

# What's Bugging You?

## The Human Microbiome and Health

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- <http://www.christopherhobbs.com>

# A few astounding facts

- 10 times more bacteria than our own cells
- (We are 90% bacteria, 10% us)
- 100 x more microbial genes than our own
- Our resident microflora pick up many genes from “wild-type” bacteria from our environment, including drug resistance from pathogenic bacteria
- Bacteria are actively participating in our normal physiology
- Transplanted bacteria from obese donors—more fat

# Human Microbiome Project (HMP)

- [www.hmpdacc.org](http://www.hmpdacc.org)
- “The aim of the HMP is to characterize microbial communities found at multiple human body sites and to look for correlations between changes in the microbiome and human health.
- More information can be found in the menus above and on the [NIH Common Fund site](#).”

# Topics—Human Microbiome Conference

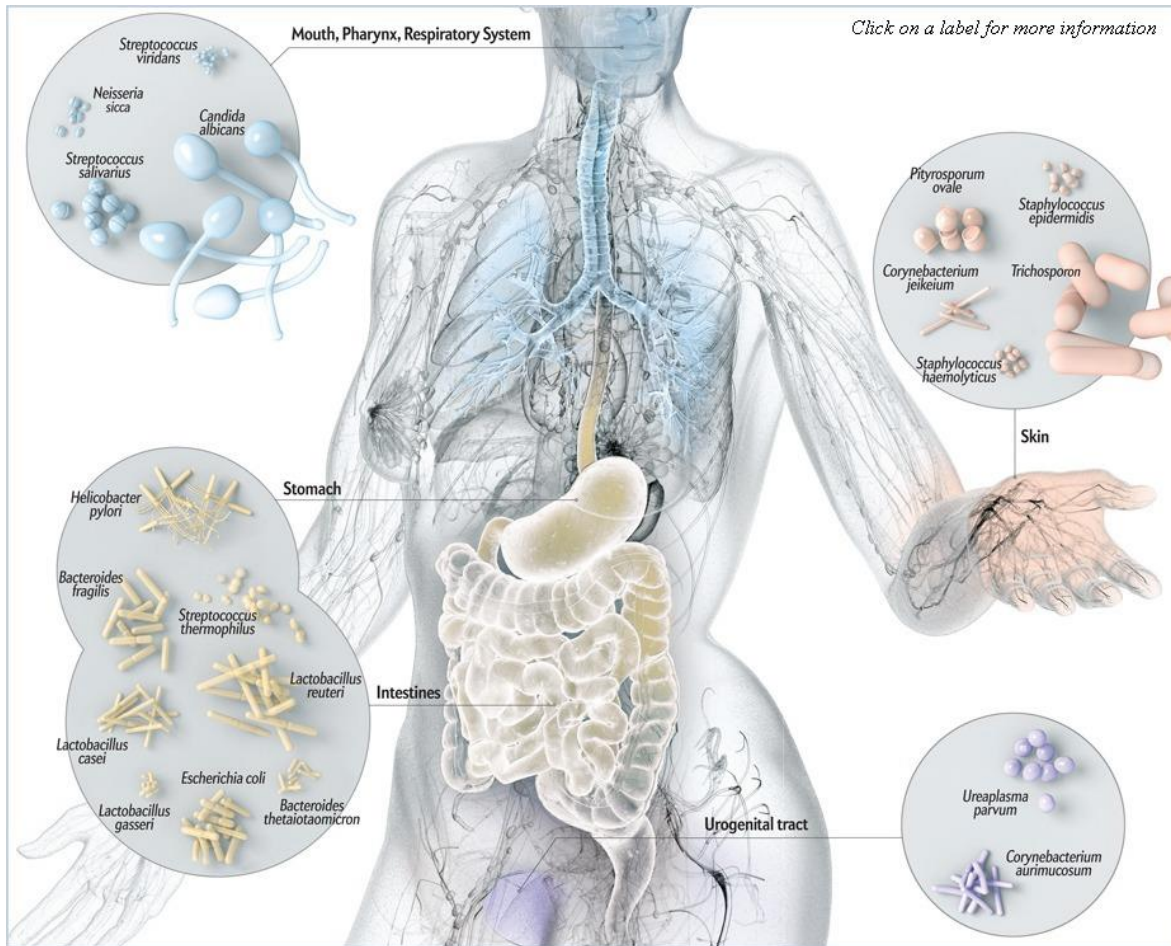
- EMBL Conference (European Molecular Biology Lab)
  - Microbes and us: A complex ecosystem
  - Bioinformatics for the microbiome
  - Microbiome in nutrition and metabolism
  - Microbiome: Interactions with the immune system
  - Moving microbiome research into human populations

# New Projects—NIH Funded

- Cutaneous microbiome (Mb) in psoriasis
- The vaginal Mb
- Urethral Mb in adolescents
- Crohn's disease
- Role of gut Mb in obesity
- Skin Mb and acne
- Atopic dermatitis and immunodeficiency



# Human Microbiome—What is it?



## Microbes that live on and within us

- Hair (even inside)
- Gut
- Stomach (especially as we age)
- Mouth, teeth, gums
- Sinuses
- Vaginal
- Urogenital
- Skin (actually embedded)

# The Human Biome

- Tremendous potential to impact our physiology, both in health and in disease
- They contribute metabolic functions, protect against pathogens, educate the immune system
- Through these basic functions, affect directly or indirectly most of our physiologic functions
- Dysbiosis: microbial imbalance inside or outside our body—compared with our “native” microbiota (eubiosis)
- the relationship between dysbiosis and disease pathogenesis is mostly uncertain at present

# *Origins of the Gut Flora*

- *Genetic factors*
- *Age*
- *Gender*
- *Mother's microbiology*
- *Mode of delivery*
- *Feeding practices*
  - *Breast-fed – Bifidobacterium*
  - *Bottle-fed - Lactobacillus*
- *Medications*
- *Diet*





# Human Biome/Probiotics



- Traditional fermented foods
  - Sauerkraut, yogurt, kefir, sourdough bread, many fermented breads (nan) in India, kim chee, miso, pickles, olives
  - “Intestinal gardening”
- Prebiotics
- Probiotics
- An estimated 100 trillion microorganisms representing more than 500 different species inhabit every normal, healthy bowel

# Stability of Microbiome

- An individual's microbiome is quite stable over time
  - Composition depends on birthing method, mother's MB
- Variability at the extremes of age and among different individuals
- Diet and other environmental factors also affect the composition of the microbiome

# Stability of gut microflora—Study

- 37 healthy American adults followed for 5 years
- Greater than 70% of species were stable for over 1 year
- 60% stable after 5 years
- May be mostly stable over an individual's lifetime
- Early gut colonizers, such as those acquired from our parents and siblings
  - have the potential to exert their physiologic, metabolic, and immunologic effects for most, and perhaps all, of our lives.

Source: Faith *et al.*, 2013. The long-term stability of the human gut microbiota. *Science* 341(1237439).

