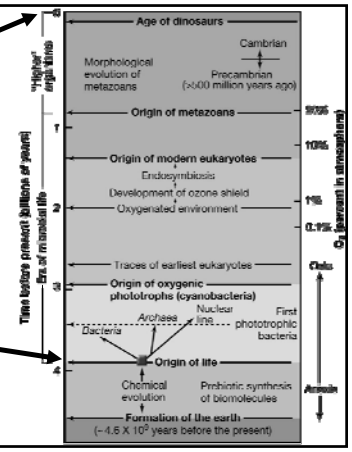
Primates have been around for 3 million years



Prokaryotes have been around for 3.8 billion years

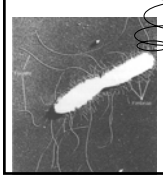
Co-evolved symbiotic relationships between microbes and multicellular organisms are a prominent feature of life on Earth.

Why not use humans as another habitat?

-climate-controlled (37°C)

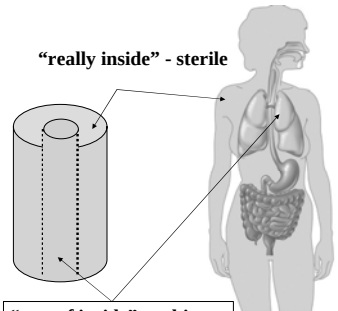
-stable pH (varies by organ)

-steady supply of nutrients



General aspects of indigenous microbiota

"really inside" - sterile



"sort of inside" – a big test tube

- **found in body regions exposed to the outside world**
- **specific to each body region**
- **occurrence in urine, blood or body organs is unusual and is usually an indication of infection.**
- **we benefit from our microbiota**

Inside the "tube", various microbes find a niche that fits.

Acquisition of Normal Microbiota

- The womb is generally free of microorganisms (axenic)
- Microbiota begins to develop during the birthing process, acquired during passage through vagina
- Much of one's resident microbiota established during the first months of life
  - Food
  - Contact with other humans/animals

Animal microbiota: related but not the same as ours

Microbial colonization of 14 baby guts

-16S rRNA microarray, 10,500 DNA probes *(who is there?)*

-clone libraries (n = 4,100) *(who is there?)*

-PCR using universal Bacterial primers *(how many are there?)*

Palmer et al., 2007. PLoS 5(7): 177

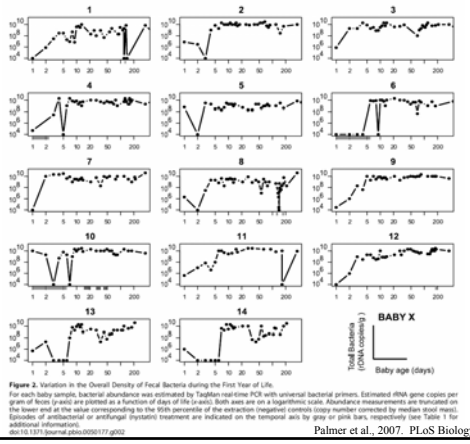
Table 1. Relevant Characteristics of the Infants in This Study

Baby	Sex	Delivery	Birth Weight	Hospital Stay	Formula Feedings	First Food	Antimicrobials
1	F	C-section	3,660 g		None noted	No data	Ax/Cl week 18
2	M	C-section	3,570 g		None noted	20 wk	None noted
3	F	Vaginal	3,490 g		Week 10–11	No data	None noted
4	M	Vaginal	2,380 g	3 d in NICU	None noted	17 wk	Day 1–2 Ap + Gm
5	M	Vaginal	4,480 g		Formula day 4–5	No data	None noted
6	M	Vaginal	3,570 g	5 d in SC	Day 1–12 and after 2.5 months	22 wk	Day 1–6 (uAb)
7	F	Vaginal	3,230 g		None noted	No data	None noted
8	F	Vaginal	3,740 g		None noted	No data	Week 19–20 Ax: month 6 Ax/Cl, then Az
9	M	Vaginal	3,520 g		None noted	22 wk	None noted
10	M	Vaginal	4,060 g	1 wk in NICU	Day 6–7, day 30	No data	Day 1–6 Ap + Gm; Nystatin ointment day 14–21, day 28–35, week 6; Oral nystatin day 28–35.
11	M	C-section	2,950 g		Day 1–14, week 15 through month 6 (+breast)	12 wks	
12	F	Vaginal	3,550 g		None noted	18 wk	Month 6 Ax
13	M	C-section	2,640 g		Started day 1 (+breast)	No data	None noted
14	M	C-section	2,980 g		Started day 1 (+breast)	No data	None noted

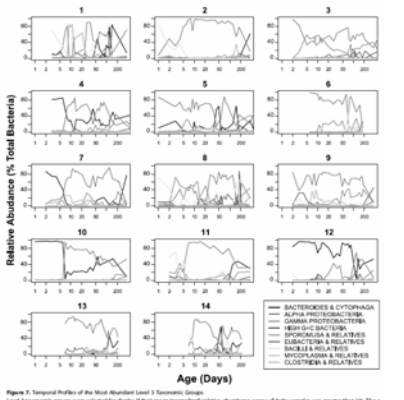
Ap, ampicillin; Ax, amoxicillin; Ax/Cl, amoxicillin/clavulanic acid; Az, azithromycin; C-section, caesarean section; Gm, gentamicin; NICU, neonatal intensive care unit; SC, special care nursery; (+), supplement; uAb, unspecified antibiotic.

doi:10.1371/journal.pbio.0050177.t001

Palmer et al., 2007. PLoS biology 5(7): 177



Palmer et al., 2007. PLoS Biology 5(7): 177



Babies 13 & 14 are fraternal (dizygotic) twins

Palmer et al., 2007. PLoS Biology 5(7): 177

Points of interest:

By one year: mainly Proteobacteria, *Bacteroides*, Firmicutes, Actinobacteria, and Verrucomicrobia.

First few days: chaos, influenced by maternal signature

Maternal signatures do not persist indefinitely

Twins: all aspects of triangle similar – genetics, environment, development. Microbes similar too.

Periods of relative stability punctuated by abrupt shifts
-antibiotic treatment
-phage?
-invasion by more fit species?
-diet change?
-developmental change?

Normal flora: refers to the organisms that colonize the body's surfaces without normally causing disease

- Resident microbiota (autochthonous species):
  - Are a part of the normal microbiota throughout life
  - Comprise Bacteria, Archaea, Eukarya
  - Most described as **commensal** *(what does that mean?)*
- Transient microbiota (allochthonous species)
  - "Tourists", remain in the body for only hours to months before disappearing
  - Found in the regions also occupied by resident microbiota
  - May be autochthonous in another part of body
  - May be ingested in food/water, etc.
  - Cannot persist in the body
    - Competition from other microorganisms
    - Elimination by the body's defenses cells
    - Chemical or physical changes in the body
- Opportunistic pathogen: normal flora includes opportunistic pathogens, which can cause disease if host resistance is lowered. Host resistance is not a static trait!

