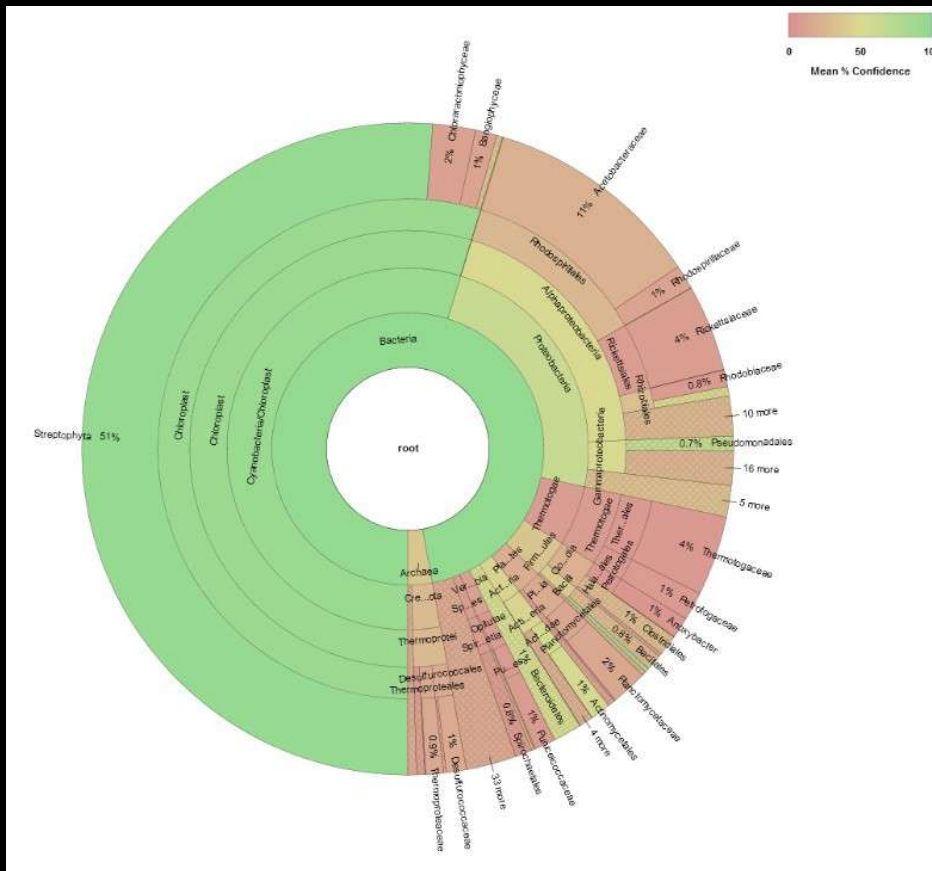


The Complex Microbiology of Bee Pollen

Collaborators: I. K. Nagy, A. Varga, I. Miklóssy, E. E. Rosenberg, K. Kovács, M. Antoniadou, E. Huszárné Pásztor, E. Takács, B. Kocsis, T. Kovács



Peter HANTZ
Project Leader
Senior Research Scientist
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peter.hantz@protonmail.com

