SUPPORTING INFORMATION

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

Short title: Oxygen gradient and the gut microbiota

Albenberg, LA[1],[a]Esipova, TV[3],[a] Judge, CP[1], Bittinger, K[4], Chen, J[5], Laughlin, A., Grunberg, S[4], Baldassano, RN[1], Lewis, JD[2, 5], Li, H[5], Thom, SR[6], Bushman, FD[4], Vinogradov, SA[3],* and Wu, GD[2]*

[1]Division of Gastroenterology, Hepatology, and Nutrition, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA;
[2]Division of Gastroenterology, [3]Department of Biochemistry and Biophysics, [4]Department of Microbiology, [5]Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104; [6]Dept. of Emergency Medicine, University of Maryland, Baltimore, MD 21201

Phosphorescent probe for intraluminal oximetry. To prepare probe OxyphorMicro, polymethylmetacrylate (PMMA, Aldrich, 2 g) was dissolved in dichloromethane (CH_2Cl_2, 10 ml). Pd *meso*-tetraaryl-tetrabenzoporphyrin (1) (Fig. 1C main text) was added to a final concentration of ~20 μM, and the solution was added drop-wise to rigorously stirred hexane (50 ml). The initially formed fine precipitate coagulated, yielding a viscous mass, which was separated, washed on a filter with hexane (3x50 ml) and dried under vacuum. The resulting solid was transferred into a porcelain cup and ground to yield a greenish powder that was subsequently dried in vacuum and used directly in intraluminal oxygen measurements.

Calibration of probe Oxyphor G4 in solution. Probe Oxyphor G4 in aqueous solutions was calibrated according to the previously published method (1), using a setup developed specifically for oxygen titrations of phosphorescent probes (2, 3) and a home-built phosphorometer (1, 4).

Calibration of probe OxyphorMicro in viscous (e.g. intraluminal) materials. The setup used for calibration of semi-solid samples containing probe OxyphorMicro is shown in Fig. S1. A glass vial (~2 cm in diameter) with a gas-impermeable stopper and magnetic stirring bar was positioned inside a thermostat chamber. Temperature inside the chamber was controlled with up to ±0.1°C accuracy. The chamber was equipped with a magnetic stirrer. An aqueous solution of Oxyphor G4 (5 μM) was added to the vial to make up a ~5 mm-high liquid layer. Above that layer, a plastic porous disk was positioned in order to support another small open-top glass vial (~5 mm in diameter). The small vial was placed next to the wall of the large vial, near the entry port for a pair of optical fibers (3 mm in diameter) for excitation and collection of phosphorescence. The fibers were ran through a channel in the thermostat and connected to the optical ports of the phosphorometer. A similar pair of fibers ran through another channel at the level of the liquid solution (Oxyphor G4) and attached to another phosphorometer.

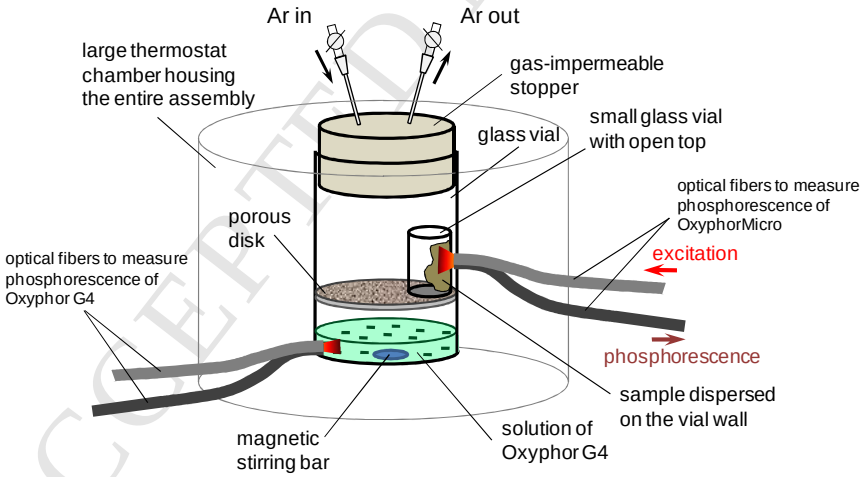

Figure S1. Setup used for calibration of phosphorescent probe OxyphorMicro in semi-solid samples.

