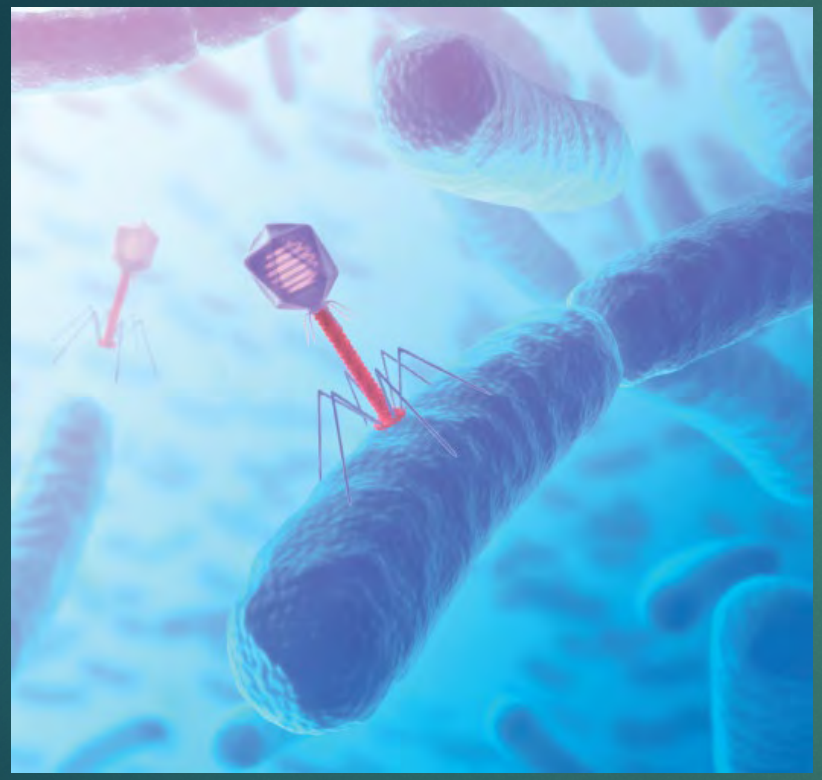
How do your gut microbes compare to others?



Sampling the gut microbiota

- It is difficult to say positively the exact composition of the human gut microbiota.
- Sampling from one area of the gut may not detect species or diversity from another area.
- A small sample may not be characteristic of the whole. (think sampling the Brazilian Rainforest for plant life by taking a house sized chunk of it. Or football sized chunk. Or a square mile.
- Species which may be physiologically significant but occur as a small percentage may not be detected at all.
- Consider the significance of this when the next social media meme appears stating positive claims for microbiome effects.

Human gut virome

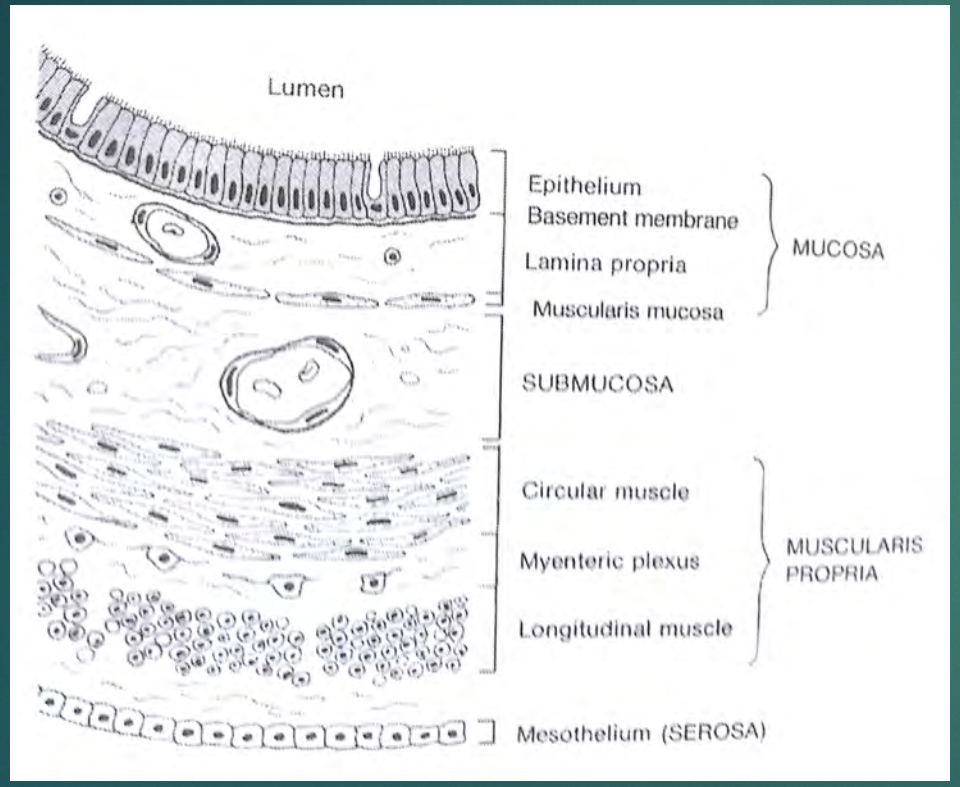


- Humans are colonized by immense populations of viruses, which metagenomic analysis shows are mostly unique to each individual.
- “This study did not yield any clear examples of known DNA viruses infecting animal cells.”
- Most of the human virome are bacteriophages, and may serve as important controls on the bacterial population

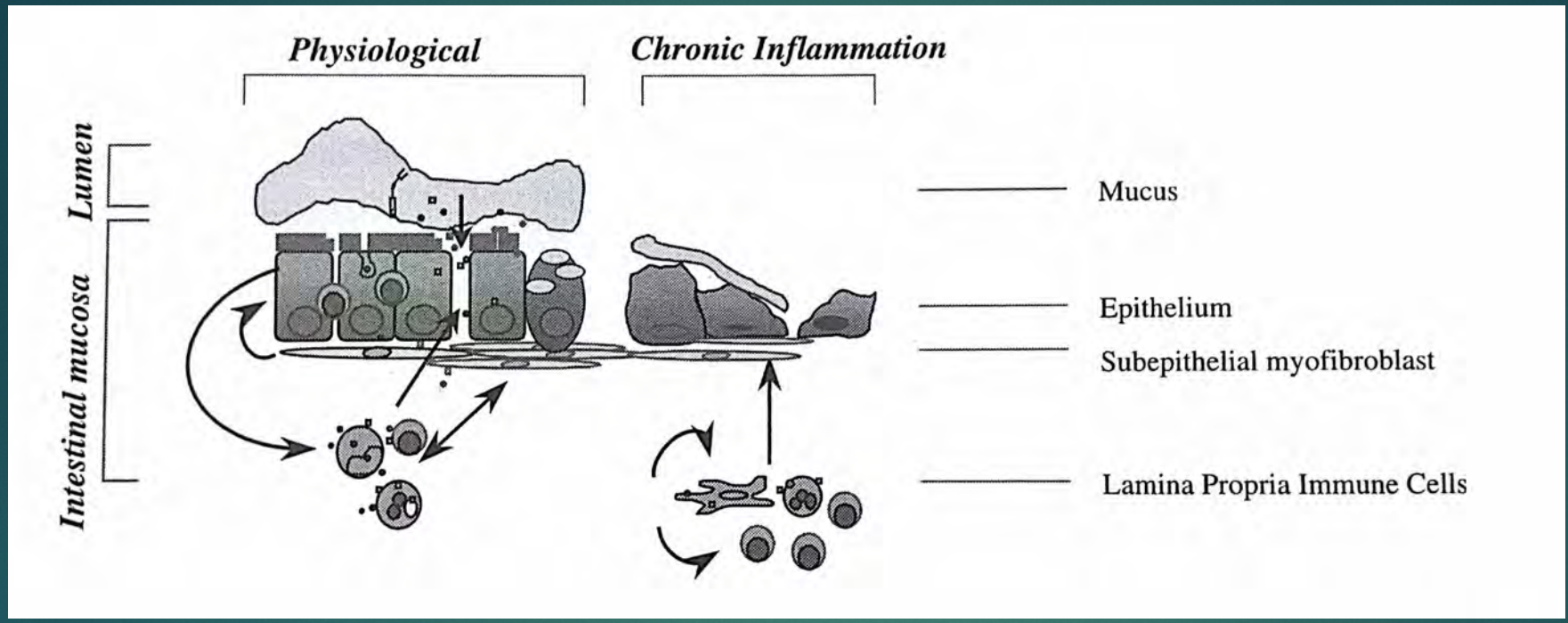
Minot S, Bryson A, Chehoud C, Wu GD, Lewis JD, Bushman FD. Rapid evolution of the human gut virome. Proc Natl Acad Sci U S A. 2013 Jul 23;110(30):12450-5.

Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, Lewis JD, Bushman FD. The human gut virome: inter-individual variation and dynamic response to diet. Genome Res. 2011 Oct;21(10):1616-25.

Microbiome as autonomous organ



- The microbiome is surrounded by the lining of the gut.
- Its secretions help to trigger and maintain tight junctions between the cells and surface immune activation
- Immune cells in and below the gut lining trigger and regulate systemic immunity.
- The cells lining the tract are dependent on the microbiome for nutrition.



Myofibrocytes in basement membrane below enterocytes are antigen-presenting and cytokine-secreting cells.

Chesney J, Bacher M, Bender A, Bucala R. The peripheral blood fibrocyte is a potent antigen-presenting cell capable of priming naive T cells in situ. Proc Natl Acad Sci U S A. 1997 Jun 10;94(12):6307-12.

Gut lumen and microbiome

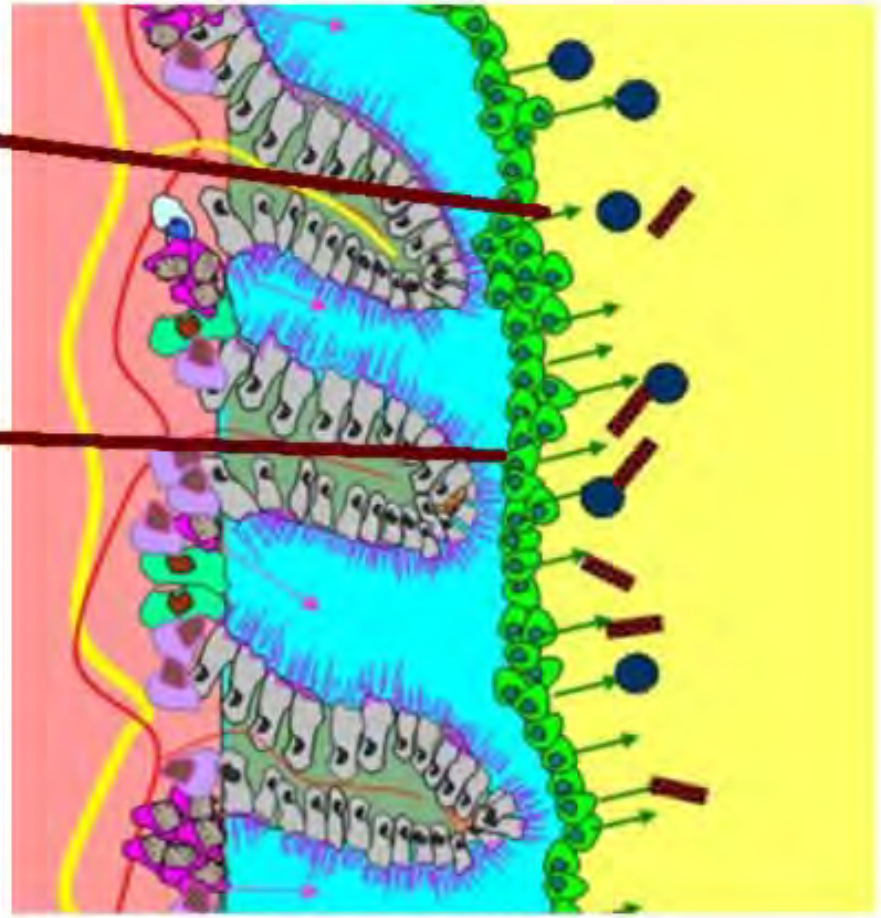
- The lumen contains a robust and diverse bacterial civilization, and a bacterial biofilms also line the mucin layer.
- The bacterial colony collectively metabolizes fiber, starch, pectin, inulin, and cellulose to short-chain fatty acids which are required by the enterocytes for ***fuel*** and ***immune signaling*****.** They have local and **systemic *anti-inflammatory*** properties, and also affect insulin metabolism and brain function.
- *Acidophilus* and *Bifidobacterium* species (and other) are ***adherent*** friendly bacteria and in biofilm contribute a layer of protection to the gut, and also produce SCFA as above.