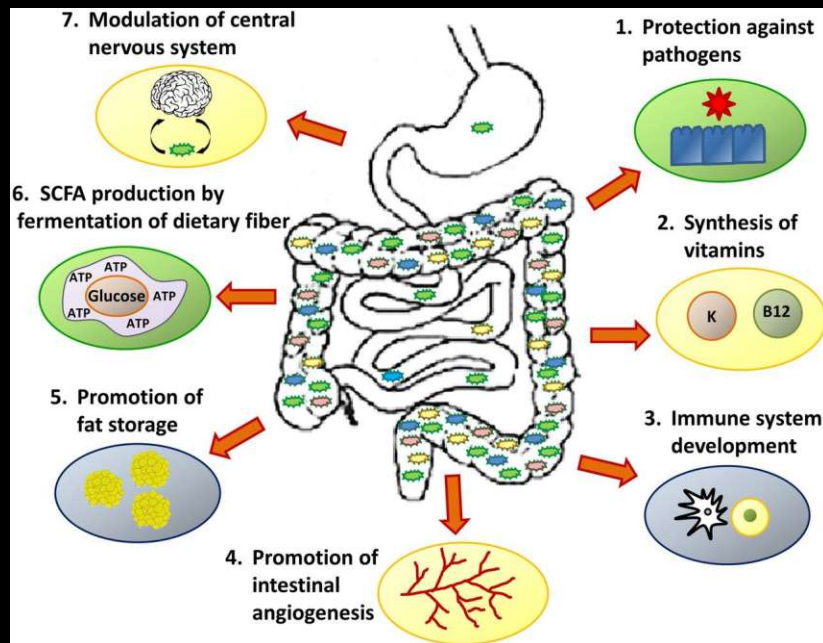
The Importance of Microbe Communities

Glyphosate perturbs the gut microbiota of honey bees

Erick V. S. Motta^{a,1}, Kasie Raymann^{a,2}, and Nancy A. Moran^{a,1}

^aDepartment of Integrative Biology, University of Texas at Austin, Austin, TX 78712

Bees



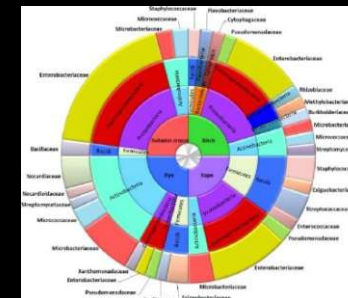
Human Large Intestine

environmental microbiology

Environmental Microbiology (2016) 00(00), 00–00

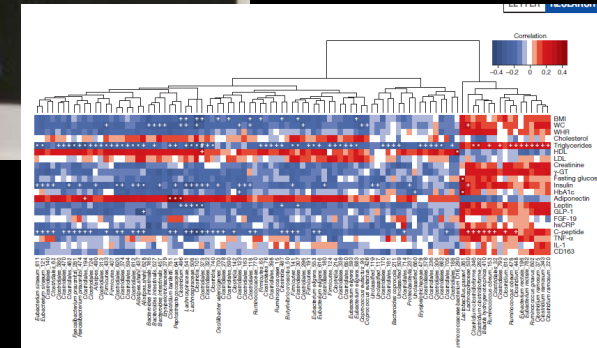
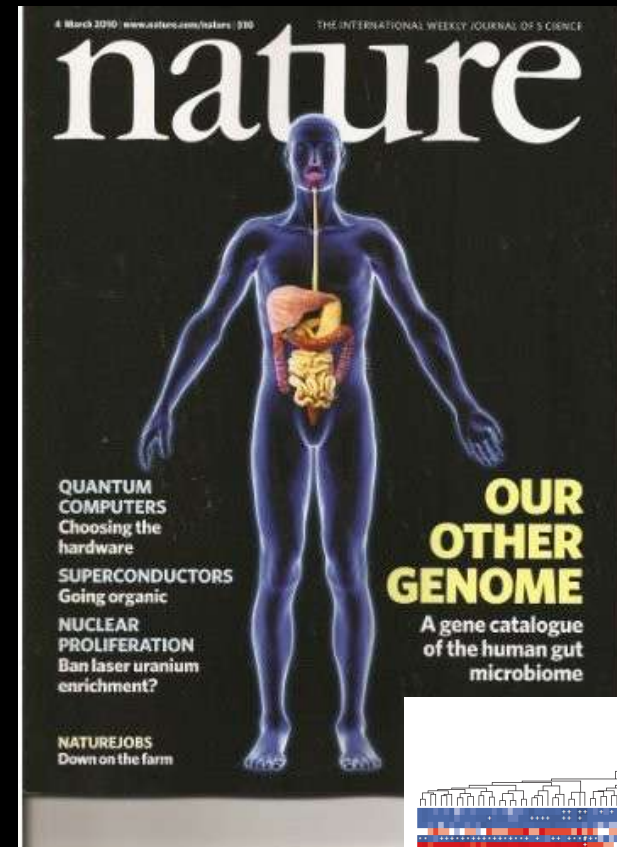
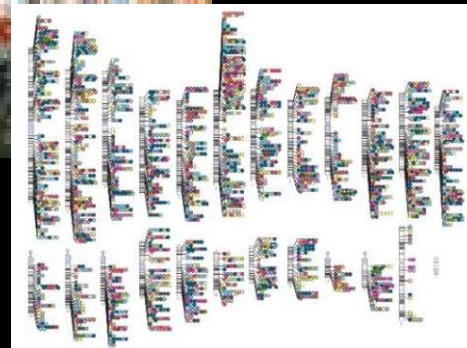
doi:10.1111/1462-2920.13524