A sample (e.g. a material extracted from the cecum of a mouse fed previously with chow containing OxyphorMicro), was deposited onto the wall of the small vial and spread on the wall to give a thin layer. The layer had to be thin in order to insure quick equilibration of the probe immersed in the material with the gas phase in the chamber. The vial was closed with the stopper containing two needle gas ports with stopcocks and left to equilibrate at 36.5°C , while the phosphorescence lifetime in both the sample and Oxyphor G4 solution was continuously monitored (at e.g. 10 s intervals), until the readings became stable. Importantly, because the sample at all times was inside the closed vial containing aqueous solution, it did not dry during

the calibration. Drying could cause changes in the sample viscosity, which, in spite of relative insensitivity of OxyphorMicro to the medium properties, still could affect the calibration.

After lifetime readings reached a stable baseline, both OxyphorMicro in the sample and Oxyphor G4 in aqueous solution were considered to be at equilibrium with air (pO_2~155 mmHg, i.e. 21% O_2 in the atmosphere at total pressure of 760 mmHg, minus the water vapor pressure at 36.5^{o}C). This was confirmed by phosphorescence lifetime value of Oxyphor G4, which was pre-calibrated at exactly the same conditions (see above). The stopcock on the Ar inlet port was then opened for ca 5 s, after which both stopcocks were tightly closed. Letting in some Ar led to a rapid (seconds) rise in the phosphorescence lifetime of G4 to a new steady state, indicating that equilibration of the thin (~5 mm) stirred solution with the gas phase was very fast. In contrast, relatively slow rise in the lifetime of OxyphorMicro confirmed that diffusion of O_2 in the viscous material and equilibration with the gas phase was significantly slower. Once the equilibrium was reached, and the phosphorescence lifetimes of both probes were stable, a new portion of Ar was let in, replacing some more oxygen; and the procedure was repeated many times, until all oxygen was replaced from the vial. At each steady state, readings from Oxyphor G4 provided independent and accurate measurements of pO_2. An example trace recording is shown in Fig. S3, while superimposed calibration traces of three samples from three different mice are shown in Fig. 1 (main text). Similar curves were obtained when OxyphorMicro was mixed with artificial viscous material, e.g. toothpaste AquaFresh, and titrated in the same manner. The obtained data were fit to an arbitrary analytical function (e.g. hyperbola), which was used to convert phosphorescence lifetimes to oxygen pressures in *in vivo* experiments.

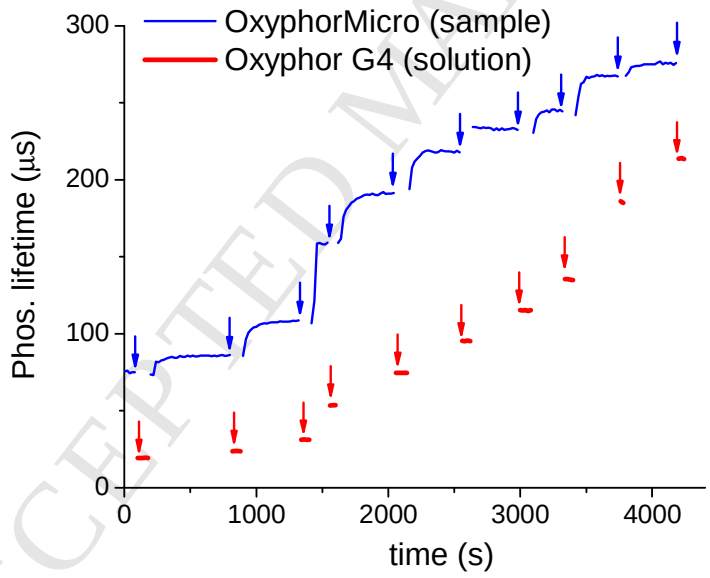

Figure S2. Typical calibration plot obtained using the setup shown in Fig. S1. Blue arrows indicate time points when readings of phosphorescence lifetimes of OxyphorMicro were taken, while red arrows show the matching lifetimes for solution of Oxyphor G4, from which pO_2 values were calculated.

