Probiotics
Nutrition Masters Course

Noelle Patno, PhD

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Learning Objectives

- Review early pioneers of research on the intestinal microbiome and its re-emergence as a focal point of immunology
- Understand how the microbiome relates to health and disease
- Clearly define and select a quality probiotic based on WHO/FAO guidelines
- Identify key aspects of a probiotic strain as described in published literature
- Explore emerging microbiome-related research

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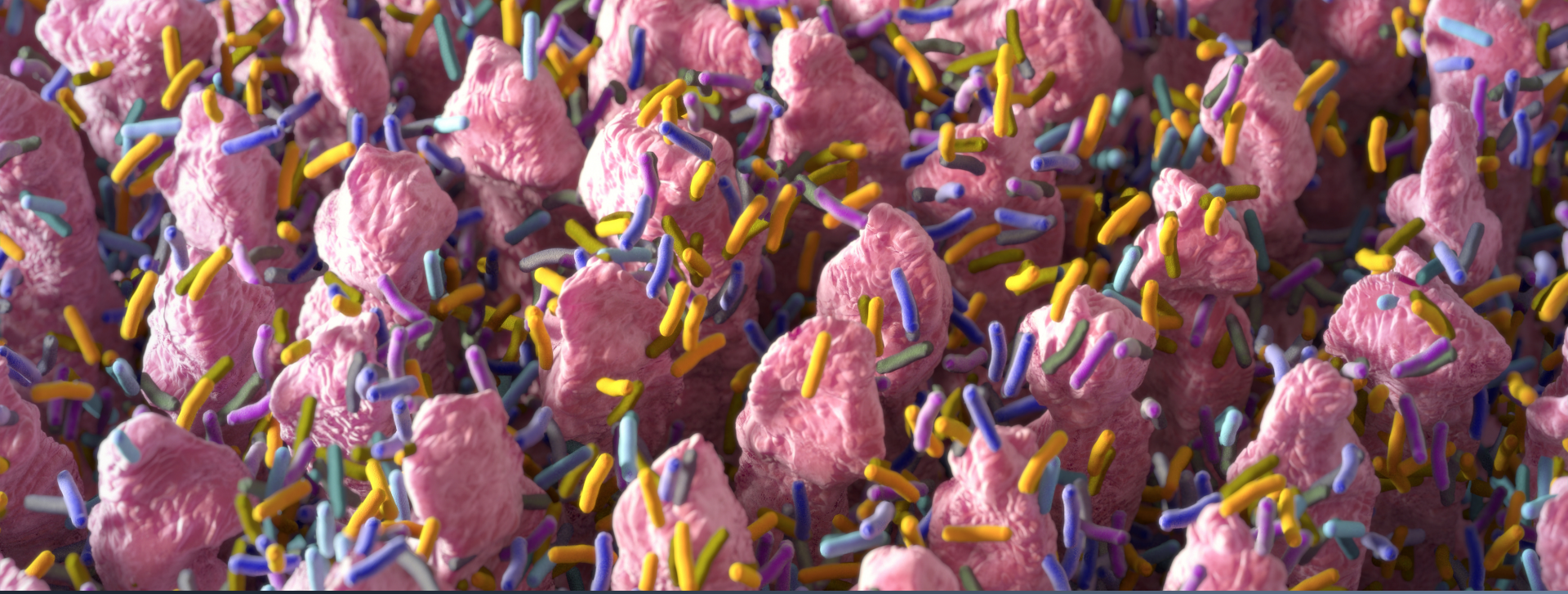

Early Pioneers of Microbiome-Related Research

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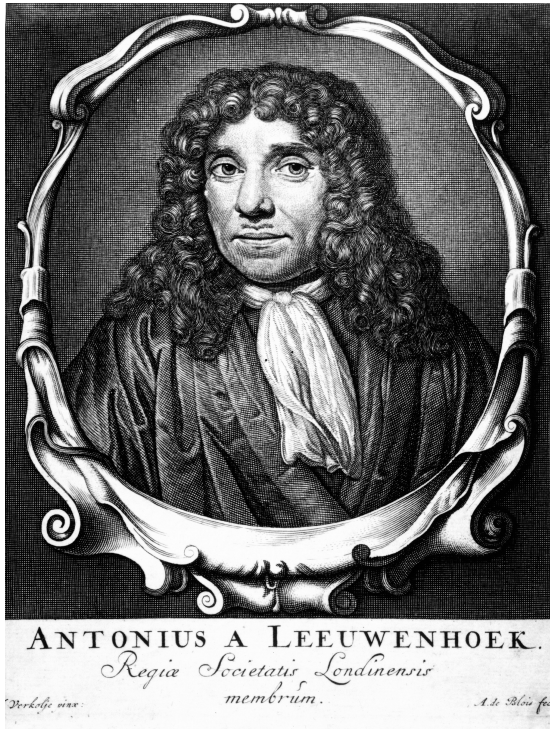
How Long Have Microorganisms Been Studied?

- In 1590, Zacharia Janssen, a spectacle maker, discovered that two lenses magnified better than one—inventing the first microscope[1]
- During the late 17th century, Antonie van Leeuwenhoek was the first to connect the use of a microscope with microbiology[2]
- Between 1665-1683, the existence of microscopic organisms was discovered by Robert Hooke and Antonie van Leeuwenhoek[3]



1. Gest H. *Notes Rec R Soc Lond.* 2004;58(2):187-201.
2. Tan SY. *Singapore Med J.* 2003;44(11):557-558.
3. Lane N. *Philos Trans R Soc B.* 2015; 370(1666):20140344.