Bacterial microbiota associated with flower pollen is influenced by pollination type, and shows a high degree of diversity and species-specificity



Bee Pollen

The Importance of Microbe Communities



The Importance of Microbe Communities

- Our gut microbiome contains at *least 1000 species of known bacteria* with more than 3 million genes (150 times more than human genes).
- The microbiome in your intestine: a (momentary) individual ID card
- *Diversity* of species of the gut microbiome plays an important role in the *metabolic balance* of the host.
- Large scale testing of the microbiome of different organs revealed important *correlation with a series of common diseases*

The Importance of SCFA like butyrate

Microbial modulation of diabetes

Short-chain fatty acids (SCFAs) are produced by various human gut microbes. SCFAs act as an energy source to the colonic epithelium and are also sensed by host signaling pathways that modulate appetite and inflammation. Deficiency of gut SCFAs is associated with type 2 diabetes. Zhao *et al.* found that adopting a high-fiber diet promoted the growth of SCFA-producing organisms in diabetic humans. The high-fiber diet induced changes in the entire gut microbe community and correlated with elevated levels of glucagon-like peptide-1, a decline in acetylated hemoglobin levels, and improved blood-glucose regulation.

Zhao et al., Science, Mar 2018

Altered microbiota in diabetes

LETTER

Gut metagenome in European women with normal, impaired and diabetic glucose control

Frederik H. Kuchner²⁰, Valentin Trzaskowski²⁰, Isaac Atwood²¹, Citran Bergerson¹, Carl Johan Böker², Björn Egerberg¹, Joni Moberg² & Fredrik Riklund^{1,2,†}

ARTICLE

A metagenome-wide association study of gut microbiota in type 2 diabetes

[illegible]

Consensus: Butyrate-producing bacteria are reduced in T2D



Identifying microorganisms

Culturing: about 20% only

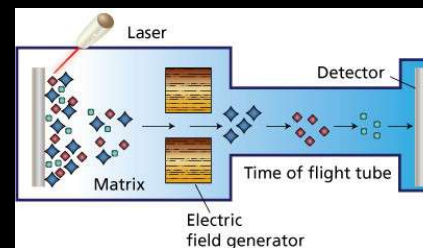


Special media, Enzyme or Chemical tests, Serology, Phage typing

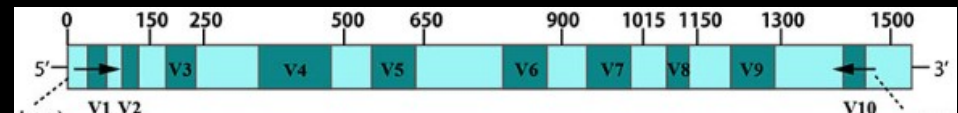


16S rDNA:
highly conserved primer binding sites +
*hypervariable regions that can provide
species-specific signature* sequences