Important roles for microbial symbionts: turf battle with pathogens

-Competitive exclusion (cover attachment sites)
-Compete for nutrients
-Produce antimicrobials

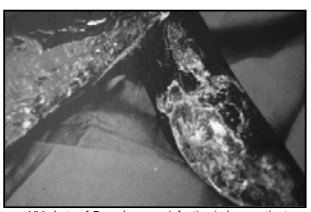
1. streptococci in nasal passages produce H_2O_2 which inhibits *Corynebacterium diphtheriae*, the causal agent of diphtheria)

2. *Staphylococcus epidermidis* and *Propionibacterium* spp. on skin break down lipids into fatty acids that inhibit other bacteria

3. Some strains produce antibiotics

Opportunistic Pathogens

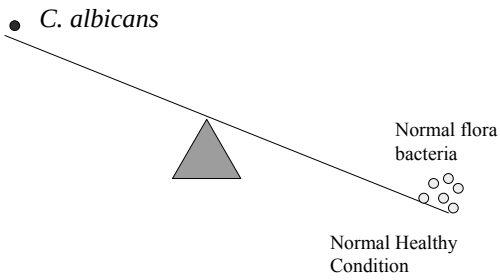
- Normal microbiota that can cause disease under certain circumstances
- Conditions that provide opportunities for pathogens:
  - Immune suppression
  - Changes in the normal microbiota- changes in relative abundance of normal microbiota may allow opportunity for a member to thrive and cause disease
  - Introduction of normal microbiota into unusual site in the body

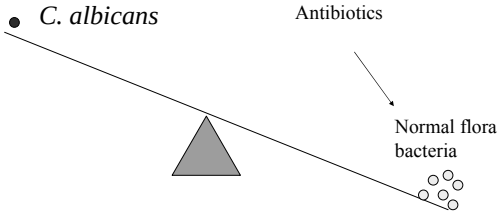


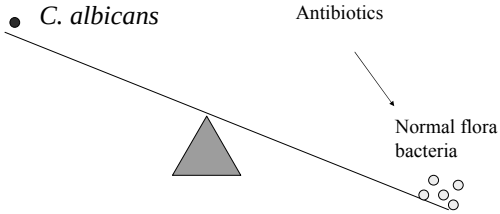
UV photo of *Pseudomonas* infection in burn patient
http://aci.mta.ca/Courses/Biology/Images/bacterial%20folder/PseudomonasInfections.html

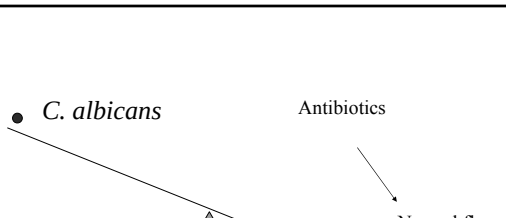
Candida albicans
(an example of an opportunistic pathogen)

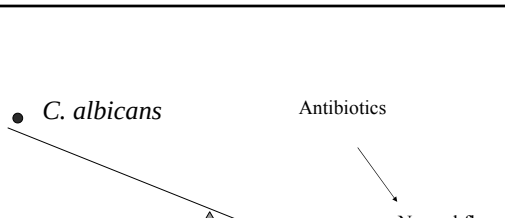
- Part of the vaginal normal flora in over 50% of females.
- Scenario for a *Candida* infection:
  - Woman takes antibiotics for a bacterial infection… "collateral damage" by antibiotics eliminates the vaginal normal flora bacteria.
  - Antibiotics have no effect on yeast (a eukaryote – remember "selective toxicity")
  - *Candida albicans* increases in numbers and causes a vaginal yeast infection (vaginal candidiasis)

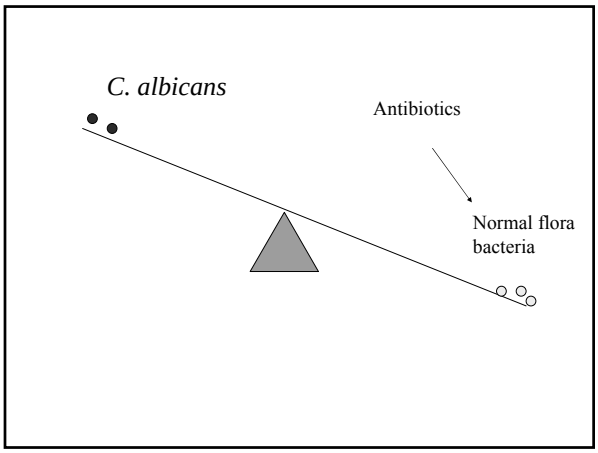


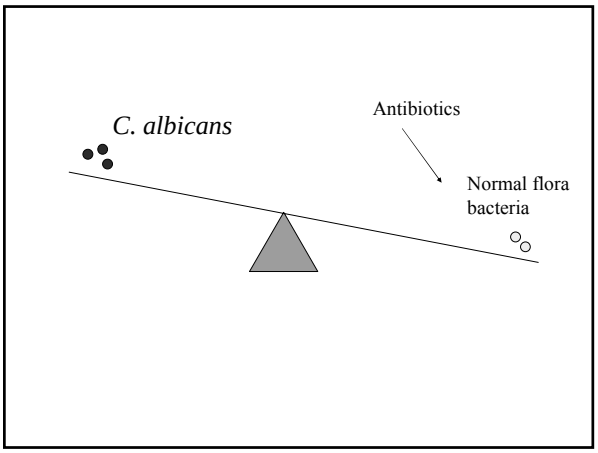


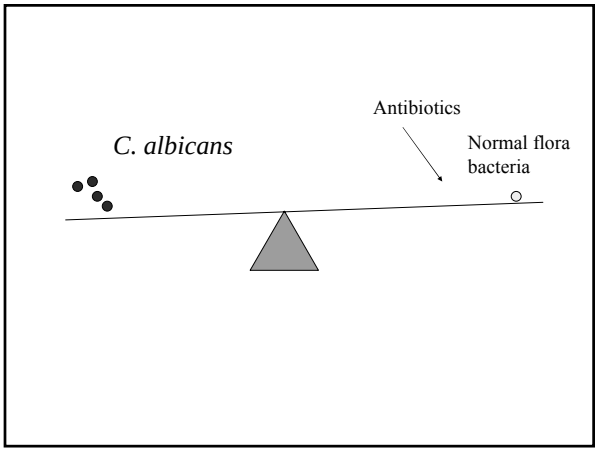


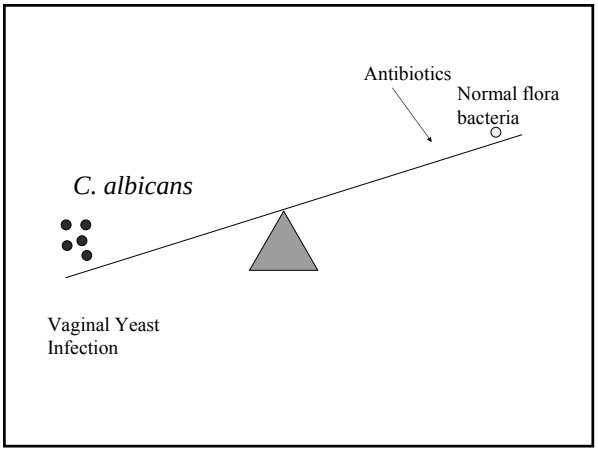












Important roles for microbial symbionts: "set up" and maintain immune system

Mucosal epithelia

-All metazoans have mucosal epithelia
-One of the most ancient and universal modules of innate immunity
-**Skin & mucosal epithelia** are the main interface between the host and the microbial world