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Genetics highlights

1866—Gregor Mendel discovers inheritance in pea plant experiments

1953—Discovery of double-helix model of DNA structure
1986—First semi-automated DNA sequencing machine
1988—Quantitative/real-time PCR (qPCR) method developed

2000—First commercially available sequencing method
2001—Human genome unraveled
2004—Next Generation Sequencing (NGS)

Microbiome highlights

1676—Discovery of bacteria by Antonie van Leeuwenhoek
1881—Culture-dependent methods for stool analysis
1885—First human gut microbiome studied

1959—Germ-free mice reared
1996—First human fecal sample sequenced

2006—Study of microbiome in age and country
2007—Start human microbiome project (HMP)
2009—First study of microbiome in health and disease:
lean vs. obese

See also Cenit MC et al. *Biochem Biophys Acta.* 2014;1842(10):1981-1992.

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1800s: Pioneers of the Immune System

Élie Metchnikoff, PhD

- Commonly referred to as the father of cellular innate immunity[1,3]
- Discovered phagocytosis by macrophages and microphages as a critical host-defense mechanism[2]

Paul Ehrlich, PhD

- Considered to be one of the fathers of humoral adaptive immunity[3]
- Described the role of antibodies in the immune response to bacterial infection[3]

1. Mackowiak PA. *Front Public Health.* 2013;1:52.
2. Gordon S. *J Innate Immun.* 2016;8(3):223-227.
3. Kaufmann SH. *Nat Immunol.* 2008;9(7):705-712.

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Early Use of Probiotics by Metchnikoff

- In the late 1890s, Élie Metchnikoff spent time in remote villages of Eastern Europe where the majority of villagers were centenarians. He noted they drank a fermented yogurt that contained *Lactobacillus bulgaricus.*
- "For suppressing putrefactive colonic bacteria" Metchnikoff recommended daily doses of probiotics in the form of "soured milk (i.e., yogurt) prepared by a group of lactic bacteria, or of pure cultures of the *Bulgarian bacillus* (*Lactobacillus bulgaricus*), but in each case (accompanied by) a certain quantity of milk, sugar, or sucrose."

Mackowiak PA. *Front Public Health.* 2013;1:52.

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Contemporary Research
Abigail Salyers, PhD

- Abigail Salyers, PhD from University of Illinois first drew attention to the overuse of antibiotics (in the early 1990s) and helped raise awareness of the importance of diverse intestinal microbiome (and attention to the damage caused by overuse of antibiotics)
- Her research focused on the ecology of microorganisms in the human body and the transfer of antibiotic-resistance genes, particularly genes among the *Bacteroides* species

https://www.asm.org/index.php/podcasts/meet-the-microbiologist/item/3005-mts33-abigail-salyers. Accessed May 30, 2018.

Patrice Cani, PhD

- Dr. Cani's main research involves the investigation of interactions among the gut microbiota, the host, innate immune system and others related to metabolic disease.[1,2]
- His more recent publications discussed *Akkermansia muciniphila*—one of most abundant species in the human intestinal microbiota.[3]
- It is a novel candidate to improve metabolic disorders associated with:[3]
  1. Liver diseases
  2. Obesity
  3. Cardiometabolic disorders

1. Plovier H et al. *Endocr Dev.* 2017;32:139-164.
2. Tilg H et al. *Gut.* 2016;65(12):2035-2044.
3. Cani PD et al. *Front Microbiol.* 2017;8:1765.

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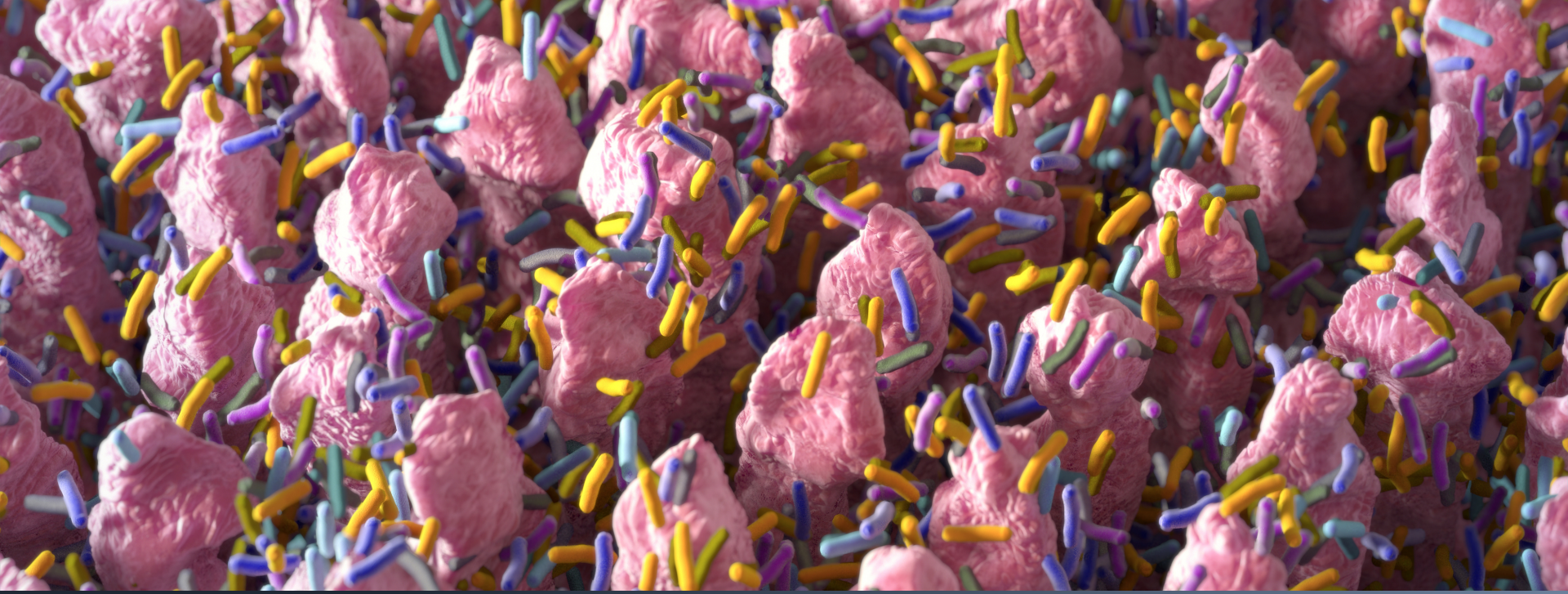

Relationship Between the Microbiome and Health

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Human Microbiome Project (HMP)

https://hmpdacc.org/. Accessed May 30, 2018.

