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Correlation Between Intraluminal Oxygen Gradient and Radial Partitioning of Intestinal Microbiota in Humans and Mice

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Abstract

Background & Aims—The gut microbiota is a complex and densely populated community in a dynamic environment determined by host physiology. We investigated how intestinal oxygen levels affect the composition of the fecal and mucosally adherent microbiota.

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Methods—We used the phosphorescence quenching method and a specially designed intraluminal oxygen probe to dynamically quantify gut luminal oxygen levels in mice. 16S rRNA gene sequencing was used to characterize the microbiota in intestines of mice exposed to hyperbaric oxygen, human rectal biopsy and mucosal swab samples, and paired human stool samples.

Results—Average pO_2 values in the lumen of the cecum were extremely low (<1 mmHg). In altering oxygenation of intestines of mice, we observed that oxygen diffused from intestinal tissue and established a radial gradient the extended from the tissue interface into the lumen. Increasing tissue oxygenation with hyperbaric oxygen altered the composition of the gut microbioita in mice. In humans, 16S rRNA gene analyses revealed an increased proportion of oxygen-tolerant organisms of the Proteobacteria and Actinobacteria phyla associated with the rectal mucosa, compared with the feces, indicating an effect of oxygenation on the microbiota. A consortium of asaccharolytic bacteria of the Firmicute and Bacteroidetes phyla, which primarily metabolize peptones and amino acids, was associated primarily with mucus. This could be due to the presence of proteinaceous substrates provided by mucus and the shedding of the intestinal epithelium.

Conclusions—In an analysis of intestinal microbiota of mice and humans, we observed a radial gradient of microbes linked to distribution of oxygen and nutrients provided by host tissue.

Keywords

aerobic; anaerobic; microbe; spatial gradient of oxygen

Introduction

The bacterial microbes that inhabit the intestinal tract form a complex community dominated by obligately anaerobic organisms from both the *Firmicutes* and *Bacteroidetes* Phyla.[1] Carbohydrates are a major source of energy for the microbiota that relies heavily on fermentative metabolism in the anaerobic environment. Considerable attention has focused on the mechanisms by which the saccharolytic microbes digest glycans to produce short chain fatty acids that, in turn, influence host physiology.[2] Although the gut microbiota in humans is generally stable in composition, alterations of the host induced, for example, by a change in diet[3, 4] or intestinal inflammation,[5] can alter both the microbiota composition and function.[6] Within a healthy host, there are differences in the gut microbiota along the longitudinal axis of the gut[7] as well as between different regions within a single fecal sample.[8] In this report, we examined the radial distribution of the gut microbiota and attempted to identify host-derived factors in the intestinal environment that could influence its composition. Identification of such factors could improve our understanding of the taxonomic differences between the mucosally-associated and fecal microbiota in health as well as the development of the "dysbiotic" microbiota associated with intestinal inflammation.

The intestinal niche is largely devoid of oxygen. Several lines of evidence implicate both aerobic and facultative anaerobic bacteria in the development of the anaerobic environment.[9] Culture-dependent surveys of the newborn microbiota suggest that the initial community of aerobic and facultative anaerobic bacteria might consume oxygen in the

intestine, allowing the population of obligate anaerobes to develop.[10, 11] This observation is supported by more recent studies, showing predominance of oxygen tolerant *Gamma-Proteobacteria* in the gut microbiota of newborns.[12] In addition, analysis of flatus composition in humans reveals a mixture of gases produced by microbial metabolism, namely hydrogen, carbon dioxide, methane, and hydrogen sulfide. Abundant levels of nitrogen are also present, but oxygen levels are very low.[13] However, direct evidence for the role of the gut microbiota in regulating oxygen levels in the intestinal lumen is still lacking.

The mechanism responsible for maintaining the anaerobic environment of the gut lumen is unclear, due in part to the difficulty in quantifying intestinal oxygen levels. Although there are several reports on measurements of oxygenation of the intestinal tissue,[14, 15] very few describe measurements of oxygen directly in the lumen of the gut. Most experiments relied on Clark-type oxygen electrodes,[16-18] showing that the pO_2 (partial pressure of oxygen) in the lumen is less than 0.5 mmHg.[16] However, use of electrodes is complicated by their invasiveness and potential leaks of oxygen into the measurement environment. Recently, EPR (Electron Paramagnetic Resonance) oximetry was applied to non-invasively image gut oxygen levels in mice.[19] A spin probe (charcoal) was delivered orally, and the decay of the spin polarization was used to assess oxygen concentration.[20] The measured pO_2 levels decreased from 58 mmHg in the stomach to 3 mmHg near the distal sigmoid colon, significantly exceeding those measured by electrodes.[16, 18]. One possible source of the discrepancy is that the charcoal probe was calibrated using aqueous suspensions, while the measurements were performed when the probe was in viscous fecal material. Large differences between the rates of oxygen diffusion in these two environments may have caused errors, since the signal in EPR oximetry (EPR line width) is a function of both oxygen concentration and oxygen diffusion coefficient.