# Practical Benefits of the Microbiota

- Microbes liberate short-chain fatty acids (SCFA) from indigestible dietary fibers
    - Important energy source for intestinal mucosa
    - Critical for modulating immune responses and tumorigenesis in the gut (numerous, complex, bidirectional)
  - Many interactions with the immune system
  - Protects against inherently pathogenic bacteria
  - Metabolic and weight-regulation functions
- A better understanding of the functional interactions between the human host and the microbiome is very likely to lead to new diagnostic, prognostic, and therapeutic capabilities



# Human microbiota composition

- Combined set of metagenomic sequencing data from 1267 gut metagenomes from 1070 individuals was published
- Nonredundant gene catalog of 9.8 million microbial genes.
- Each sample contained about 750,000 genes or about 30 times the number of genes in the human genome
- Less than 300,000 genes were shared by greater than 50% of individuals

# Diversity of Microbiome

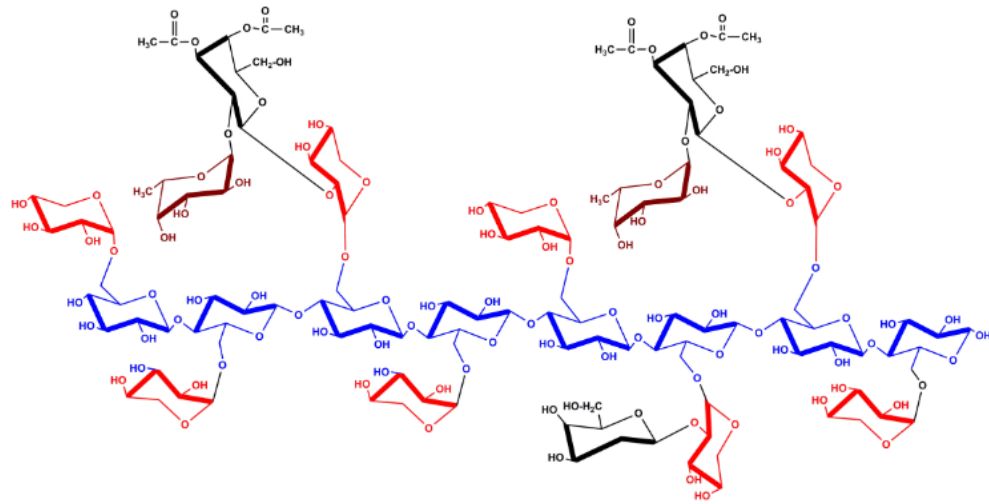
- Healthy adult humans each typically harbor more than 1000 species of bacteria belonging to a relatively few known bacterial phyla
- The microbiota of the gut is quite diverse compared to other body sites, and there is considerable variation in the constituents of the gut microbiota among apparently healthy individuals

Source: Shreiner *et al.*, 2015. The gut microbiome in health and in disease. *Curr Opin Gastroenterol*.

# Distinct gut microflora community types

- Statistical analysis of community types in health people not taking antibiotics
- Four distinct communities were found in the stool
- Metadata factors associated with community types included breastfeeding, gender, and education
- Community types in the oral cavity were predictive of those in the stool though the specific constituents were different (Shreiner *et al.*, 2014. Gut Microbiome in health and disease.)

# Metabolic functions of the microbiota



Xyloglucan: Common vegetable cell wall component



- Gut microbiota is central to host digestion and nutrition
- nutrients generated from substrates that are otherwise indigestible by the host
- Example
  - Xyloglucans (XyGs) are a ubiquitous family of highly branched plant cell wall polysaccharides found in many vegetables (insoluble fiber)
  - Undigestible, except by rare species of gut microflora
  - Ca. 92% of individuals tested contained these few rare species
  - Adaptation and co-evolution with bacteria

Source: Larsbink *et al.*, 2014. *Nature* 506:498.



# Effects on the Immune System

- Appropriately selected probiotics, prebiotics, or their combination exert potent effects on the immune system
- SCFAs are one example of the many bacterial metabolites which influence immune cells
- Microflora interactions with dendritic cells, epithelial cells, T regulatory cells, effector lymphocytes, natural killer T cells, and B cells (Frei et al., 2015)

# Interspecies Communication

- Nonredundant gene catalog of 9.8 million microbial genes.
- Bacteria as a source of new genes—DNA Exchange
- Massive pool of unique genes—and they SHARE!
- Our endemic bacteria has a reservoir of drug-resistant genes, that are often the same as found in pathogens
- Contact with and exchange during active disease
- DNA sex in dental plaque

# Traditionally-Fermented Foods



Amasi	South Africa	fermented milk
Amazaki	Japan	rice
sourdough	Europe	wheat, other grains
Chicha	South America	Corn
Dosa	India	rice, lentils
Fish	Indonesia, China	Fermented fish
Injera	Ethiopia	teff flour
Kefir	Asia	Milk, water
Kimche	Asia	vegetables
Kombucha	Europe	Tea, sugar, etc.
Miso	Japan	Soybeans
Natto	Japan	Soybeans
Olives	Europe	olives--salt, fermentation
Pickles	Europe, etc.	cucumbers
Rejuvelac	n. America	grains (wheat, millet, etc.)
Salami		
Sauerkraut	n. Asia to Europe, etc.	cabbage
Sour Cream	Europe	cream
Tabasco	Mexico	peppers, etc.
Tempeh	Indonesia, Asia	Soybeans
Wine	Everywhere	grapes, malted barley, etc.
Worcestershire	British	vinegar, molasses, sugar, salt, anchovies, tamarind
Yogurt	European	milk



# Kimchi



"Korea-Kimchi-Ancient.form-Museum-01" by by ayustety - <http://flickr.com/photos/ayustety/12743814/>. Licensed under CC BY-SA 2.0 via Wikimedia Commons - <https://commons.wikimedia.org/wiki/File:Korea-Kimchi-Ancient.form-Museum-01.jpg#/media/File:Korea-Kimchi-Ancient.form-Museum-01.jpg>





# Kimchi—benefits

- Kimchi is a traditional Korean food
  - manufactured by fermenting vegetables with probiotic lactic acid bacteria (LAB)
  - Many bacteria are involved
  - LAB become dominant while the putrefactive bacteria are suppressed during salting of baechu cabbage and the fermentation
- Cabbage main ingredient
- Other functional foods
  - Ginger, cayenne, garlic
- typically served with steamed rice at every Korean meal
  - Antiobesity, anticonstipation, colorectal health promotion
  - probiotic properties, cholesterol reduction, fibrolytic effect, antioxidative and antiaging properties, brain health, immune promotion, skin health

# Probiotics on Fruits, Vegetables

- Significant differences in community composition between conventional and organic analogs within produce types.
- Humans are exposed to substantially different bacteria depending on the types of fresh produce they consume with differences between conventionally and organically farmed varieties contributing to this variation.
- Method: DNA analysis, not cultures



Jonathan W. Leff, Noah Fierer. **Bacterial Communities Associated with the Surfaces of Fresh Fruits and Vegetables.** *PLoS ONE*, 2013; 8 (3): e59310 DOI:[10.1371/journal.pone.0059310](https://doi.org/10.1371/journal.pone.0059310)



# Sauerkraut—traditional fermented food



- Sauerkraut has predominantly lactic-acid producers (*Lactococcus lactis*, *Lactobacillus plantarum*, *Leuconostoc mesenteroides*)
- Two species produced nisin, an antibacterial compound known to inhibit pathogenic species (Harris et al., 1992)
- Salt, temperature, days of fermentation affects species composition (up to 15)
- Starter affects species mix (Halász et al., 1999)

# Sauerkraut as a Probiotic Product

## A True Superfood!

- Highly variable results with spontaneous fermentation (vs. starter)
- Use starter for low-salt fermentation
- Shredded cabbage—lower salt concentration (1.5%)
- *Leuconostoc mesenteroides* produces a mild, pleasantly aromatic flavor; *L. mesenteroides* and *L. plantarum* best starter (commercially available)
- In one study (Beganović et al., 2011), a finished product with  $>10^6$  CFU /gram (2.5% NaCl)

