Correlation Between Intraluminal Oxygen Gradient and Radial Partitioning of Intestinal Microbiota

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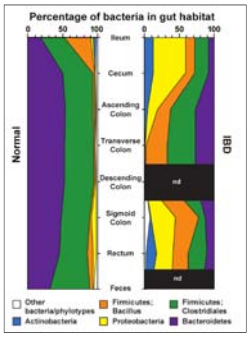
Dysbiosis in Inflammatory Bowel Disease (IBD)



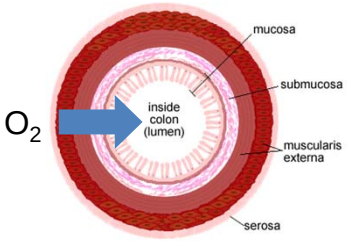
Peterson et al. *Cell Host & Microbe*. 2008.

- Increases in Proteobacteria and Actinobacteria
  - Generally aerotolerant
  - Better able to manage oxidative stress in the setting of inflammation?
- Host inflammatory response leads to production of oxidation products which serve as electron acceptors supporting anaerobic respiration by facultative anaerobes (Winter et al. *EMBO Reports*. 2013.)

The Anaerobic Intestinal Lumen

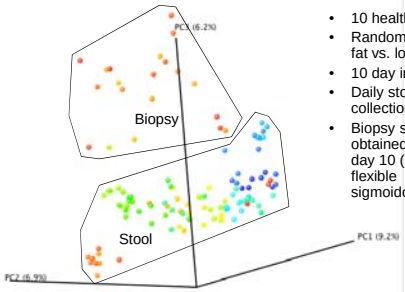
- The intestinal lumen in humans is thought to be strictly anaerobic, but the reason for this largely unknown
- Current technology is unable to dynamically quantify oxygen in the intestinal tract, so the mechanisms that maintain this anaerobic environment remain unclear

Oxygen Gradient



Biopsy and Stool Communities Cluster Separately Independent of Individual, Diet, and Time

CAFÉ Study Days 1 and 10
(Stool and Biopsy Samples)



Unweighted Unifrac

- 10 healthy volunteers
- Randomized to high fat vs. low fat diet
- 10 day inpatient stay
- Daily stool sample collection
- Biopsy specimens obtained on day 1 and day 10 (un-prepped flexible sigmoidoscopy)

Wu et al. *Science* 2011;334:105-8

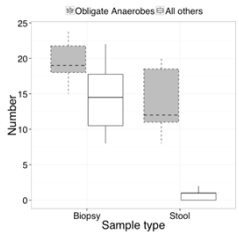
CAFE biopsy vs. stool analysis

Hypothesis: Bacteria adherent to the rectal mucosa is enriched in aerotolerant bacteria relative to the feces where most organisms are obligate anaerobes.

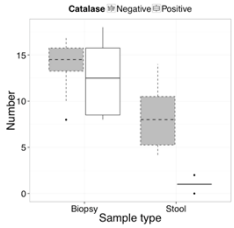
Classify the genera based on oxygen preference

- Focus on 73 bacterial genera with maximum proportion > 0.002
- Classify each genus as either "facultative anaerobe or aerotolerant", "aerobe or microaerophile" or "obligate anaerobe"
- Two groups
  - Obligate anaerobe
  - All others
    - Classify the genera into Stool or Biopsy-dominant

Enrichment of "Aerotolerant" Catalase Positive Bacteria on the Rectal Mucosa



p=0.002



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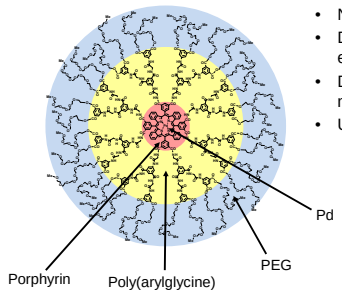
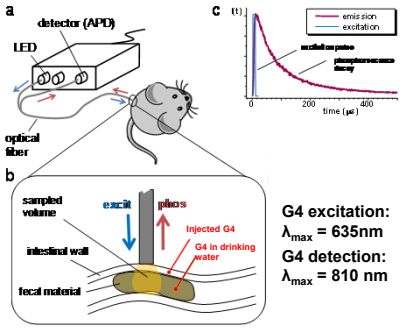
Phosphorescence Quenching: A form of Biological Oximetry

Phosphorescent Nanoprobe Oxyphor G4



- Non-toxic
- Does not interact with the environment
- Does not cross biologic membranes
- Unlimited water solubility