MALDI-TOF identification



16S rDNA PCR and Sanger sequencing



Second generation Sequencing: “massively parallel” *de novo* and *targeted*

Illumina
Ion Torrent
SOLiD



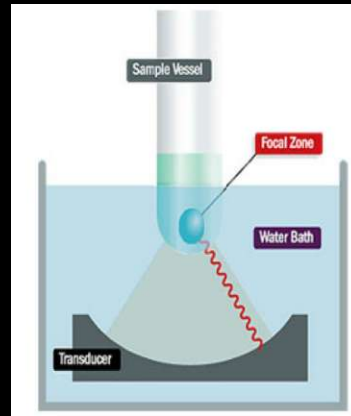
targeted: 16S rDNA PCR-amplicon

de novo: Shotgun sequencing-no PCR step

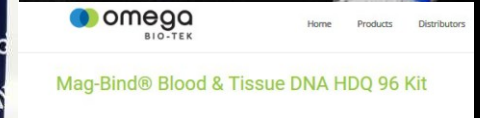
Third generation Sequencing: Single Molecules

Massively Parallel Sequencing

Sample Preparation



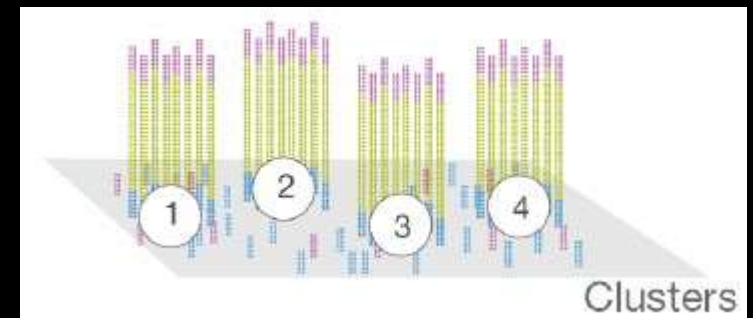
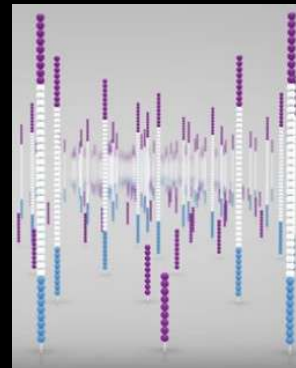
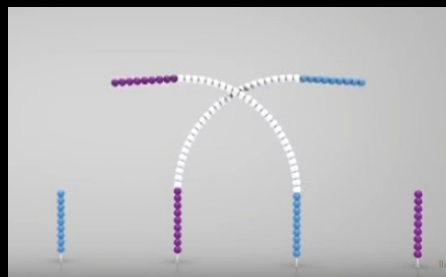
Get purified and unbiased DNA (!!!)