Probiotic Benefits

ACIDOPHILUS AND OTHER PROBIOTIC BACTERIA SECRETE: ANTIVIRAL ANTIBACTERIAL AND ANTIFUNGAL CHEMICALS.

PROBIOTICS FORM A PHYSICAL BARRIER TO HINDER INVASION OF BACTERIA AND YEASTS

PROBIOTICS LIKE ACIDOPHILUS CREATE AN ACIDIC MICROENVIRONMENT WHICH PROMOTES IRON AND OTHER MINERAL ABSORBTION.

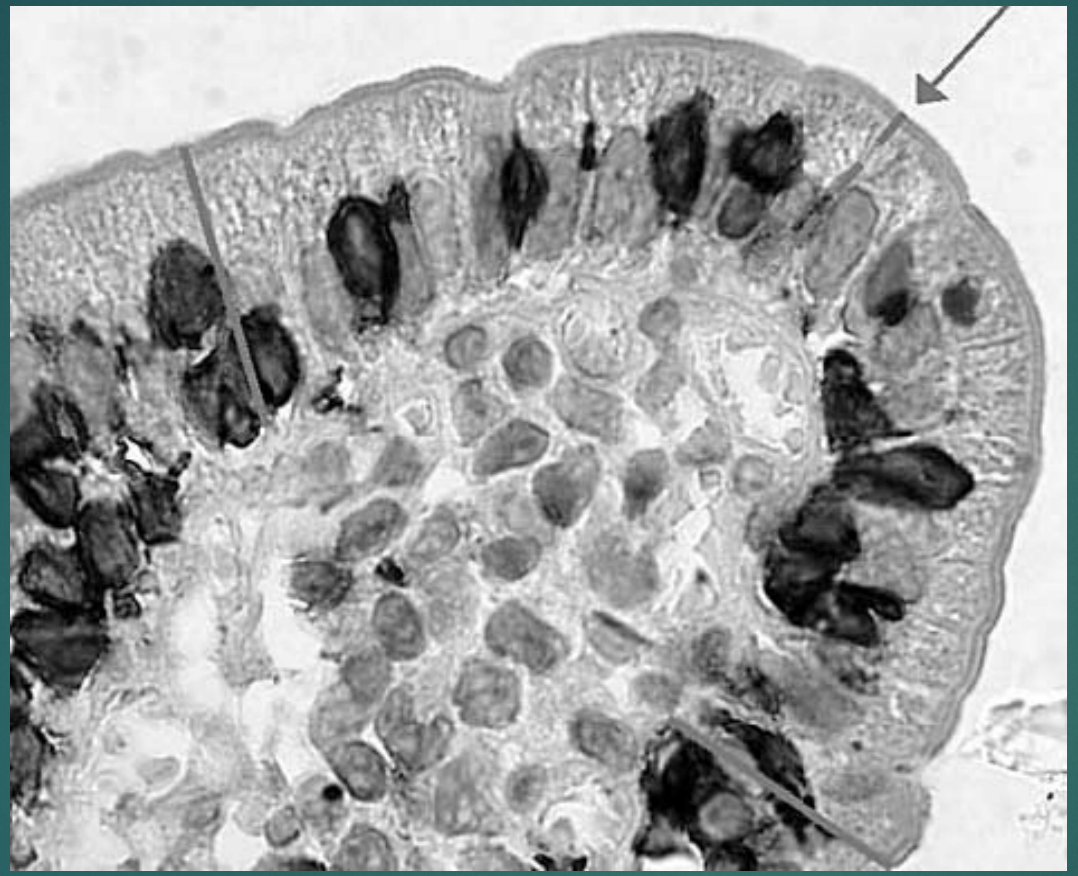


Microbiome as a metabolic organ

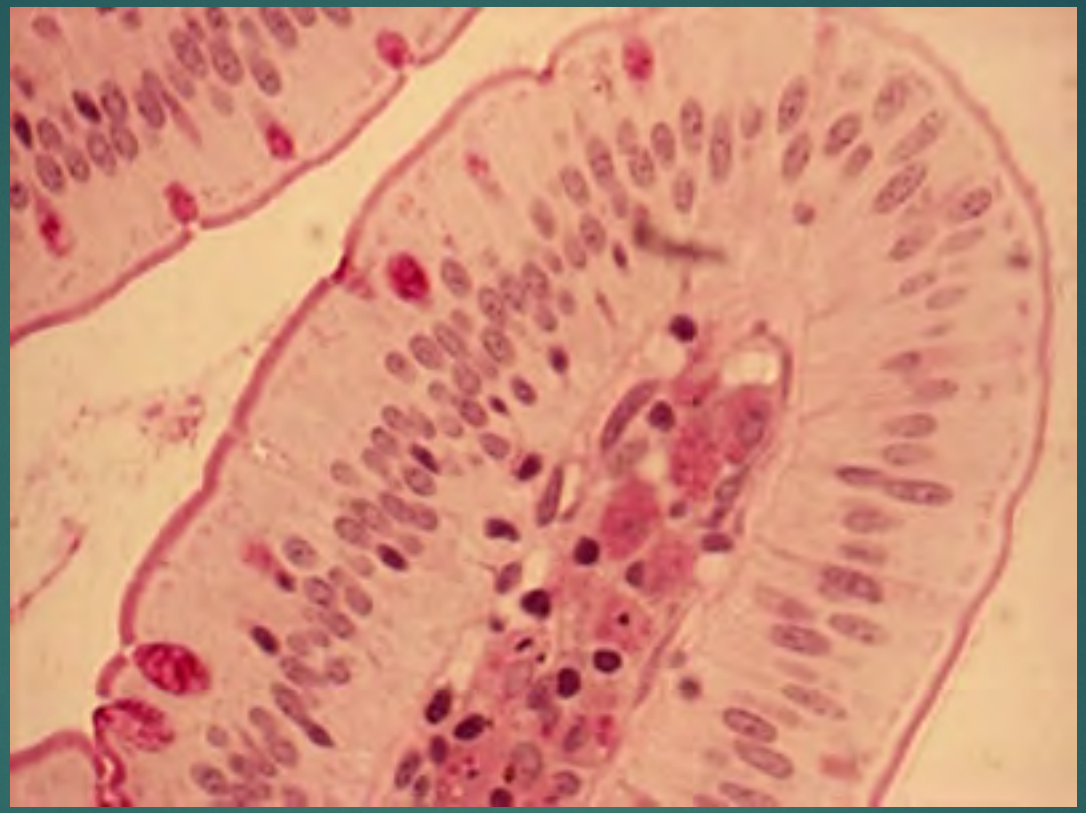
- Microorganisms of the gut microbiome process portions of both digestible and indigestible foods and metabolize them to produce ***short-chain fatty acids.***
- Butyrate, acetate, and propionate.
- SCFA provide essential calories (10% of total mitochondrial fuel)
- SCFA promote tight junction in the gut, and surface immunity.
- SCFA have systemic metabolic effects, and promote insulin sensitivity.
- SCFA have systemic anti-inflammatory effects
- Metabolic byproducts of the microbiome are essential immune and other effects on the brain

Microbiome as an immune organ

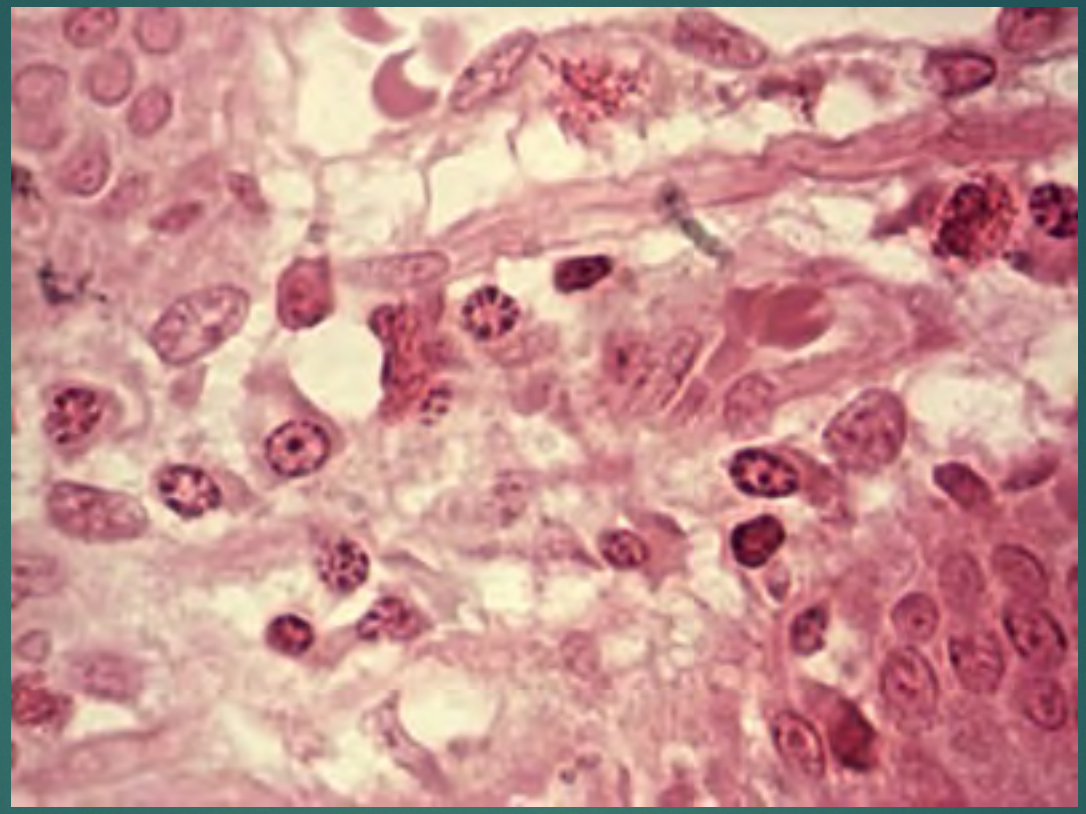
- Some microbiome organisms act to stimulate immune T-regulatory cells, which can affect reactivity to antigens and allergens throughout the system. Most of these effects are a ***Great Unknown***.
- A loss of microbiome diversity in the gut results in lowered reactivity to immune challenges throughout the system, through unknown mechanisms.
- Immune cells in the gut lining form antibodies to members of the microbiome as well as to food and other antigens, and send antibody producing cells and cytokines throughout the system.



