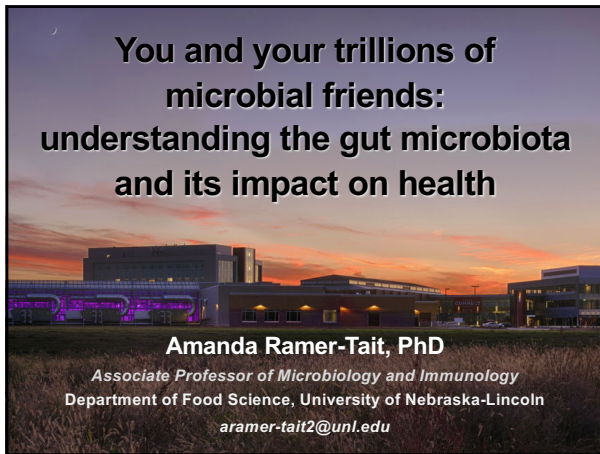


You and your trillions of microbial friends: understanding the gut microbiota and its impact on health



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Department of Food Science, University of Nebraska-Lincoln
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You and your gut microbes



100 trillion
microbial cells in your body

1,000
species of gut bacteria identified to date

~200
times as many microbial genes in your body than human genes

Two-thirds
of the species of gut bacteria in your body are unique to you

6 pounds
is the combined weight of all the microbes in your body (twice that of your brain)

Why are gut microbes important?



You + Microbes = Holobiont

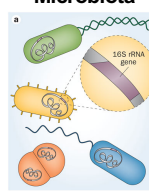
Digestion
Immune System Development
Gut Barrier Integrity

Vitamin Production
Colonization Resistance

What do we call our gut microbes?

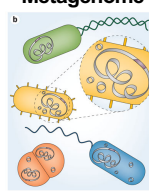
What's the difference between the microbiota, the metagenome and the microbiome?

Microbiota



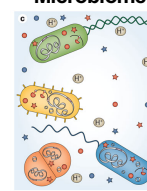
The community of microbes in an environment

Metagenome



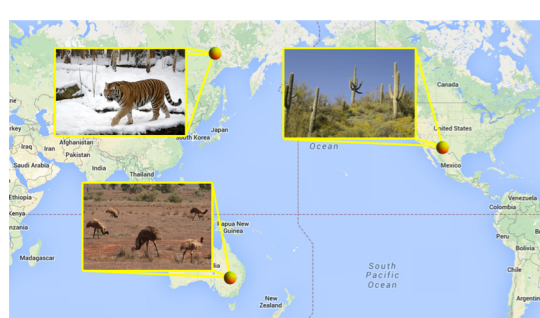
Genes and genomes of the microbiota
(the genetic potential of the population)

Microbiome



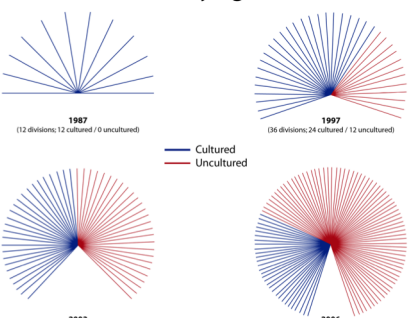
Total collection of genes, genomes and products of the microbiota

How do we know who's there?



Can we culture them?

Known Bacterial Phylogenetic Divisions



1987 (12 divisions; 12 cultured / 0 uncultured)

1997 (36 divisions; 24 cultured / 12 uncultured)

2003 (53 divisions; 26 cultured / 27 uncultured)

2006 (~100 divisions; 30 cultured / ~70 uncultured)

Legend: — Cultured — Uncultured

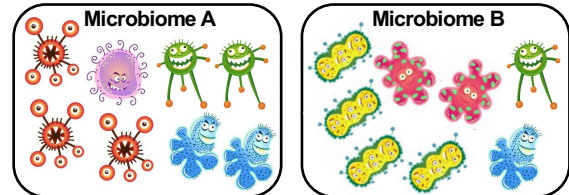
So what do we do?