Fracture in 200-600 bp
Add adapters

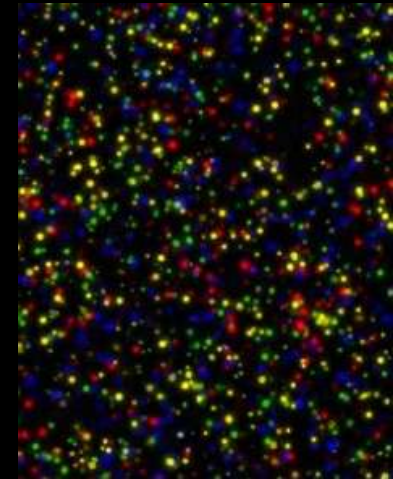


Cluster generation: PCRs



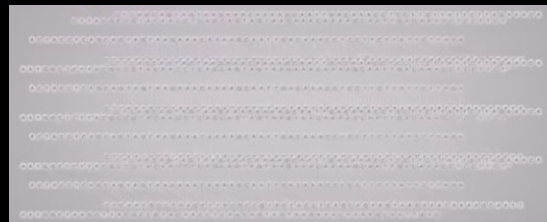
Massively Parallel Sequencing

Sequencing by Synthesis → Excitation/Fluorescence after each cycle



Data Processing

Index-based separation



Usually: Assembly - contigs



Researcher's work: analysis



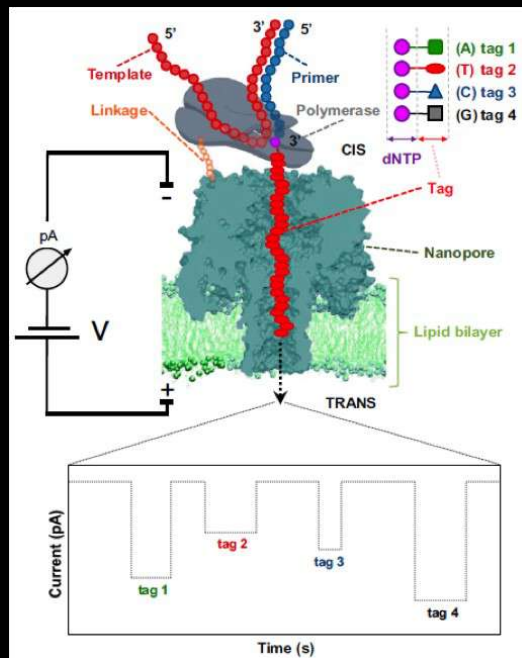
Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences.

Geneious is a powerful and comprehensive suite of molecular biology and NGS analysis tools