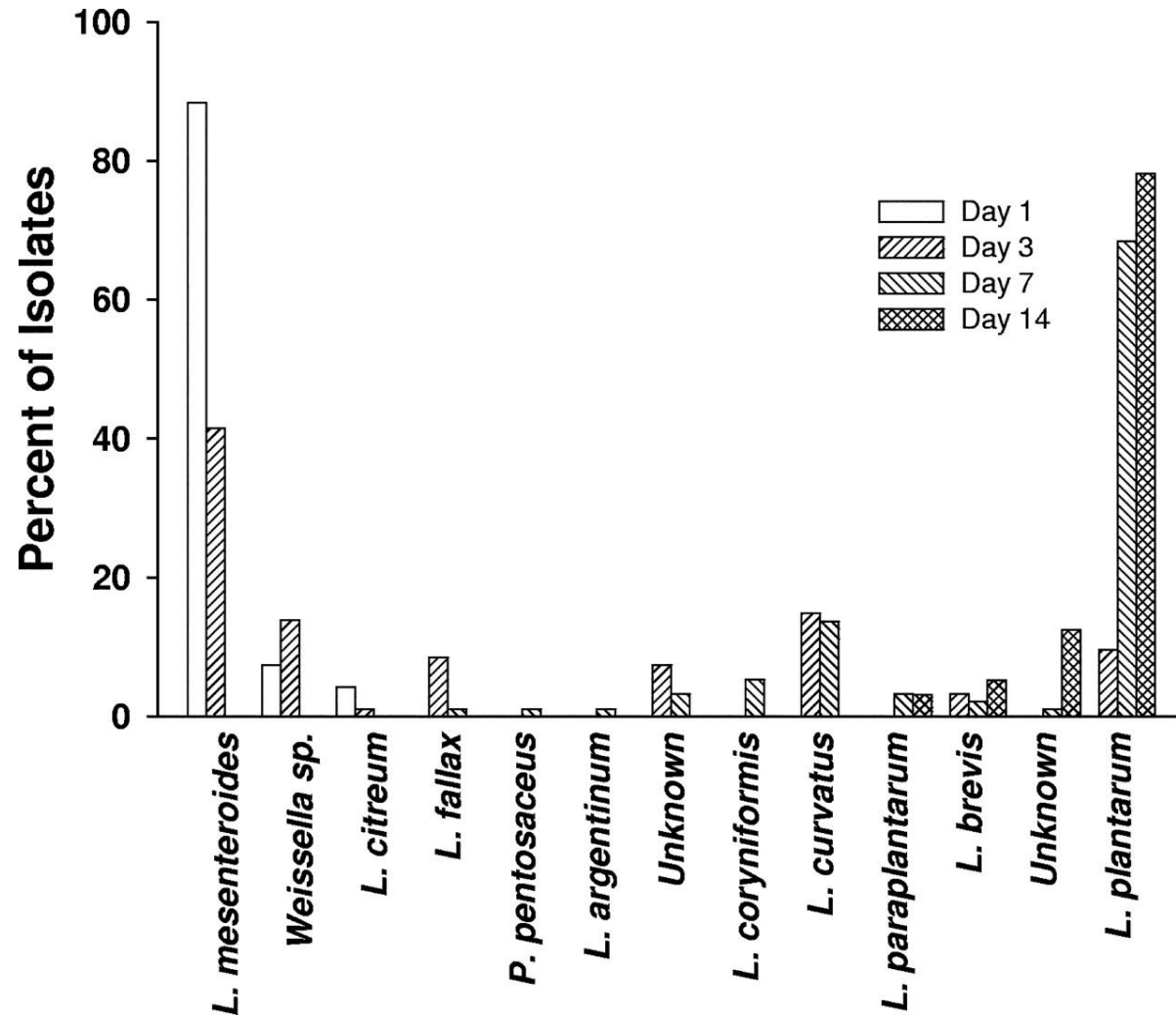


# Making 'Kraut



- Wash, Chop (or dice) cabbage
- Pound with a mallet, stone, etc.—extract some juice
- Layer in jar
  - Cabbage
  - Salt (1/2 tsp sea or earth salt—minerals!)
  - Starter (*Leuconostoc mesenteroides*)
  - Caraway seed or other flavor (juniper cones)
  - Keep layering—don't leave much space at the top
  - Ferment to taste, perhaps 4 weeks

Microbial diversity of the Y1 fermentation as determined by ITS-PCR and 16S rRNA gene sequencing.



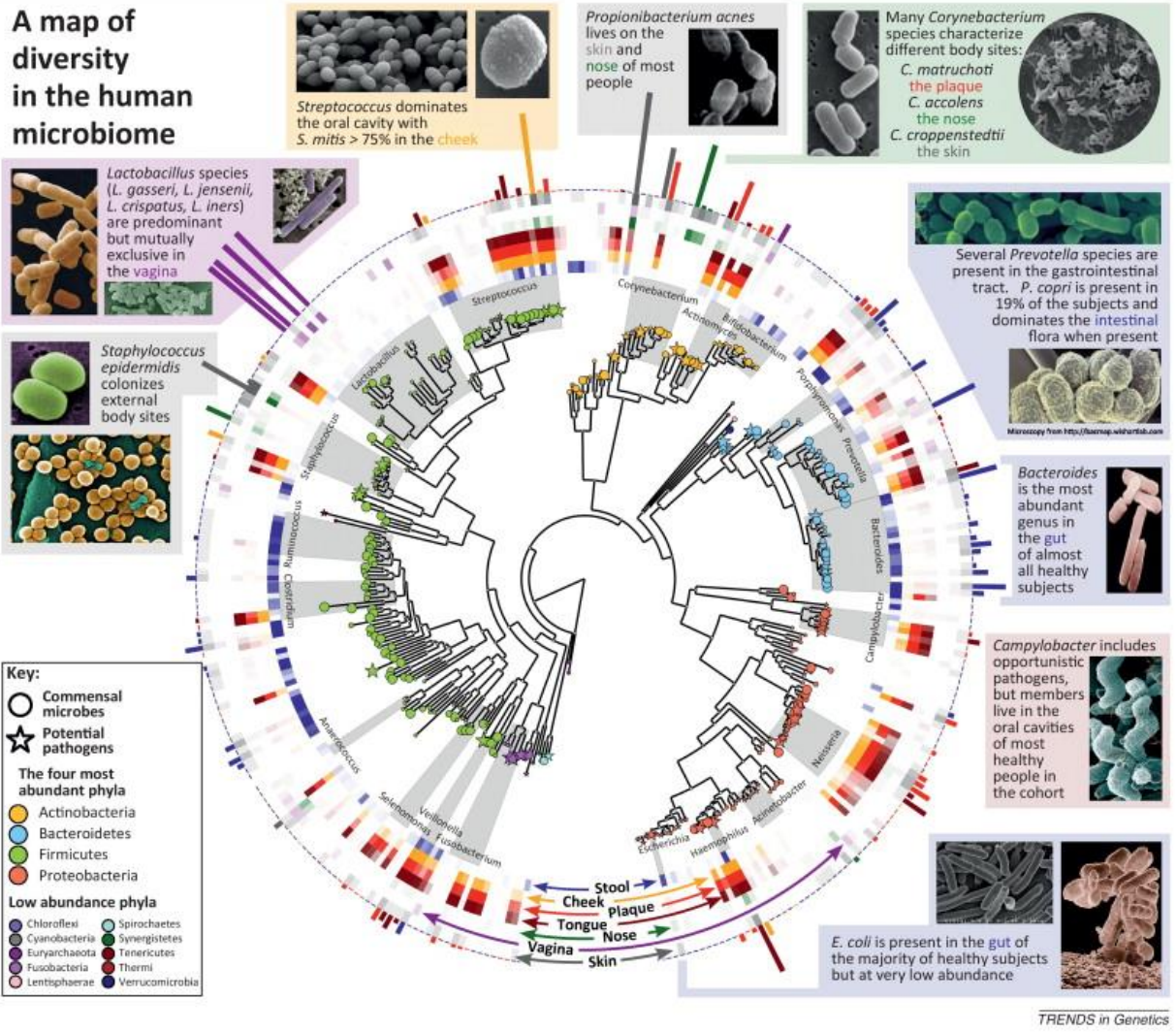
Plengvidhya V et al. Appl. Environ. Microbiol. 2007;73:7697-7702