-Accordingly, mucosal epithelial cells and skin keratinocytes have specialized antimicrobial functions: for example, producing antimicrobial peptides, which limit the viability and multiplication of pathogens and symbiotic microorganisms that colonize these sites.

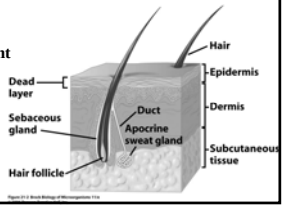
-The production of these antimicrobial molecules is induced by engagement of TLRs and NOD proteins and, presumably, other PRRs.

-Epithelial cells at the mucosal surface also produce mucins, which help to prevent the attachment and entry of pathogens.

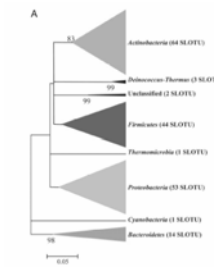
Indigenous Microbiota of the Skin

- **Dry – favors growth of microbes with barriers to dehydration**
- **pH depends on secretions: ranges between pH 4 and pH 6**
- **Most microbes are associated with "wet" areas:**
  -apocrine (sweat) glands and ducts
  -urea, amino acids, salts, lactic acids & lipids
  -acidic pH (4-6)

-hair follicles
-sebaceous glands secrete lubricant



Indigenous Microbiota of the Skin



Sequencing of 1,345 clones from human forearm skin of 6 subjects

Harbors transients (unable to multiply) or residents (multiply & divide).

Non-resident bacteria inhibited:

- **-low moisture**
- **-low pH**
- **-fatty acids generated by *Propionibacterium* spp.**

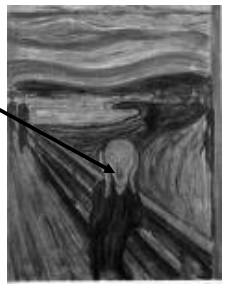
Residents include:

- **-*Staphylococcus epiderimidis***
- **-*Propionibacterium acnes* (associated with acne)**
- **-*Corynebacterium* spp. [Gram$^+$ bacteria]**
- **-*Acinetobacter***
- **-*Candida* can proliferate in immunocompromised patients**

Gao et al., 2007. PNAS 104(8):2927

There are more bacteria in this mouth than there are people in the world.

There are ~600 different microbial species in the oral cavity.



Human Oral Microbiome Database (HOMD)
➔ public, comprehensive database

http://www.homd.org/index.php

Indigenous Microbiota of the Oral Cavity

Enamel = calcium phosphate crystals

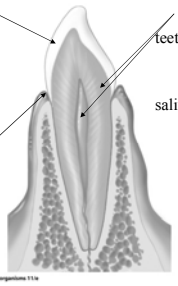
Dentin and pulp = living tooth tissues

Before baby teeth emerge, streptococci and lactobacilli (aerotolerant anaerobes).

After teeth: anaerobes adapted for growing in gingival crevices

teeth - surface, not a direct source of nutrients

saliva - low nutrients; antibacterial substances (e.g. lysozyme) and lactoperoxidase (kills bacteria via free radical generation)



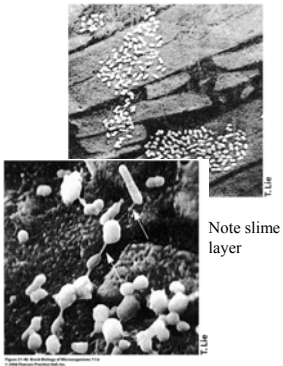
Indigenous Microbiota of the Oral Cavity

Saliva coats teeth with acidic glycoproteins: sticky

Several streptococci can colonize this layer:

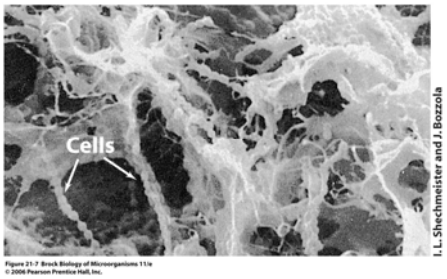
- *S. sanguis*
- *S. sobrinus*
- *S. mutans*
- *S. mitis*

Next, filamentous *Fusobacterium* spp. can colonize. So can spirochetes, actinomycetes, and others.



Note slime layer

A biofilm has formed.
Oxic layer at oral cavity surface (air) and anoxic at tooth surface.

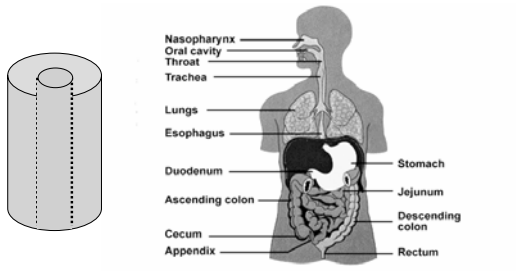


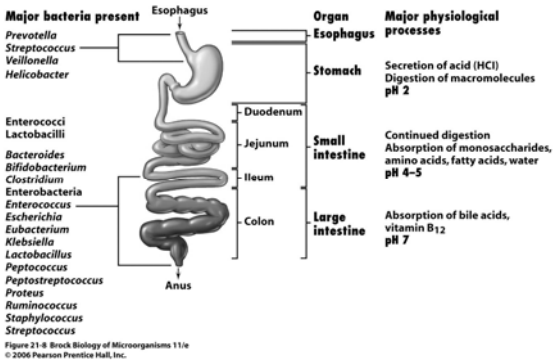
SEM of the cariogenic bacterium *Streptococcus mutans*. The sticky dextran material holds cells together as filaments.

Oral microbial ecology…

More later from Leslie and Sasha!!