Single Molecule Sequencing

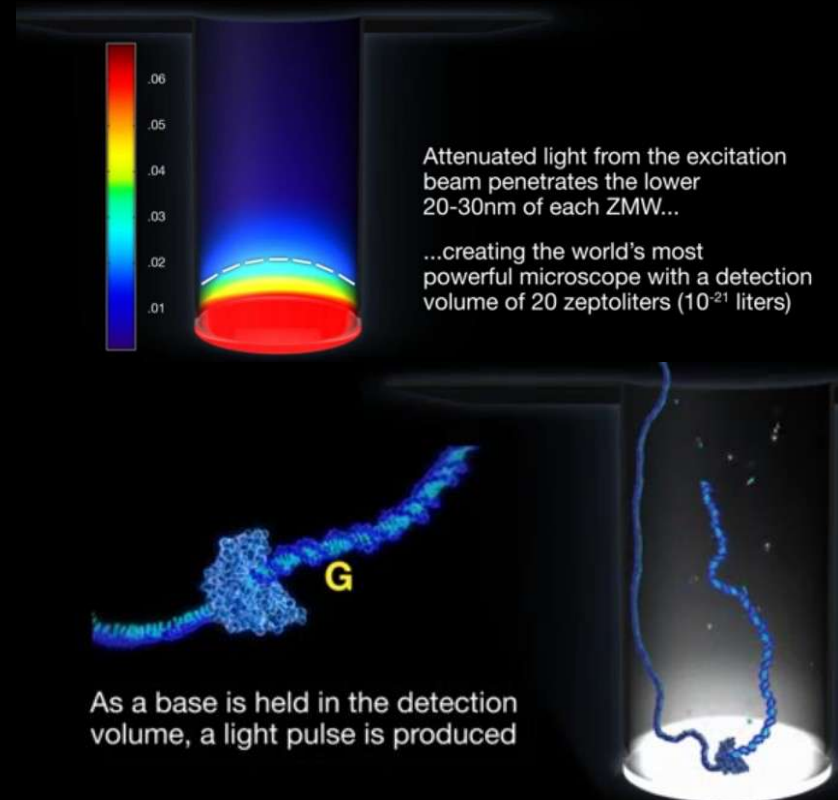
Oxford Nanopore



Long reads!
Problem: accuracy only 96%

PACific biosciences

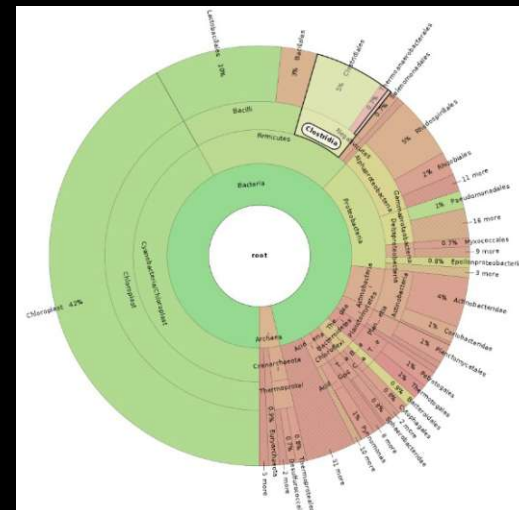
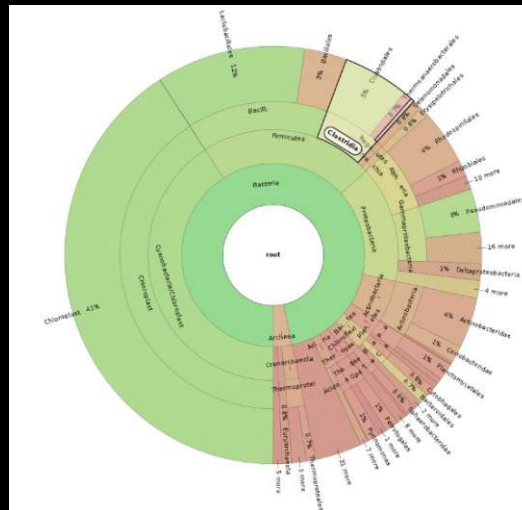
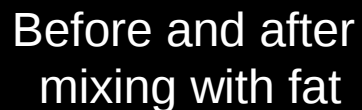
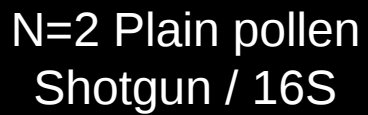
A zero-mode waveguide is an optical waveguide that guides light energy into a volume that is small in all dimensions compared to the wavelength of the light.



Long reads!
Expensive...