Applied and Environmental Microbiology



# Phylogenetics

## A map of diversity in the human microbiome



- Extraction, amplification (PCR), and sequencing of genes found in organisms
- Alignment, and statistical algorithms to infer ancestral relationships in the form of bifurcated trees (cladistics)

# *Probiotics*

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

A bacterial strain that:

- Survives the stomach acid and bile
- Adheres to intestinal lining
- Grows and establishes temporary residence in the intestines
- Imparts health benefits

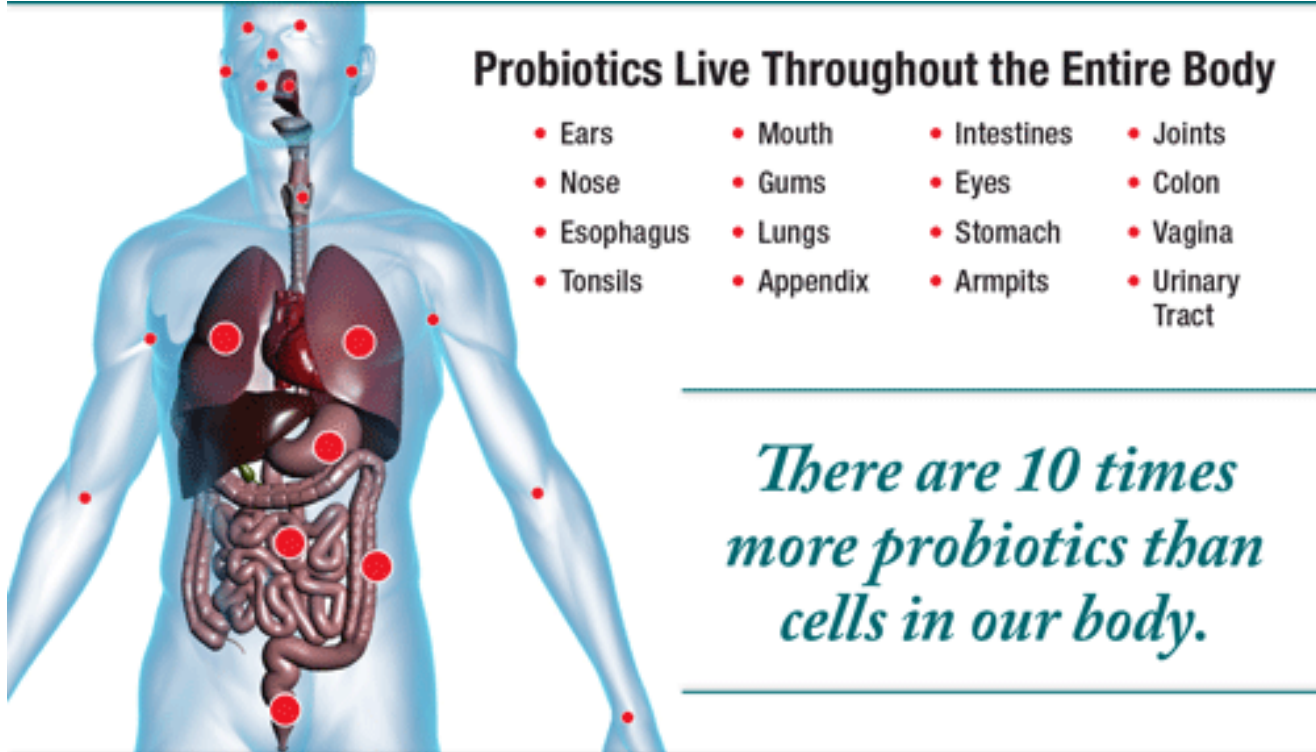


# Products

- Single or mixtures of beneficial (lactic acid-forming) species
- Capsules, tablets, drinks, yogurt, cultured foods
- Old dose from 1980s, 1990s, 20 million organisms
- New dose, based on current research, 10 billion minimum; 25 billion, several times a day up to 125 billion or more
- Refrigerated, except for spore-forming types like *Bacillus coagulans* (= *Lactobacillus sporogenes*)

# Common Species Used in Probiotics

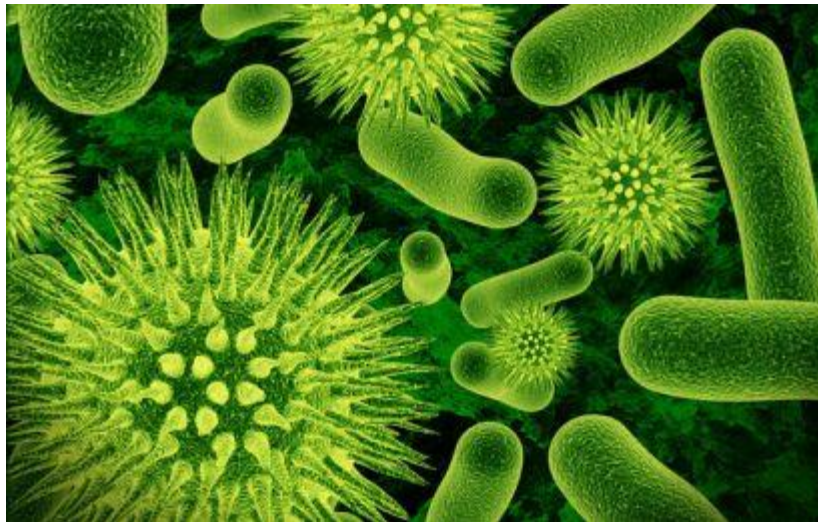
Causing a “shift” with individual species



- *S. sporogenes*
- *L. caseyi*
- *L. rhamnosus*
- *L. plantarum*
- *L. acidophilus*
- Spore-forming species (heat stable; *S. sporogenes*)