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Relevant Definitions

Term	Definition
Microbiota	Microorganisms of a particular site or habitat (such as the gut microbiota)
Microbiome	Refers to the entire habitat of a human, including the microorganisms (bacteria, archaea, lower and higher eukaryotes, and viruses) and their genomes (i.e., genes)

Marchesi JR et al. *Microbiome.* 2015;3:31.

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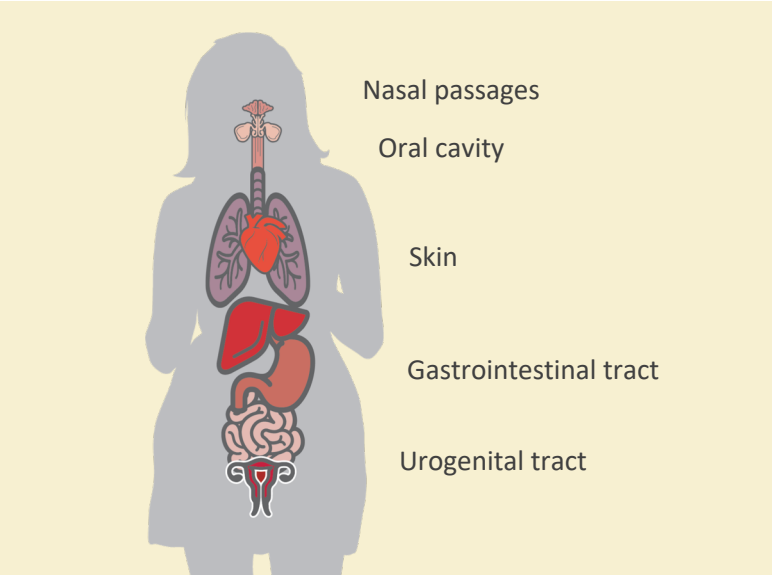
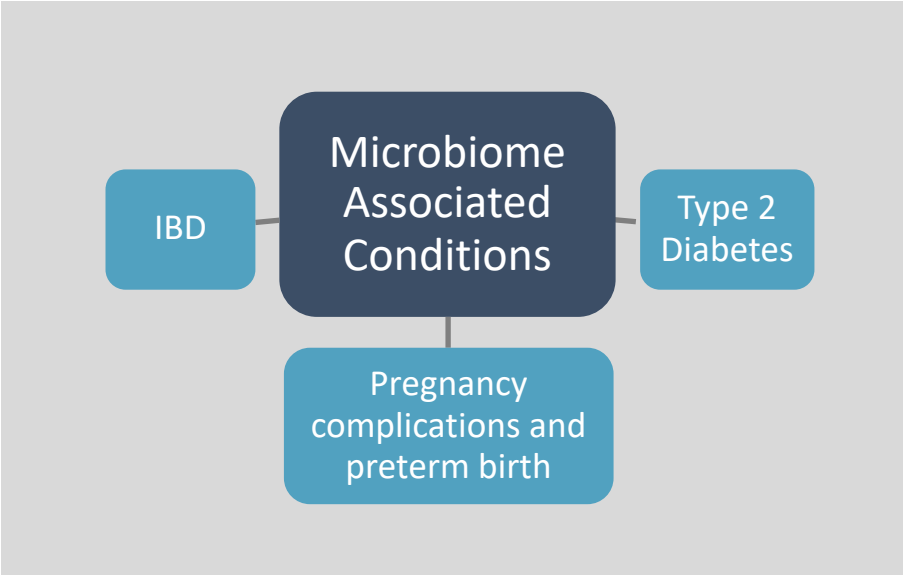
Two Parts of the Human Microbiome Project



5 major body sites characterized



3 different microbiome-associated health conditions

https://hmpdacc.org/. Accessed May 30, 2018.

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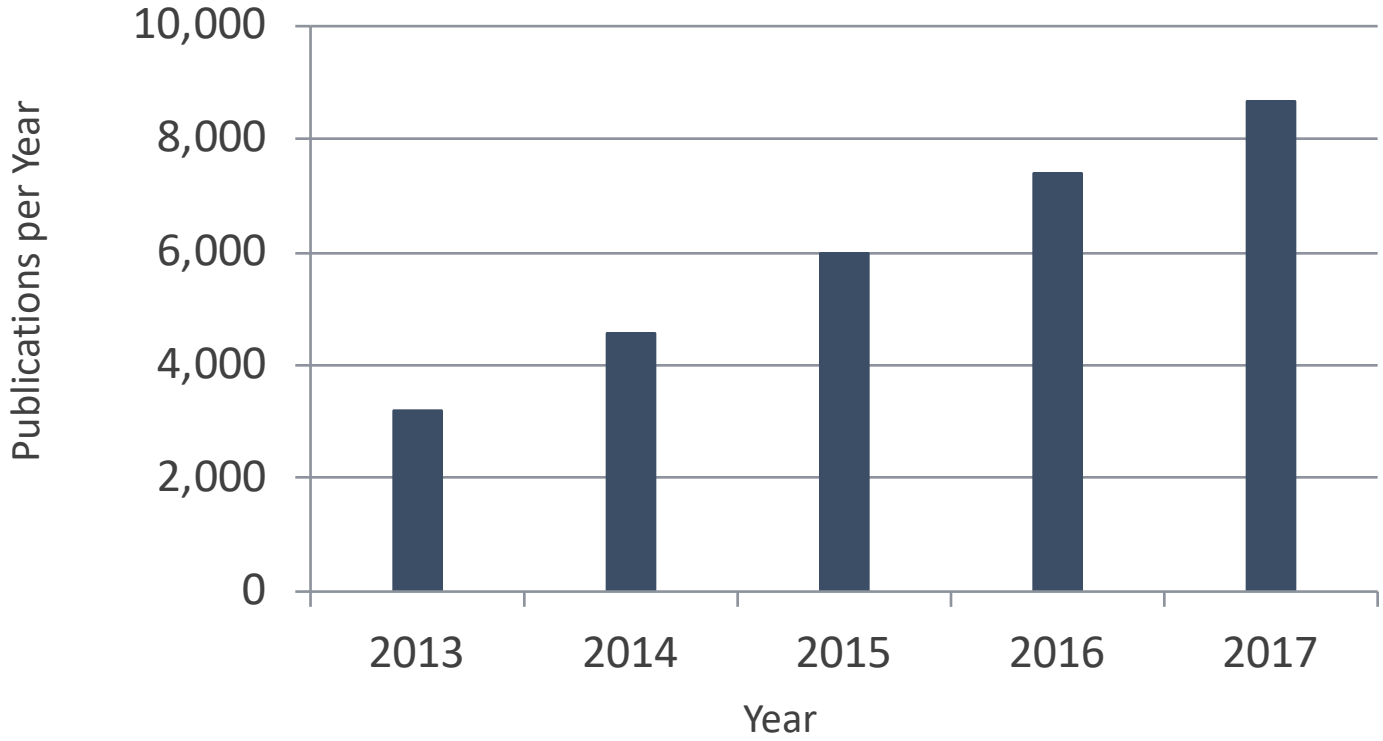
Significance of the Human Microbiome Project