Oxygen measurements in intestinal tissue and lumen of the gut. Oxygen measurements in the intestinal tissue of mice followed the protocols described previously (1). Oxyphor G4 (200 μM) was injected into the tail vein to an estimated final concentration in the blood plasma of ~2 μM. OxyphorMicro was admixed with chow to measure luminal oxygenation. In order to examine the effect of the host tissue oxygenation on intraluminal oxygen content, mice with the probe in the vasculature or in the lumen were subjected to brief periods of inhalation of pure oxygen through the anesthesia apparatus.

The optical configuration (Fig. 1A) resembled that used in our previous studies (1). A pair of closely positioned optical fibers (2 mm in diameter) for excitation and collection of phosphorescence were positioned right at the extraluminal surface of intestinal tissue. The probe in the tissue or lumen was excited by 10 μs-long pulses from an LED (635 nm) near the maximum of the absorption Q-band of Pd tetrabenzoporphyrin (Fig. 1B and C), and the phosphorescence was digitized (333 kHz) during 2 ms-long acquisition period. 100-400 decays (0.5-2 s total acquisition time) were averaged to obtain adequate signal-to-noise ratios (SNR). The resulting decays (Fig. 1B) were analyzed on-the-fly by the least-squares method using single-exponential model. The recovered decay times were converted into oxygen concentrations by applying Stern-Volmer calibration curves obtained independently (Fig. 1D; see SI for details of calibration experiments).

Human samples. Oxygen tolerance of bacterial taxa found in human biopsy specimens versus stool samples used samples collected during a controlled inpatient feeding study(5). Stool samples and rectal biopsy samples, through un-prepped flexible sigmoidoscopy, were collected on days 1 and 10. Paired rectal swab and stool samples were collected within 24 hours of each other from an additional 7 pediatric patients, ages 3-12, seen at The Children's Hospital of Philadelphia. Exclusion criteria included subjects with perianal disease and the use of antibiotics or probiotics between the two sample collections. Rectal swab samples, obtained during a routine clinical exam used a dry swab (CopanFlock Technologies) that was inserted 2-3 cm into the rectum, turned 360^{o}, removed, placed into a coded sterile tube, and frozen at -80℃ until analysis. The stool samples from the sub jects in this study were collected and stored using previously described methods (5).

Hyperbaric Oxygen Therapy (HBOT) of mice. 5 female C57B6/J mice, 8 weeks of age underwent hyperbaric oxygen therapy (HBOT). Pure oxygen (medical grade, 98%) at 2.0 atmospheres absolute pressure (ATA) was delivered daily for 2 hours per day for 5 consecutive days using a Bethlehem Steel Corp. Model G15-APSP hyperbaric chamber following a published protocol (6). After 48 hours of recovery time, the animals again underwent HBOT using the same 2 hour cycle for an additional 4 consecutive days. A second group of 5 control mice (same strain, sex, and age) were also placed into HBOT chambers for the same period of time but ambient oxygen and atmospheric pressure were maintained. During the study period, all animals were fed the same, normal chow diet (AIN-76, Research Diets). Fecal pellets were collected for 16S rRNA gene sequencing from each animal on day 1 (before therapy), and after 4, 6, and 9 days of therapy.

16S rRNA gene sequencing and bioinformatics. Sequence reads were acquired using the 454/Roche or Illumina platforms, and are available at the SRA. Reads were analyzed using QIIME version 1.6.0-dev(7). To control for quality, reads were required to have a perfect match to the molecular barcode of a sample and to the leading 16S primer sequence. Reads were removed from the analysis if they were less than 200bp in length, had more than 1 ambiguous base, or contained a homopolymer run longer than 6bp. OTUs were selected by clustering the sequences at 97% similarity with UCLUST version 1.2.22. Taxonomic assignments were generated with the RDP Classifier version 2.5, using the default RDP taxonomy. A phylogenetic tree was constructed with FastTree version 2.1.1, using the representative sequences for each OTU as input. Weighted and unweightedUniFrac distances were calculated for each pair of samples using the implementation in the PyCogent python library. To estimate the functional profile for each sample, the reads were processed with PICRUSt version 1.0.0, using the instructions included with the software(8). For presence-absence analysis, we tested taxa that were present in at least 10 animal/time point combinations and absent in at least 10 animal/time point combinations.