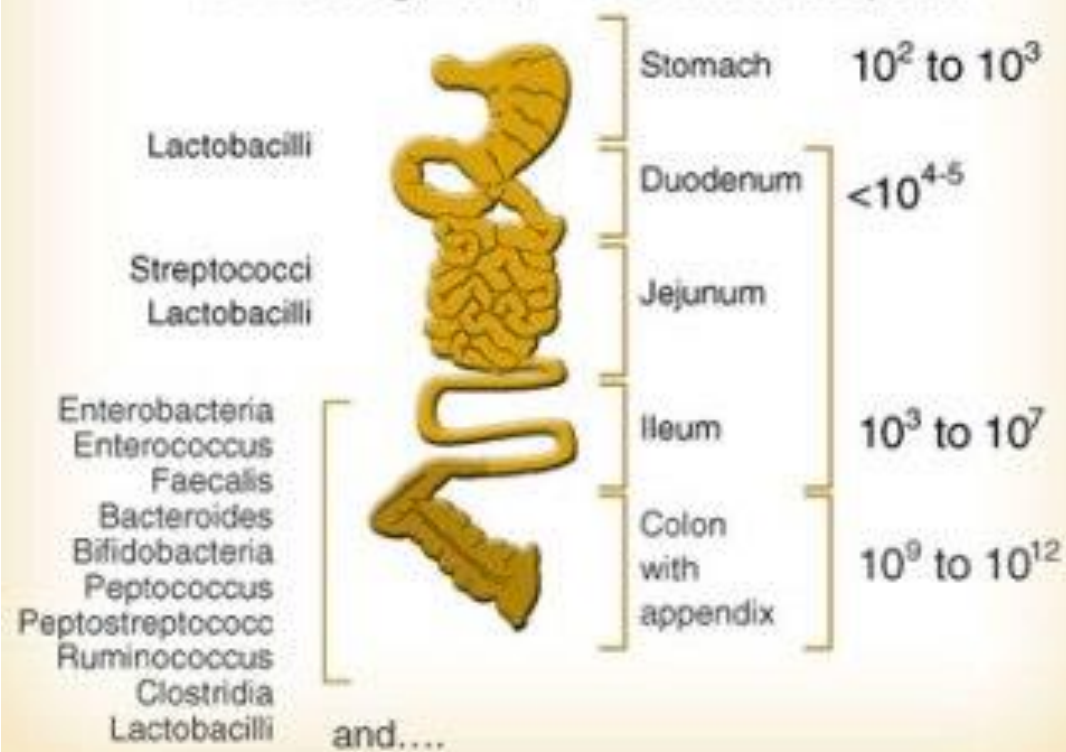
# Probiotics: product variability

- ConsumerLab.com tested probiotic products off the shelf
  - In 2009, 85% failed to deliver labeled number of live organisms
  - In 2012, only 17% failed testing (likely due to increased refrigeration)



# INTESTINAL MICROFLORA

$10^{14}$  micro-organisms, >500 differentes species





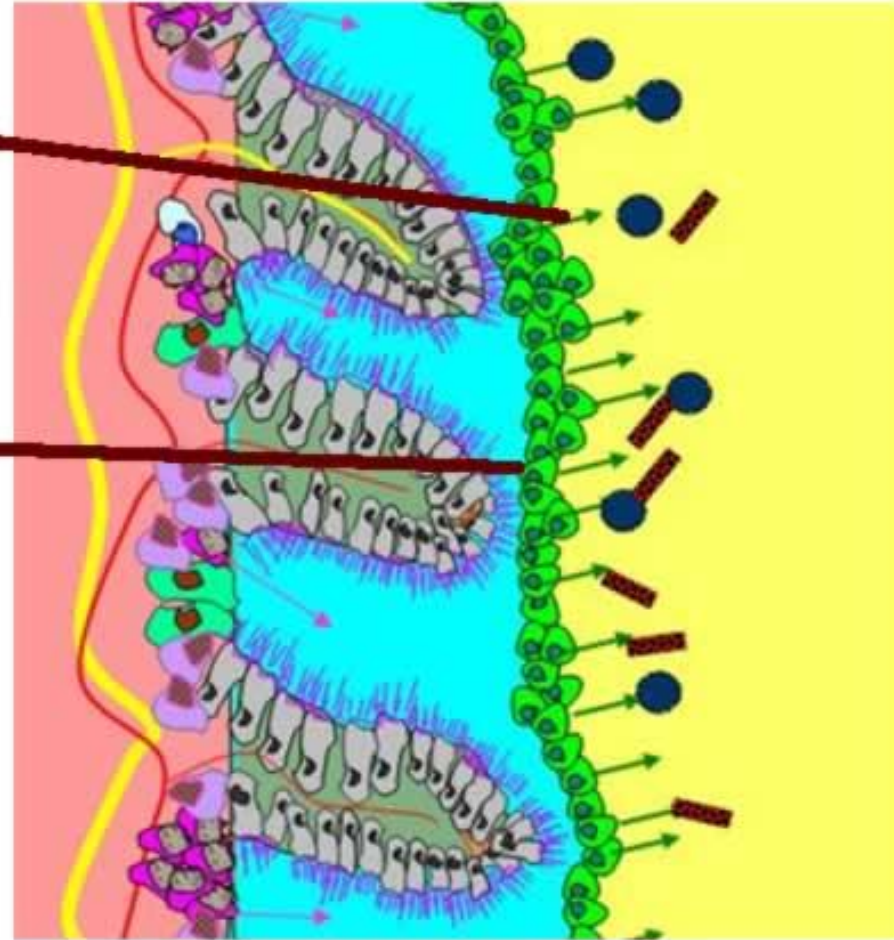
## Probiotic Benefits

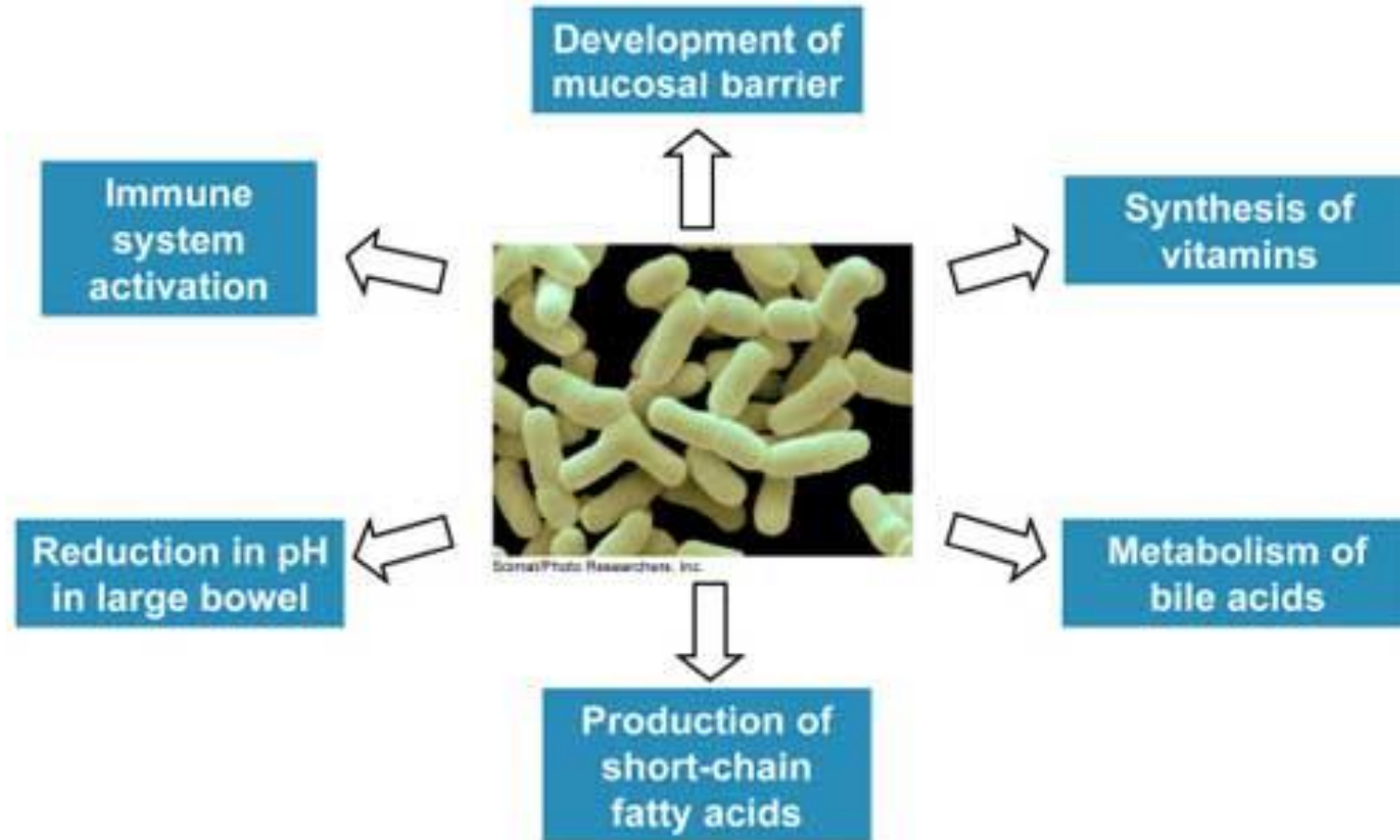
ACIDOPHILUS AND OTHER  
PROBIOTIC BACTERIA  
SECRETE:  
ANTIVIRAL  
ANTIBACTERIAL AND  
ANTIFUNGAL CHEMICALS.