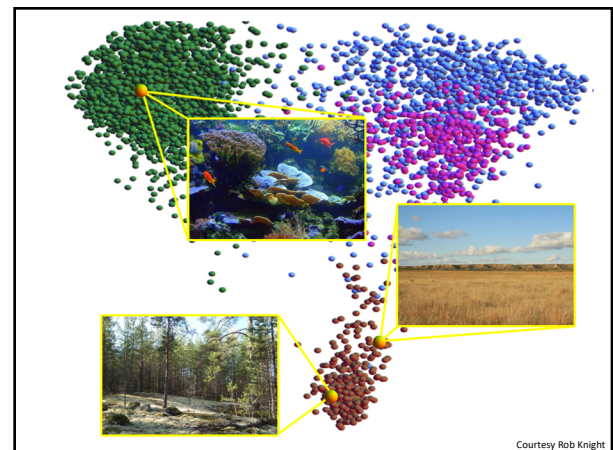
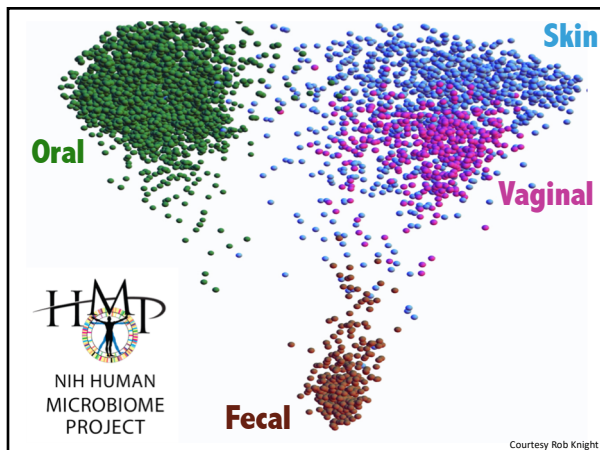
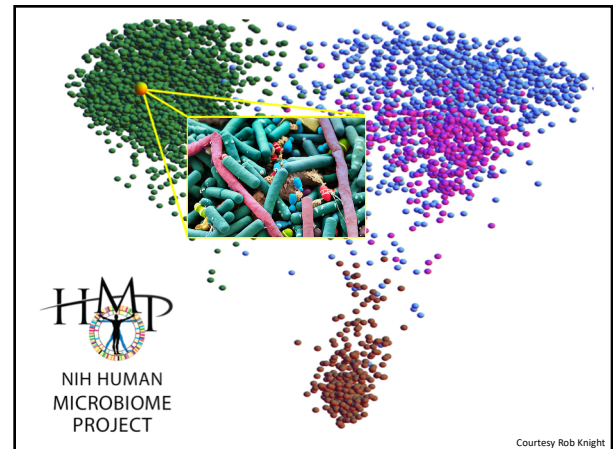
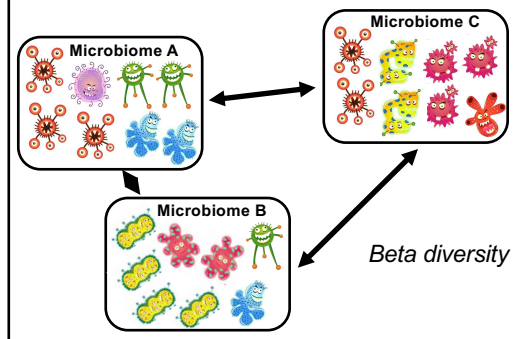
Metagenomics:
the study of the genes and genomes
present in the microbiota

What do we do with the DNA data?

ATGGCACTTA... : 
TCCGAAAGCC... = 
GGGTCTAGCG... = 



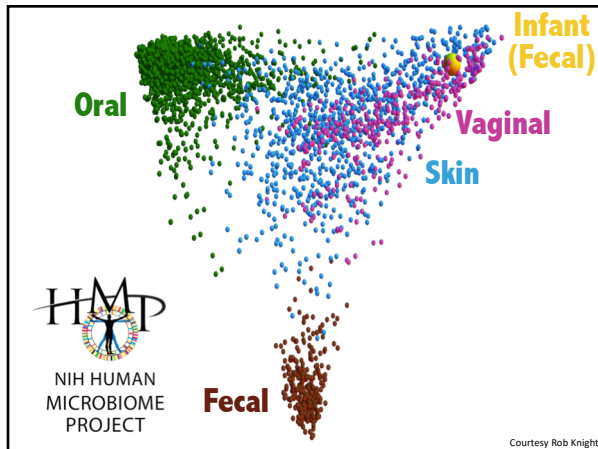
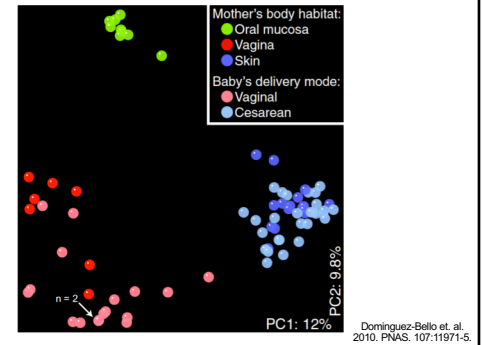
How do we visualize microbiome differences?



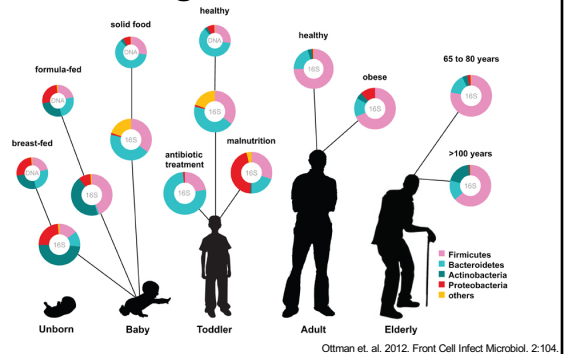
When does our gut microbiota develop?



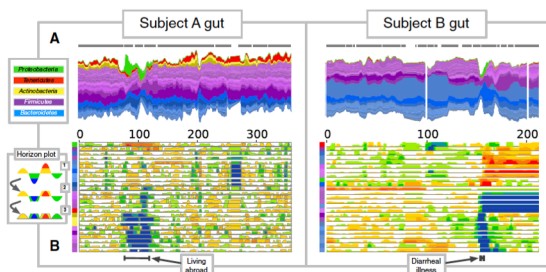
When does our gut microbiota develop?