Back to the "you" tube…





Indigenous microbiota of the stomach

pH 2 (acidic microbial barrier)

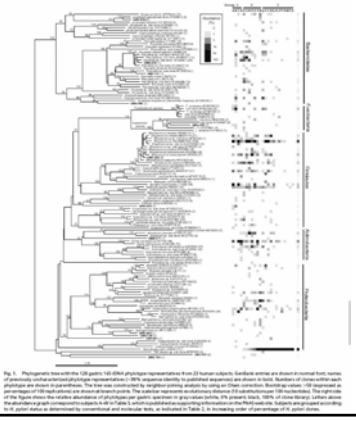
Conventional wisdom: too acidic for most species besides *Helicobacter*

Experiment: 16S rRNA sequencing of 1,833 clones from 23 subjects (gastric endoscopic biopsy)

Findings: 128 phylotypes

Major sequences: *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes,* and *Fusobacteria*

Of interest: 10% of phylotypes previously uncharacterized, including a *Deinococcus* relative (not previously reported from humans)



How to tell transients from residents?

Patients:
22 males, only 1 female

13 Caucasians
5 Hispanics
5 African-Americans

Food prior to sample not stated

Bik et al., 2006. PNAS 103(3): 732

Indigenous microbiota of the stomach

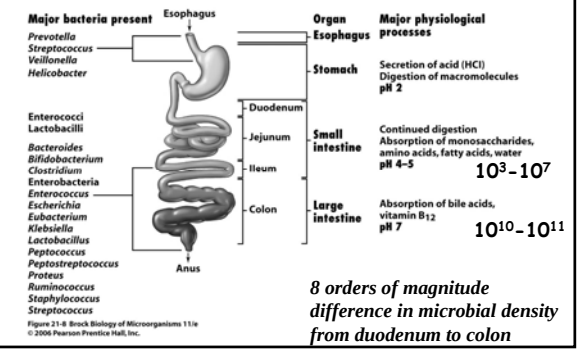
Table 1. Previously uncharacterized phylotypes found in gastric clone libraries

Gastric sequence			Closest neighbor present in public database	
Clone name	Assigned accession no.	Sequence similarity, %	Accession no.	Description
GMC-T94	AY582897	93.6	AJ549111	*Deinococcus indicus* from arsenic-contaminated water from aquifer
GMC-P27, GMC-U27	AY582895	94.2	AF173818	Uncultured Antarctic bacterium from permanent Antarctic lake ice
GMA-C11	AY582888	95.5	AY043855	Uncultured actinobacterium isolated from forest mineral soil
GMA-N01/N26/N40	AY582894	95.3	AY494619	Uncultured delta-proteobacterium from salmonid gill
GMC-T47	AY582896	97.2	AF385509	*Prevotella* sp. from tongue dorsa
GMA-H46/H63	AY582893	97.3	AJ318110	Uncultured bacteroidetes from waste-gas biofilter
GMA-E44	AY582889	97.4	AY038612	Uncultured eubacterium from lacustrine subsurface sediments
GMA-E91	AY582890	98.2	AY162043	Uncultured alpha proteobacterium from soil
GMA-A52, GMA-B11/B19/B27/B81	AY582886	98.6	AY207059	*Peptostreptococcus* sp. from human mouth
GMA-A36, GMA-B65, GMC-W25	AY582885	98.7	AF385518	*Leptotrichia* sp. from tongue dorsa
GMA-F28	AY582891	98.8	AF131297	*Sphingomonas aquatilis*
GMA-B32	AY582887	98.9	L16469	*Prevotella melaninogenica*
GMA-G25/G61	AY582892	98.9	AF432137	*Streptococcus* sp. from tongue dorsa

Previously uncharacterized phylotypes were defined as sequences or groups of sequences having <99% sequence similarity to sequences present in public databases. The NCBI GenBank accession number and a short description of the closest neighbor is given, as well as sequence similarity (%) to that neighbor. Clone designations indicate site, patient, and clone number, e.g., GMA-E91 represents gastric mucosal biopsy from antrum, patient E, clone 91. GMC, gastric mucosal biopsy from corpus. From each phylotype, one representative sequence (underlined in the case of multiple clones) was deposited into the NCBI GenBank.

Bik et al., 2006. PNAS 103(3): 732

Indigenous microbiota of the gastrointestinal tract



8 orders of magnitude difference in microbial density from duodenum to colon

Your largest collection of microbes is in your intestine:

-500 to 1000 different species

-1.5 kg *(how many pounds?)*

-most are refractory to cultivation

-If 1000 species, at average genome size of *E. coli*, then the aggregate size of all microbial genomes ("microbiome") is ≈ to human genome in size, but 100X more genes *(why??)*

-we found only ~20,000 genes in human genome, similar to *Drosophila,* but think of ourselves as more complex... this brings us closer to the pre-genome estimate of ~100,000 genes that have to do with "being human".

Small intestine:

- pH gradually increases
- # bacteria increases
- Lower ileum 10^5-10^7 cells per gram

***Fusiform anaerobes* in large intestine**

Large Intestine:

- A **chemostat**: 1-2 doublings of bacteria/day in colon, continually displaced and replaced by new growth. 1/3 of fecal mass is bacteria.
  (In addition, you shed ~ 20-50 million epithelial cells per minute from your small intestine and 1/10th that amount from the colon.)
- Facultative anaerobes contribute < 10^7 cells per gram
- **Obligate anaerobes** contribute 10^{10}-10^{11} cells per gram; 99.9% of cultured isolates
- >90% are from two of the 70 known Bacterial divisions (phyla): **Firmicutes & Bacteroidetes**

Gut microbiota functions as multifunctional organ to provide metabolic traits that we have not evolved in our own genome:

-Breakdown of plant polysaccharides

-Fat breakdown and deposition (obesity in gnotobiotic mice)

-Biotransformation of conjugated bile acids & xenobiotics

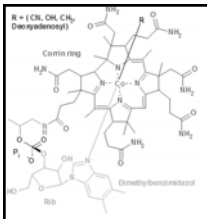
-Degradation of dietary oxalates (kidney stone prevention – kidney stones may be varied in composition but 80% of them are calcium oxalate)

-Synthesis of vitamins (B_{12}, K)

-Steroid metabolism (esterification, dehydroxylation, oxidation, reduction, inversion)

-Stimulation of renewal of gut epithelial cells

-Others??? Heart size, locomotor activity less in gnotobiotic mice

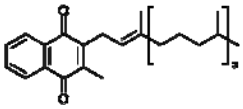


Vitamin B_{12} (a.k.a. cyanocobalamin): Only made by Bacteria and Archaea. Naturally found in foods that harbor B_{12}-producing bacteria: meat, eggs, milk.

Function in humans:

1. acts as a cofactor for methylmalonyl-coenzyme A mutase, which catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA.

2. acts as a cofactor for 5-methyltetrahydrofolate homocysteine methyltransferase, which is part of the S-adenosyl methionine (SAM) cycle and produces methionine from homoscysteine.



Vitamin K: Collective name for a group of related compounds sharing a methylated naphthoquionone ring structure, and which vary in the aliphatic side chain attached at the 3-position.