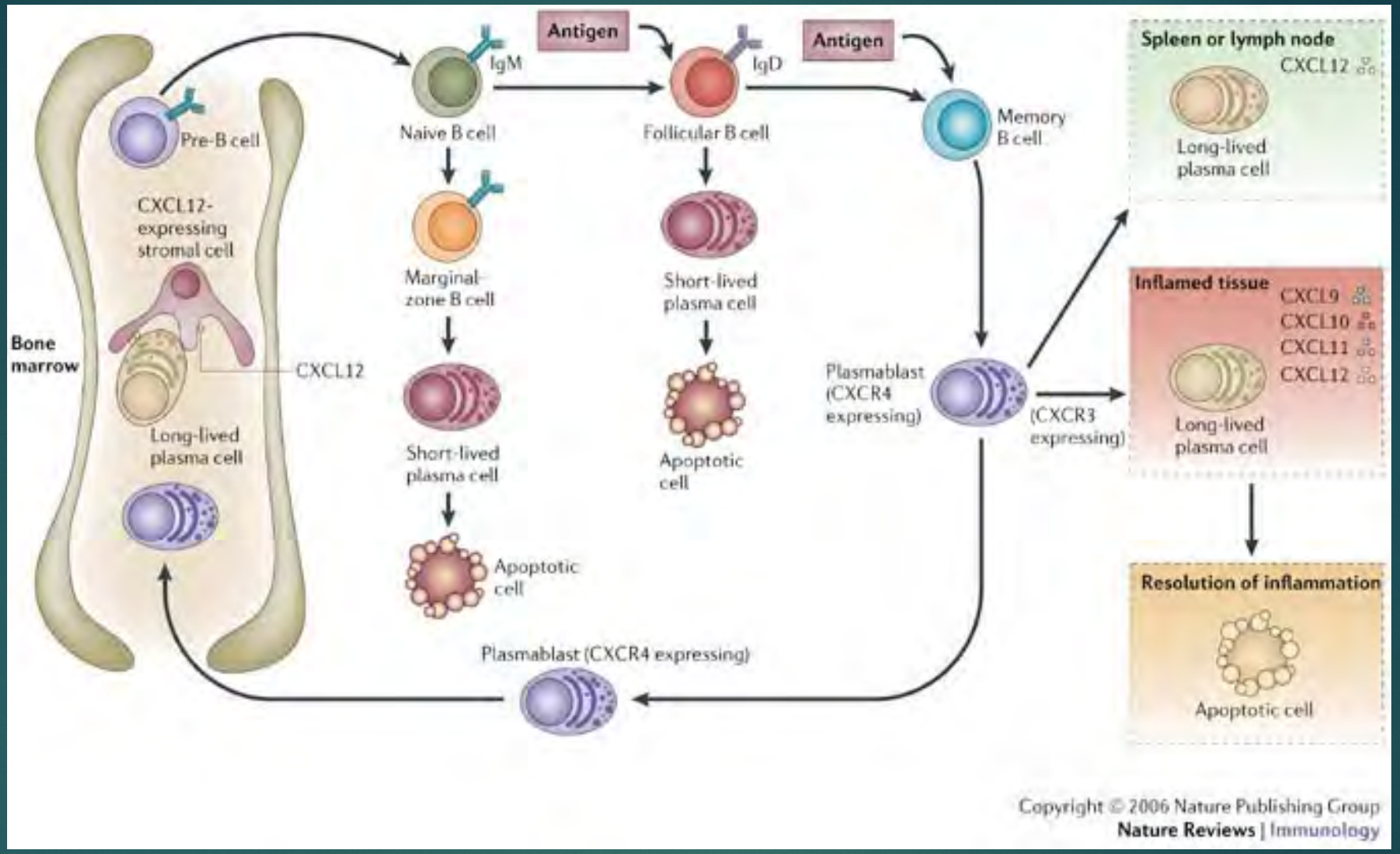
Lymphocytes in villus tip are stained in black



Macrophages in villus are stained pink

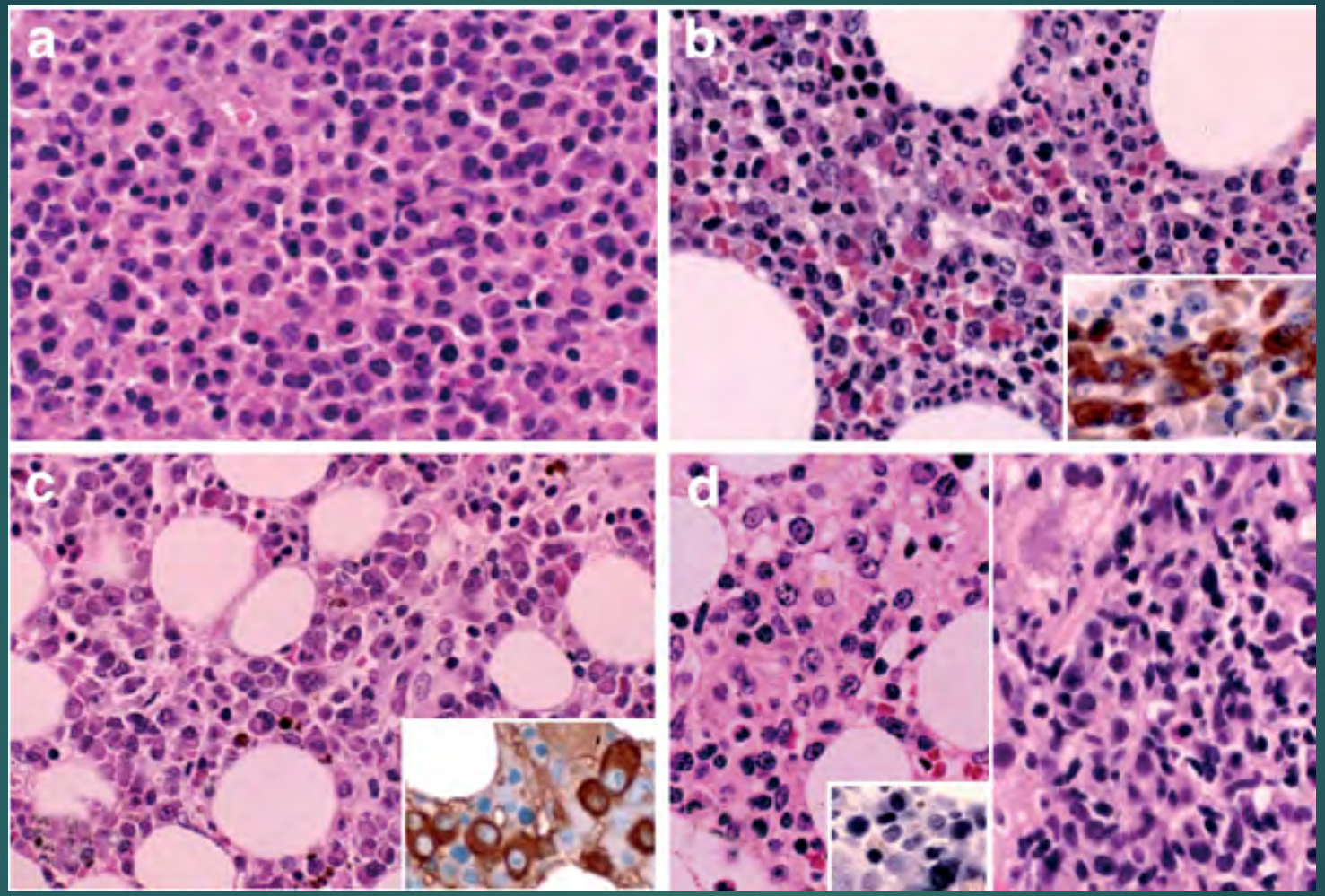


Plasma cells in villus stained dark pink



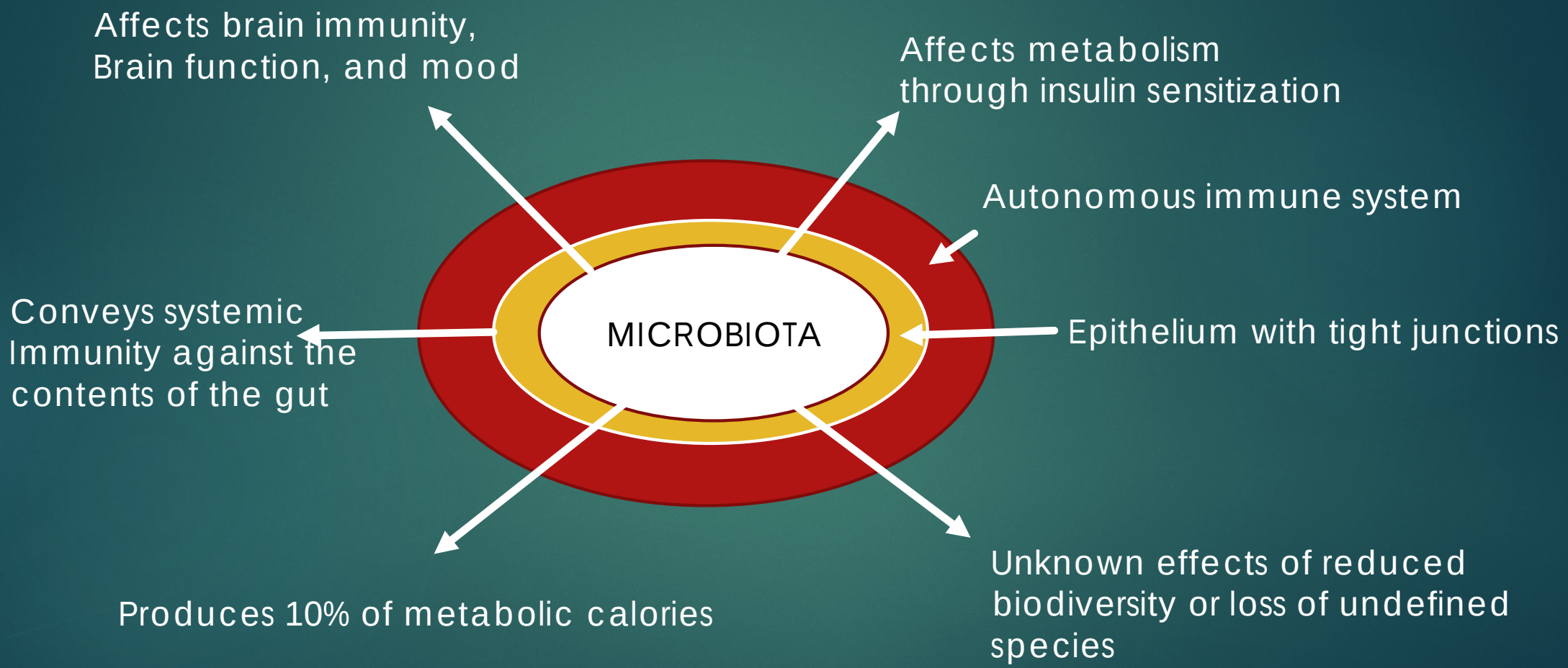
Plasma cells migrate to bone marrow, spleen or nodes.
They may be attracted to and sequestered in any area of inflammation