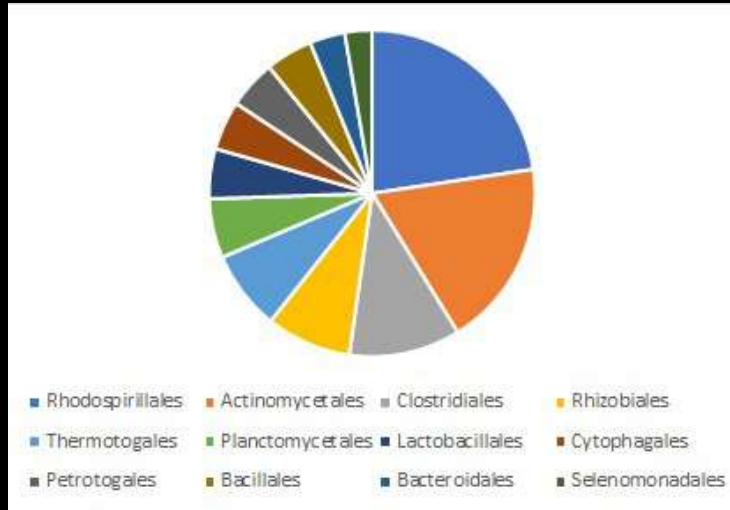
- **Sunflower Pollen Microbiome, N=2**

- **Sunflower Pollen Microbiome, N=2**

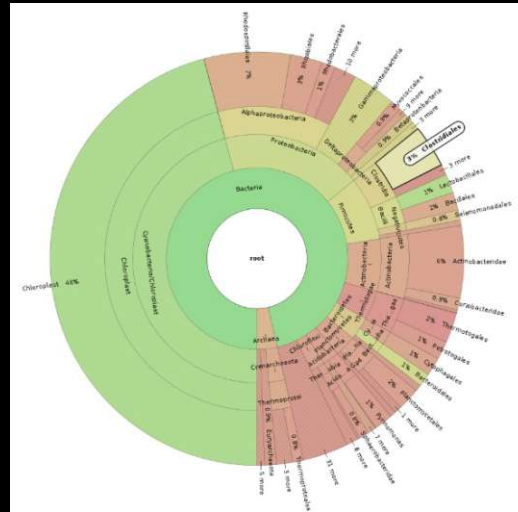


Our Work in Progress

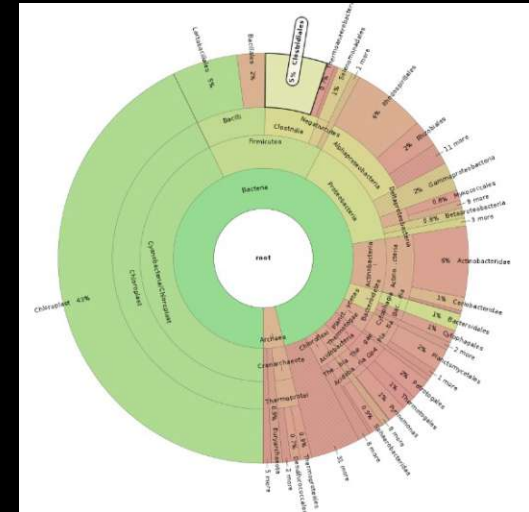
•Certain Mixed Pollen Microbiome, N=3



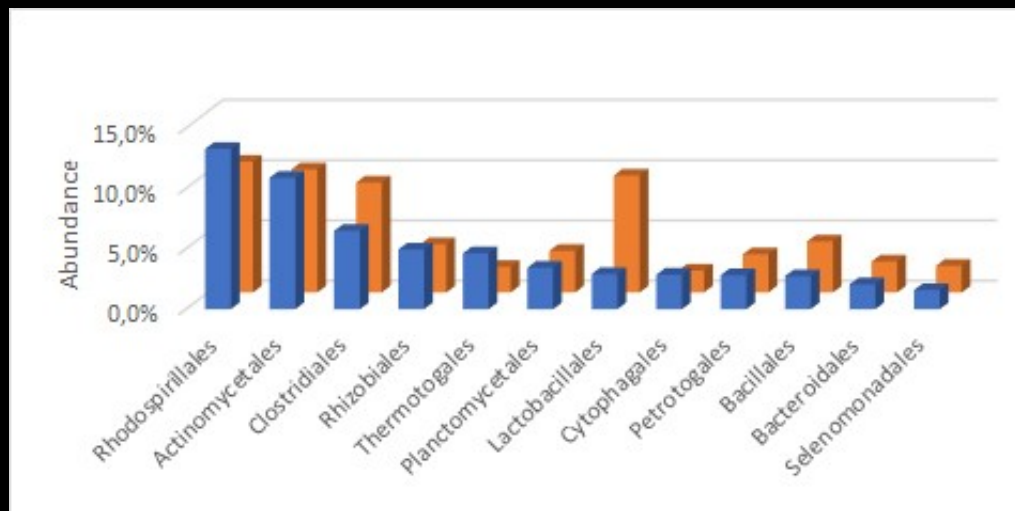
Shotgun, Plain Pollen