PROBIOTICS FORM A  
PHYSICAL BARRIER TO  
HINDER INVASION OF  
BACTERIA AND YEASTS

Such as *Candida*

PROBIOTICS LIKE  
ACIDOPHILUS CREATE  
AN ACIDIC  
MICROENVIRONMENT  
WHICH PROMOTES  
IRON AND OTHER  
MINERAL  
ABSORPTION.





# Fecal Microbiota Transplants



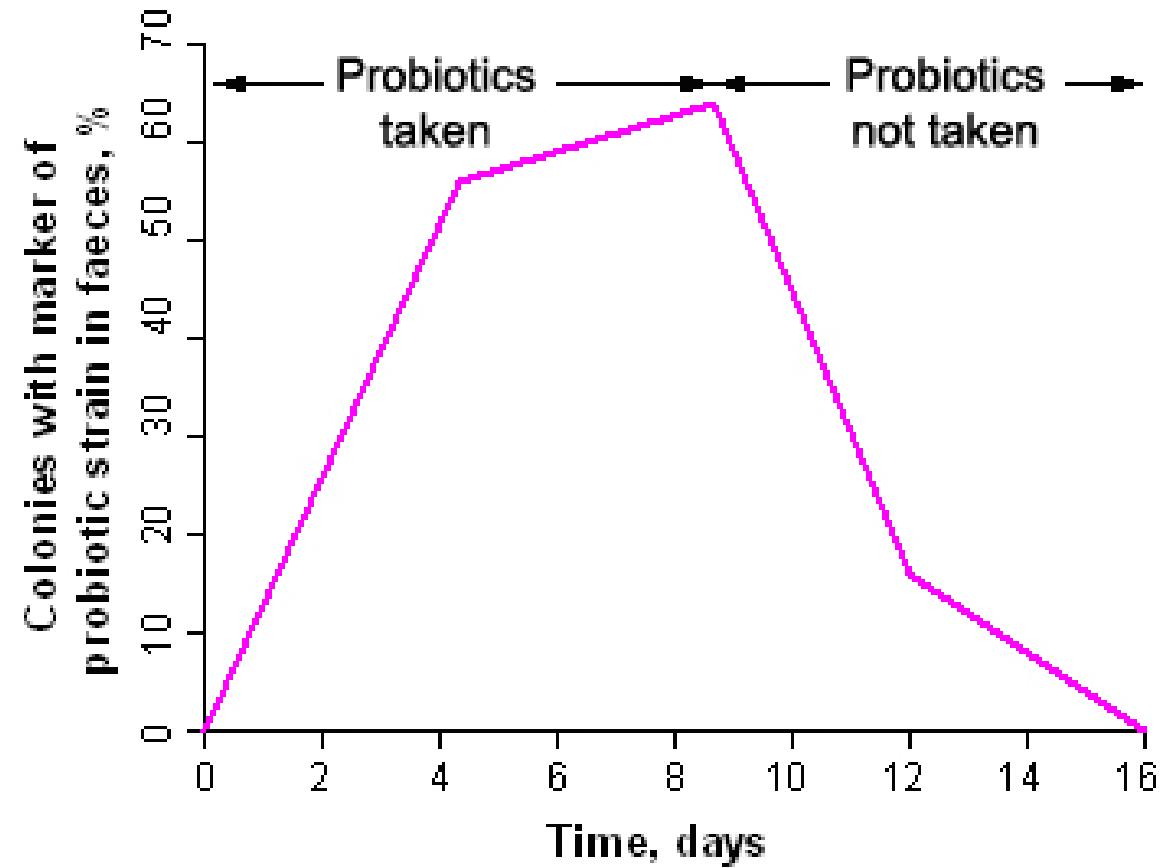
Moayyedi *et al.*, 2015

The master probiotic:  
Enjoying a S---- shake for health!

- RCT = 70 active UC patients
- 24% of those receiving FMT were at remission at 7 weeks
- 5% for those receiving placebo
- Stool from patients receiving FMT had greater microbial diversity, compared with baseline, than that of patients given the placebo

## Fate of Ingested Probiotics

The appearance of ingested probiotics bacteria in faeces





# *Lactobacillus rhamnosus* GG

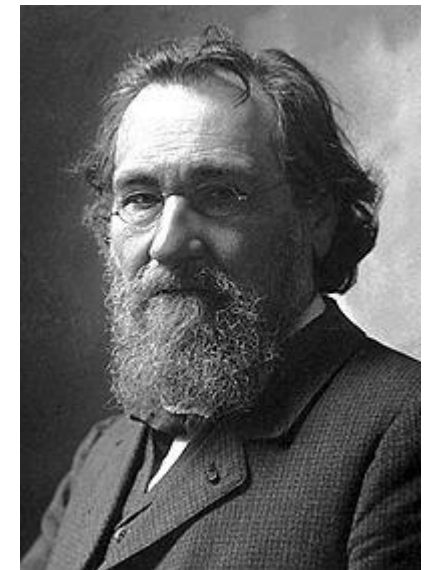
- Isolated from a healthy human's intestinal flora
- Over 800 studies
- Highly resistant to stomach acid, and bile
- Likely transient in gut

# *Lactobacillus rhamnosus* GG, Uses

- Some uses:
  - Alleviate peanut allergy (Scott S., 2015)
  - Diarrhea in children (Canaani et al.)
  - Reduction of risk for URIs in children (Hojsak et al., 2010)
  - Good evidence for reducing atopic dermatitis in children with high risk of allergy (Kalliomäki et al., 2007)
  - Bacterial vaginosis (mixed results, but some positive studies for implantation and oral; (Falagas et al., 2006)

# *History Of Probiotics*

- *In 76 BC the Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis .*
- *Russian scientist Eli Metchnikoff in early 20<sup>th</sup> century suggested that it would be possible to modify the gut flora and to replace harmful microbes by useful microbes*
- *The term “**Probiotics**” was first introduced in 1965 by Lilly and Stillwell, when it was described as growth promoting factors produced by microorganisms.*





# Prebiotics

- General term for food components that encourage growth of beneficial microorganisms to enhance human health
- In the diet, and in dietary supplements, this refers mostly to soluble and insoluble fibers (especially oligosaccharides)
- Make it through the upper GI tract intact to feed microorganisms in the lower tract
- Can change the composition of the gut microflora

Dahlia Tubers--Inulin



Source: [www.dahlias.net](http://www.dahlias.net)