Stained plasma cells in bone marrow tissue



The bone-marrow plasma cells secrete antibodies to circulate throughout the system

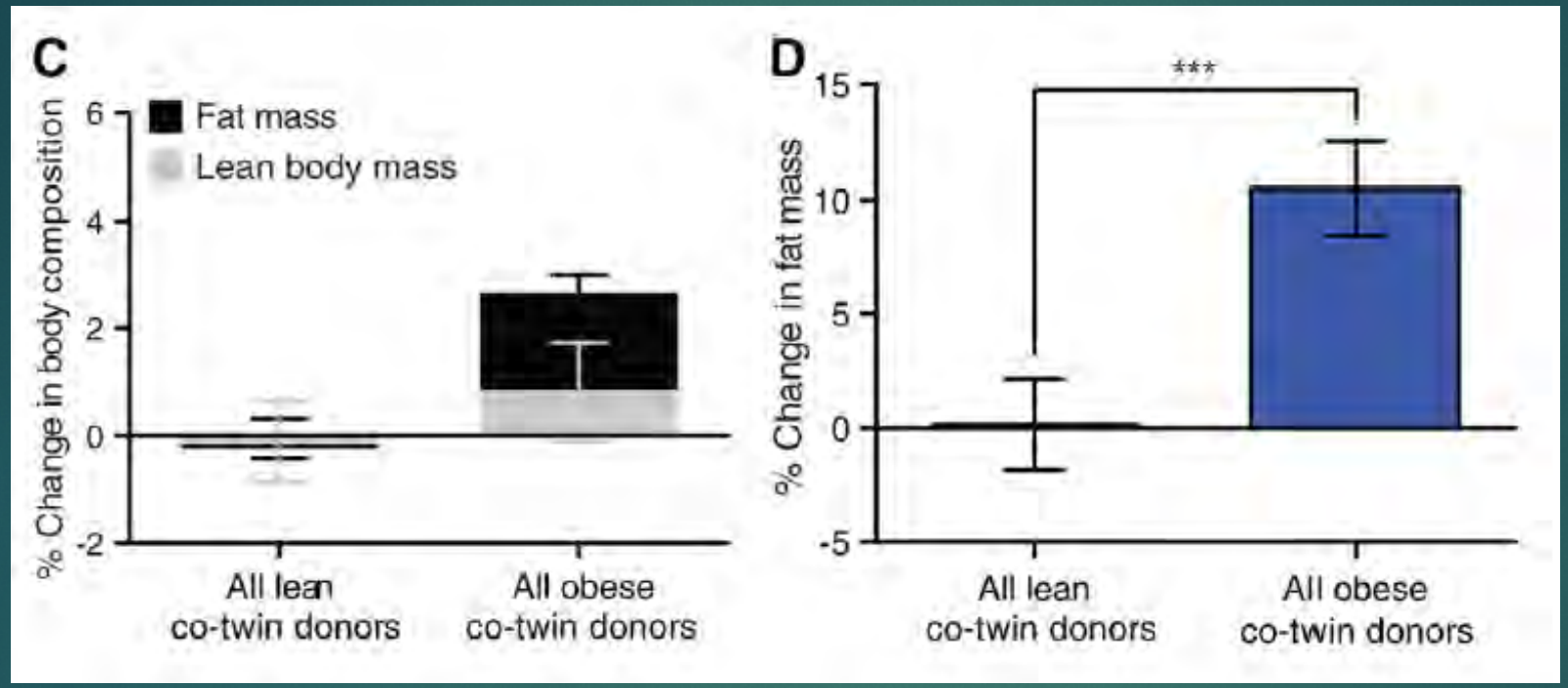
Gut Microbiota as an autonomous organ



Microbiome as organ of the endocrine, immune, and neurological systems.

Microbiome and obesity

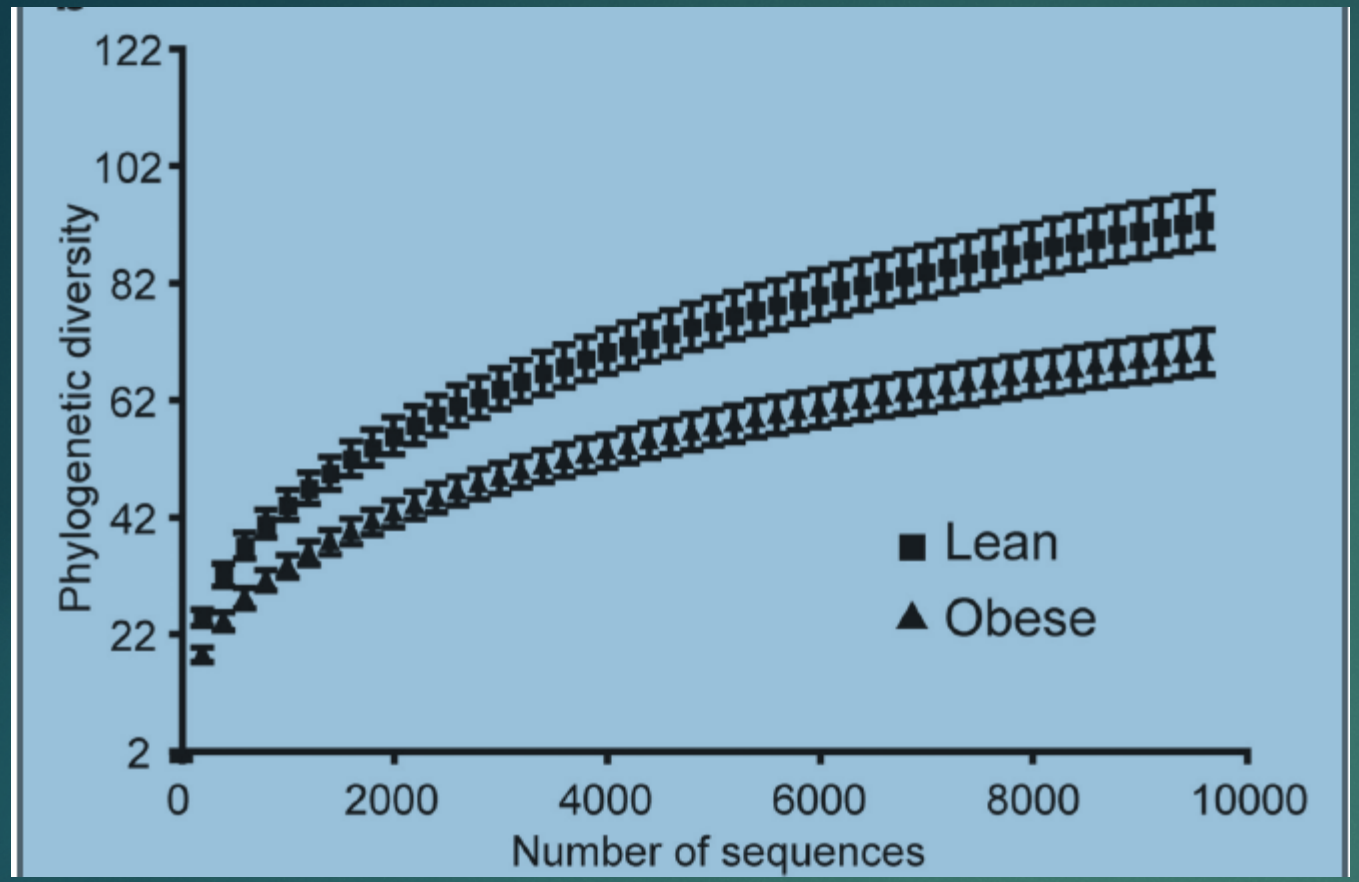
- Four pairs of Human identical twins discordant for obesity
- Fecal sample implanted into guts of germ-free mice
- After colonization, microbiota matched donor profiles
- The mice developed the obesity profile of their donors
- Protection against weight gain appeared to be associated with the large category of Bacteroidetes and with the production of short chain fatty acids
- Co-housing coprophagic lean mice with mice given the obese microbiota protected against weight gain.



Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013 Sep 6;341(6150):1241214.

Twin Study

- Comparison of microbiota and its metabolic functions in mothers and identical and fraternal twins
- Assessed Phyla were widely variable, but genes and metabolic functions similar despites phyla differences.
- ***Reduced diversity*** of gut bacteria in obese individuals.
- A lower proportion of Bacteroidetes and a higher proportion of Actinobacteria in obese versus lean individuals
- Specific diets may correct or improve the imbalance, specific nutrients are meat protein, high fiber, and fruits and vegetables



Turnbaugh, P. J., Hamady, M., Yatsunenko, T., Cantarel, B. L., Duncan, A., Ley, R. E., … Gordon, J. I. (2008). *A core gut microbiome in obese and lean twins. Nature, 457(7228), 480–484.*

Metabolic syndrome

- Individuals with Metabolic Syndrome have an increased Firmicutes/Bacteriodetes ratio and a reduced capacity to degrade carbohydrates to short-chain fatty acids.
- Firmicutes favored by low fiber carbohydrates
- Bacteroidetes favored by meat, fiber, fruits and vegetables
- Prebiotics or probiotics can increase short-chain fatty acid production.

Santos-Marcos JA, Perez-Jimenez F, Camargo A. The role of diet and intestinal microbiota in the development of metabolic syndrome. J Nutr Biochem. 2019 Aug;70:1-27.

Fecal Implantation in obese humans

- Review of 3 clinical trials
- Results were equivocal between the trials for improvements in insulin sensitivity or blood sugar control, some successful some not
- None of the trials showed that the obese patients lost weight.