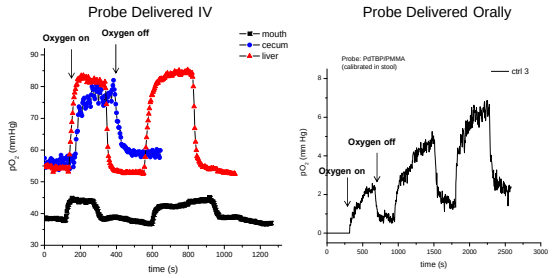
Methods: Phosphorescence Quenching



G4 excitation:
λ_{max} = 635nm
G4 detection:
λ_{max} = 810 nm

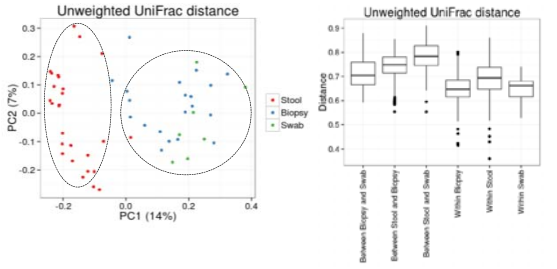
Vinogradov, SA *et al.* Rev Sci Instrum, 2001. 72(8): 3396-3406.

Oxygenation of the Host and in the Gut Lumen



Albenberg, L *et al.* Gastroenterology, 2014. 147(5): 1055-63.

Bacterial Taxonomy in Human Stool is Different from Either Rectal Biopsies or Swabs



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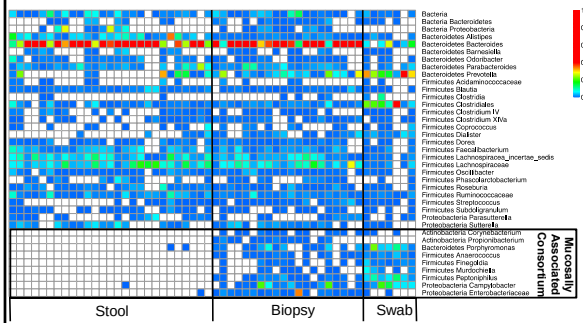
The Mucosally-Associated Microbiota in Humans



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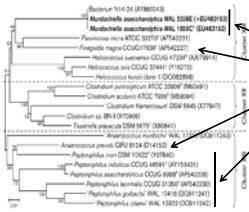
The Assacharolytic and Oxygen Tolerance Bacterial Signature at the Mucosal Interface



Rectal Biopsy/Swab Specific Taxa

- Murdochiella (Firmicute)
- Finegoldia (Firmicute)
- Anaerococcus (Firmicute)
- Peptoniphilus (Firmicute)
- Porphyromonas (Bacteroidetes)
- Campylobacter (Proteobacteria)
- Propionibacterium (Actinobacteria)
- Corynebacterium (Actinobacteria)
- Enterobacteriaceae (Proteobacteria)

Assacharolytic

Aerobic and Facultative Anaerobic

Ulger-Toprak et al. Int. J. System and Evol. Micro. 2010;60:1013

The Mucosally-Associated Signature Consortium

Phylum	Genus	Oxygen Tolerance	Catalase	Asaccharolytic	
Actinobacteria	*Corynebacterium*	Aerobic and Facultative Anaerobes	Positive	No	<0.0001*
Bacteroidetes	*Porphyromonas*	Obligate Anaerobe	Negative	Yes	<0.0001*
Firmicutes	*Anaerococcus*	Obligate Anaerobe	Negative	Yes	<0.0001*
Firmicutes	*Finegoldia*	Obligate Anaerobe	Negative	Yes	<0.0001*
Firmicutes	*Murdochiella*	Obligate Anaerobe	Negative	Yes	<0.0001*
Firmicutes	*Peptoniphilus*	Obligate Anaerobe	Negative	Yes	<0.0001*
Proteobacteria	*Campylobacter*	Microaerophilic	Positive	Yes	<0.0001*
Proteobacteria	*Enterobacteriaceae*	Facultative Anaerobes	Positive	No	<0.0001*

*Compared to stool, FDR corrected

Albenberg, L *et al.* Gastroenterology, 2014. 147(5): 1055-63.

Spatial Segregation of the Intestinal Microbiota at the Mucosal Interface

- Using phosphorescence quenching, we confirm the oxygen-poor environment of the gut lumen
- Composition of the gut microbiota is spatially-segregated along the radial axis of the gut
- The intestinal mucosal surface is enriched for oxygen tolerant organisms that may serve as "founding" communities for the development of the dysbiotic microbiota associated with intestinal inflammation
- By comparing the microbiota in rectal biopsies and swabs to the feces, we also show that a consortium of asaccharolytic bacteria that primarily metabolize amino acids are associated with mucus.

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