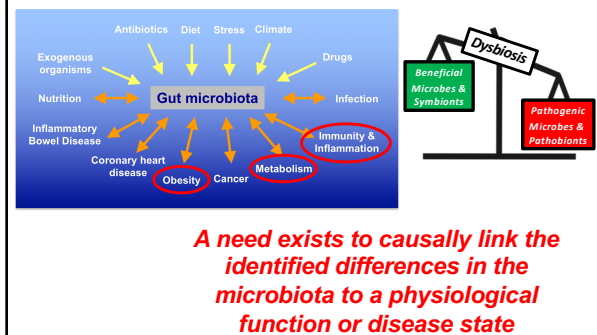
Does our gut microbiota change throughout our lives?



Does our gut microbiota change throughout our lives?



Are changes in the gut microbiota linked to disease?



How can we establish causation?

Gnotobiotic: from Greek gnōtos "known" + biotē "life"

The status of the microbial community is known



Germ-free:
No microbes

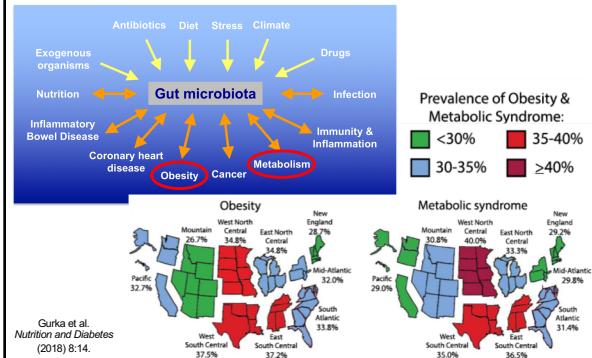


Mono-associated:
Germ-free mouse colonized
with one bacterial species



Conventional:
Complex microbiota from
birth—*NOT gnotobiotic*

Obesity and metabolic syndrome: A connection to the gut microbiota

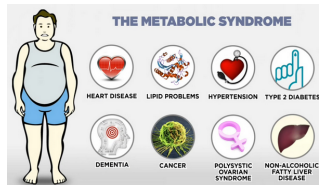


Metabolic Syndrome

A cluster of conditions that raises the risk for heart and metabolic diseases

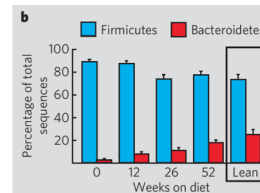
A diagnosis of MetS requires three or more of the following:

- A large waistline
- High triglyceride level
- Low HDL-cholesterol level
- High fasting blood sugar
- High blood pressure

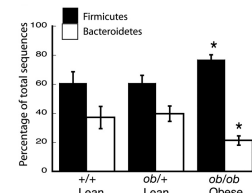


Obesity and metabolic syndrome: A connection to the gut microbiota

Observations in people...



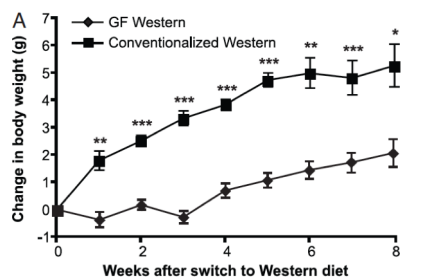
observations in conventional mice...



Ley et al. Nature, 2006.
Ley et al. PNAS, 2005.

Obesity and metabolic syndrome: A connection to the gut microbiota

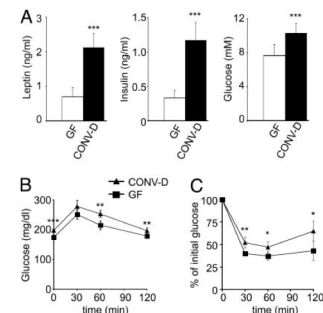
...and studies in germ-free mice.



Backhed et al. PNAS, 2004 and 2007.

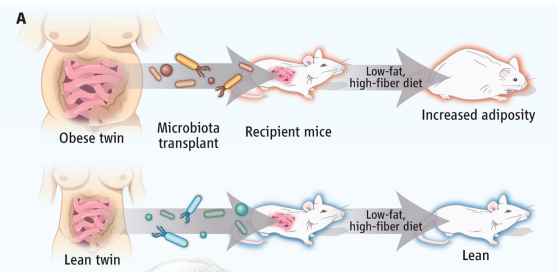
Obesity and metabolic syndrome: A connection to the gut microbiota

...and studies in germ-free mice.



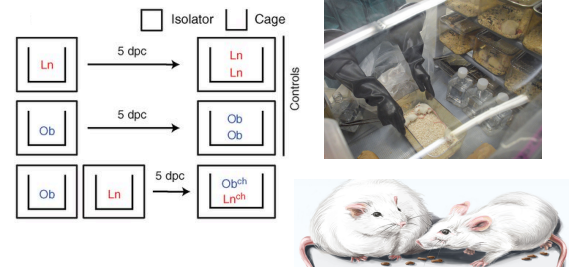
Backhed et al. PNAS, 2004 and 2007.

Gut microbes from twins discordant for obesity modulate mouse metabolism

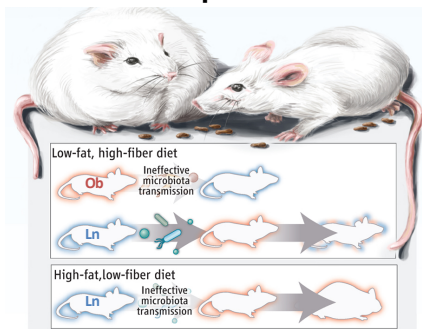


Ridaura et al. Science 2013;341:1241214

How well do “lean” and “obese” microbiomes compete with each other?