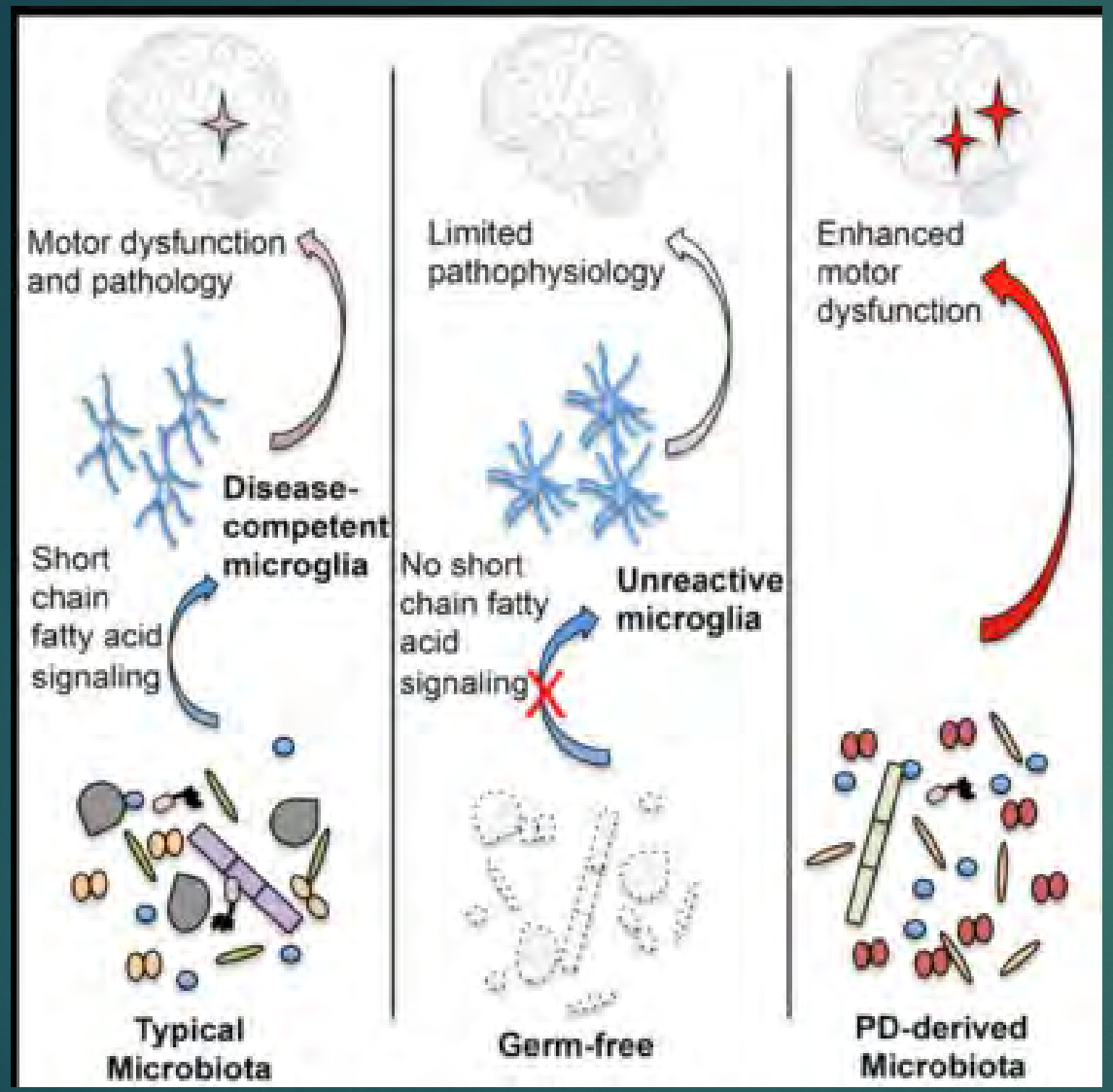
Zhang, Mocanu, Cai, Dang, Slater, Deehan, … Madsen. (2019). *Impact of Fecal Microbiota Transplantation on Obesity and Metabolic Syndrome—A Systematic Review. Nutrients, 11(10), 2291.*

Allegretti, J. R., Kassam, Z., Mullish, B. H., Chiang, A., Carrellas, M., Hurtado, J. Thompson, C. (2019). *Effects of Fecal Microbiota Transplantation With Oral Capsules in Obese Patients. Clinical Gastroenterology and Hepatology.*

Parkinson’s Disease

Discovery of gut-brain link

- Genetically bred mice with Parkinson's disease
- When the mouse microbiota was removed to make gut-sterile mice, their PD was in remission.
- When short-chain fatty acids, a normal metabolic by product of the microbiome, was given, the PD symptoms returned
- When human microbiota from humans with PD was given, the symptoms returned
- When human microbiota from healthy humans without PD was given, no symptoms appeared



Short-chain fatty-acids: acetate, butyrate, propionate

Total SCFA or dominance or deficiency of one or more may be involved.

Does not implicate the microbiome as the *root cause* of PD, only that the immune cells in the brain are activated by SCFA and dysbiosis can increase the activation of pre-existing inflammatory cells.

Quantitative analysis of SCFA concentrations in fecal samples revealed a significant decrease in absolute concentrations for acetate, propionate and butyrate (Fig. 2a) and also a significant relative reduction for butyrate (Fig. 2b) in PD patients compared to age matched controls.

Does not implicate the microbiome as the *root cause* of PD, only that the immune cells in the brain are activated by SCFA and dysbiosis can increase the activation of pre-existing inflammatory cells

Unger, M. M., Spiegel, J., Dillmann, K.-U., Grundmann, D., Philippeit, H., Bürmann, J., … Schäfer, K.-H. (2016). *Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. Parkinsonism & Related Disorders, 32, 66–72.*

Two recent studies showed that the abundance of certain gut microbiota differs between PD patients and controls, with relative dominance or absence of certain families

F. Scheperjans, V. Aho, P.A. Pereira, K. Koskinen, L. Paulin, E. Pekkonen, E. Haapaniemi, S. Kaakkola, J. Eerola-Rautio, M. Pohja, E. Kinnunen, K. Murros, P. Auvinen, Gut microbiota are related to Parkinson's disease and clinical phenotype, Mov. Disord. (2015) 350e358.

A. Keshavarzian, S.J. Green, P.A. Engen, R.M. Voigt, A. Naqib, C.B. Forsyth, E. Mutlu, K.M. Shannon, Colonic bacterial composition in Parkinson's disease, Mov. Disord. (2015) 1351e1360.

Autism

Gut Microbiome of Children with Autism

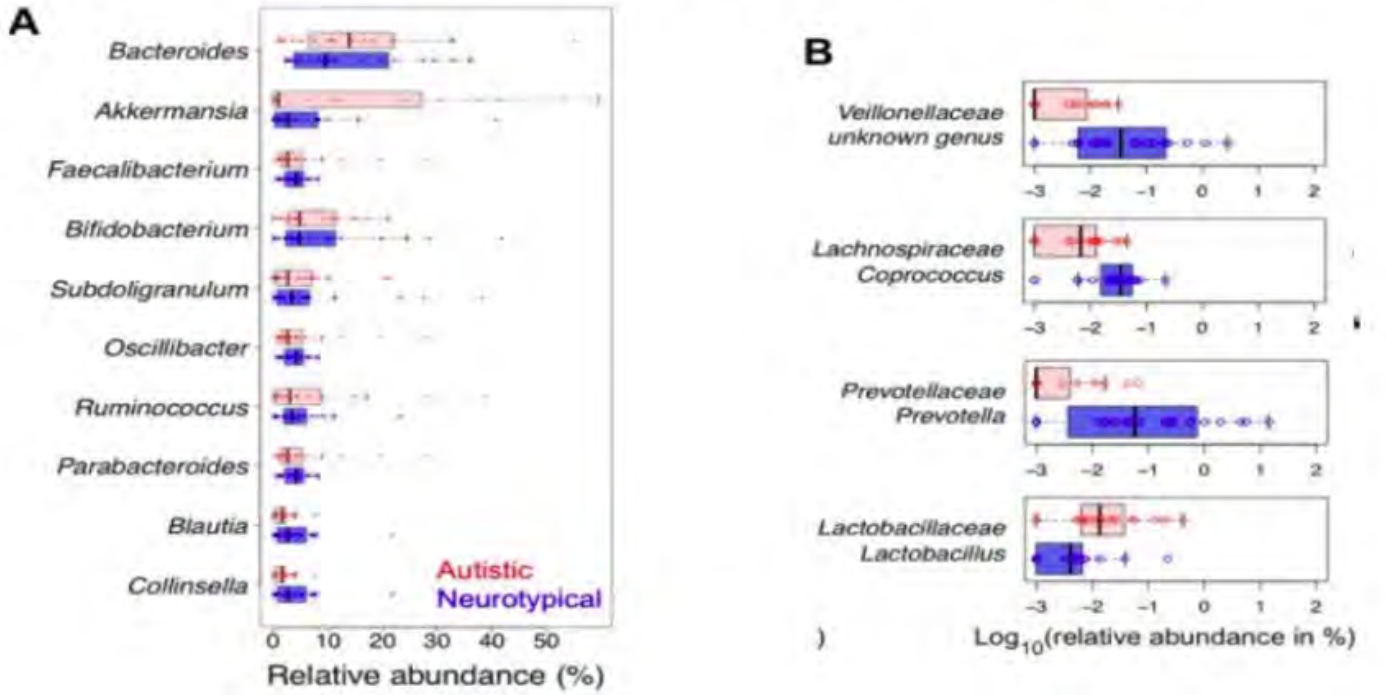
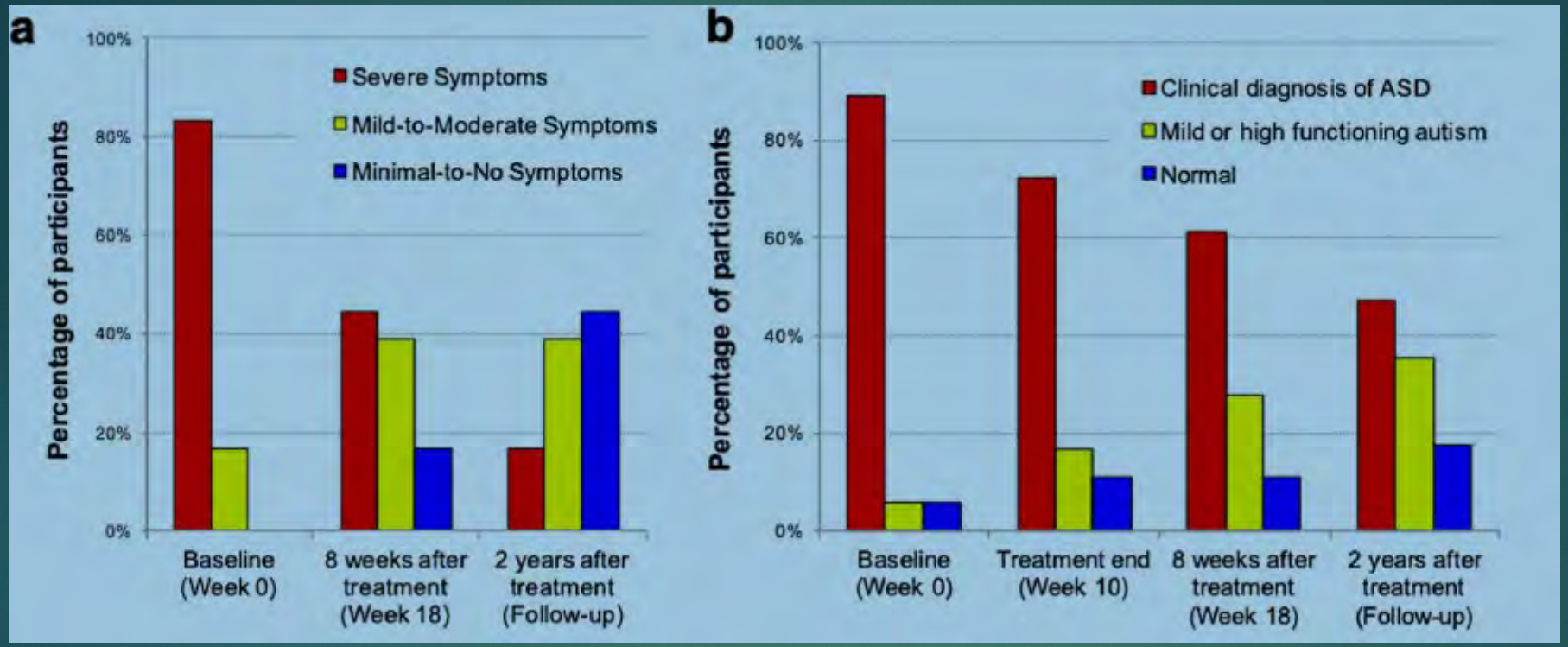