Function in bacteria (*E. coli*): part of electron transport chain

Function in humans: carboxylation of glutamate residues to gamma-carboxyglutamate residues in certain proteins; these are involved in binding calcium.

"Pinches" from cleaned guts: mucosal surface-associated bacteria

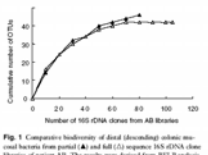


Fig. 1 Comparative biodiversity of distal (descending) colonic mucosal bacteria from partial (Δ) and full (Δ) sequence 16S rDNA clone libraries of patient AB. The results were derived from RFLP analysis. The cumulative OTU is expressed as a function of the total number of clones that have been analysed

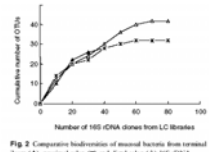


Fig. 2 Comparative biodiversities of mucosal bacteria from terminal ileum (▲), proximal colon (■) and distal colon (Δ) 16S rDNA clone libraries of patient LC. The results were derived from RFLP analysis. The cumulative OTU is expressed as a function of the total number of clones, which have been analysed

Table 1 Summary of bacterial diversity from mucosa of human terminal ileum and colon (proximal and distal) obtained by 16S rDNA analysis

Group	Sample LC*	AB†	Mean
Alphaproteobacteria (%)	0	67	33
Betaproteobacteria (%)	14	62	38
Gammaproteobacteria (%)	1	267	134
Bacteroides (*Bacteroides CFB*) (%)	38	173	106
Clostridium cluster I (%)	0	13	06
Clostridium cluster IV (%)	0	179	89
Clostridium cluster IX (%)	1	18	14
Clostridium cluster XI (%)	137	0	69
Clostridium cluster XIVa (%)	141	153	297
Clostridium cluster XVIII (%)	107	0	54
Bacillus-Lactobacillus-Streptococcus (%)	1	13	11

*Samples taken from terminal ileum, proximal and distal colon of a healthy 35-year-old female. Partial 350 bp sequences.
†Samples taken from the descending colon of a 68-year-old female with mild sigmoid colonic diverticulosis. Sequence lengths were both 350 and 1100 bp.

Wang et al., 2003. J. Appl. Microbiol. 95: 508

Figure 1 | The concept of a core human microbiome. The core human microbiome (red) is the set of genes present in a given habitat in all or the vast majority of humans. Habitat can be defined over a range of scales, from the entire body to a specific surface area, such as the gut or a region within the gut. The variable human microbiome (blue) is the set of genes present in a given habitat in a smaller subset of humans. This variation could result from a combination of factors such as host genotype, host physiological status (including the properties of the innate and adaptive immune systems), host pathobiology (disease status), host lifestyle (including diet), host environment (at home and/or work) and the presence of transient populations of microorganisms that cannot persistently colonize a habitat. The gradation in colour of the core indicates the possibility that, during human micro-evolution, new genes might be included in the core microbiome, whereas other genes might be excluded.

Note: host physiology and immune system will depend on development (ontology)

Turnbaugh et al., 2007. Nature 449: 804

Bacteroides thetaiotaomicron = prominent component of the normal mouse and human intestinal microflora

Gnotobiotic (germ-free, "known life") mice were colonized with *B. thetaiotamicron*

Global intestinal transcriptional responses to colonization were observed with DNA microarrays. *B. thetaiotamicron* modulated expression of genes involved in:

-mucosal barrier fortification
-xenobiotic metabolism
-postnatal intestinal maturation

Hooper et al., 2001. Science 291: 881

Bacteroides thetaiotaomicron

Also:
-Directs synthesis of glycans with α-linked fucose in epithelium; these are "sign posts" of friendly territory to Bt, which then eats the fucose (β-fucosidases)
(*Bt* colonizes niche first: keystone species)

-within 10d of colonization, induces complex angiogenesis at submucosal epithelium

-enhances triacylglyceride absorption
-enhances triacylglyderide import/storage by repressing ANGPLT4, a repressor of the key lipase

-stimulates gut innate immune system (recognize certain pathogens, e.g. *Listeria*, and ignore symbionts) → directs its own microbial neighborhood

Reviewed in Xu and Gordon, 2003. PNAS 100(18): 10452

What does *B. thetaiotamicron* do in the gut?

Table 1. Glycosylhydrolases encoded by the genomes of selected sequenced members of the adult human distal intestinal microbiota

Gene	*B. thetaiotaomicron* VPI 5482	*E. coli* K12, MG1655	*Bifidobacterium longum* NCC2705	*C. perfringens* strain 13	*Enterococcus faecalis* V583	*P. aeruginosa* PAO1
Amylase	8	2	0	2	1	0
Arabinase	2	0	0	0	0	0
α-Arabinofuranosidase	4	0	5	0	0	0
α-Arabinosidase	7	0	5	0	0	0
Chitinase	3	0	0	0	0	1
β-Fructofuranosidase (levanase)	2	0	1	0	0	0
α-Fucosidase	3	0	0	1	0	0
α-Galactosidase	8	1	2	2	0	0
β-Galactosidase	31	3	6	5	4	0
α-Glucosidase	14	0	3	4	3	0
β-Glucosidase	10	8	7	1	10	1
α-Glucuronidase	1	0	0	0	0/1	0
β-Glucuronidase	2	1	1	1	1/0	0
β-Hexosaminidase	14	0	2	3	0	1
α-Mannanase	8	0	0	0	0	0
α-Mannosidase	14	1	3	2	0	0
β-Mannosidase	5	0	0	0	0	0
α-*N*-Acetylglucosaminidase	3	0	0	1	0	0
β-*N*-Acetylglucosaminidase	6	0	1	2	1	0
α-Rhamnosidase	5	0	0	0	0	0
α-Xylosidase	11	1	2	5	0	0
β-Xylanase	3	0	1	0	1	0
β-Xylosidase	8	1	0	0	0	1
Total	172	18	39	29	21	4
Genome size, Mb	6.26	4.64	2.26	3.03	3.22	6.26

Estimated numbers of genes for each category are based on genome annotation files in GenBank, as well as analysis of functional domains by using INTERPRO. *P. aeruginosa*, a member of the Gamma branch of Proteobacteria whose genome size and coding potential are similar to those of *B. thetaiotaomicron*, is included to illustrate features in a Gram-negative bacterium with considerable ecological versatility.

What does *B. thetaiotamicron* do in the gut?