1. Developed a new database system—much more efficient, and organized for searching, storing and accessing data
2. Developed analytical tools to compare patterns in datasets
3. Established a catalog of reference bacterial genomes to compare across multiple body sites

Publications on Microbiome: 2013-2017



https://www.ncbi.nlm.nih.gov/pubmed/. Accessed May 30, 2018.

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The Impact of the Microbiome on Health Begins Early

Initial colonization of the gut in infants affects health later in life[1]

- Factors influencing the development of the infant microbiota include[1]
  - Diet
  - Family environment
  - Caesarean vs. vaginal birth
  - Diseases and therapies
- Alterations in the establishment of a healthy gut microbiota and factors that *decrease* microbial diversity in infants and young children may affect the risk of developing disease[2]

1. Matamoros S et al. *Trends Microbiol.* 2013;21:167-173.
2. Adlerberth I et al. *Acta Paediatr.* 2009;98:229-238.

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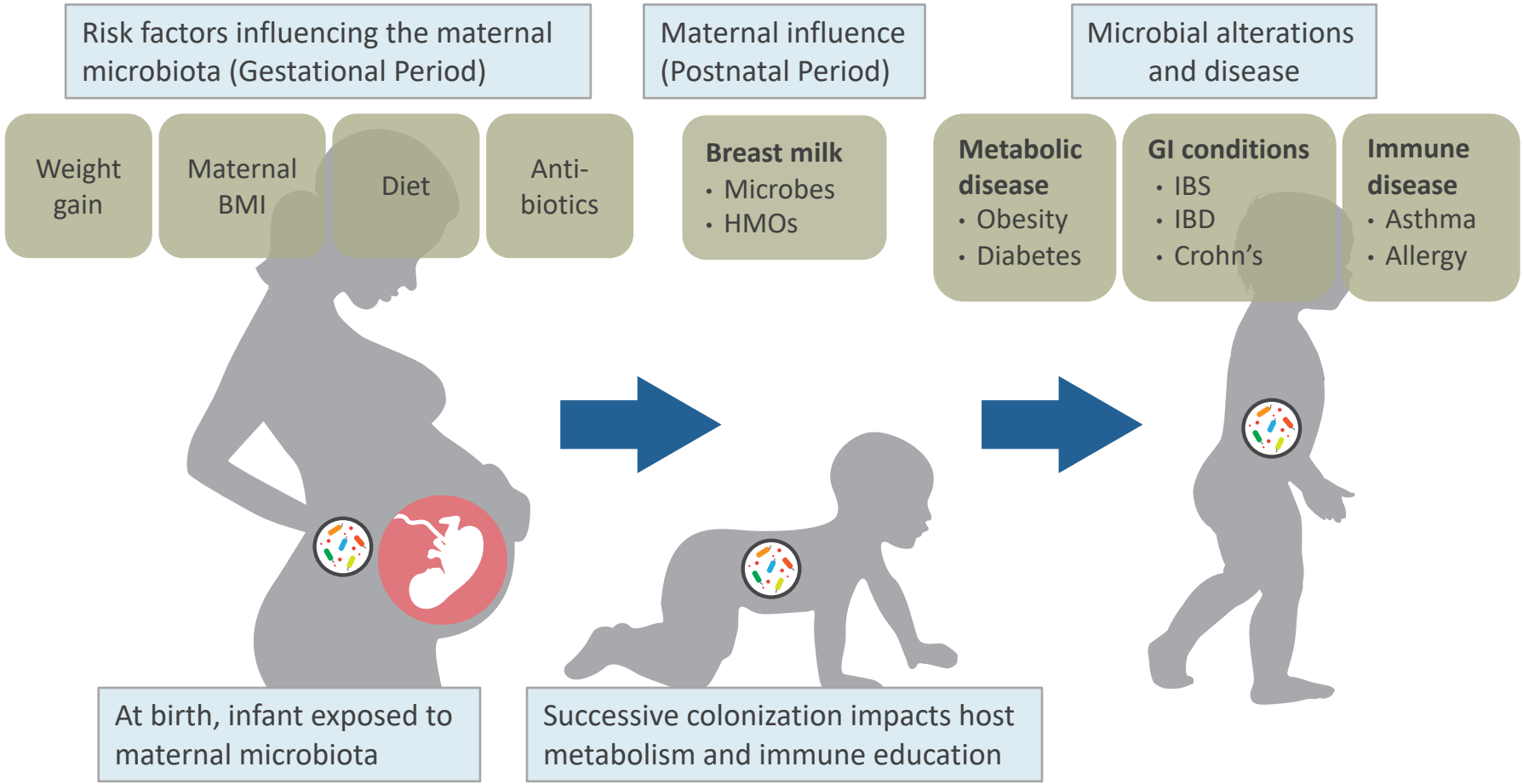

See also Mulligan CM et al. *J of Endocrin.* 2017;235(1):R1-R12.