Figure credit: Kang et al. PLOS ONE, 2013

Trial of fecal transplant in autistic children

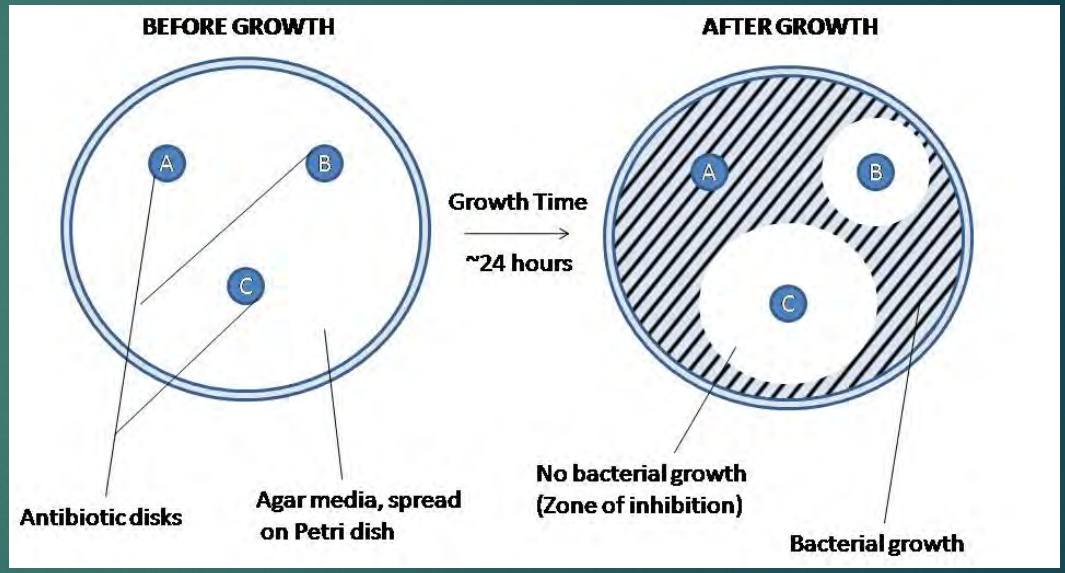
- We confirmed lower gut microbial diversity and reduced relative abundances
- The data in this study support that children with ASD have altered metabolite profiles in feces when compared with neurotypical children
- Children received fecal transplant and symptoms were followed for two years
- **ASD fecal bacterial diversity was higher two years after the MTT stopped**



Kang, D.-W., Adams, J. B., Coleman, D. M., Pollard, E. L., Maldonado, J., McDonough-Means, S., … Krajmalnik-Brown, R. (2019). Long-term benefit of Microbiota Transfer Therapy on autism symptoms and gut microbiota. Scientific Reports, 9(1).

Antibiotics and the microbiome

- Terms: Antibiotic, antimicrobial, antiseptic
- Mechanisms of antibiotic activity
- Broad spectrum antibiotics
- Measurement of effectiveness.
  - MIC = minimum inhibitory concentration
  - MBC = minimal bactericidal activity



Effect of antibiotics on gut microbiota

- Depletion of the microbiota diversity
- Production and proliferation of antibiotic resistant microbes.
- Depletion of microbiota led to downregulation of immunity.
- Enhanced quorum-sensing and increased formation of biofilms.

Morgun A, Dzutsev A, Dong X, Greer RL, Sexton DJ, Ravel J, Schuster M, Hsiao W, Matzinger P, Shulzhenko N. Uncovering effects of antibiotics on the host and microbiota using transkingdom gene networks. Gut. 2015 Nov;64(11):1732-43.

Microbiota and influenza (mouse)

- Test mice were given antibiotics for several weeks, some broad spectrum and some specific to certain types of bacteria.
- The treatment resulted in significant changes in the culturable gut microbiota
- The mice were then exposed to the influenza A virus.
- Anti-influenza antibody titers were reduced in antibiotic-treated mice
- The loss of commensals specifically sensitive to Vancomycin were responsible for the loss of immune activation against influenza.
- Immunity to a variety of other pathogens was not affected.
- ***Unforeseen and unintended consequences***
- Neomycin depleted gram-positive bacteria in the gut and impaired immune responses to respiratory influenza virus infection,

Ichinohe T, Pang IK, Kumamoto Y, Peaper DR, Ho JH, Murray TS, Iwasaki A. Microbiota regulates defense against respiratory tract influenza A virus infection. Proc Natl Acad Sci U S A. 2011 Mar 29;108(13):5354-9.

A single course of clindamycin

- Seven days duration clindamycin
- Fecal microbiota followed for 2 years
- highly significant disturbances persisted throughout the sampling period.
- Sharp decline in diversity of Bacteroides isolates,
- Long-term persistence of highly resistant clones
- The Bacteroides community never returned to its original
- A dramatic and persistent increase in levels of resistance genes in feces.
- Persistent long term impacts on the human intestinal microbiota that remain for up to 2 years post-treatment.

Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. ISME J. 2007 May;1(1):56-66.

A single course of double-antibiotic therapy

- The combination of metronidazole and clarithromycin is frequently prescribed for Helicobacter pylori infection in the stomach.
- Researchers studied the short and long-term effects of such a course on the microbiota of the lower intestine and the throat.
- There was a dramatic decline in Actinobacteria in both throat and feces immediately after treatment.
- On average, the diversity of the microbiota recovered to resemble its original state
- In some individuals the microbiota was still deranged at four years.
- Also at four years, on average, the patient had high levels of microbial resistance to the macrolide type of antibiotic used.

Jakobsson, H. E., Jernberg, C., Andersson, A. F., Sjölund-Karlsson, M., Jansson, J. K., & Engstrand, L. (2010). *Short-Term Antibiotic Treatment Has Differing Long-Term Impacts on the Human Throat and Gut Microbiome. PLoS ONE, 5(3), e9836.*

Cipro short and long term

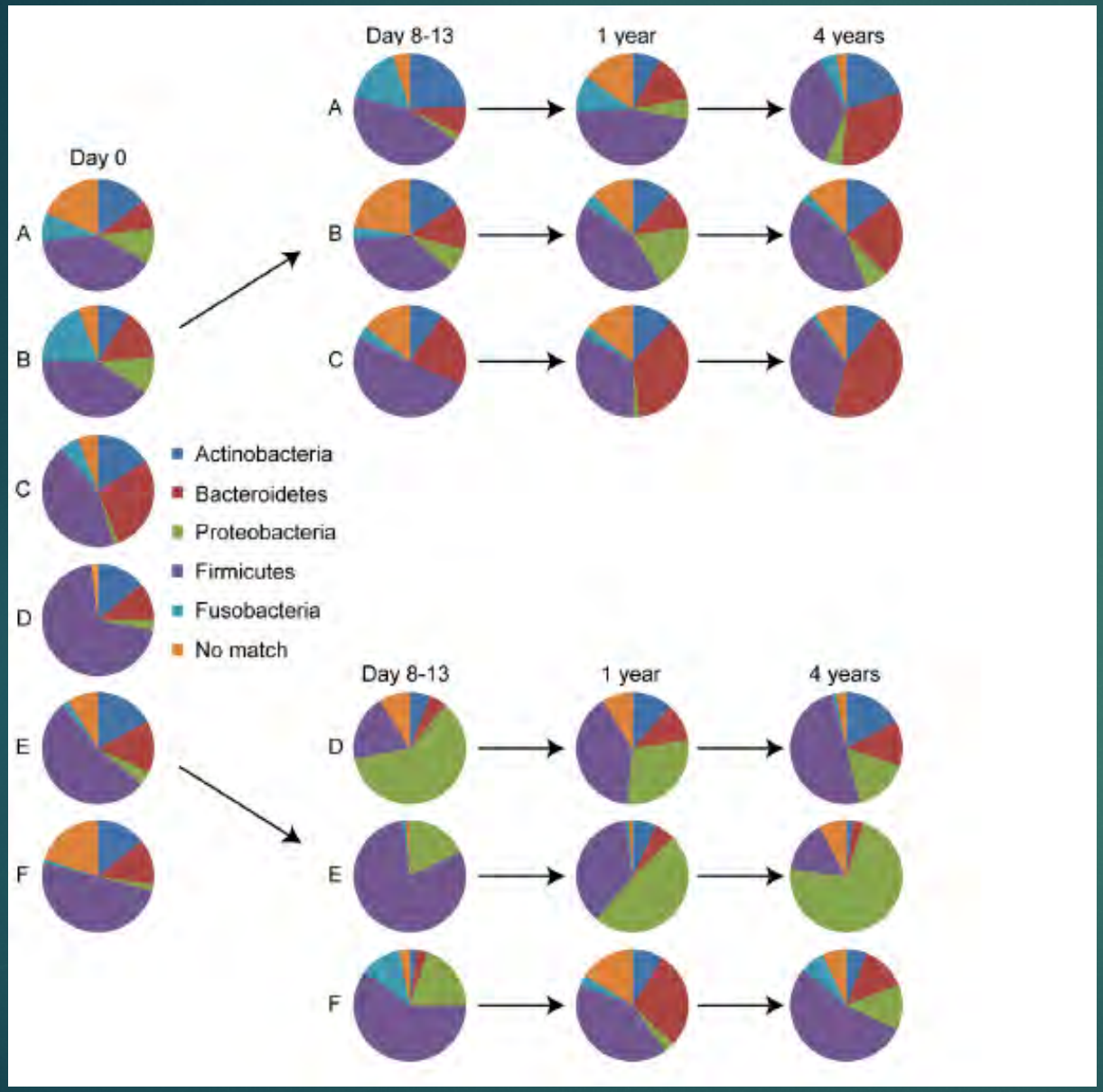
- After a short course of ciprofloxacin(500 mg twice a day for 5 d), typical of the treatment prescribed, e.g., for an uncomplicated urinary tract infection
- Ciprofloxacin reduced the diversity of the intestinal microbiota, with significant effects on about one-third of the bacterial taxa. decreasing the taxonomic richness, diversity, and evenness of the community.
- Despite this pervasive disturbance, the membership of the communities had largely returned to the pretreatment state within 4 weeks.
- Several taxa failed to recover within 6 months.

Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS Biol. 2008 Nov 18;6(11):e280.