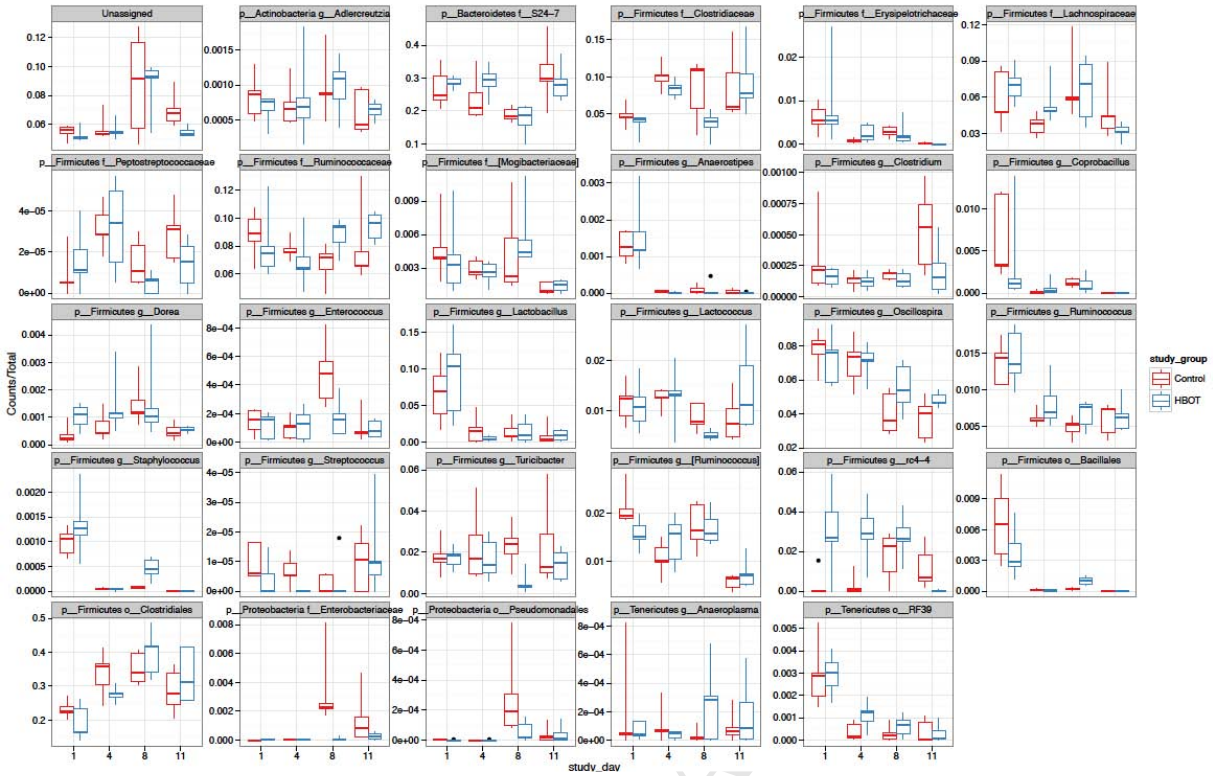

Figure S3. Bacterial lineages showing significant changes over time after HBOT versus controls (n=5 in each group). Longitudinal behavior was analyzed using a generalized linear mixed effects model, allowing a random intercept for each mouse, and tested for an interacton between treatment and time point. Shown are lineages achieving p<0.05 after correction for multiple comparisons.