# Good sources of prebiotics

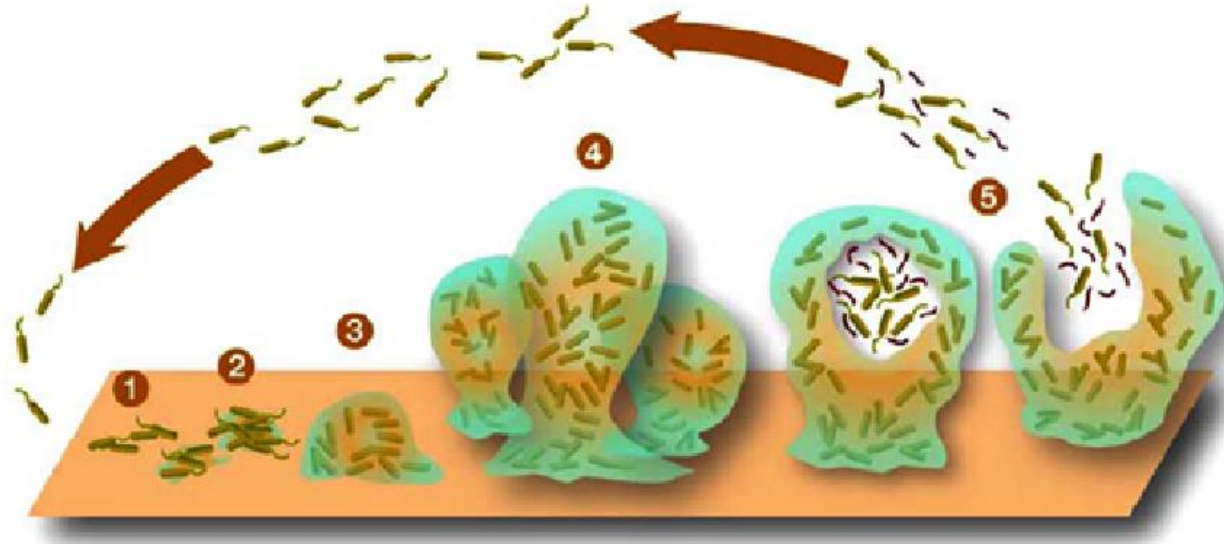
- Dahlia tubers, Dandelion, chicory (64%), burdock roots (inulin)
- Jerusalem artichokes (32% inulin)
- Garlic (about 18%)
- Leeks and onions (9-12%)
- Asparagus (5%)
- Beans (soy, mung, lentils): source of oligosaccharides; (Carlsson et al., 1992)
- Raw oats (Kedia et al., 2009) MUSELI!

# Biofilms

- Biofilm is the preferred form of life for the vast majority of microorganisms
- Microbe communities residing within biofilms may consist of:
  - one or more species that communicate and collaborate with one another
  - heterogeneous community
- Kombucha popular example of a biofilm

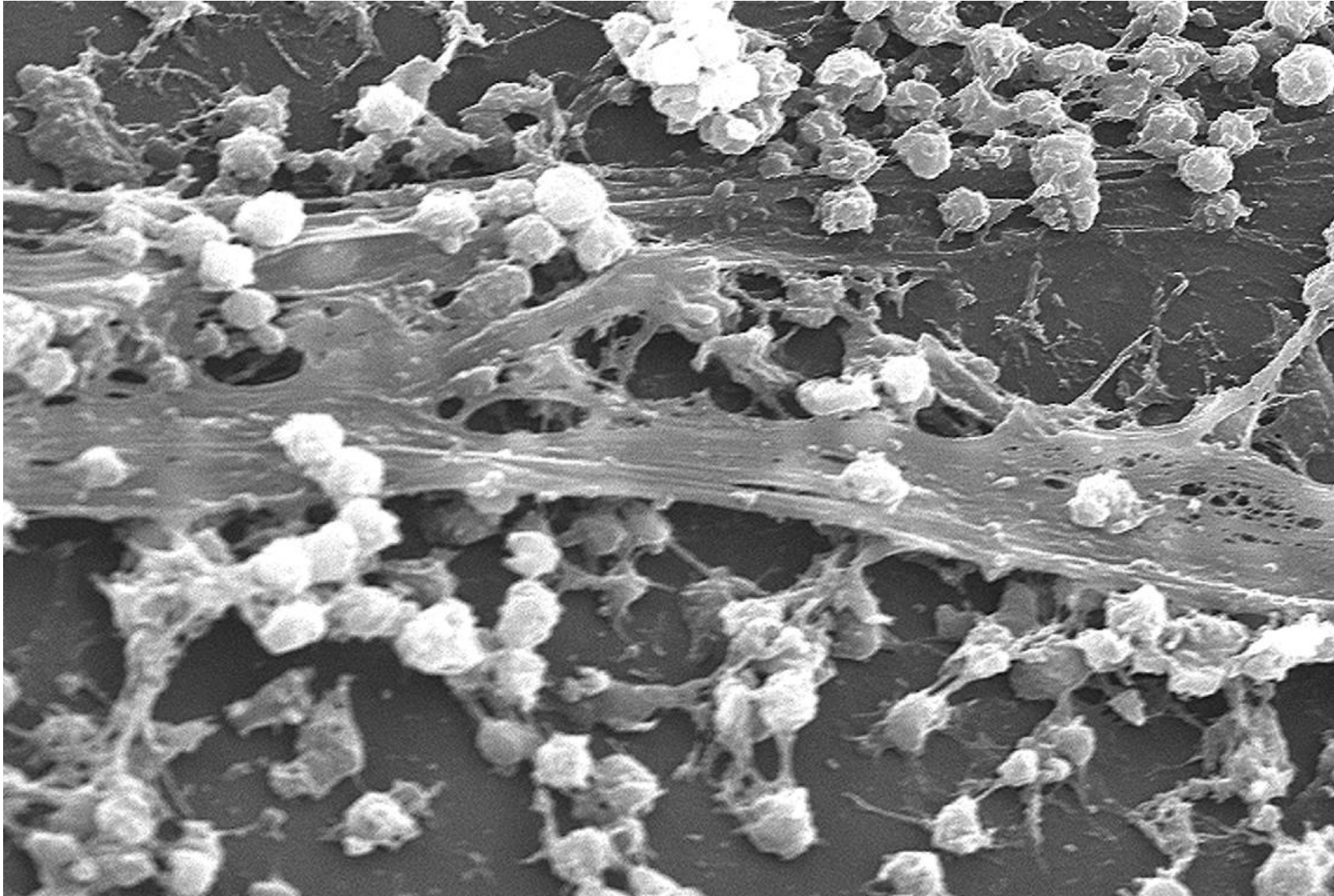


# Heterogeneous Community of Microorganisms in Biofilms



1. Original infection or introduction of microorganisms
2. Colonization, start of biofilm production
3. Biofilm
4. Development of heterogeneous community of microorganisms
5. Release of individual organisms to spread colony

# Biofilm



# More on Biofilms

- Survival adaptation
- The presence of calcium, iron, and magnesium is essential for biofilm production and serves to cross-link the anionic regions of polymers
- confers significant survival advantages to bacteria and yeasts
- Biofilms strongly adhere to interfaces and resist dislodgement.



# Biofilms 3

- Sessile biofilm microorganisms are 100 to 1000 times more resistant to antibiotics compared to planktonic forms of the same strain
- Biofilm induces resistance factors, impenetrable to some drugs
- Increased protection from host immune effector cells
- Disruption or eradication of biofilms is a key to successful treatment of infections
- However, beneficial, some resident bacteria also form biofilms

# Biofilm treatment strategies

- Antibiofilm activity is assessed by minimum biofilm eradication concentration (MBEC™)
- Developed by the Biofilm Research Group at the University of Calgary
- InterFase® (contains naturally-occurring enzymes to break down biofilm, InterFase Plus® adds EDTA to help bind metals that strengthen biofilms )
- note: author has no association with Klaire Labs

# Biofilm: Frequently Recommended Agents

- Monolaurin or Lauricidin [AKA Glyceryl laurate or glycerol monolaurate]
- Nattokinase (a potent oral fibrinolytic enzyme supplement)
- InterFase Plus™ (broad-spectrum enzyme formula w/EDTA)
- NAC (N-Acetyl-Cysteine)
- Xylitol (sugar alcohol)
- Extra-Virgin Coconut Oil (42-52% Medium Chain Fatty Acids [MCFA], lauric acid, by volume)
- Turmeric, Neem oil, Reishi Mushroom
- BFB-1™ & BFB-2™
- Carbonized Bamboo