Follow up with second course of Cipro

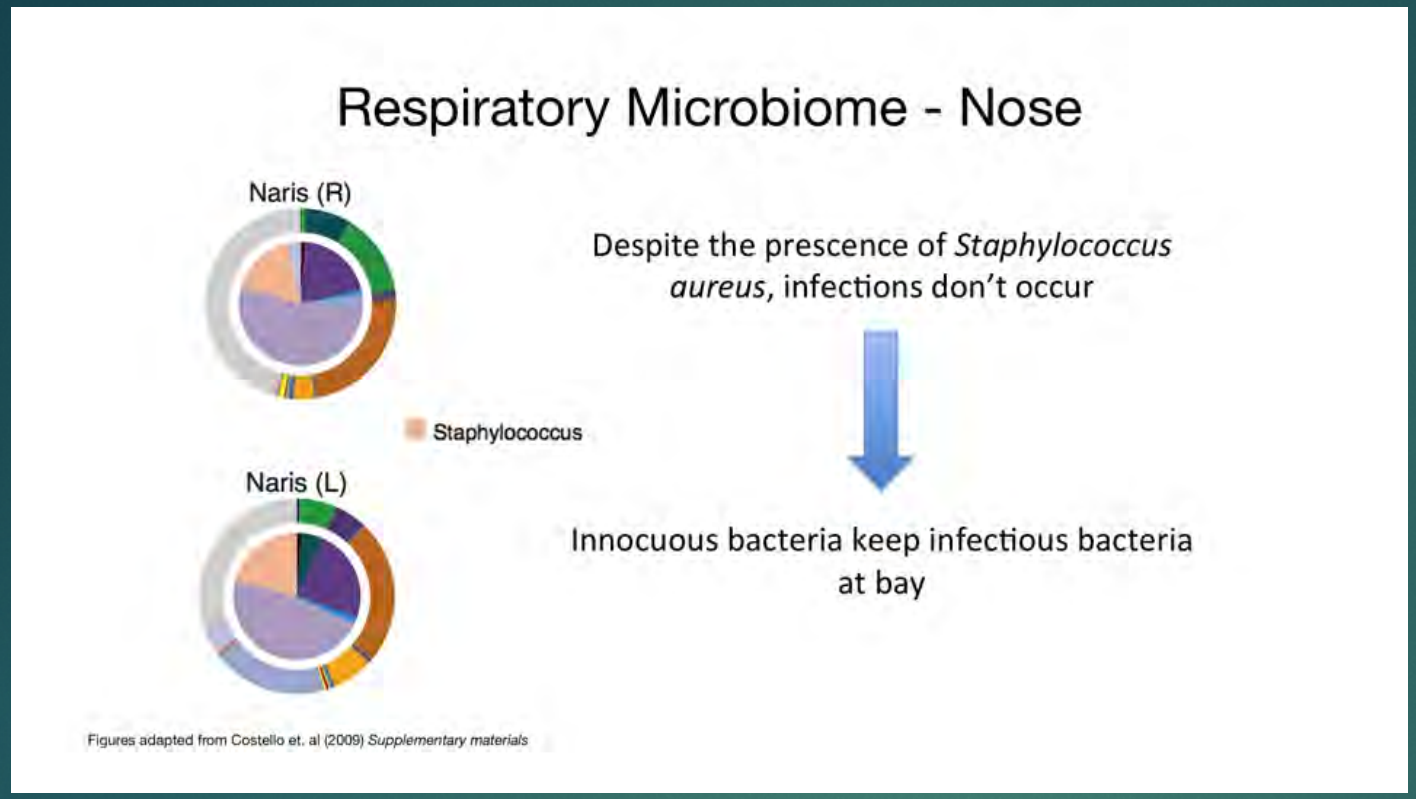
- A second 5-day course of Cipro was given at the six month point.
- Patients were followed for ten months.
- After the two courses none of the patient microbiomes
- None of the patient-microbiomes returned to resemble their original state even at the four year point of follow up.

Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 1:4554-61.



Patients A,B, and C are controls, they received now antibiotics, and demonstrate the normal range of evolution of the nasal microbiome over 4 years.

The bottom three show the shifts in bacterial ecology after a single course of the double-antibiotic treatment.



In the nasal area, the Actinobacteria which were reduced or eradicated by the treatment are commensals that keep Staphylococcus in check.

Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, Pace NR. The human nasal microbiota and Staphylococcus aureus carriage. PLoS One. 2010 May 17;5(5):e10598.

3-7 days with either amoxicillin or azithromycin followed for six months

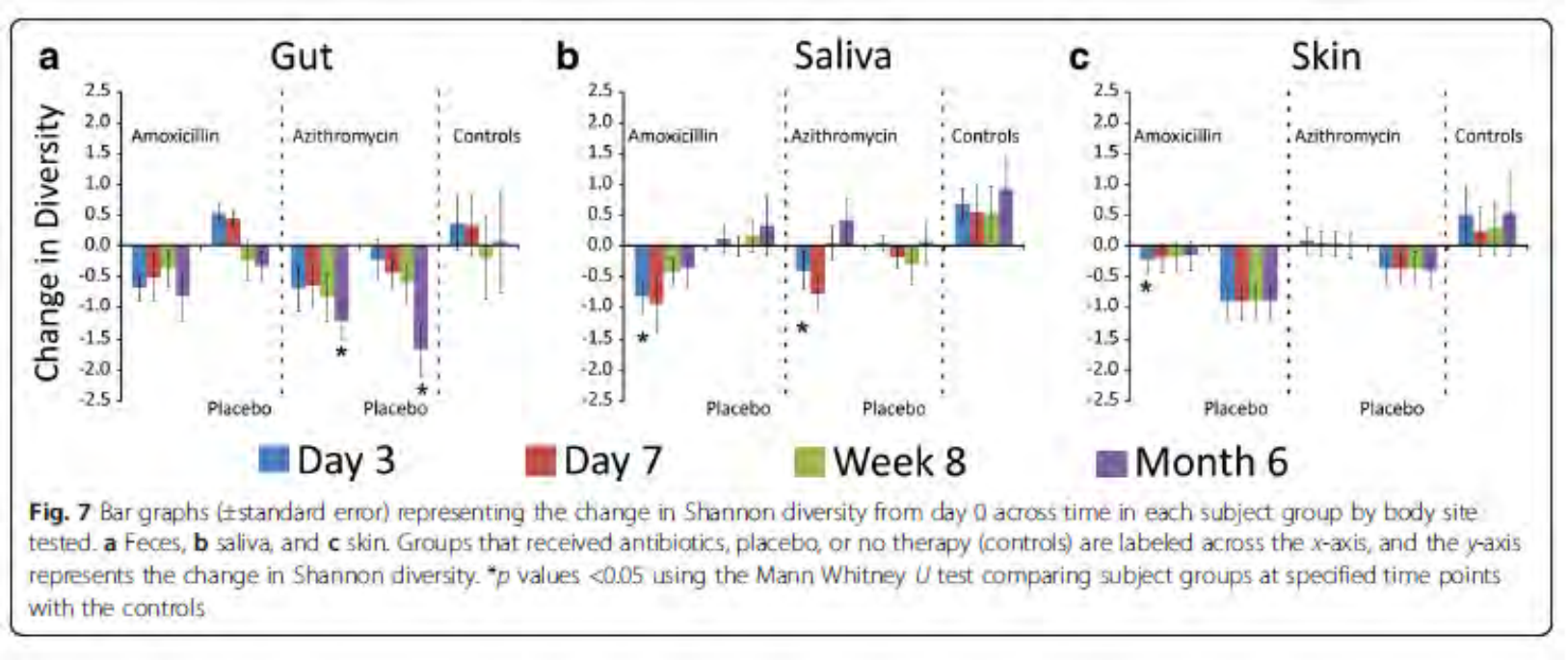


Fig. 7 Bar graphs (±standard error) representing the change in Shannon diversity from day 0 across time in each subject group by body site tested. **a** Feces, **b** saliva, and **c** skin. Groups that received antibiotics, placebo, or no therapy (controls) are labeled across the *x*-axis, and the *y*-axis represents the change in Shannon diversity. **p* values <0.05 using the Mann Whitney *U* test comparing subject groups at specified time points with the controls

Abeles SR, Jones MB, Santiago-Rodriguez TM, Ly M, Klitgord N, Yooseph S, Nelson KE, Pride DT. Microbial diversity in individuals and their household contacts following typical antibiotic courses. Microbiome. 2016 Jul 30;4(1):39.

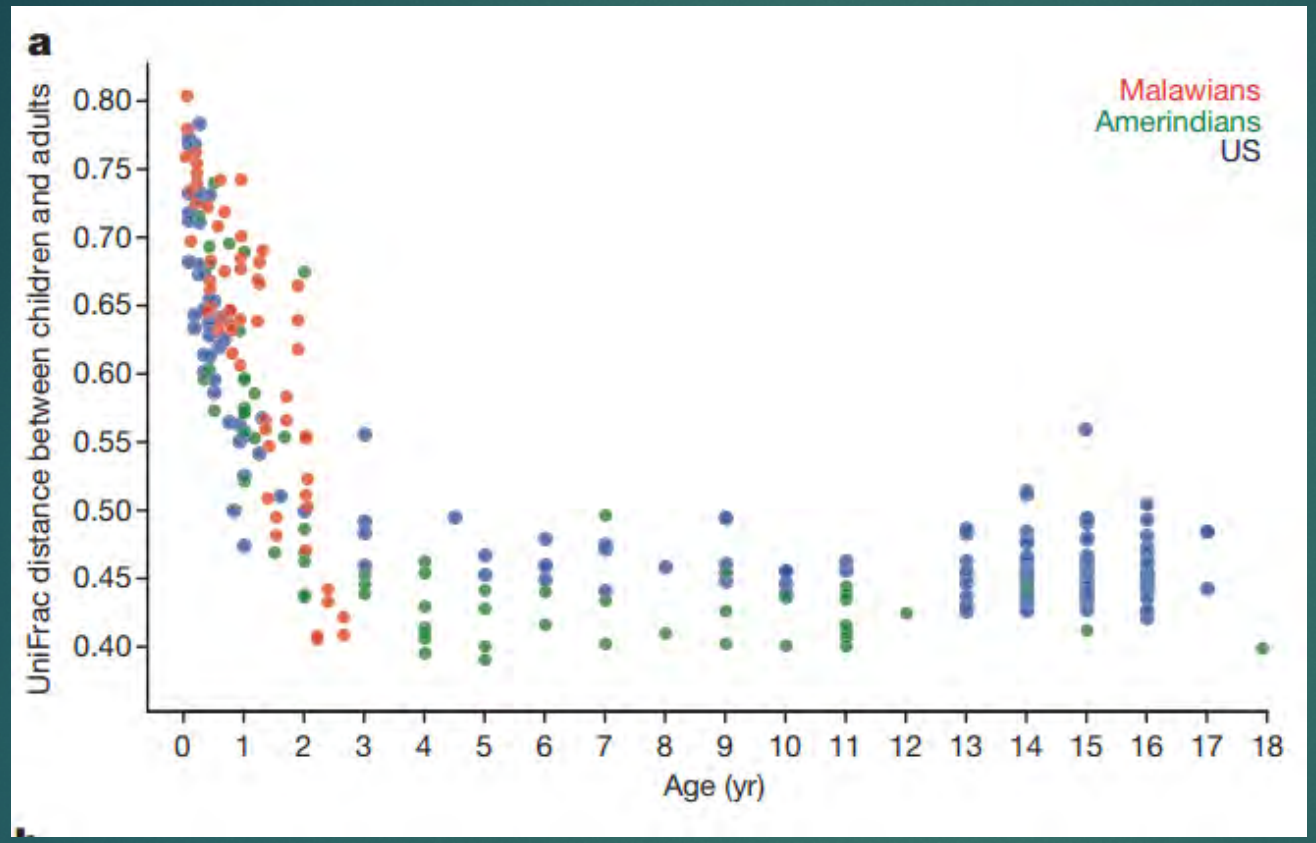
Antimicrobial effect on the microbiome

- Systemic antibiotics can promote the development of resistance in every compartment of the microbiome.
- Systemic antibiotics can force the metabolic evolution of the microbiome to produce more energy (SFCA)
  - Relationship to the fattening of farm animals with SIC of antibiotics
  - Relationship to obesity in U.S. population.
- Systemic antibiotics can reduce the diversity in every compartment of the microbiome.