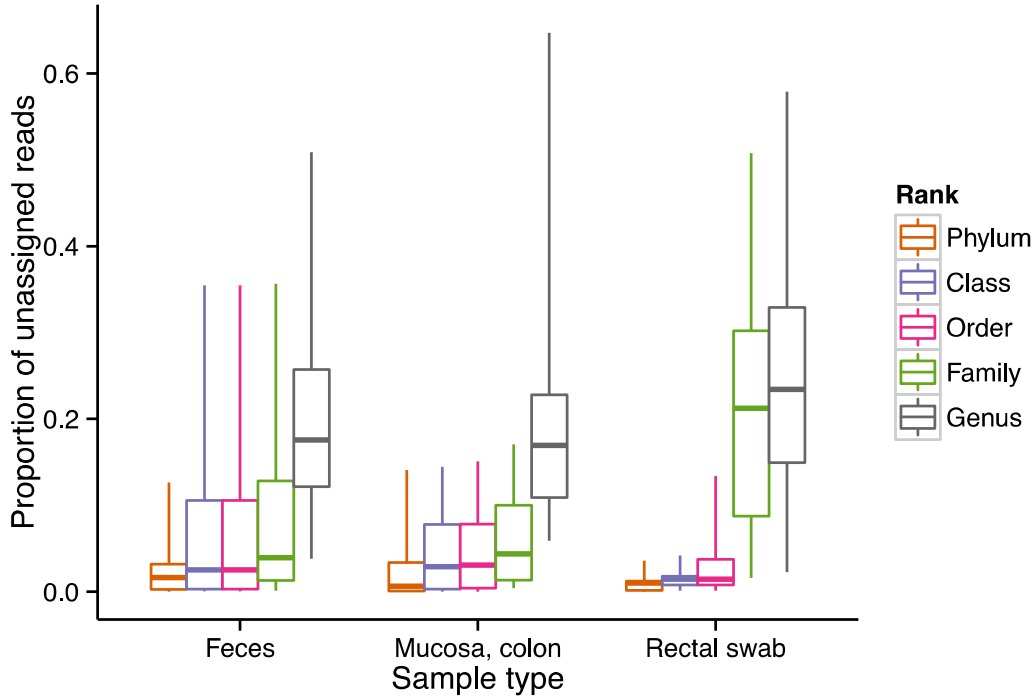

Figure S4. Unassigned reads at each rank in taxonomy based on sample type. An analysis using a Kruskal-Wallis test of ranked proportions was performed to determine if there was a difference in unassigned reads between sample types. There was no statistically significant difference (p-value = 0.4848).

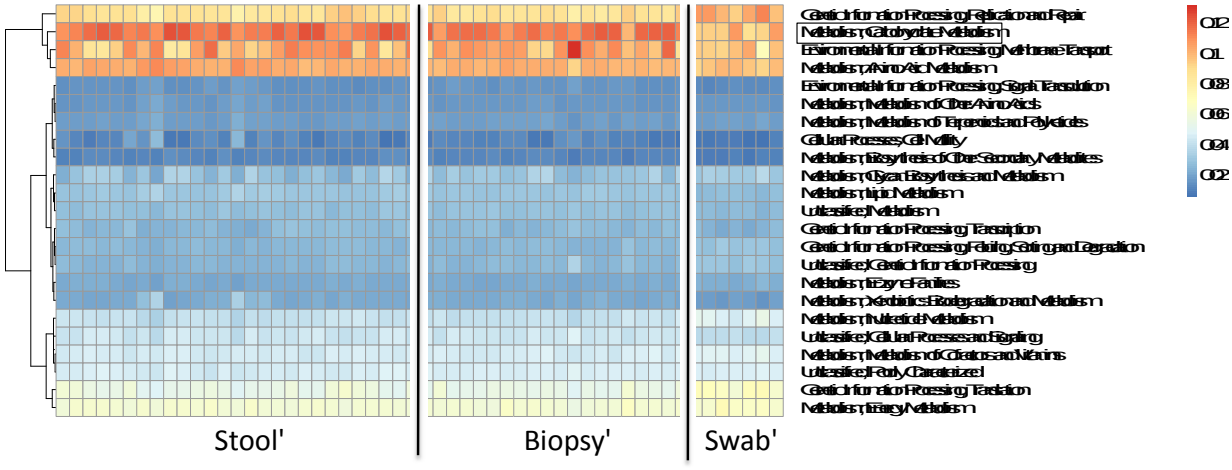

Figure S5. Heatmap of gene abundance assigned to major metabolic pathways inferred from 16S rRNA gene sequence information in stool, biopsy, and swab samples using PICRUSt.