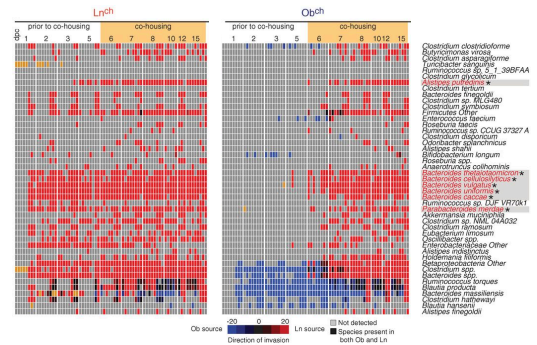


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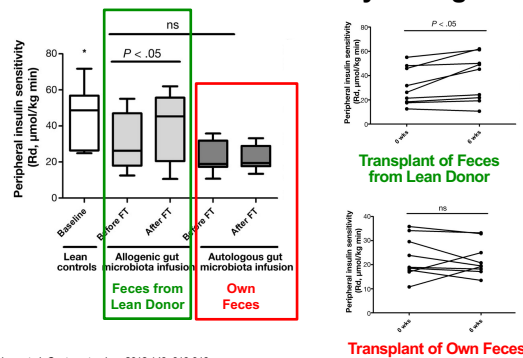
Ridaura et al. Science 2013;341:1241214

**“Lean” microbiomes outcompete
“obese” microbiomes**

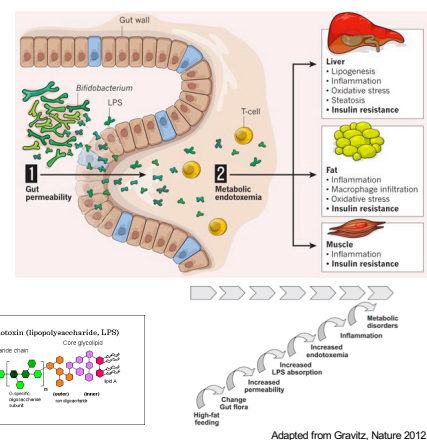


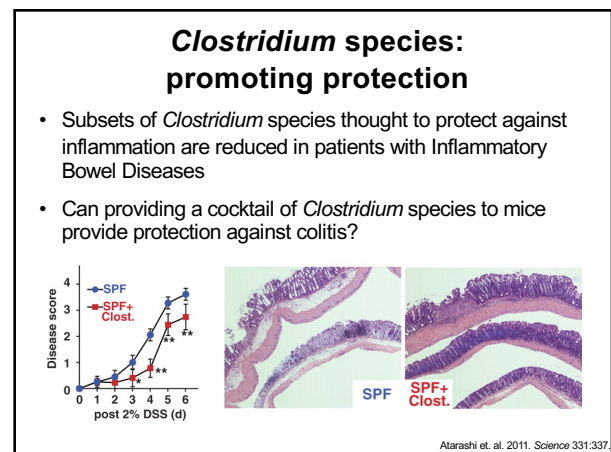
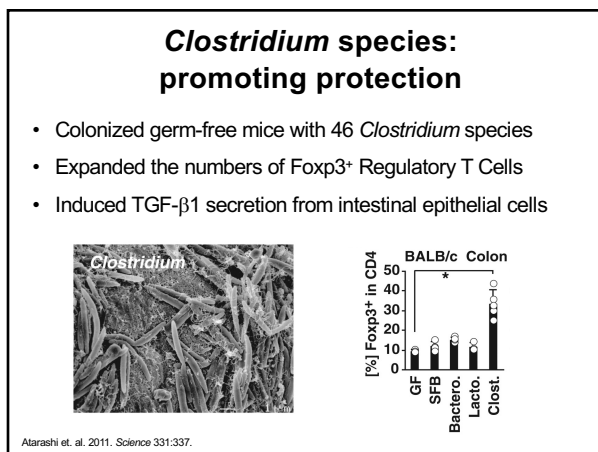
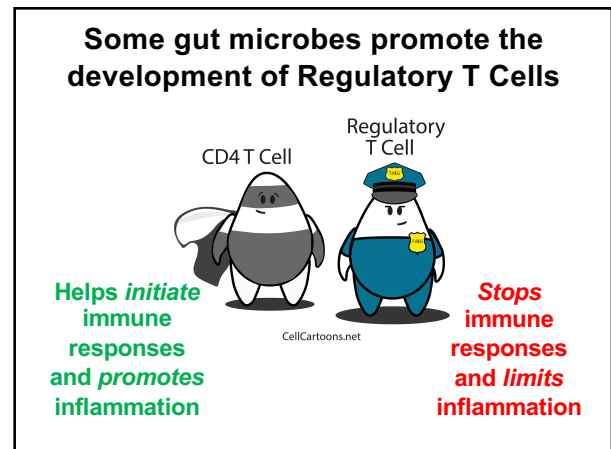
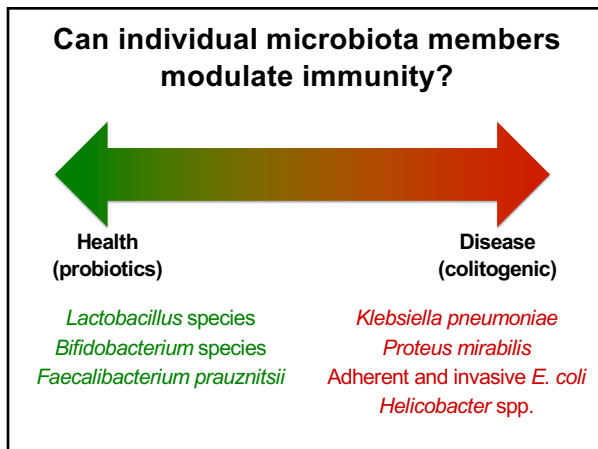
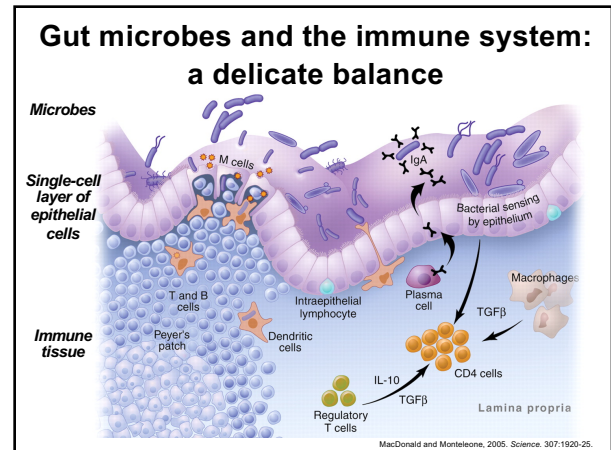
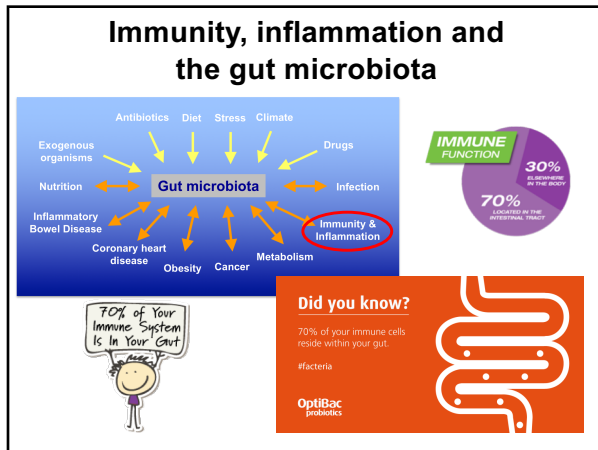
Ridaura et al. Science 2013;341:1241214