C diff

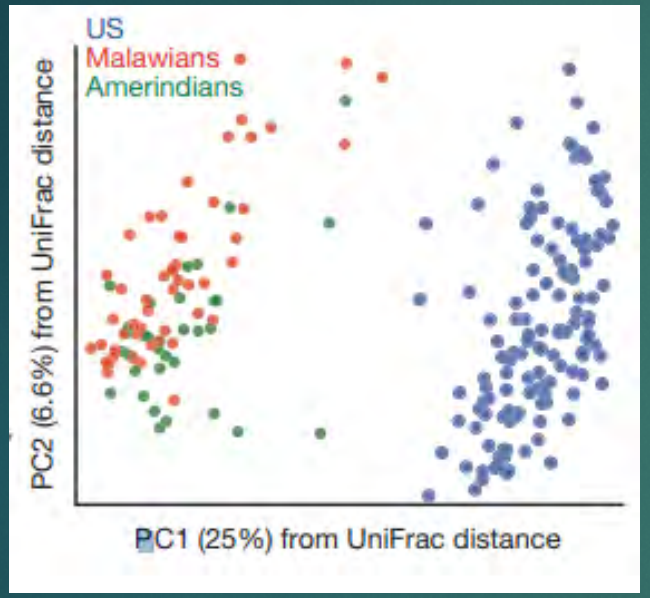
Age and geography

Microbiome matures by about age 3

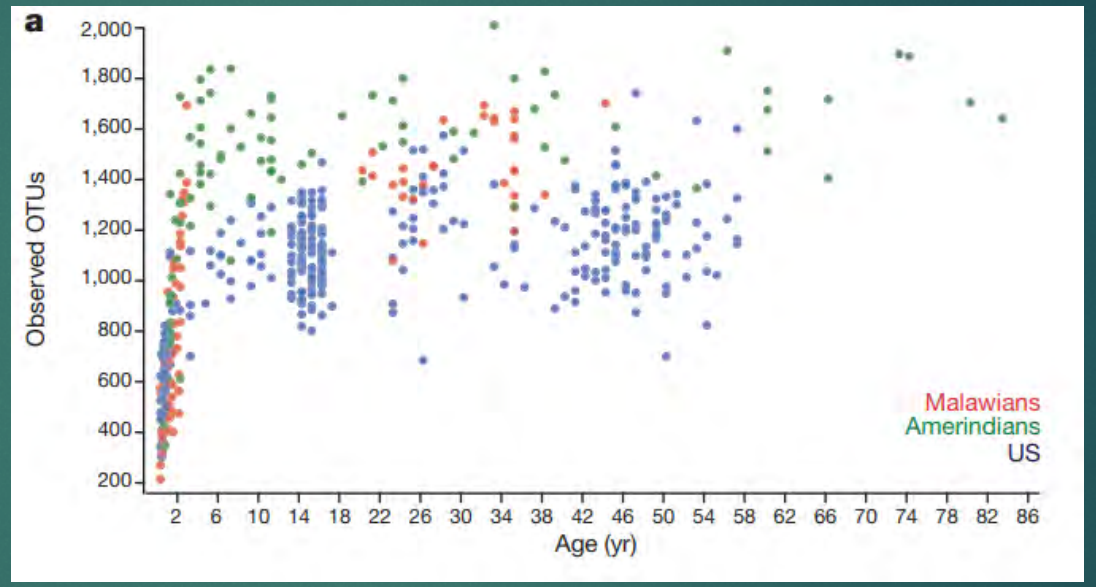


Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2012). *Human gut microbiome viewed across age and geography. Nature, 486(7402), 222–227.*

Modern humans vs indigenous.

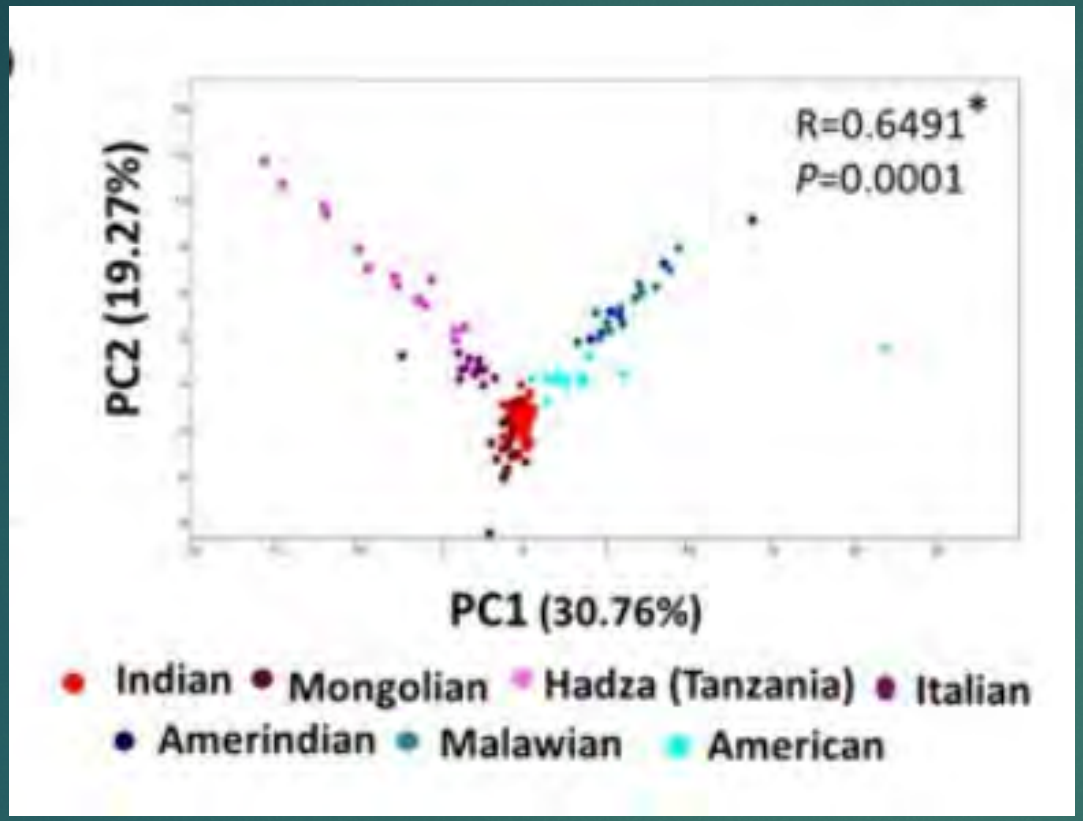


Species



Diversity

Yatsunenko, T., Rey, F. E., Manary, M. J., Trehan, I., Dominguez-Bello, M. G., Contreras, M., … Gordon, J. I. (2012). *Human gut microbiome viewed across age and geography. Nature, 486(7402), 222–227.*



Dehingia M, Devi KT, Talukdar NC, Talukdar R, Reddy N, Mande SS, Deka M, Khan MR. Gut bacterial diversity of the tribes of India and comparison with the worldwide data. Sci Rep. 2015 Dec 22;5:18563.

All-animal vs all-plant diet

- Relative abundance of various bacterial species shifted within one day after the food hit the gut.
- Within 3 days, the altered microbiota began to affect behavior.
- Different genes in the microbiota were upregulated.
- Meat eggs and cheese (exclusive) diet increased flora that tolerate bile including Bacteriodes.
- The same diet decreased Firmacutes.
- Vitamin synthesis was also increased

David, L. A., Maurice, C. F., Carmody, R. N., Gootenberg, D. B., Button, J. E., Wolfe, B. E., … Turnbaugh, P. J. (2013). *Diet rapidly and reproducibly alters the human gut microbiome. Nature, 505(7484), 559–563.*

Probiotics

- *Acidophilus, Bifidobacter*, others
- Adherent, lactic acid producing, also produce SCFA
- “Super strains” developed in various laboratories for beneficial effects.
- Consider renewable yoghurts, kefirs, coconut kefirs, etc fortified with a variety of superstrain capsules.

Prebiotics

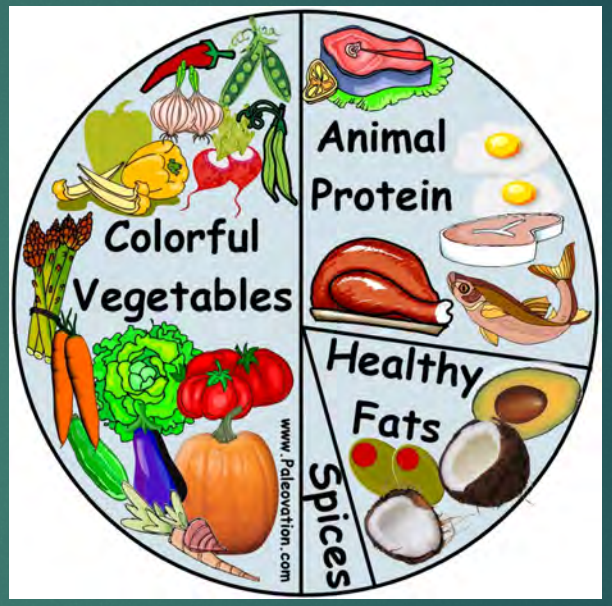
- Inulin, a fructo-oligosaccharide, is a common storage starch in sunflower family plants. An indigestible starch but metabolized to SCFA by many bacteria.
- 50% of *Arctium* root by weight; 40% of *Inula helenium;* 25% of *Taraxacum*
- Of the 3 herbs, only *Arctium* can be taken in regular food-like quantities.
- Inulin is highly soluble in hot water: Recipe from Wm Cook: 2 ounces burdock root in pint of water, simmer to ½ pint. Taken in 1-3 ounce doses.
- Alternately sprinkle burdock root powder on food, or mix it into fermented foods.

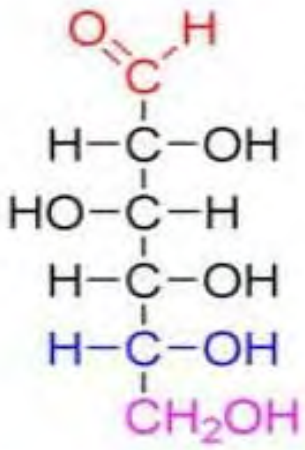
Probiotic/prebiotic strategy

- Flooding strategy: 150 billion organisms per day for 4-7 days. *Acidophilus, Bifidobacter*, etc (with concentrated products or fermented foods)
- Maintain with 10 billion organisms per day intermittently.
- With each dose, take 1-2 tsp of *Arctium* powder in water.
- Keep an influx of the probiotic and the prebiotic present in the ecology most of the time.
- Arctium powder is inexpensive, about $8 per pound at wholesale, and has a mildly sweet taste.

Food for the microbiome

- Proteins
- Starches
- Polysaccharides
- Pectins
- Inulin
- Insoluble fiber
- Flavonoids





Carbohydrates: Not all created equal

Paul Bergner
Director, North American Institute of Medical Herbalism
Editor, *Medical Herbalism* Journal

http://naimh.com/nunm/infection