In the present study we adapted the phosphorescence quenching method[21] to measure oxygen levels in the intestinal lumen. Using a newly developed probe, we quantified oxygen levels in the intestinal lumen, gaining evidence that bacteria in the gut consume oxygen delivered by the colonic tissue. Furthermore, we demonstrate enrichment of oxygen-tolerant bacteria in the vicinity of the rectal mucosa in healthy human subjects as well as an unanticipated signature of asaccharolytic bacteria, which are dependent upon the metabolism of proteinacious substrates in the rectal mucus. Together, the compendium of bacteria exhibiting aerotolerance and protein-based metabolism distinguish the mucosally-associated microbiota from the feces. These bacterial taxa may play a role in the development of the dysbiotic microbiota associated with the inflammatory response in Crohn's disease and ulcerative colitis.[5]

Materials and Methods

Molecular oxygen (O_2) quenches phosphorescence originating from excited triplet electronic states of molecules. The dependence of the phosphorescence lifetime (τ) on the pO_2 in the environment throughout the range of biological oxygen concentrations follows the Stern-Volmer model (Eq. 1): $1/\tau=1/\tau_0+k_q\times pO_2$, where τ is the phosphorescence lifetime, and τ_0 and k_q are probe-specific parameters. By exciting an object containing a probe with a pulse of light and measuring the phosphorescence decay, pO_2 in the environment can be

quantified. The measurements have millisecond response, high specificity and are independent of the probe distribution throughout the environment. The synthesis of the oxygen probe, the details of *in vivo* phosphorescence measurements as well as the methods for acquisition of human samples, storage, and sequencing can be found in the Supporting Information.

Animals

C57/B6 mice 8-12 weeks of age were used. All had free access to chow (AIN76, Research Diets). Anesthesia was induced through a nose cone *via* inhalation of isoflurane 2.5% mixed with air, after which isoflurane proportion was decreased to 1.5%. Low levels of isoflurane (<3%) do not cause significant changes in oxygenation[22]. Animals were anesthetized, and a laparotomy performed in some cases (see Discussion for details). At the end of the experiments, the mice were euthanized according to guidelines established by the American Veterinary Medical Association Panel on Euthanasia. Experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania.

Results

Oxygen measurements

The synthetic phosphorescent probes for tissue oxygen measurements [23-26] do not interact with proteins or other endogenous molecules, and the calibration parameters of these probes remain unchanged in any aqueous environment, ensuring absolute oxygen quantification.[27] In the present work, we used one such probe, Oxyphor G4,[24] to measure pO_2 in the intestinal tissue. The probe was injected into the tail vein in mice, and measurements were performed in reflectance-type geometry (Fig. 1A). Excitation photons (λ_{ex}=635 nm) are able to diffuse several cm into the tissue,[24, 28] while excitation efficiency decreases exponentially with depth. The largest contribution to the signal is from the probe-containing layers closest to the fiber tip; however, in mice phosphorescence could be considered averaging over the thickness of the entire intestinal wall, which is ~300 μm thick.

For quantitative oxygen measurements, the probe constants have to be determined in the same medium as encountered by the probe during measurements. In particular, quenching constant k_q depends on the rate of oxygen diffusion that differs between viscous intraluminal substances and water. Thus, calibration parameters obtained in solutions/suspensions are not suitable for measurements in the lumen.[19] Furthermore, properties of molecular probes, such as Oxyphor G4, may be affected during their transit through the intestinal tract, e.g. due to actions of gut enzymes, bile acids and/or interactions with processed food.

The quenching constant k_q is a function of the viscosity of the medium through which oxygen must diffuse during the probes' excited triplet lifetime (τ_0=200-300 μs for probes like Oxyphor G4). We reasoned that probe molecules dispersed in a large particle would predominantly encounter oxygen that traveled only within that particle. For example, in a 20 μm particle (~10^8 probe molecules at 50 μM concentration) less than 1% of the probe will experience collisions with oxygen entering from the outside, assuming oxygen diffusion coefficient in the particle is 2×10^{-7} cm^2s^{-1} (i.e. 100x lower than that in water, a typical value

for solid polymers).[29, 30] Hence, calibration of such a particle should be minimally affected by the properties of the environment, while oxygen equilibrium between the inside and outside of the particle would be established in milliseconds.

Based on the above, a probe was prepared, abbreviated OxyphorMicro, comprising polymethylmetacrylate (PMMA) particles (~10-20 μm in diameter) containing co-dissolved phosphorescent Pd tetrabenzoporphyrin (PdTBP)[24] (Fig. 1C). The oxygen diffusion coefficient in PMMA is 4×10^{-8} cm^2s^{-1} at 25°C.[30] OxyphorMicro was calibrated in aqueous suspensions as well as directly in fecal material extracted from the mouse cecum (see SI). The resulting plots (Fig. 1D) revealed good animal-to-animal reproducibility and almost no alterations between environments of different viscosity, suggesting that phosphorescence quenching is minimally affected by the medium and can be a robust indicator of luminal oxygenation.

OxyphorMicro was mixed with chow, and after ingestion it remained only in the intestinal tract due to its hydrophobicity and inability to diffuse into the tissue. Indeed, large PMMA particles cannot be endocytosed by cells at the mucosal interface and/or distributed throughout the tissue by the lymph flow. At the same time, hydrophobic PdTBP dye cannot leach from the nanoparticles into a hydrophilic environment. Measurements in the gut lumen were performed in the same fashion as in tissue (Fig. 1A). After laparotomy the fiber tip was positioned directly by the organ of interest. Furthermore, because the probe phosphorescence was very strong, measurements could also be accomplished in intact animals by placing the fiber against the shaved abdomen. In this configuration, excitation light diffuses through the abdominal wall and intestinal tissue before reaching the probe. As a result, origin of the signal could not be attributed to any specific part of the intestine. However, there was very little variation in the measured oxygen values regardless of the fiber position (*vide infra*). All values revealed the luminal pO_2 levels below 1 mmHg. The pO_2 in laparotomized mice was always somewhat higher, probably due to diffusion of oxygen from the air after surgery (Fig. 2B,C).