# Lauric acid—Coconut oil

- After sensing external signals, *Proteus mirabilis* undergoes a multicellular behavior called swarming which is coordinated with the expression of virulence factors
- Lauric acid (LA) was effective for inhibiting swarming (Liaw *et al.*, 2004)
- Coconut oil contains about 50% LA!
- Potential uses for disrupting bacterial biofilms in the gut, and in dental plaque

# Monolaurin

- Lauric acid is converted into monolaurin in the body
- Monoglyceride which exhibits antiviral, antimicrobial, antiprotozoal and antifungal properties
- Breast milk is the only other natural source (besides coconut oil; Marina *et al.*, 2009)
  - high concentration of lauric acid, which could explain the decrease of infections of all types in breast-fed babies (Gibson & Kneebone, 1981)
- Available in capsules and microtablets (monolaurin)

# Lauricidin

- Dissolves biofilms associated with chronic allergic rhinitis; sinusitis
- Taken orally





# Probiotic Research Summary

- 1,475 clinical trials (Pubmed)
- 151 meta-analyses
- 6,047 studies published in the last 5 years
- 3,027 review articles

[Source: [scholar.google.com](https://scholar.google.com)]

# Probiotic Clinical Trials

- So far, none of the tested probiotics has proven successful in Crohn's disease
- For ulcerative colitis a recent meta-analysis on 12 clinical trials (1 of them in children) showed efficacy for the probiotic mixture VSL#3 in contributing to induce and to maintain remission (Guandalini, 2014)

# More studies

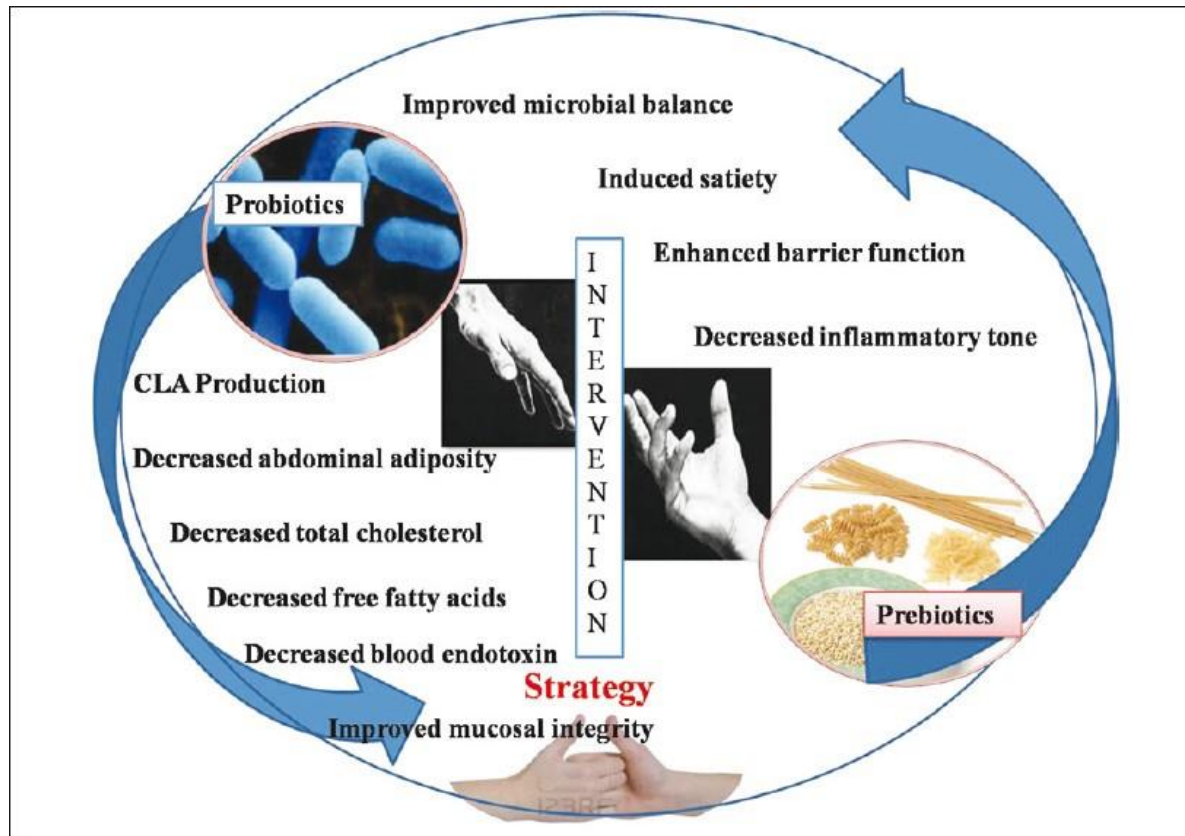
- Twenty-three randomized controlled trials with a total of 1,763 participants met the inclusion criteria.
- Probiotics significantly increased the remission rates in patients with active ulcerative colitis
- (Shen *et al.*, 2014)

# Current Areas of Research

- Digestive health (poor absorption of nutrients, reduction of gas, bloating, loose stools, constipation, diarrhea)
- Cardiovascular disease
- Irritable bowel syndromes
- Urogenital health (vaginal infections, UTIs)
- Allergies
- Immune support (preventing and treating infections)
- Antibiotic-associated diarrhea (positive results from systematic review and meta-analysis) (Hempel *et al.*, 2012)
- Psychiatric conditions, plus more



# Microflora and Obesity



Source: Mallappa *et al.*, 2012

CLA = conjugated linoleic acid

- Dynamic link between obesity and gut flora demonstrated in humans (Source: Ley *et al.*, 2006. Nature 444)
- Possible mechanisms of improved microbial balance, decreased food intake, decreased abdominal adiposity and increased mucosal integrity with decreased inflammatory tone (Mallappa *et al.*, 2012)

# Probiotics for Colds and Flu

- Probiotic Supplement Reduces Cold/Flu Days in Acutely Stressed Individuals
- Researchers reported that supplementation with the probiotic *Bifidobacterium* in stressed individuals resulted in more healthy days and less days with colds or flu.
  - researchers randomly assigned 581 academically stressed undergraduate students to receive 1) *Lactobacillus helveticus*, 2) *Bifidobacterium longum* ssp. *infantis*, 3) *Bifidobacterium bifidum*, or 4) placebo for six weeks.
  - Supplementation with *B. bifidum* resulted in a greater proportion of healthy days. Significantly fewer subjects receiving *B. bifidum* (and *B. longum*) had one or more days of cold/ flu when compared to those receiving placebo.

# When to take probiotics

- Take with food
- The original mode of delivery of probiotics was in cultured foods such as yogurt or kefir
- Human beings have also naturally ingested micro-organisms along with foods throughout evolutionary history
- Study shows that a commonly-consumed *Lactobacillus* strain survived in the passage through the gut well when consumed with food

Source: Klaire labs

# How much to take?

- 10 billion organisms/day minimum for maintenance
- Better taken with food, twice daily
- Try different products for best results
- Different organisms for specific symptoms/ailments
- 25 billion twice daily from my clinical experience
- Up to 50 billion twice daily, or even 100 billion b.i.d.



# How long to take probiotics?

## How long before significant results?

- Results might be obvious within a week
  - Skin problems (acne)
  - Allergies
- Within a day or two for some digestive upsets
- Typically for well-established chronic conditions, results are obvious within a week, but a permanent change (“cure”) might not be seen for at least **9 months**
  - Acne rosacea; chronic acne, some bowel syndromes, allergies
  - **Consistency (daily use) is vital to success**

# What species are best?

- Research is still emerging
- Most commercial products go with combinations of organisms that have research behind them
- Especially well-established companies

# Acknowledgements

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- David Becker, M.D. (with permission)