Table S1. Oxygen tolerance and catalase activity in human rectally-associated and fecal genera.

Genus	Oxygen Tolerance	Catalase production	Reference
Actinobacteria|Actinobacteria|Actinomycetales|Actinomycetaceae|Actinomyces	Facultative anaerobe	Variable	Bergey's
Actinobacteria|Actinobacteria|Actinomycetales|Corynebacteriaceae|Corynebacterium	Facultative anaerobe	Pos	Bergey's
Actinobacteria|Actinobacteria|Actinomycetales|Propionibacteriaceae|Propionibacterium	Facultative anaerobe but with variable aerotolerance	Pos	Bergey's
Actinobacteria|Actinobacteria|Coriobacteriales|Coriobacteriaceae|Atopobium	"Anaerobic"	Neg	Rodriguez Jovita et al IJSB 2009
Actinobacteria|Actinobacteria|Coriobacteriales|Coriobacteriaceae|Collinsella	"Obligate anaerobe"	Neg	Kageyama et al IJSB 1999
Actinobacteria|Actinobacteria|Coriobacteriales|Coriobacteriaceae|Eggerthella	"Obligately anaerobic"	Neg	Wade et al IJSEM 1999
Bacteroidetes|Bacteroidetes|Bacteroidales|Bacteroidaceae|Bacteroides	Anaerobe or microaerophile	Neg	Bergey's
Bacteroidetes|Bacteroidetes|Bacteroidales|Bacteroidaceae|Megamonas	"Obligately Anaerobe"		Bergey's; Chervrot et al. Int J Syst Evol Microbiol 2008
Bacteroidetes|Bacteroidetes|Bacteroidales|Porphyromonadaceae|Parabacteroides	"Obligately anaerobic"		Sakamoto and Benno IJSEM 2006
Bacteroidetes|Bacteroidetes|Bacteroidales|Porphyromonadaceae|Porphyromonas	"Obligately anaerobic"		Bergey's
Bacteroidetes|Bacteroidetes|Bacteroidales|Prevotellaceae|Hallella	"Obligately anaerobic"	Neg	Moore et al IJSEM 1994
Bacteroidetes|Bacteroidetes|Bacteroidales|Prevotellaceae|Prevotella	"Obligately anaerobic"	Neg	Bergey's
Bacteroidetes|Bacteroidetes|Bacteroidales|Prevotellaceae|Xylanibacter	"Strictly anaerobic"	Neg	Ueki et al IJSEM 2006
Bacteroidetes|Bacteroidetes|Bacteroidales|Rikenellaceae|Alistipes	"Strictly anaerobic"	Pos	Rautio et al IJSEM 2003
Bacteroidetes|Flavobacteria|Flavobacteriales|Flavobacteriaceae|Cloacibacterium	Facultative anaerobe	Pos	Allen et al IJSEM 2006
Deinococcus-Thermus|Deinococci|Deinococcales|Deinococcaceae|Deinococcus	Aerobe	Pos	Bergey's
Firmicutes|Bacilli|Lactobacillales|Aerococcaceae|Aerococcus	Aerobe	Neg	Bergey's
Firmicutes|Bacilli|Lactobacillales|Lactobacillaceae|Lactobacillus	Facultative anaerobe or microaerophile	Neg	Bergey's
Firmicutes|Bacilli|Lactobacillales|Streptococcaceae|Streptococcus	Facultative anaerobe	Neg	Bergey's
Firmicutes|Clostridia|Clostridiales|Clostridiaceae|Clostridium	"Obligately anaerobic"	Neg	Bergey's

Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XI|Anaerococcus	"Strictly anaerobic"		Ezaki et al IJSEM 2001
Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XI|Finegoldia	"Obligately anaerobic"		Murdoch et al IJSEM 2000
Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XI|Parvimonas	"Obligately anaerobic"		Tindall et al IJSEM 2006
Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XI|Peptoniphilus	"Obligately anaerobic"	Neg	Ezaki et al IJSEM 2001
Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XIII|Anaerovorax	"Strictly anaerobic"	Neg	Matthies et al IJSEM 2000
Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XIII|Mogibacterium	"Strictly anaerobic"	Neg	Nakazawa et al IJSEM 2000
Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XV|Dethiosulfovibrio	Aerotolerant		Magot et al IJSEM 1997
Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Bryantella	"Anaerobic"	Neg	Wolin et al IJSEM 2004
Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Butyrivibrio	"Anaerobic"	Neg	Bergey's
Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Coprococcus	"Obligately anaerobic"	Neg	Bergey's
Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Dorea	"Obligately anaerobic"	Neg	Taras et al IJSEM 2002
Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Lachnospira	"Anaerobic"	Neg	Bergey's
Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Roseburia	"Obligately anaerobic"	Neg	Bergey's
Firmicutes|Clostridia|Clostridiales|Peptococcaceae|Peptococcus	"Anaerobic"		Bergey's
Firmicutes|Clostridia|Clostridiales|Peptostreptococcaceae|Peptostreptococcus	"Obligately Anaerobic"	Variable	Bergey's
Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Acetanaerobacterium	"Anaerobic"	Neg	Chen et al IJSEM 2001
Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Anaerotruncus	"Strictly anaerobic"	Neg	Lawson et al IJSEM 2004
Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Faecalibacterium	"Obligately anaerobic"		Duncan et al IJSEM 2002
Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Papillibacter	"Strictly anaerobic"		Defnoun et al IJSEM 2000
Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Ruminococcus	"Anaerobic"	Neg	Bergey's
Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Subdoligranulum	"Strictly anaerobic"	Neg	Holstrom et al Anaerobe 2004
Firmicutes|Clostridia|Clostridiales|Veillonellaceae|Acidaminococcus	Obiligate anaerobe	Neg	Bergey's: J Bacteriol. May 1969; 98(2): 756–766
Firmicutes|Clostridia|Clostridiales|Veillonellaceae|Dialister	"Anaerobic"	Neg	Moore et al IJSEM 1994
Firmicutes|Clostridia|Clostridiales|Veillonellaceae|Megasphaera	"Fermentative metabolism"	Neg	Bergey's
Firmicutes|Clostridia|Clostridiales|Veillonellaceae|Succinispira	"Obligately Anaerobic"		Janssen et al IJSEM 1999;