Phosphorescence oximetry reveals exchange of oxygen between the host and gut microbiota

Intestinal tissue measurements in the cecum revealed baseline pO_2 levels of approximately 40 mmHg (Fig. 2A), which is in good agreement with earlier measurements by oxygen electrodes.[15] Inhalation of pure oxygen led to a rapid increase in tissue pO_2, which reached a new steady state and, as expected, decreased to the baseline once the mouse was returned to ambient air. Similar changes in tissue pO_2 upon inhalation of pure oxygen have been observed previously in other tissues.[31]

Remarkably, inhalation of pure oxygen also led to an increase in the luminal pO_2, which dropped upon return to ambient air. Similar changes were observed using either a trans-abdominal (Fig. 2B) or after-laparotomy measurements (Fig. 2C). The increase in luminal oxygenation due to the inhalation of pure oxygen is slower than the concurrent increase in the tissue pO_2 (Fig. 2D), and a steady state is not reached, as oxygen continues to diffuse slowly into the lumen over more than 500s (Figs. 2B,C). Furthermore, upon return to ambient air, luminal oxygen levels failed to reach the baseline (Fig. 2B, dotted line). This

behavior may be rationalized considering that diffusion of oxygen through the fecal substrate is significantly slower than through liquid. Based on the diffusion theory, if the oxygen diffusion coefficient in the intraluminal medium is 2×10^{-6} cm^2s^{-1} (only 10 times lower than in water), complete tissuelumen equilibrium should take ~30-50 min to establish in an intestinal tube 2-3 mm in diameter.

At the initial state, the oxygen gradient extends from the tissue throughout the mucosal interface into the lumen (Fig. 1A), whereby microorganisms adjacent to the tissue consume most of the available oxygen, keeping the bulk of the lumen deeply anaerobic. The fact that phosphorescence measurements in intact animals show overall very low baseline intraluminal pO_2 (Figs. 2B,C) suggests that the oxygen-containing layer, which is the closest to the tissue and to the fiber tip, is very thin and contributes minimally to the overall signal. Therefore, we conclude that the oxygen gradient in the lumen must be very steep. Additional studies will be needed to quantify the dynamics of oxygen consumption at the mucosal interface. Overall, these results demonstrate that changes in host oxygenation alter oxygen content of the luminal environment, raising the question of whether oxygen levels may thereby modulate the composition of the gut microbiota.

Hyperbaric Oxygen Therapy (HBOT) results in alterations in gut microbiota composition

Having demonstrated that enhanced host oxygenation alters luminal oxygenation in the gut, we examined the effect of HBOT on the composition of the gut microbiota in mice. HBOT (100% O_2 at 2.0 atm) was delivered to mice for 2 h daily for 9 days, similar to clinical protocols.[32, 33] Under these conditions, HBOT increases tissue oxygenation by up to 5-fold.[32, 33] Control animals were also placed into HBOT chambers for the same period of time, but ambient oxygen pressure was maintained. Fecal pellets were collected at days 1 (baseline), 4, 6, and 9, and DNA was extracted and prepared for 16S rRNA gene sequencing. Sequence data from control and HBOT mice were compared using UniFrac. Abundance-weighted analysis showed no differences among groups. However, a non-weighted analysis of community membership showed differences after HBOT treatment [p=0.043 (day 4), p=0.008 (day 6), p=0.021 (day 9); day 6 data in Fig. 3A], but no significant difference at day 1 (p=0.17). A second experiment (data not shown) also demonstrated a difference in the gut microbiome after HBOT. Analysis of bacterial abundance using a generalized linear mixed effects model (Fig. S3) showed that 28 lineages changed detectably after HBOT, most with complex temporal behavior. An analysis of community membership showed that one lineage--*Anaerostipes*, a catalase-negative obligately anaerobic Firmicute--was detected in fewer samples after HBOT (p<10e-10), consistent with selective loss of an oxygen-intolerant lineage (Fig 3B). These results demonstrate that host oxygenation can alter the composition of the gut microbiota, but the short-term effects are both modest and complex.

The gut microbiota in rectal biopsies are enriched for oxygen tolerant organisms with catalase activity

Our results suggest that a fraction of the gut microbiota consumes oxygen, leading to extremely low oxygen levels at the baseline and causing a decrease in luminal pO_2 after an oxygenation burst (Figs. 2B,C). Using samples collected from a controlled feeding

experiment in ten healthy human subjects over a ten-day inpatient stay,[3] we searched for such a bacterial population at the mucosal interface. Fecal samples were collected daily, and an unprepped flexible sigmoidoscopy was performed on days 1 and 10 of the study to obtain rectal biopsies. Although the composition of the fecal microbiota reached a new steady state within 24 h of the dietary intervention, there were no consistent taxonomic alterations associated with this dietary intervention, i.e. intersubject variability remained the largest source of variance.[3] However, the mucosally-associated microbiota in the rectal biopsies was found to be distinct (Fig. 3E). We quantified the unassigned reads in each rank in taxonomy based on sample type to exclude the possibility that this difference was due to a bias in reads assigned to the different groups (Fig. S4).