Fecal transfer of lean donor microbiomes increases insulin sensitivity during MetS

Vrieze et al. *Gastroenterology* 2012 143, 913-916

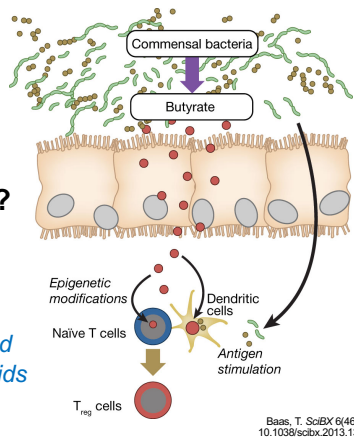
How does the microbiota contribute to obesity and metabolic disease?





How do gut microbes provide protection against inflammation?

By producing metabolites and by-products of fermentation called short-chain fatty acids (butyrate)



Should we change the composition of the gut microbiota? How?

Antibiotics
Bacteriophages (viruses)
Fecal transplantation
Diet
Probiotics
Prebiotics
Fiber and whole grains
Other food components



Probiotics

dietary supplements containing potentially beneficial bacteria or yeasts

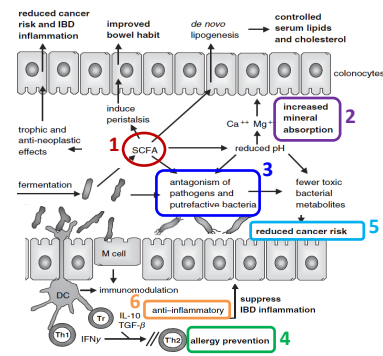
According to the currently adopted definition by FAO/WHO, probiotics are:

“Live microorganisms which when administered in adequate amounts confer a health benefit on the host.”

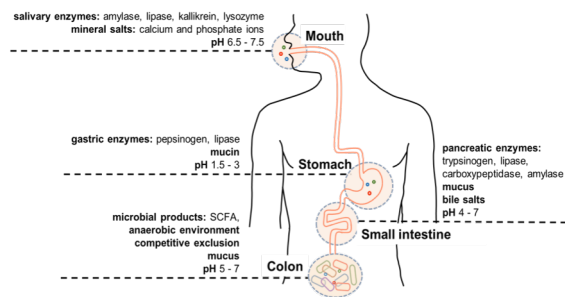


Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powdered Milk with Live Lactic Acid Bacteria (October 2001)