SusC & SusD: Starch Utilization System; acquisition of polysaccharides - bind to cell surface and break into medium-sized oligosaccharides

In genome:

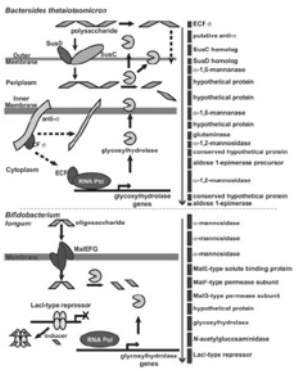
56 homologs of *susD*
106 homologs of *susC*
47 of the *susC* homologs are next to glycosylhydrolases

In contrast, *Bifidobacterium longum* has no susC homologs, but has 8 ABC transporters for oligosaccharides, and a PTS, which is lacking in Bt.

-Adaptive grazing? Stabilization of foodweb
-Keystone species: break down varying plant polysaccharides for other species to absorb?

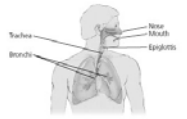
Also in Bt genome: unprecedentedly high number of **ECF sigma factors** and **one-component** systems. Quick response to changing environment?

What does *B. thetaiotamicron* do in the gut?



Summary of resident microbiota

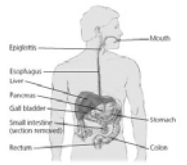
Table 14.2 Resident Microbiota[a] *(1 of 2)*



Upper Respiratory Tract

Genera
Staphylococcus, Streptococcus, Moraxella, Haemophilus, Lactobacillus, Veillonella, Fusobacterium, Candida (fungus)

Notes
The nose is cooler than the rest of the respiratory system and has some unique microbiota. The trachea and bronchi have a sparse microbiota compared to the nose and mouth. The alveoli of the lungs, which are too small to see at this magnification, have no natural microbiota.



Upper Digestive Tract

Genera
Lactobacillus, Haemophilus, Actinomyces, Bacteroides, Treponema, Neisseria, Corynebacterium, Entamoeba (protozoan), *Trichomonas* (protozoan)

Notes
Microbes colonize surfaces of teeth, gingiva, lining of cheeks, and pharynx, and are found in saliva in large numbers. Dozens of species have never been identified.

Lower Digestive Tract

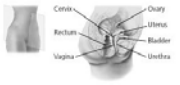
Genera
Bacteroides, Fusobacterium, Escherichia, Lactobacillus, Clostridium, Bifidobacterium, Enterococcus, Proteus, Shigella, Candida (fungus), *Entamoeba* (protozoan), *Trichomonas* (protozoan)

Notes
Bacteria are mostly strict anaerobes, though some facultative anaerobes are also resident.

[a]Genera are bacteria unless noted.

Summary of resident microbiota

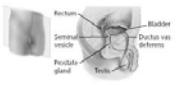
Table 14.2 Resident Microbiota[a] *(2 of 2)*



Female Urinary and Reproductive Systems

Genera
Lactobacillus, Streptococcus, Staphylococcus, Bacteroides, Clostridium, Candida (fungus), *Trichomonas* (protozoan)

Notes
Microbiota change as acidity in the vagina changes during menstrual cycle. The flow of urine prevents extensive colonization of the urethra.



Male Urinary and Reproductive Systems

Genera
Staphylococcus, Streptococcus, Mycobacterium, Bacteroides, Fusobacterium, Peptostreptococcus

Notes
The flow of urine prevents extensive colonization of the urethra.

Eyes and Skin

Genera
Skin: Propionibacterium, Staphylococcus, Corynebacterium, Micrococcus, Malassezia (fungus), *Candida* (fungus)
Conjunctiva: Staphylococcus

Notes
Microbiota live on the outer, dead layers of the skin and in hair follicles and pores of glands. The deeper layers (dermis and hypodermis) are axenic.
Tears wash most microbiota from the eyes, so there are few compared to the skin.

[a]Genera are bacteria unless noted.

"The human can be thought of as a human-microbe hybrid…"

Molecules

↓

Communities

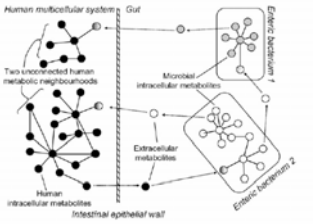


Figure 2 A complex metabolic network from a superorganism showing metabolites derived from the enzymatic action of proteins encoded by genes in the human genome (*black circles*). One of these metabolites has been secreted into the gut, where it has been used as a substrate by a microorganism resident in the gut (*enteric bacterium 2*). This bacterium has metabolically transformed this metabolite (*white circles*) using its own microbially derived enzymes. Two of these products are secreted; 1 crosses the intestinal barrier and is used by the human, while the other is absorbed by a second enteric microbe (whose metabolites are represented by *gray circles*), leading to so called cross-feeding. Note in the schematic shown that areas of metabolism in humans that are not connected could become linked by microbial transformation.

Goodacre, 2007. J. Nutrition
Supplement: Int. Research Conf. on Food, Nutrition, and Cancer. 259S.

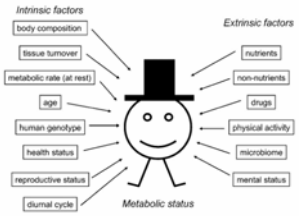


Figure 3 Intrinsic and extrinsic factors that affect the metabolic status of the human. The status of these can be measured using metabolomics.

Goodacre, 2007. J. Nutrition
Supplement: Int. Research Conf. on Food, Nutrition, and Cancer. 259S.

Human Microbiome Project

-huge collaborative effort

http://nihroadmap.nih.gov/hmp/index.asp

"The HMP… has the potential to break down the artificial barriers between medical microbiology and environmental microbiology."

--Peter J. Turnbaugh, Ruth E. Ley, Micah Hamady, Claire M. Fraser-Liggett, Rob Knight & Jeffrey I. Gordon, 2007

Ecosystem-level functions:

The first gut "microbiome" showed that, compared with all previously sequenced microbial genomes and the human genome, gut microbiomes of these (2) adults showed significant enrichment for genes involved in:

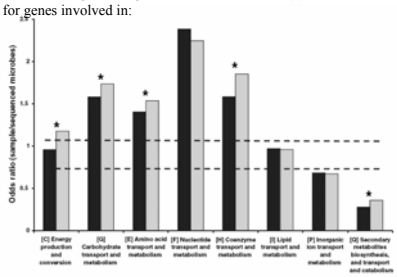


Fig. 2. COG analysis reveals metabolic functions that are enriched or underrepresented in the human distal gut microbiome (relative to all sequenced microbes). Color code: black, subject 7; gray, subject 8. Bars above both dashed lines indicate enrichment, and bars below both lines indicate underrepresentation ($P < 0.05$). Asterisks indicate categories that are significantly different between the two subjects ($P < 0.05$). Secondary metabolites biosynthesis includes antibiotics, pigments, and nonribosomal peptides. Inorganic ion transport and metabolism includes phosphate, sulfate, and various cation transporters.