Based the notion of the host-gut microbiota oxygen gradient, we hypothesized that the mucosally-adherent microbiota would have a higher representation of oxygen tolerant bacterial taxa when compared to the bacteria residing in feces. Bacteria can be classified into five groups by their ability to utilize oxygen for cellular functions: obligate aerobes, microaerophiles, facultative bacteria, aerotolerant bacteria, and obligate anaerobes. Based on the literature,[34-36] obligate anaerobic bacteria can be classified at the genus level,[37] and each of the 70 bacterial genera in the stool and biopsy samples with proportion greater than 0.002, were classified into one of these categories (Table S1). For each participant, we then determined whether the predominant organisms in each stool sample and biopsy specimen were obligate anaerobes or “all others” based on their averaged abundance on days 1 and 10. For this analysis, we classified bacterial described as “anaerobic” in the literature as being “obligate anaerobes” because they were also catalase negative. For each participant, we computed an odds ratio of obligate anaerobic bacteria being more abundant in the stool sample than in the biopsy sample. The one-sample Wilcoxon signed-rank test was used to test for the null hypothesis of no enrichment, that is, population median odds ratio equals one. This analysis, shown in Fig. 3C, revealed that bacteria that are capable of tolerating oxygen were greatly enriched in the mucosal biopsy samples relative to the stool ($p=0.0001$).

Survival of oxygen tolerant bacteria requires the expression of ROS (reactive oxygen species) detoxifying enzymes, such as catalases, peroxidases and superoxide dismutases.[38] Catalase activity in the 52 genera (Table S1) was found to be more commonly expressed in the mucosally-associated microbiota than in the microbes residing in the stool (Fig. 3D, $p=0.002$). In total, these results demonstrate that the mucosally adherent microbiota is better adapted for residence in an oxygen-containing environment relative to the microbiota in the feces.

Aerotolerant and asaccharolytic protein metabolizing bacterial taxa distinguish rectal mucosally-associated microbiota from feces

Mucosally-adherent bacteria reside primarily within the outer intestinal mucus layer[39] where many of the bacterial taxa are saccharolytic, i.e. capable of harvesting energy through the digestion of carbohydrates.[2, 40] To compare mucus-associated gut microbiota relative to the stool, we collected rectal mucus using a swab inserted through the anus, in parallel with stool from several healthy human subjects and performed 454 16S gene sequencing from the

isolated DNA. PCoA of a non-weighted Unifrac analysis examining the presence vs. absence of bacterial taxa revealed that microbiota obtained by rectal swab and biopsy were more similar to each other than to the microbiota of the stool (Fig. 3E). PERMANOVA with permutation revealed significant differences between groups (p=0.0001) with differences between swab and stool (p=0.009) and biopsy and stool (p=0.001), dominating the difference between the swab and biopsy samples (Fig. 3F). The similarity between the biopsy and swab specimens predominated despite the strong contribution of intersubject variability.

Taxa from these three sampling sources are visualized in a heat map (Fig. 4). The greater similarity between the rectal swab and biopsies relative to the feces on the unweighted Unifrac analysis (Figs. 3E,F) is due the consistent presence of nine low-abundance bacterial taxa in the mucosal samples that are absent in most stool samples (Fig. 4). These included four genera from cluster XIII of the Clostridium in the Firmicutes phylum (*Anaerococcus*, *Finegoldia*, *Murdochiella*, and *Peptoniphilus*),[41] one Bacteroidetes (*Porphyromonas*), two Proteobacteria (*Campylobacter* and *Enterobacteriaceae*) and one Actinobacteria (*Corynebacterium*) (Table S2). Despite their divergence in phylogeny, these six genera of the Firmicutes, Bacteroidetes, and Proteobacteria phyla contain asaccharolytic organisms that require proteinaceous substrates as carbon and energy sources.[41-45] In addition, four taxa in the Actinobacteria and Proteobacteria phyla are either aerobic, facultatively anaerobic, or microaerophilic organisms that exhibit tolerance to oxygen. These results show that the mucosally-adherent microbiota can be distinguished from those in the stool by the presence of a consortium of asaccharolytic and aerotolerant bacteria.

Genes for carbohydrate metabolism are decreased in abundance in the mucosally-adherent microbiota

Analysis of gene representation using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt), which infers full gene composition from 16S rRNA sequence data,[46] allowed us to perform metabolic reconstruction of the stool and mucosally-associated microbiome.[47] A heatmap of gene abundance assigned to major metabolic pathways revealed a lower abundance of carbohydrate metabolism genes in the microbiome obtained by swab (Fig. S5). Although the percent of genes associated with carbohydrate metabolism was 10-12% in the stool and paired biopsy specimens, the percentage obtained by rectal swab was significantly lower (Fig. 5, p=0.016 by exact binomial test) consistent with the maximal separation between rectal swab and the other two sample sources (Fig. 3F). This observation supports the idea that asaccharolytic bacteria are associated with the rectal mucus.

Discussion

Previous studies established that the composition of the gut microbiota differs along the longitudinal axis of the gut. Here we report that the gut microbiota is also segregated radially and correlated with radial oxygen gradient and distribution of the tissue-associated mucus, which provides a nutrient source. Using the phosphorescence quenching method, we provide the first direct evidence that oxygenation of the host influences gut luminal

oxygenation. Luminal oxygenation increased upon increase in host oxygenation, demonstrating diffusion of oxygen from the host tissue into the intestinal lumen and suggesting the presence of a spatial oxygen gradient. The decrease in the luminal pO_2 upon return of the host tissue to normoxia supports the notion that oxygen is being consumed by the gut microbiota residing close to the mucosal interface. Our data show an increase in the abundance of oxygen-tolerant organisms adherent to the mucosal surface, consistent with this idea. A luminal oxygen gradient might play a role in “aerotaxis” of motile bacteria, through bacterial energy sensing pathways[48], into the mucus layer where they are able to survive and proliferate. This resembles the ecology of environmental microoxygenic zones, such as activated sewage sludge,[49] marine snow and soil aggregates that develop oxygen gradients, whereby microbes juxtaposed to the oxygenated environment consume oxygen before it reaches the interior of the microbial community.[50] The presence of a spatial gradient of oxygen in the lumen provides an explanation for the enrichment of aerobic and facultative anaerobic organisms from the Proteobacteria and Actinobacteria phyla in rectal biopsy and swab samples.