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Some Factors Affecting the Microbiome

Early Life

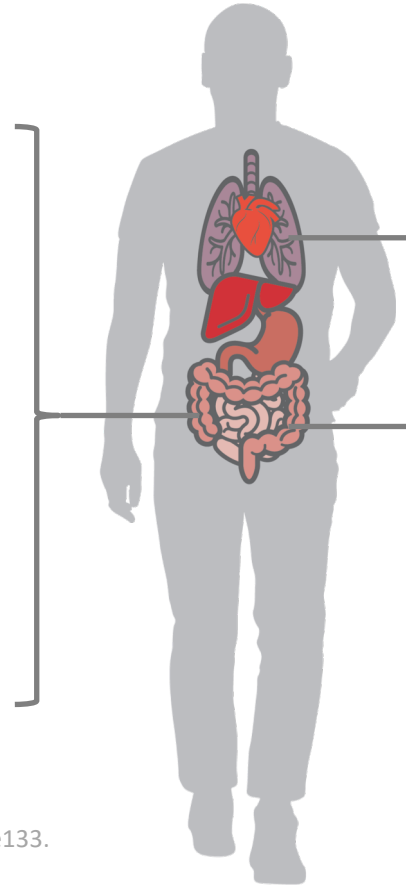
Maternal BMI

Pre-term
Pharmacotherapy (e.g., antibiotics, steroids)

0-3 months
Caesarean
vs.
Vaginal Birth

1-3 years
Breastfeeding cessation

Day care attendance



Adult Life

Cigarette smoke

Allergens

Infections

Air pollution

- Age
- Gender
- BMI
- Onset of chronic diseases
- Diet
- Pharmacotherapy (e.g., antibiotics, steroids)

See also Shukla SD et al. *Clin Transl Immunology*. 2017;6(3):e133.

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Different Diets Impact the Microbiome

Western diet

Mediterranean diet

Gluten-free diet

- Different diets affect microbial dysbiosis and diversity[1]
- Dysbiosis, an imbalance in bacterial composition, can be categorized into one or more of the following[2]:
  1. Loss of beneficial organisms
  2. Excessive growth of potentially harmful organisms
  3. Loss of overall microbial diversity
- Dysbiosis is associated with diseases[2]

1. Singh et al. *J of Transl Med.* 2017 15:73.
2. DeGruttola AK et al. *Inflamm Bowel Dis*. 2016;22(5):1137-1150.

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The Diversity of the Microbiome is Associated with Disease

Increased diversity is linked to lower risk for metabolic or immunological diseases.[1]

Decreased diversity has been associated with human diseases.[2,3]

Diversity Refers to the Different Types of Bacteria and Abundance of Each Type

Adapted from Finotello F et al. *Brief Bioinform*. 2016;26.

1. Manichanh C et al. *Gut.* 2006;55(2):205-211.
2. Mosca A et al. *Front Microbiol.* 2016;7:455.
3. Lozupone CA et al. *Nature*. 2012;489(7415):220-230.
4. Finotello F et al. *Brief Bioinform*. 2016;26.

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Activity of the Microbiota

- Gut bacteria benefit the host in a variety of ways, such as[1]
  - Regulating gut motility
  - Producing vitamins
  - Transforming bile acid and steroids
  - Metabolizing foreign substances
  - Absorbing minerals
  - Activating and destroying toxins
- Bacteria produce metabolites including short-chain fatty acids (SCFAs), such as acetic, propionic, and butyric acids, which are potentially therapeutic[2]

1. Zhang Y-J et al. *Int J of Mol Sci*. 2015;16(4):7493-7519.
2. Soldavini J et al. *Dig Dis Sci*. 2013;58(10):2756-2766.