Gill et al., 2006. Science 312: 1355

*KEGG ➔ uses gene and genome information to predict potential enzymatic functions and metabolic pathways

Compared to:

Microbiome = blue
Human genome = red
All Archaea = yellow

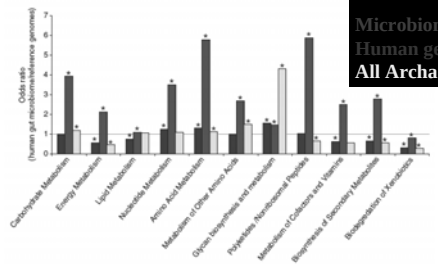


Fig. 3. KEGG pathway reconstructions reveal metabolic functions that are enriched or underrepresented in the human distal gut microbiome as follows: both samples compared with all sequenced bacterial genomes in KEGG (blue), the human genome (red), and all sequenced archaeal genomes in KEGG (yellow). Asterisks indicate enrichment (odds ratio > 1, $P < 0.05$) or underrepresentation (odds ratio < 1, $P < 0.05$). The KEGG category, "metabolism of other amino acids," includes amino acids that are not incorporated into proteins, such as β-alanine, taurine, and glutathione. Odds ratios are a measure of relative gene content based on the number of independent hits to enzymes present in a given KEGG category.

Gill et al., 2006. Science 312: 1355

454 technology

Sample preparation. Fragments of DNA are ligated to adapters that facilitate their capture on beads (one fragment per bead). A water-in-oil emulsion containing PCR reagents and one bead per droplet is created to amplify each fragment individually in its droplet. After amplification, the emulsion is broken, DNA is denatured and the beads, containing one amplified DNA fragment each, are distributed into the wells of a fiber-optic slide.

Pyrosequencing. The wells are loaded with sequencing enzymes and primer (complementary to the adapter on the fragment ends), then exposed to a flow of one unlabeled nucleotide at a time, allowing synthesis of the complementary strand of DNA to proceed. When a nucleotide is incorporated, pyrophosphate is released and converted to ATP, which fuels the luciferase-driven conversion of luciferin to oxyluciferin and light. As a result, the well lights up. The read length is between 100 and 150 nucleotides.

The general principle behind different pyrosequencing reaction systems

$$(NA)_n + \text{Nucleotide} \xrightarrow{\text{Polymerase}} (NA)_{n+1} + PPi$$

$$PPi + APS \xrightarrow{\text{ATP sulfurylase}} ATP + SO_4^{2-}$$

$$ATP + \text{Luciferin} + O_2 \xrightarrow{\text{Luciferase}} AMP + PPi + \text{Oxyluciferin} + CO_2 + \text{Light}$$

Polymerase = *E. coli* DNA polymerase
ATP sulfurylase = *S. cerevisiae* enzyme that converts PPi to ATP
Luciferase = American firefly *Photinus pyralis* enzyme that uses ATP in the oxidation of luciferin to generate light.

Reaction = 3-4 seconds per nucleotide
One pmol of DNA in a **pyrosequencing** reaction yields 6×10^{11} ATP molecules which, in turn, generate more than 6×10^9 photons at a wavelength of 560 nm, easily detected by photomultiplier tube.

In **pyrosequencing**, the most critical reactions are DNA polymerization and nucleotide removal by either washing or enzymatic degradation. Nucleotide removal (descending curve) competes with the polymerization reaction (ascending curve). Therefore, slight changes in the kinetics of these reactions directly influence the performance of the sequencing reaction.

Discussion Papers

Dethlefsen et al., 2007. Nature 449: 811

Table 1 | Model systems for animal–microbe symbioses

Type of symbiosis	Specific system (Host/symbiont species)	Host phylogenetic affiliation	Host tissue colonized	Reference
Highly complex consortia (10^2–10^3)*	*Mus musculus* (mouse)	Vertebrate chordate	Intestine	19
	Danio rerio (zebrafish)	Vertebrate chordate	Intestine	86
	Microcerotermes spp. and *Reticulitermes* spp. (termites)	Insect arthropod	Hindgut	87
Relatively simple consortia (~2–25)*	*Hirudo medicinalis* (leech)	Oligochaete annelid	Intestine	88
	Lymantria dispar (gypsy moth)	Insect arthropod	Larval midgut	89
	Drosophila melanogaster (fruitfly)	Insect arthropod	Intestine	90
	Hydra oligactis and *Hydra vulgaris*	Hydrozoan cnidarian	Not determined	91
Monospecific (1)*	*Euprymna scolopes* (sepioloid squid)/*Vibrio fischeri*	Cephalopod mollusc	Light organ	92
	Eisenia fetida (earthworm)/*Acidovorax* spp.	Oligochaete annelid	Excretory tissues	93
	Steinernema spp./*Xenorhabdus* spp. and *Heterorhabditis* spp./*Photorhabdus* spp.	Entomopathogenic nematodes	Gut-associated vesicle or region	94

*Number of bacterial–symbiont phylotypes found reproducibly.

Dethlefsen et al., 2007. Nature 449: 811

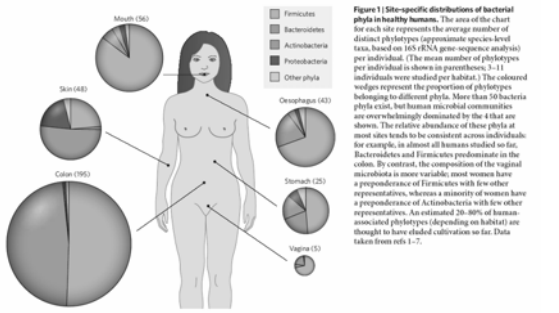


Figure 1 | Site-specific distributions of bacterial phyla in healthy humans. The area of the chart for each site represents the average number of distinct phylotypes (approximate species-level taxa, based on 16S rRNA gene-sequence analysis) per individual. (The mean number of phylotypes per individual is shown in parentheses; 3–11 individuals were studied per habitat.) The coloured wedges represent the proportion of phylotypes belonging to different phyla. More than 50 bacteria phyla exist, but human microbial communities are overwhelmingly dominated by the 4 that are shown. The relative abundance of these phyla at most sites tends to be consistent across individuals: for example, in almost all humans studied so far, Bacteroidetes and Firmicutes predominate in the colon. By contrast, the composition of the vaginal microbiota is more variable; most women have a preponderance of Firmicutes with few other representatives, whereas a minority of women have a preponderance of Actinobacteria with few other representatives. An estimated 20–80% of human-associated phylotypes (depending on habitat) are thought to have eluded cultivation so far. Data taken from refs 1–7.

… What does this tell you about human/symbiont co-evolution?
… what functional information does this survey give us?
… how representative is the dataset?