The results of our studies of HBOT in mice show that alteration of host oxygenation can modify the composition of the gut microbiota. These results are in agreement with previous culture-based studies on the effect of HBOT in a model of intestinal obstruction[51]. Nevertheless, the results suggest that the effects, at least in the short-term, may be modest and complex. Increased tissue oxygenation could directly influence microbes, such as reducing *Anaerostipes*, but could also have an effect on host immune system,[33] that could indirectly influence the gut microbiota composition. In addition, alterations in the host oxygenation might alter mucosallyadherent bacterial populations differently than those in the feces.

In addition to the presence of mucosally-associated oxygen tolerant taxa, there were six additional taxa found exclusively in biopsies and rectal swab samples, but not in the feces. Remarkably, all six represent assacharolytic bacteria that utilize proteinaceous substances rather than carbohydrates. Because all but one were also found in rectal swab samples, we infer that these assacharolytic organisms reside in the intestinal mucus layer, which is a richer source of proteinaceous substrates than feces. The protein source might be shedding of the intestinal epithelium into the mucus,[52] or the protein may come from the mucus itself, since mucus is composed of glycoproteins.[2, 52, 53] The ability of saccharolytic bacteria to digest glycans in mucus[2, 40] may help polypeptides to become more accessible for assacharolytic bacteria, enhancing the syntrophic interactions between Bacteroides and Clostridial genera.[2] Taken together these results highlight both mutualistic and syntrophic interactions between bacteria that play a role in the coaggregation of taxa residing together in a spatially-defined architecture such as have been described for biofilms.[39, 52] Such interactions have been recently suggested by dense metabolomic and taxonomic profiling of endoscopic mucosal lavage samples from the colon.[54]

The observed enrichment of Actinobacteria and Proteobacteria in the mucosally-associated microbiota in healthy human subjects may also be relevant to intestinal disease states. Both of these phyla contain taxa, such as enterobacteriaceae, that are consistently observed in the dysbiotic microbiota associated with inflammatory bowel disease.[55] This supports the notion

that dysbiosis is the result of the oxidative nature of the host inflammatory response.[47, 56] These aerotolerant organisms, normally residing at low levels in intestinal mucus[8] in a healthy host, could serve as keystone communities that expand during intestinal inflammation facilitated by the greater flux of oxidative metabolites and oxygen into more highly hydrated feces with the development of diarrhea.[55] In this manner, the oxidative nature of the intestinal inflammatory response may affect the community membership, gene expression, and function of the gut microbiota[47, 50, 57, 58] in a way that perpetuates the inflammatory condition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

pO_2	partial pressure of oxygen
EPR	Electron Paramagnetic Resonance
SNR	signal-to-noise ratio
PMMA	polymethylmetacrylate
PdTBP	Pd tetrabenzoporphyrin
ROS	reactive oxygen species
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
HBOT	hyperbaric oxygen therapy