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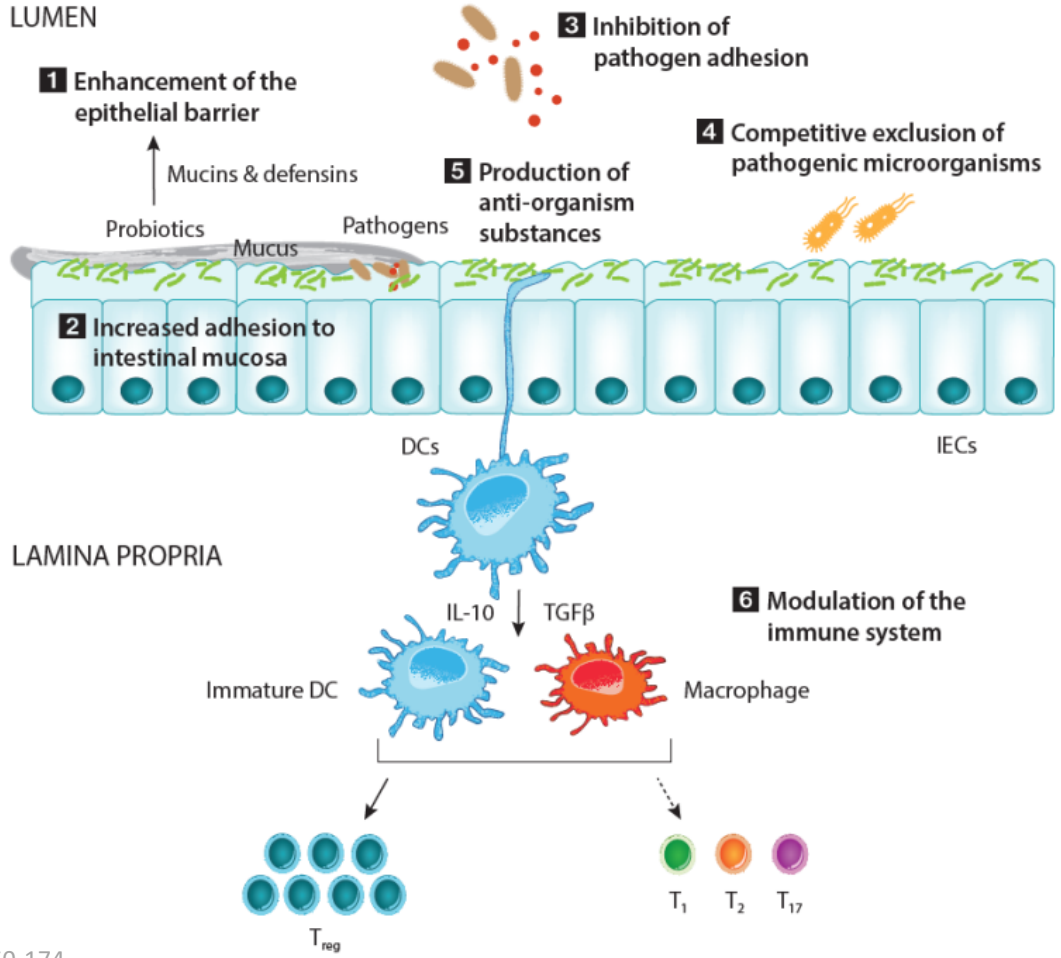
Potential Mechanisms of Bacteria in the Intestine

Mechanisms

1. Enhance epithelial barrier
2. Adhere to intestinal mucosa
3. Inhibit pathogen adhesion
4. Competitively exclude pathogenic microbes
5. Produce anti-microbial peptides, bacteriocins, or metabolites such as lactic acid, short-chain fatty acids such as butyrate
6. Modulate the immune system



See also Bermudez-Brito M et al. *Ann Nutr Metab.* 2012;61(2):160-174.

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What Are Bacteriocins?

- Bacteriocins are peptides produced by bacteria, which can kill or inhibit other bacterial strains[1]
- They affect the changing composition of microbial communities[2]
- For example, in an *in vitro* study, *Lactobacillus salivarius* UCC118 was shown to induce bacteriocin ABP-118 gene expression upon adhesion to human intestinal epithelial cells[3]

1. Dobson A et al. *Applied and Env Microbiol.* 2012;78(1):1-6.
2. Zheng J et al. *Environ Microbiol.* 2015;17(6):2133-2143.
3. O'Callaghan J et al. *Appl Environ Microbiol.* 2012;78(15):5196-5203.

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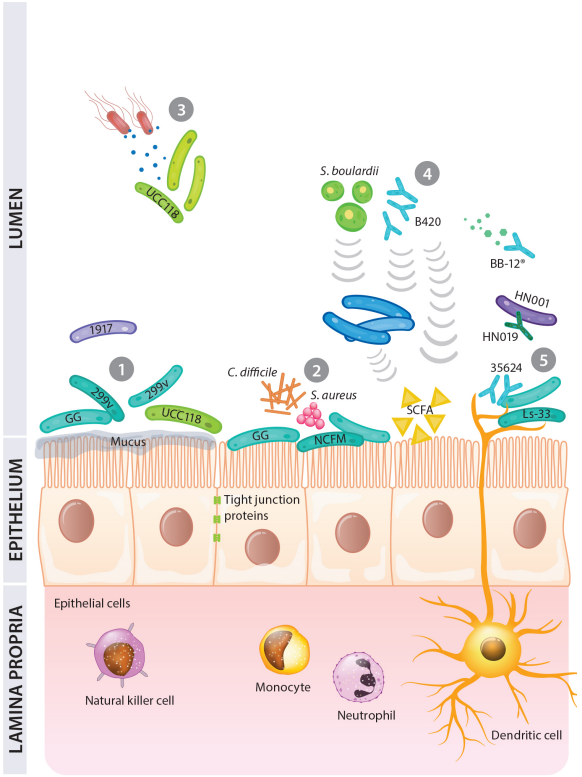
Potential Mechanisms of Specific Probiotic Strains

1. Support barrier function:
   - Increase mucin production: *L. plantarum* 299v[1]
   - Prevent barrier breakdown: *E. coli* Nissle 1917[2]
   - Prevent apoptosis: *L. rhamnosus* GG[2]
   - Support tight junction proteins: *L. salivarius* UCC118[3]
2. Adhere to cells and prevent pathogens from attachment *in vitro*[4]
3. Produce antimicrobial substances, such as bacteriocins, e.g., *L. salivarius* UCC118[5,6]



4. Produce:
   - Short-chain fatty acids (SCFA), clinical studies using *S. boulardii*,[7] or *B. lactis* B420[8]
   - or enzymes associated with bile metabolism (e.g. *B. lactis* BB-12®)[9]
5. Modulate immune system:
   - Interaction with dendritic cells: *L. salivarius* Ls-33 as well as *B. infantis* 35624 *in vitro*[10]
   - *L. rhamnosus* HN001[11,12] and *B. lactis* HN019[12,13] have been shown clinically to increase natural killer cell activity

1. Mack DR et al. *Am J Physiol*. 1999;276(4):G941-G950.
2. Bermudez-Brito M et al. *Ann Nutr Metab*. 2012;61(2):160-174.
3. Miyauchi E et al. *Am J Physiol Gastrointest Liver Physiol*. 2012;303(9):G1029-G1041.
4. Collado MC et al. *Curr Microbiol*. 2007;55(3):260-265.
5. Dunne C et al. *Antonie Van Leeuwenhoek*. 1999;76(1-4):279-292.
6. Corr SC et al. *Proc Natl Acad Sci U S A*. 2007;104(18):7617-7621.
7. Schneider SM et al. *World J Gastroenterol*. 2005;11(39):6165-6169.
8. Stenman LK et al. *EBioMedicine*. 2016;13:190-200.
9. Jungersen M et al. *Microorganisms*. 2014;2(2):92-110.
10. Gad M et al. *FEMS Immunol Med Microbiol*. 2011;63(1):93-107.
11. Gill HS et al. *Br J Biomed Sci*. 2001;58:94-96.
12. Gill HS et al. *J Clin Immunol*. 2001;21: 264-271.
13. Gill HS et al. *Am J Clin Nutr*. 2001;74: 833-839.