Role of probiotics in gut health

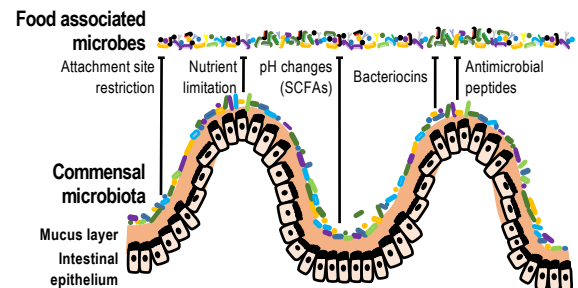


Challenges along the way



Kok and Hutkins, 2018

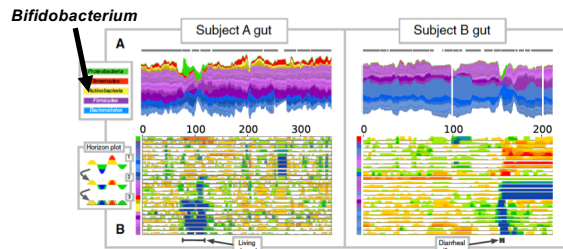
Challenges: colonization resistance



Adapted from Sassone-Corsi and Raffatellu, 2015.

How do we overcome these challenges?

Consume probiotics regularly at high doses



Regular yogurt consumption maintains bifidobacteria in the gut

David et. al. 2014. Genome Biology. 15:R89.

Probiotic consumption does not routinely alter microbiota composition...

Kristensen et al. Genome Medicine (2016) 8:52
DOI 10.1038/gim.2016.009

Genome Medicine

RESEARCH Open Access

Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials

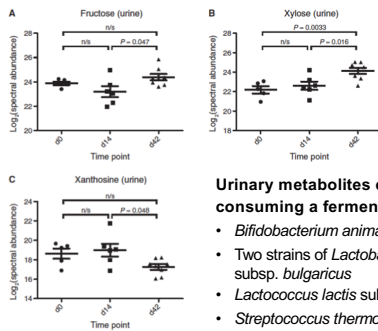
Nadja B. Kristensen¹, Thomas Bryrup, Kristine H. Allen, Tone Nielsen, Tue H. Hansen and Oluf Pedersen

Background: The effects of probiotic supplementation on fecal microbiota composition in healthy adults have not been well established. We aimed to provide a systematic review of the potential evidence for an effect of probiotic supplementation on the composition of human fecal microbiota as assessed by high-throughput molecular approaches in randomized controlled trials (RCTs) of healthy adults.

Conclusions: This systematic review of the pertinent literature demonstrates a lack of evidence for an impact of probiotics on fecal microbiota composition in healthy adults. Future studies would benefit from pre-specifying the primary outcome and transparently reporting the results including effect sizes, confidence intervals, and *P* values as well as providing a clear distinction of between-group and within-group comparisons.

Kristensen et al. 2016. Genome Medicine. 8:52.

...but probiotic consumption does alter microbiota function

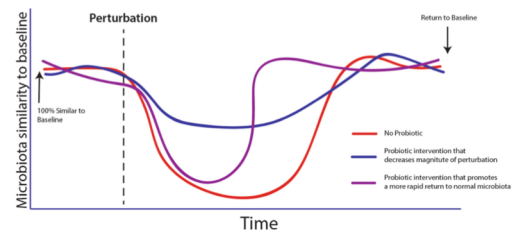


Urinary metabolites change in mice after consuming a fermented milk product:

- *Bifidobacterium animalis* subsp. *lactis*
- Two strains of *Lactobacillus delbrueckii* subsp. *bulgaricus*
- *Lactococcus lactis* subsp. *cremoris*
- *Streptococcus thermophilus*

McNulty et. al., Science Translational Medicine. 2011.

...or probiotic consumption may promote gut microbiota homeostasis (and maybe minimize the effects of microbiota disturbances)



Sanders et al. BMC Medicine (2016) 14:82
DOI 10.1186/s12916-016-0700-9

Sanders. 2016. BMC Medicine. 14:82.

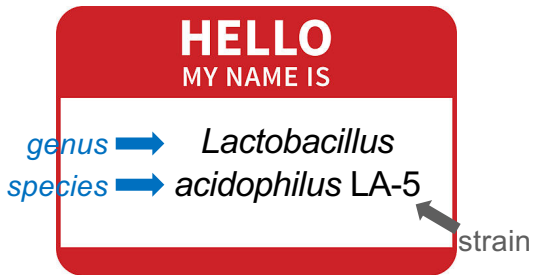
Probiotics Everywhere!



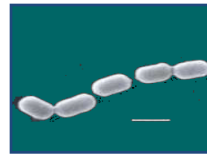
Up close and personal: The science of naming microbes and why it matters



Different species and even strains can deliver different benefits



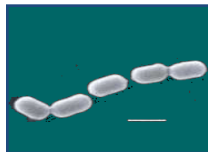
Lactobacillus



Probiotic strains that have been evaluated in clinical trials

- *Lactobacillus fermentum* RC-14 (Urex Biotech)
- *Lactobacillus acidophilus* SBT (Snow Brand)
- *Lactobacillus acidophilus* ADH (Dupont)
- *Lactobacillus rhamnosus* GG (Valio)
- *Lactobacillus rhamnosus* Lcr35 (Lycocentre)
- *Lactobacillus reuteri* DS2112 (Biogaia)
- *Lactobacillus casei* strain Shirota (Yakult)
- *Lactobacillus casei* F19 (Alra Foods)
- *Lactobacillus johnsoni* LA1 (Nestle)
- ***Lactobacillus casei* DN-114001** (Dannon)
- *Lactobacillus plantarum* 299v (Probi Foods)
- *Lactobacillus acidophilus* NCFM (Danisco)
- *Lactobacillus crispatus* CTV05 (Osel, Inc.)