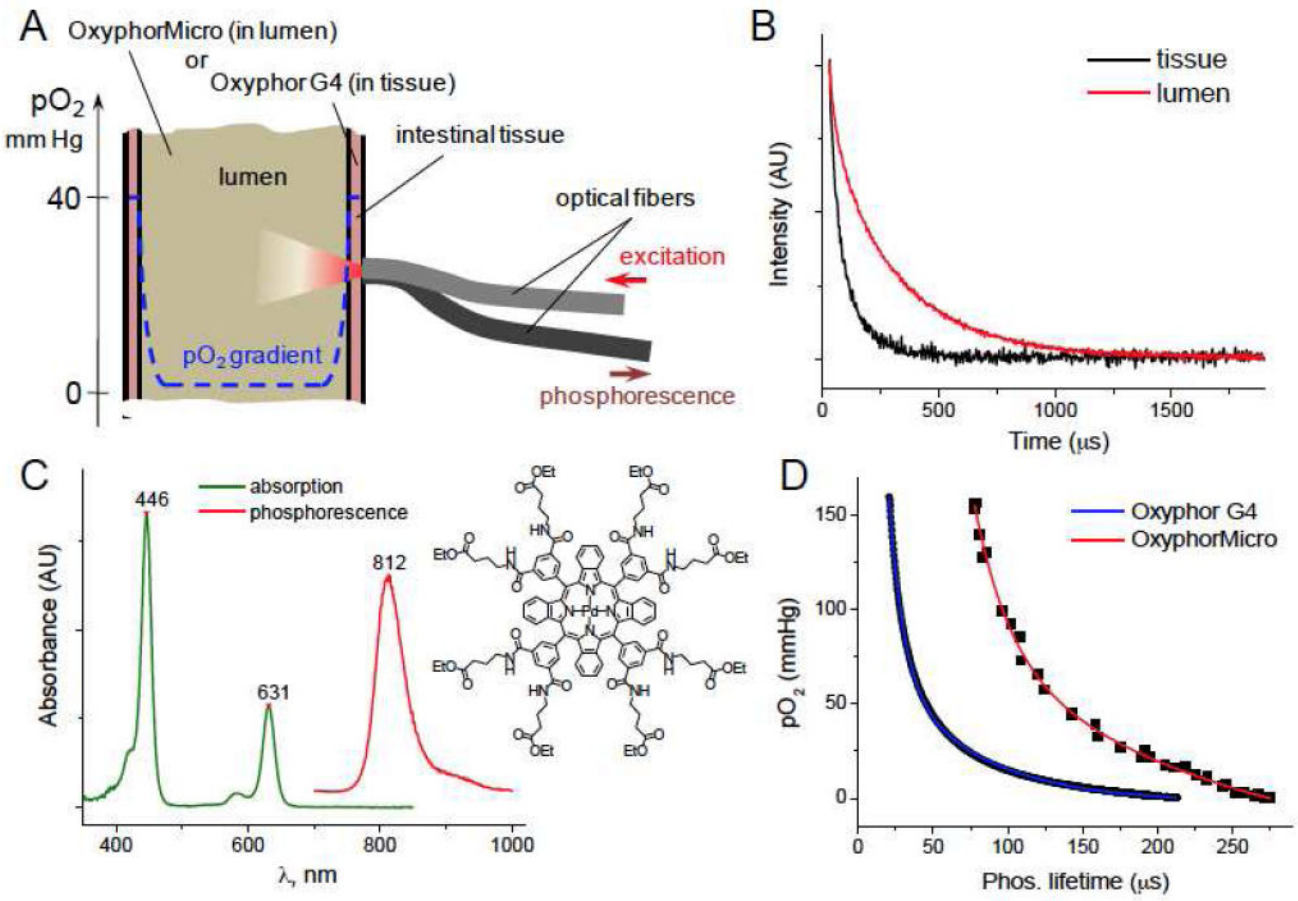

Figure 1.

Application of phosphorescence oximetry to intestinal oxygen measurements. A) Intestinal oxygen measurements by phosphorescence quenching (the scheme does not represent correct proportions of physical dimensions). B) Phosphorescence decays typically observed in tissue (Oxyphor G4) and intraluminal (OxyphorMicro) oxygen measurements. C) Structure, optical absorption, and phosphorescence spectra of Pt tetrabenzoporphyrin used as an oxygen sensing element in both Oxyphor G4 and OxyphorMicro. D) Calibration curves for Oxyphor G4 in physiological saline and OxyphorMicro directly in mouse fecal material at 36.5°C. Data for OxyphorMicro are a superposition of several animal samples.