16S rDNA, Plain Pollen



16S rDNA, 2day mix



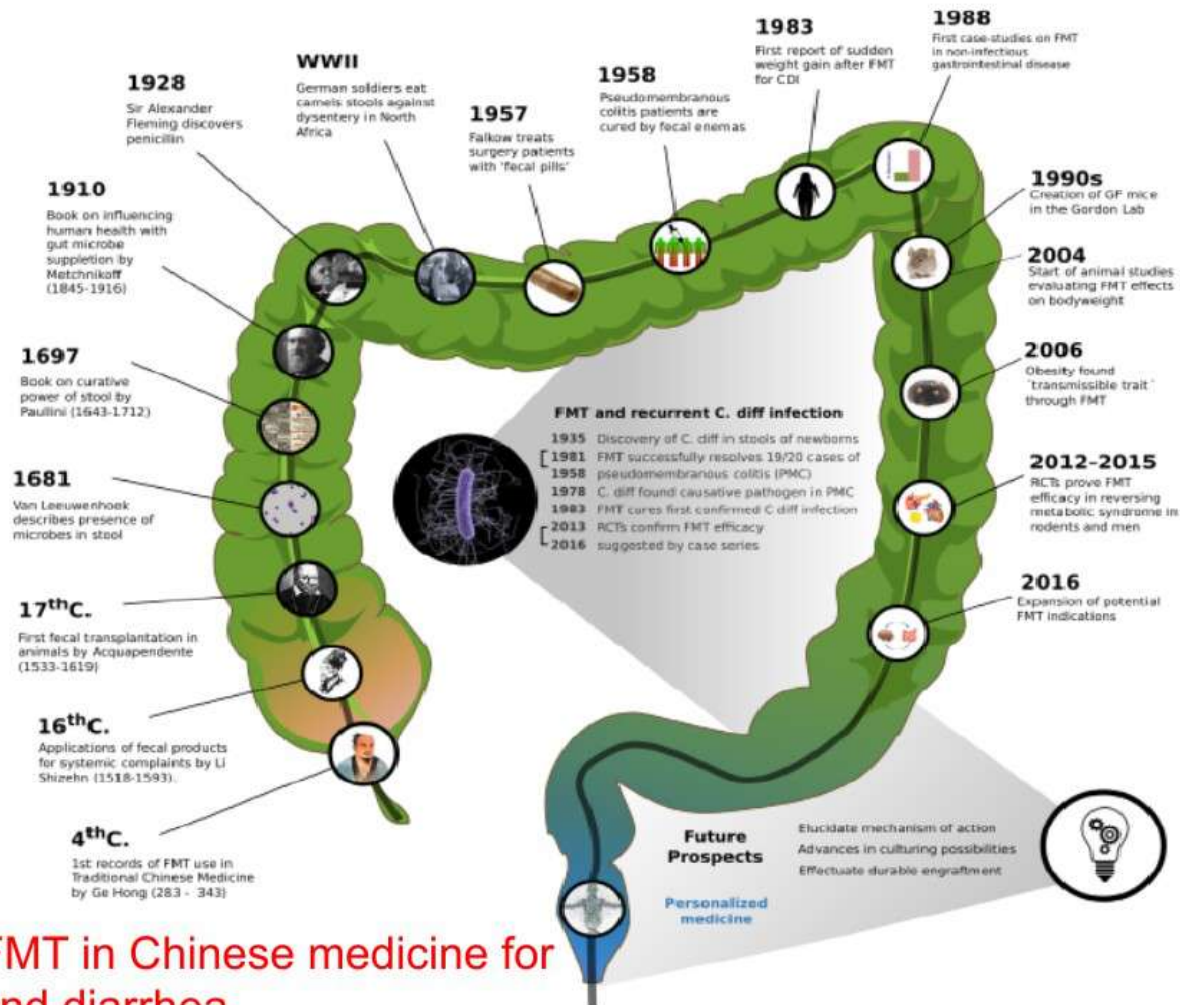
Microbiome Alteration

Thanks for your attention!

Digression: fecal transplant

1958: Eiseman, antibiotics-induced chronic diarrhea

Timeline of Fecal Microbiota Transplantation



4th Century BC: FMT in Chinese medicine for food poisoning and diarrhea