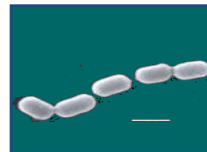
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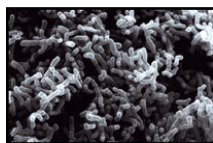
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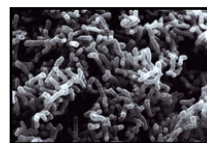
Bifidobacterium



Commercial strains that have been evaluated in clinical trials

- *Bifidobacterium longum* BB536 (Moringa)
- *Bifidobacterium bifidum* (Yakult)
- *Bifidobacterium infantis* (Shirota)
- *Bifidobacterium breve* (Yakult)
- *Bifidobacterium lactis* DS 920 (DSM Foods)
- ***Bifidobacterium animalis* DN 173010** (Dannon)
- *Bifidobacterium lactis* Bb12 (Chr Hansen)
- *Bifidobacterium essensis* (Danone)

Bifidobacterium



Commercial strains that have been evaluated in clinical trials

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- *Bifidobacterium lactis* DS 920 (DSM Foods)
- *Bifidobacterium animalis* DN 173 010 (Dannon)
- ***Bifidobacterium lactis* Bb12** (Chr Hansen)
- *Bifidobacterium essensis* (Danone)

Probiotics Q and A

Q. How to know what's in the yogurt, kefir or capsule?

A. Read the label and look for species and strains



Q. What is an appropriate dose? And in what units?

A. Usually, 100 million cells per g is the minimum.
Many products will contain 1 - 5 billion cells per g

Q. Food versus supplement?

A. Foods components may protect cells from digestion but most supplements are now well protected

Which probiotic should I choose?

Clinical guides summarize the scientific literature



<https://isappscience.org>



Clinical Guide to Probiotic Products Available in USA

Indications, Dosage Forms and Clinical Evidence to Date - 2018 Edition

Introduction

Adult Health

Women's Health

Pediatric Health

Functional Foods

References

About

INDICATIONS FOR ADULT HEALTH

Brand Name	Probiotic Strain	Dosage Form	CFU/Dose	No. of Studies	Indications (Level of Evidence)
ActoBioLife	B. infantis 6326, B. longum 3309	Enteric caps.	100milling	11 servings	C - Constipation (C) BBS - Irritable bowel syndrome (B)
Align	B. longum 3309	Capsules	100-capsule	1 capsule	BBS - Irritable bowel syndrome (B)
Align Cholesterol	B. longum 3309	Tablet	100/Tablet	1 tablet	BBS - Irritable bowel syndrome (B)
Align Extra Strength	B. longum 3309	Capsules	100-capsule	1 capsule	BBS - Irritable bowel syndrome (B)
Align Extra Strength After April 1, 2018	L. acidophilus 1.328 300,000,000 heat-killed enterococci 27mg	Capsule	10mg per cap	2 capsule	EAD: Efficacy/Adverse: Domestically (as adjunct to standard PPIs) (E)
Bio-K+™ C-120	L. acidophilus 1.328 1.0 million C-120 L. reuterus C-120	"Travel Pack" 100 capsules 500-capsule 500-capsule 500-capsule 500-capsule 500-capsule	10-capsules N/A 1.0M 10-capsules N/A 1.0M 10-capsules N/A 1.0M	AD: Antibiotic-associated diarrhea - Prevention (E) CDDU: Clostridium difficile-associated diarrhea - Prevention (E)	
BioGaia Probiotic Drops with Vitamins D	L. reuteri DSM 17930	Drops	100M/Gdrops	5-drops	AD: Antibiotic-associated diarrhea - Prevention (E) C - Constipation (C)
BioGaia Probiotic	L. reuteri DSM 17930	Drops	100M/Gdrops	5 drops	AD: Antibiotic-associated diarrhea - Prevention (E) C - Constipation (C) EAD: Efficacy/Adverse: Domestically (as adjunct to standard evaluation therapy) (E)

Which probiotic should I choose?