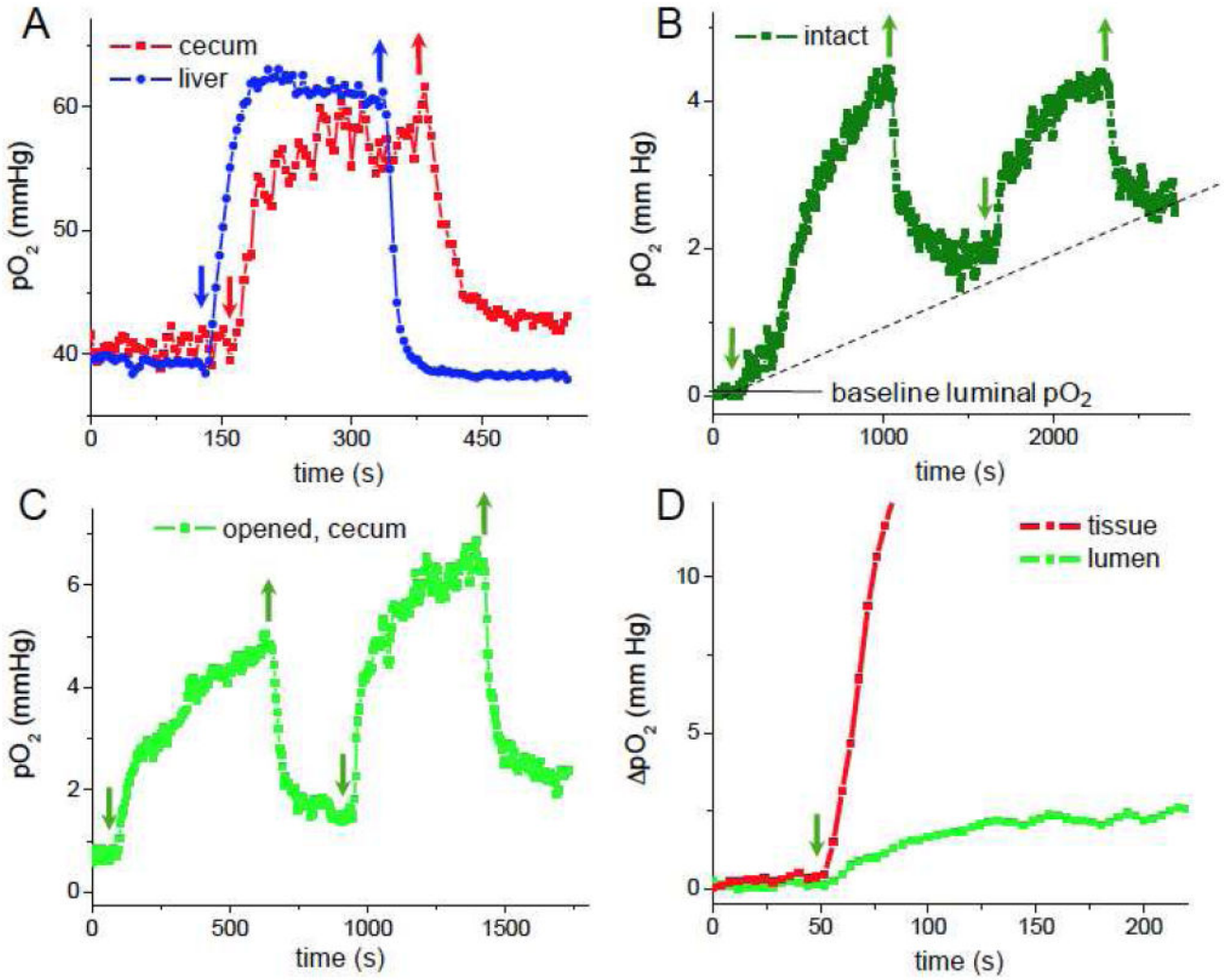

Figure 2.

Effect of altering oxygen concentration in the inhaled gas mixture on host tissue and gut luminal oxygen levels. Arrows indicate switching pure O_2 on (↓) and returning to ambient air (↑). A) Changes in intestinal tissue pO_2 in the cecum. Data for the liver tissue are shown for comparison. Changes in intestinal luminal pO_2 using trans-abdominal measurements (B) and in the cecum after laparotomy (C). D) Relative time-dependent changes in tissue and luminal (cecum) pO_2's: $\Delta pO_2 = pO_2(t) - pO_2(0)$, where $pO_2(t)$ and $pO_2(0)$ are the pO_2 values measured at time t and in the beginning of the experiment (time zero), respectively.

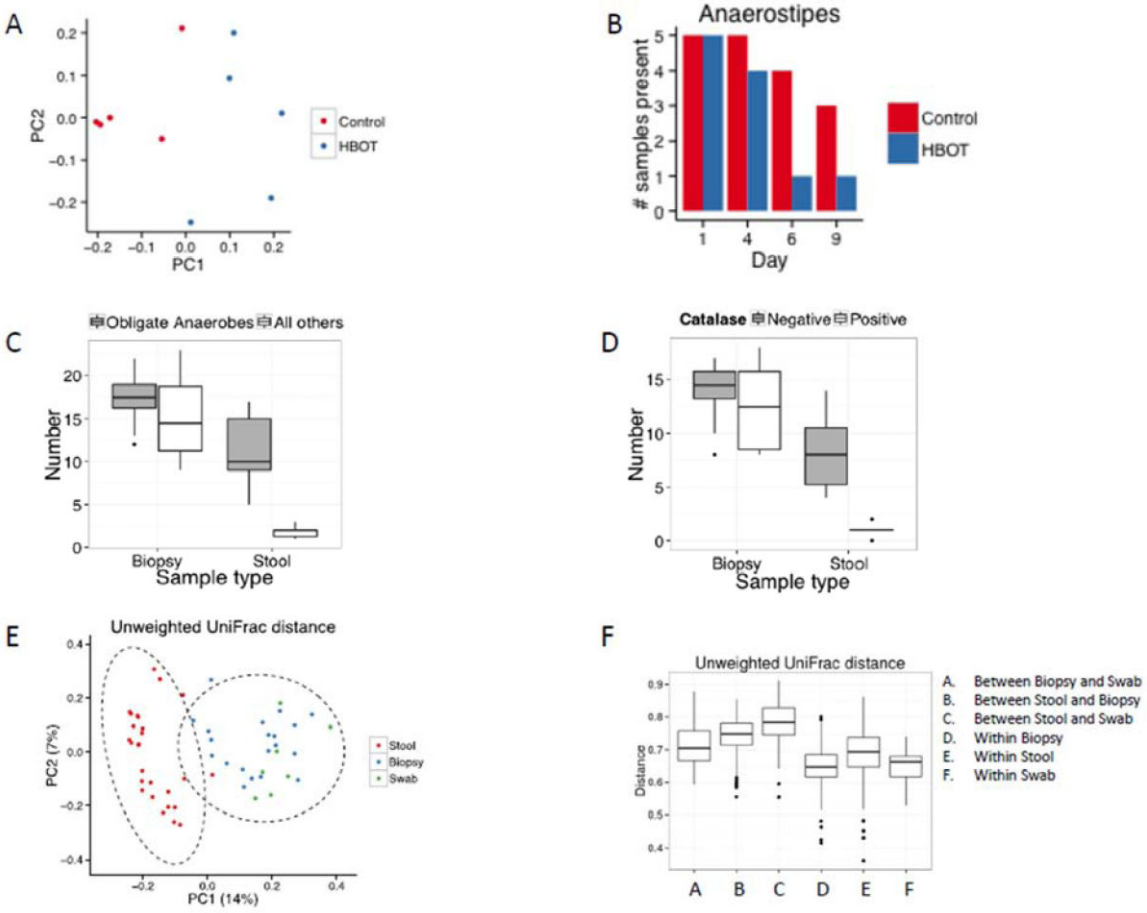

Figure 3.

Differences in oxygen tolerance and community membership between the mucosallyassociated and fecal microbiota. Bacterial communities were profiled using 16S rRNA gene sequencing. A) Separation of murine bacterial communities, analyzed using 16S rRNA gene sequencing of V1V2 tags, after day 6 of Hyperbaric Oxygen Therapy (HBOT). An unweighted UniFrac plot is shown (p=0.008; permanova). B) Number of samples with OTUs annotating as *Anaerostipes*. The difference achieves p<10e-10 (logistic mixed-effects model). C) Number of bacterial genera classified as obligate anaerobes vs. more oxygen-tolerant “all others” in paired human rectal biopsy and stool samples. D) Number of bacterial genera classified as catalase positive vs. negative in paired human rectal biopsy and stool samples. E) Relationship between mucosally- and stool-associated microbiota using an unweighted Unifrac PCoA ordination (Ellipses represent 95% confidence intervals for a multivariate normal distribution). F) Pairwise distance in microbiota composition between stool, rectal biopsy, and rectal swab samples.