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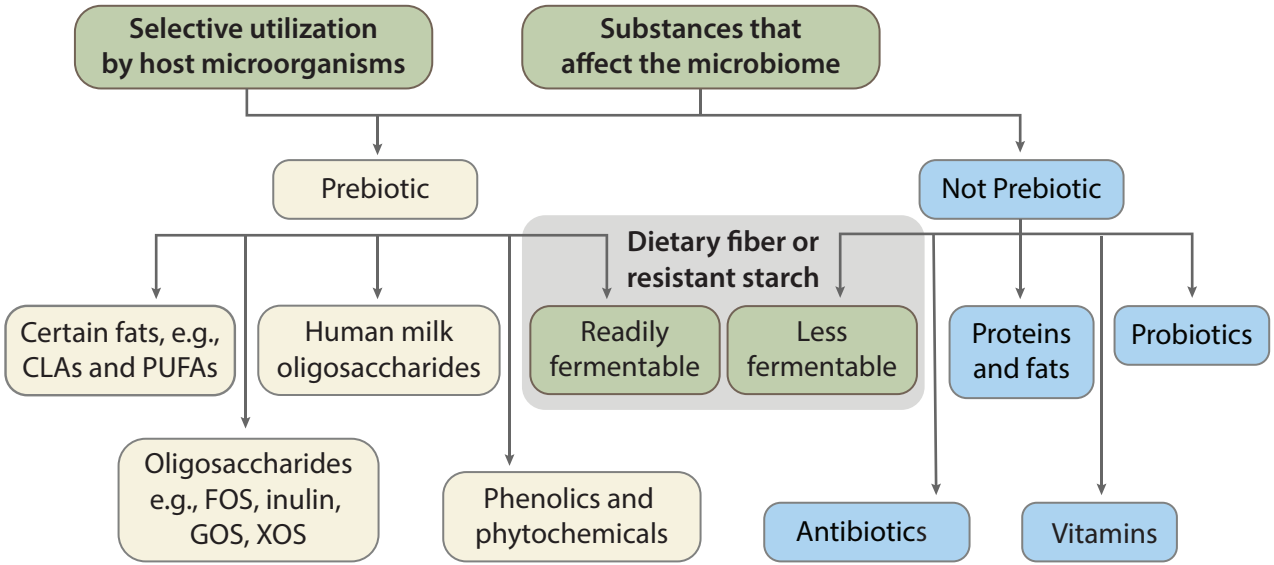
Prebiotic Definition from the ISAPP

A prebiotic is a substrate that is selectively utilized by host microorganisms conferring a health benefit



Dietary fibers (fibers from cereals, grains, fruits, vegetables, nuts and legumes, are longer than oligosaccharides) are resistant to human digestion and may or may not be fermentable by gut bacteria

See also Gibson G et al. *Nat Rev Gastroenterol and Hepatol.* 2017;14:491-502.

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Prebiotic Benefits Include *Bifidobacteria* and SCFA Increases

- Prebiotics may modify the intestinal microbiome and also impact human health, such as improve metabolic, immune and gastrointestinal health[1]
- While earlier studies focused on FOS and inulin, emerging prebiotics have been identified:
  - **IMO**s (Isomaltooligosaccharides) are well-tolerated prebiotic soluble fibers that produce high levels of *Bifidobacteria* without causing as much gas as inulin (*in vitro*)[2]
  - In human breast milk, **2'-fucosyllactose (2'FL)** is one of the most abundant human milk oligosaccharides (HMOs). HMOs, including 2'FL, support the selective growth of certain *Bifidobacteria*.[3]
  - Both IMOs[2,4] and 2'FL[3] support the production of SCFA (short chain fatty acids)

1. Holscher HD. *Gut Microbes*. 2017;8(2):172-184.
2. Rycroft et al. *J Applied Microbiology.* 2001; 91:878-887.
3. Yu ZT et al. *Glycobiology.* 2013;23(2):169-177.
4. Chen HL et al. *J Am Coll Nutr.* 2001 Feb;20(1):44-49.