Dethlefsen et al., 2007. Nature 449: 811

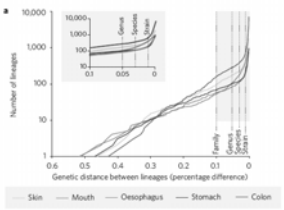


Figure 2 | Patterns of human-associated microbial diversity. a, Lineage-by-distance analysis of 16S rRNA gene-sequence data from human microbial communities in specific habitats. The *x* axis shows the percentage difference threshold (Olsen correction), over 1,241 unambiguously aligned positions of near full-length 16S rRNA gene sequences, for delineating separate lineages. The *y* axis shows the number of distinct lineages that exist at the distance threshold. If speciation and extinction occur with constant probabilities as 16S rRNA gene sequences diverge, this would result in an exponentially increasing number of lineages with diminishing evolutionary distances between them (a straight line on a semi-logarithmic plot). Such a pattern seems to hold from the phylum level (largest distances between lineages) to approximately the species level. However, relative to this trend, all sites have an excess of recently diverged lineages. The excess lineages accumulate in the range of 16S rRNA gene divergence that is typically associated with species and strains. The inset depicts a portion of the same data at a larger scale. Samples were taken from 3–11 individuals, depending on the site. Data taken from refs 1–5. **b**, When displayed as a dendrogram, 16S rRNA gene-based patterns of microbial diversity in soil and aquatic environments generally resemble the tree shape on the left, with new branches arising at all distances from the root. Patterns of diversity in vertebrate-associated communities resemble the tree shape on the right, with few branches arising close to the root and many branches arising close to the branch tips.

Dethlefsen et al., 2007. Nature 449: 811

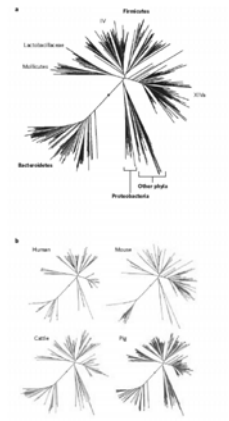


Figure 3 | Relationships between bacterial 16S rRNA gene sequences from the intestinal microbiota of animals. A set of aligned, high-quality, full-length sequences was obtained from Greengenes[36]. Sequences derived from one human stool sample and caecal samples from one mouse family were chosen to obtain approximately the same number of sequences as obtained from multiple studies of the bovine rumen and pig caecum and colon (range 617–748 sequences per host species). **a**, A neighbour-joining tree was created from 1,241 unambiguously aligned positions in all 2,735 sequences, with selected taxa indicated. Mollicutes, Lactobacillaceae and Clostridium clusters IV and XIVa are within the Firmicutes[36]. **b**, Host-specific trees were created with the same topology as the entire tree, shown in part a, but they depict only the sequences derived from the indicated host species. Branches shared with at least one other host species are shown in black, and branches specific to a single species are coloured. The same phyla and clones predominate in these animals (evident from the overlapping tree topologies and shared branches), although their relative abundances vary. By contrast, most genera and many families are specific to a single host species (coloured branches).

Dethlefsen et al., 2007. Nature 449: 811

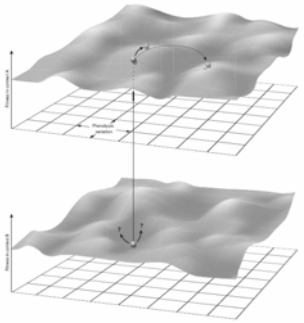


Figure 4 | Adaptive landscapes. The plane is a conceptual representation of the multidimensional phenotypes that are available to a microorganism. The height of the surface above the plane represents the fitness of the corresponding phenotypes in a given ecological context, including biotic and abiotic components of the environment. In a given environment (context A, upper panel), for mutations that have a small effect, a phenotype (circle) under natural selection will tend to evolve along the steepest path uphill towards higher fitness (solid arrow), eventually moving the mean phenotype of a population to a local fitness maximum. Mutations that have a large effect, such as horizontal gene transfer, can shift a phenotype to the slope of a different fitness peak (dashed arrow). This can markedly alter the outcome for the host; for example, it can result in pathogenesis instead of mutualism. The valley separating the peaks represents phenotypes of low fitness, such as those that are likely to elicit an immune response but lack the adaptations necessary to survive it. For a given phenotype, a change in context (for example, a change in host diet, alterations in coexisting microbial populations, or transfer to a different host or host species; context B, lower panel) can have subtle or marked effects on fitness. A phenotype near a fitness peak in context A might be in a valley of low fitness in context B. If the microorganism survives, the subsequent course of evolution might depend on the direction of phenotypic change caused by the new mutation.

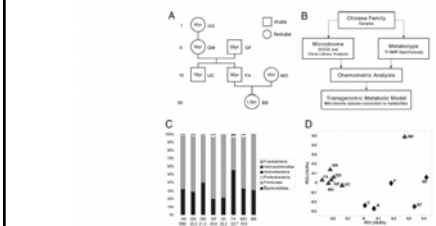


Fig. 1. Experimental procedure and structural comparison of gut microbiome between Chinese and American individuals. (*A*) Family tree diagram of the Chinese family. (*B*) Scheme of experimental procedure. (*C*) The division-level composition of gut microbiome of the Chinese family. (*D*) Species-level composition of gut microbiome of the Chinese family in comparison with reported American microbiome data (*6*, *5*). The principal coordinate score plot was generated by using UniFrac metrics. The percentages of variation described by the principal coordinates are shown in the parentheses.

Li et al., 2008. PNAS 105(6): 2117

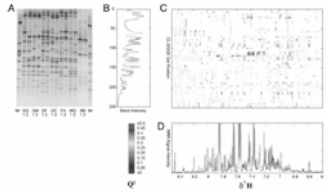


Fig. 2. Multivariate analysis for identifying associations between the gut microbiome structure and the urine metabolite profile. (*A*) DGGE gel for *C. leptum* subgroup. Mr, marker lane. (*B*) OPLS prediction of clostridia bands from the NMR urinary profile data. (*C*) Two-dimensional correlation map of NMR-derived metabolic profile variation in relation to DGGE fingerprints (only the aromatic region of urinary NMR spectra is shown); only points with absolute correlation level >0.7 are shown, red denotes positive correlation, blue denotes negative correlation. (*D*) OPLS prediction of aromatic region of the NMR spectrum from DGGE data. The color indicates the Q^2 value.

Li et al., 2008. PNAS 105(6): 2117

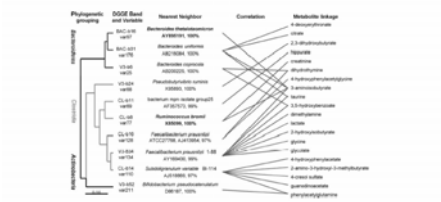


Fig. 3. Dendrogram of OTUs from DGGE bands, which are well predicted by metabolic variation, labeled as the nearest known neighbor with similarity value. Associations with specific urine metabolites are shown for each OTU with the direction of correlation indicated by red (positive) or green (negative) line. Gender-related bands predicted by OPLS-DA are denoted by bold text.

Li et al., 2008. PNAS 105(6): 2117