			Schmitz, RA et al. Prokaryotes 2006;2:86-101
Firmicutes|Erysipelotrichi|Erysipelotrichales|Erysipelotrichaceae|Catenibacterium	"Obligatory anaerobic"		Kageyama et al IJSEM 2000
Firmicutes|Erysipelotrichi|Erysipelotrichales|Erysipelotrichaceae|Coprobacillus	"Obligatorily anaerobic"		Kageyama et al IJSEM 2000
Firmicutes|Erysipelotrichi|Erysipelotrichales|Erysipelotrichaceae|Erysipelotrichaceae_Incertae_Sedis	Facultative anaerobe or aerobe		Bergey's
Firmicutes|Erysipelotrichi|Erysipelotrichales|Erysipelotrichaceae|Holdemania	"Strictly anaerobic"	Neg	Willems et al IJSEM 1997
Firmicutes|Erysipelotrichi|Erysipelotrichales|Erysipelotrichaceae|Turicibacter	"Strictly anaerobic"	Neg	Bosshard et al IJSEM 2002
Fusobacteria|Fusobacteria|Fusobacteriales|Fusobacteriaceae|Fusobacterium	"Obligately anaerobic"		Bergey's
Fusobacteria|Fusobacteria|Fusobacteriales|Fusobacteriaceae|Sneathia	"Fermentative metabolism"	Neg	Collins et al IJSEM 2002
Proteobacteria|Alphaproteobacteria|Sphingomonadales|Sphingomonadaceae|Sphingobium	Aerobe	Pos	Bergey's
Proteobacteria|Betaproteobacteria|Burkholderiales|Alcaligenaceae|Sutterella	Microaerophile or anaerobe		Wexler et al IJSB 1996
Proteobacteria|Betaproteobacteria|Burkholderiales|Burkholderiaceae|Burkholderia	Aerobe	Pos	Yabuuchi et al IJSEM 1993
Proteobacteria|Betaproteobacteria|Burkholderiales|Comamonadaceae|Diaphorobacter	Aerobe	Pos	Khan et al IJSEM 2003
Proteobacteria|Betaproteobacteria|Burkholderiales|Incertae_sedis_5|Aquabacterium	Aerobe or microaerophile	Neg	Kalmbach et al IJSEM 1999
Proteobacteria|Betaproteobacteria|Burkholderiales|Oxalobacteraceae|Janthinobacterium	Aerobe	Pos	Bergey's
Proteobacteria|Betaproteobacteria|Burkholderiales|Oxalobacteraceae|Massilia	Aerobe	Pos	La Scola et al IJSEM 2000
Proteobacteria|Deltaproteobacteria|Desulfovibrionales|Desulfovibrionaceae|Bilophila	"Obligately anaerobic"	Pos	Baron et al IJSEM 1990
Proteobacteria|Epsilonproteobacteria|Campylobacterales|Campylobacteraceae|Campylobacter	Microaerophile	Variable	Bergey's
Proteobacteria|Gammaproteobacteria|Chromatiales|Chromatiaceae|Rheinheimera	Facultative anaerobe or aerobe	Pos	Brettar et al IJSEM 2002
Proteobacteria|Gammaproteobacteria|Enterobacteriales|Enterobacteriaceae|Citrobacter	Facultative anaerobe	Pos	Bergey's
Proteobacteria|Gammaproteobacteria|Enterobacteriales|Enterobacteriaceae|Enterobacter	Facultative anaerobe	Pos	Bergey's
Proteobacteria|Gammaproteobacteria|Enterobacteriales|Enterobacteriaceae|Escherichia	Facultative anaerobe	Pos	Bergey's
Proteobacteria|Gammaproteobacteria|Pseudomonadales|Moraxellaceae|Acinetobacter	Aerobe	Pos	Bergey's
Proteobacteria|Gammaproteobacteria|Pseudomonadales|Pseudomonadaceae|Pseudomonas	Aerobe	Pos	Bergey's

Proteobacteria|Gammaproteobacteria|Vibrionales|Vibrionaceae|Vibrio	Facultative anaerobe	Pos	Bergey's
Proteobacteria|Gammaproteobacteria|Xanthomonadales|Xanthomonadaceae|Dyella	Aerobe	Pos	Xie et al IJSEM 2005
Tenericutes|Mollicutes|Mycoplasmatales|Mycoplasmataceae|Mycoplasma	Facultative anaerobe	Neg	Bergey's

Table S2. The Mucosally-AssociatedMicrobiota Consortium

Phylum	**Genus**	**Oxygen Tolerance**	**Catalase**	**Asaccharolytic**	**p value***
Actinobacteria	*Corynebacterium*	Aerobic and Facultative Anaerobes	Positive	No	0.0092
Bacteroidetes	*Porphyromonas*	Obligate Anaerobe	Negative	Yes	0.0092
Firmicutes	*Anaerococcus*	Obligate Anaerobe	Negative	Yes	0.0023
Firmicutes	*Finegoldia*	Obligate Anaerobe	Negative	Yes	0.0035
Firmicutes	*Murdochiella*	Obligate Anaerobe	Negative	Yes	0.016
Firmicutes	*Peptoniphilus*	Obligate Anaerobe	Negative	Yes	0.0023
Proteobacteria	*Campylobacter*	Microaerophilic	Positive	Yes	0.0023
Proteobacteria	*Enterobacteriaceae*	Facultative Anaerobes	Positive	No	0.068

*Compared to stool, FDR corrected

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