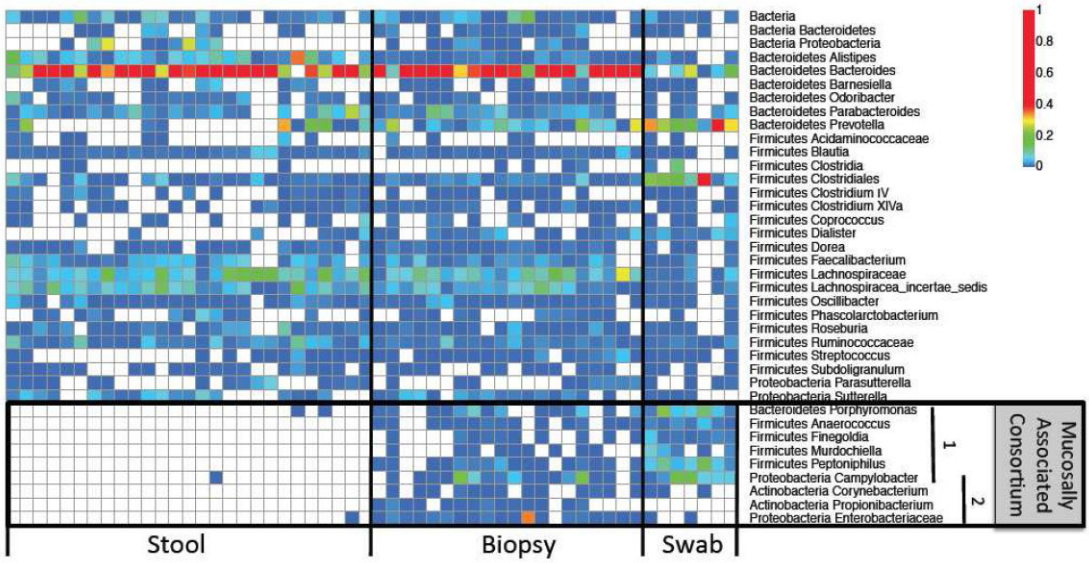

Figure 4.

Heatmap of the microbiota in stool, biopsy, and rectal swab samples from 16S rRNA gene sequencing. In the “Mucosally Associated Consortium” 1=Asaccharolytic bacteria and 2=Aerobic or facultative anaerobic bacteria.

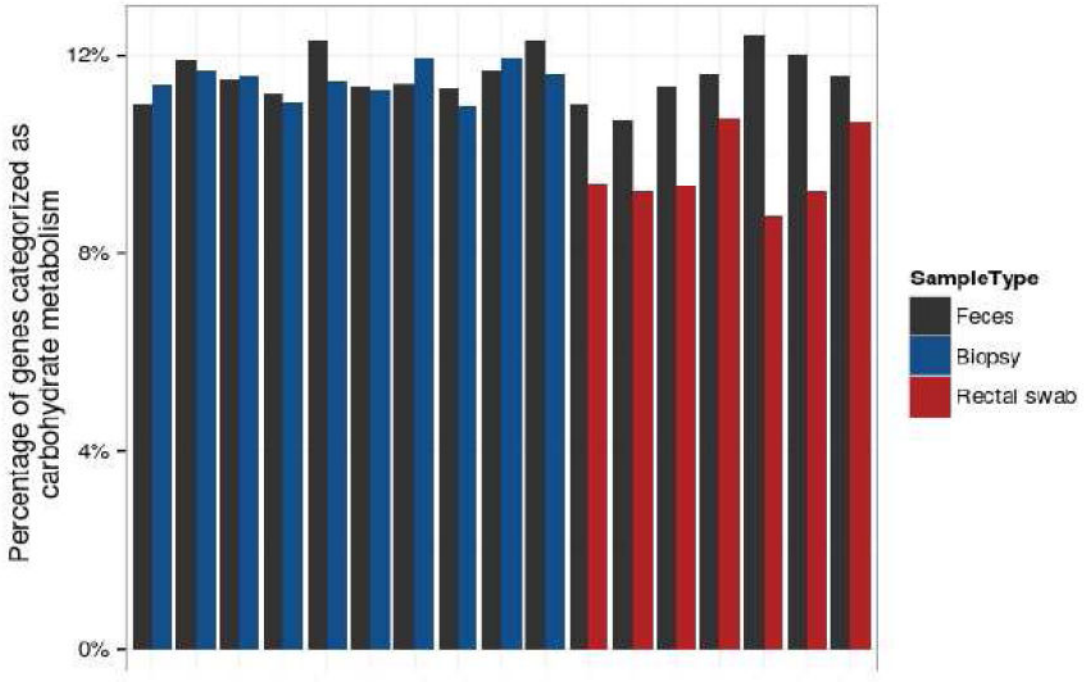

Figure 5.

Gene content in gut bacteria inferred from 16S rRNA gene data. Gene content was inferred by using 16S rRNA gene tag data to access whole genome sequences of bacterial relatives, and this information was used to infer potential gene content in the bacterial communities studied.