Clinical guides summarize the scientific literature



<https://isappscience.org>

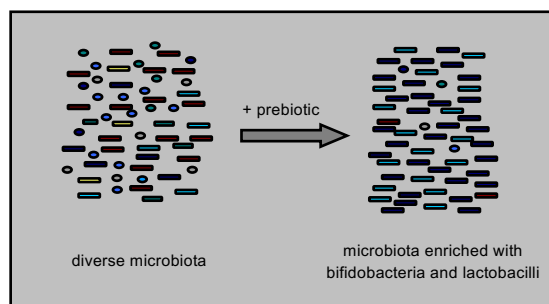
AEProbio		Clinical Guide to Probiotic Products Available in Canada				
Indications, Dosage Forms and Clinical Evidence to Date - 2018 Edition						
Introduction		Adult Health	Women's Health	Pediatric Health	Functional Foods	References
INDICATIONS FOR VAGINAL HEALTH						
Show to / entres						
Brand Name		Probiotic Strain	Dosage Form	CFU/Dose	No of Doses/Day	Indications (Level of Evidence)
Jaro-Dophilact® Women		L. casei LPV 89 L. paracasei LPV 116 L. paracasei LPV 1004 L. rhamnosus LPV 26	Oral Capsule	150 CFU/1 cap	2 capsules	BV ¹ - Bacterial vaginosis (II)
Jarvis Femibiotic® Fem-Dophilact®		L. rhamnosus GR-1	Oral capsule	2.05 each/capsule	1 capsule	BV ¹ - Bacterial vaginosis (II) V ¹ - Vaginal/vulvovaginal candidosis (II)
Proflim® (Proflimactin®) Gel		L. rhamnosus GR-1 L. reuteri RC-14	Oral capsule	2.05 each/capsule	1 capsule	BV ¹ - Bacterial vaginosis (II) V ¹ - Vaginal/vulvovaginal candidosis (II)
Urolab® Women's		L. reuteri RC-14 L. rhamnosus GR-1	Oral capsule	18 each/capsule	2 capsule	BV ¹ - Bacterial vaginosis (II) V ¹ - Vaginal/vulvovaginal candidosis (II)
Indication		Level of Evidence	References			
BV ¹ - Bacterial vaginosis		I	141. Martinez, R.C., S.A. Francisco, M.C. Patta, S.M. Quintana, R.C. G. Gomes, D.C. De Matos, and G. Best "Improved cure of bacterial vaginosis with single dose of bioactive D- Lactobacillus reuteri RC-14 and Lactobacillus reuteri RC-14. A controlled, double-blind, placebo-controlled trial." Canadian journal of microbiology 18:2 (2000): 133-138			
BV ¹ - Bacterial vaginosis		I	142. Nya, G., A.C. Pore, V.S. Sathyanarayana, and S. Sathyanarayana "Efficacy of orally applied, acidified, lactate for bacterial vaginosis and other gynecological infections: a double-blind, randomized, placebo-controlled trial." European Journal of Obstetrics and Gynaecology and Reproductive Biology 168:1 (2012): 75-79			
V ¹ - Vaginal/vulvovaginal candidosis		I	143. Martinez, R.C., S.A. Francisco, M.C. Patta, S.M. Quintana, R.C. G. Gomes, D.C. De Matos, and G. Best "Improved treatment of vulvovaginal candidosis with bioactive D- Lactobacillus reuteri RC-14 and Lactobacillus reuteri RC-14. Letter in applied microbiology 6:3 (2000): 295-294			

The *prebiotic* concept

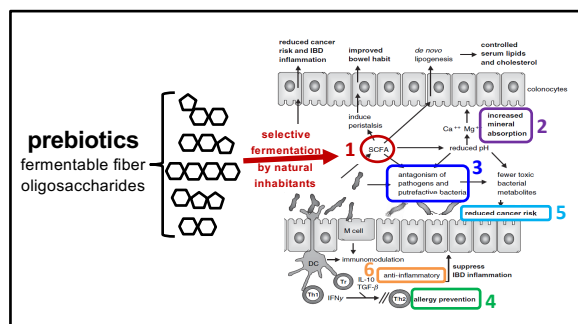
Manipulating the gut microbiota through better eating

- Prebiotics are usually non-digestible oligosaccharides that survive gastric digestion and reach the colon intact
- In the colon, these oligosaccharides are fermented, but only by **select members** of the GI tract
- The population of **oligosaccharide-fermenting strains is enriched**, at the expense of OS non-fermenting organisms
- Among the bacteria capable of metabolizing prebiotics are bifidobacteria and lactobacilli

The *prebiotic* concept



Role of prebiotics in gut health



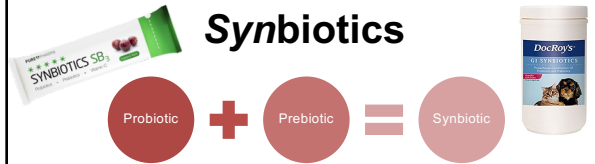
Adapted from Crittenden, 2006.

Prebiotic oligosaccharides are normal dietary components

- Inulin, fructooligosaccharides, galactooligosaccharides + others
- Extracted or synthesized and added as ingredients
- Naturally present in wide variety of fiber-rich foods



Synbiotics



- **Complementary:** components are chosen independently of one another, with each responsible for a particular effect or health benefit

Bifidobacteria + fructooligosaccharides
Lactobacillus rhamnosus GG + inulins

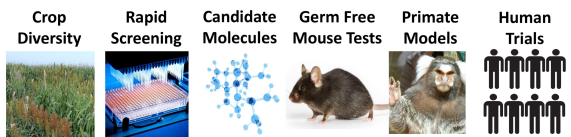
- **Synergistic:** combination is specifically designed with a prebiotic substrate synergistically supporting the competitiveness, survival or metabolic activity of a cognate probiotic strain

How synbiotics are formulated can have considerable influence on their potential effectiveness!

Diet is an excellent tool for manipulating the gut microbiome AND influencing health!



Mission: Identify food molecules with clinically proven effects



A Research Pipeline Aimed at Target Diseases and Health Outcomes

Audacious Goal:
Crops and Foods with Real Health Claims

<https://foodforhealth.unl.edu>