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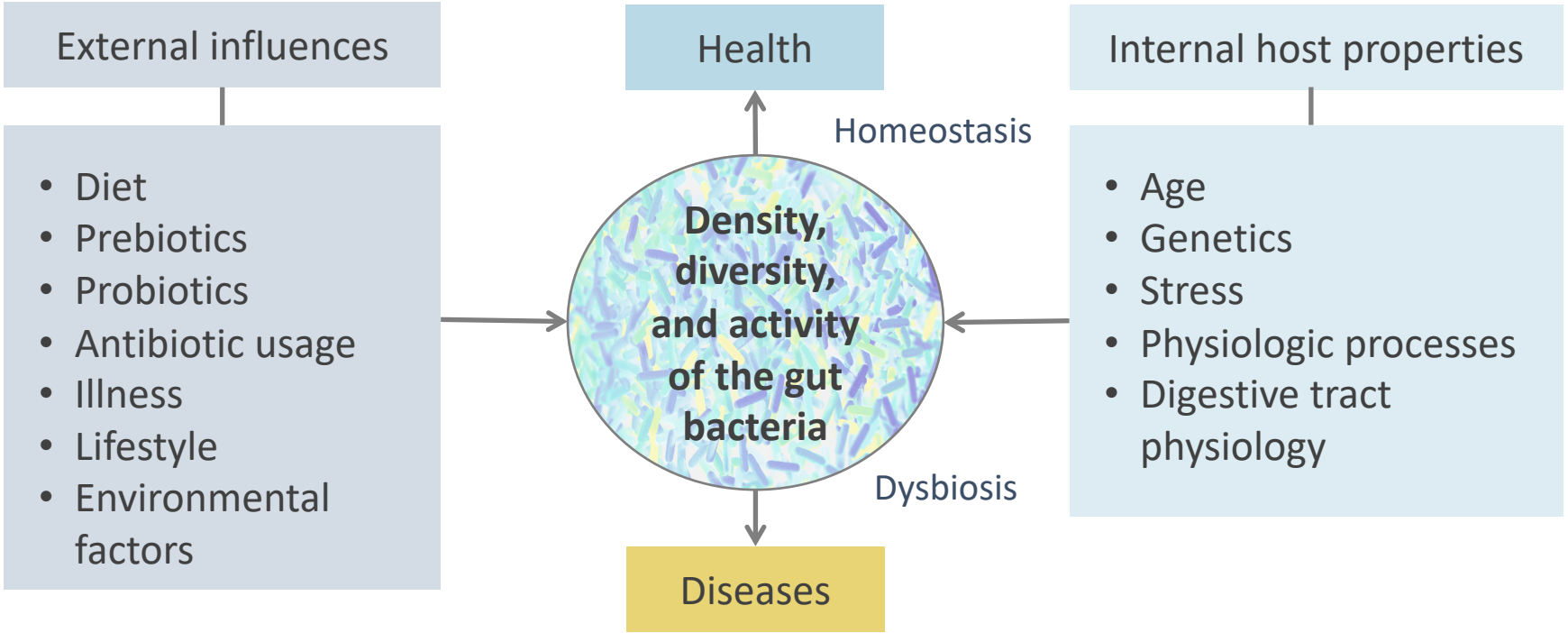
Several Factors Influence the Density, Diversity, and Activity of Gut Bacteria



See also Zhang Y-J et al. *Int J of Mol Sci.* 2015;16(4):7493-7519.

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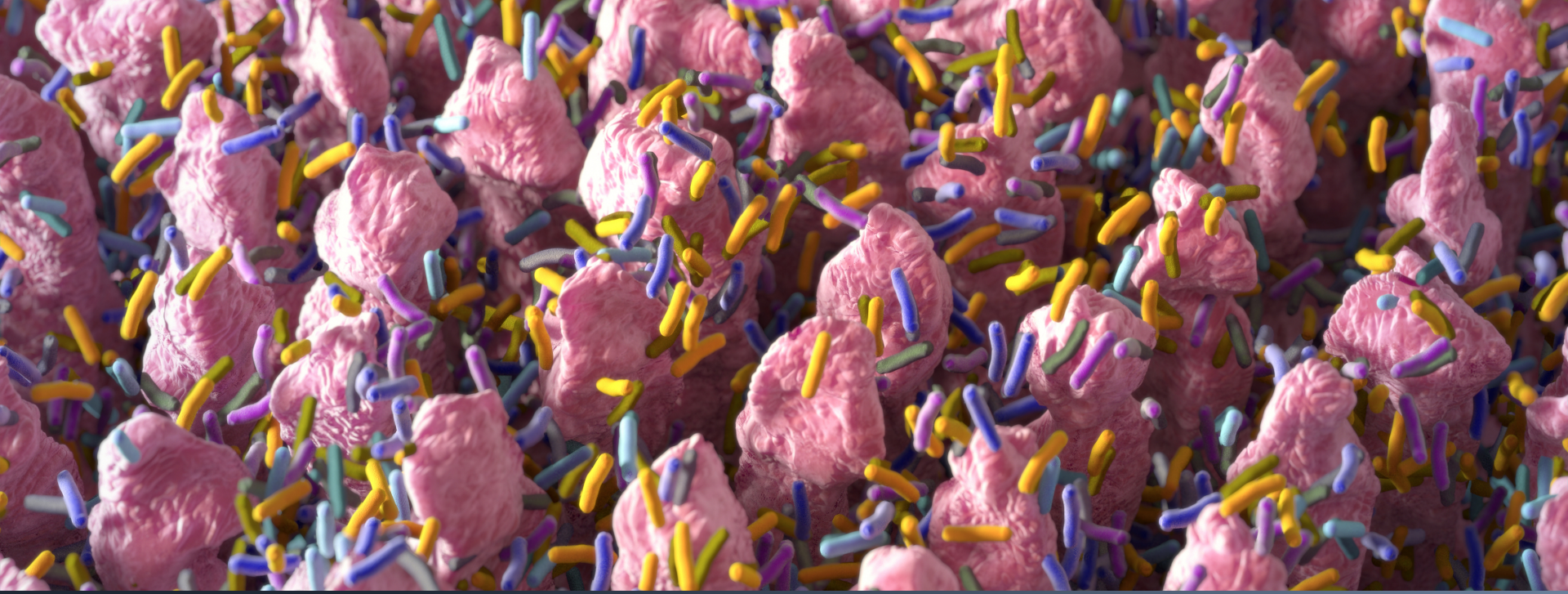

Selecting a Quality Probiotic

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Probiotic Definition

ISAPP definition:
"Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host."[1]

FAO/WHO definition:
"Live microorganisms which when administered in adequate amounts confer a health benefit on the host."[1,2]

ISAPP: International Scientific Association for Probiotics and Prebiotics
FAO: Food and Agriculture Organization of the United Nations
WHO: World Health Organization

1. http://www.fao.org/3/a-a0512e.pdf. Accessed May 30, 2018.
2. https://www.crnusa.org/sites/default/files/pdfs/CRN-IPA-Best-Practices-Guidelines-for-Probiotics.pdf. Accessed May 30, 2018.

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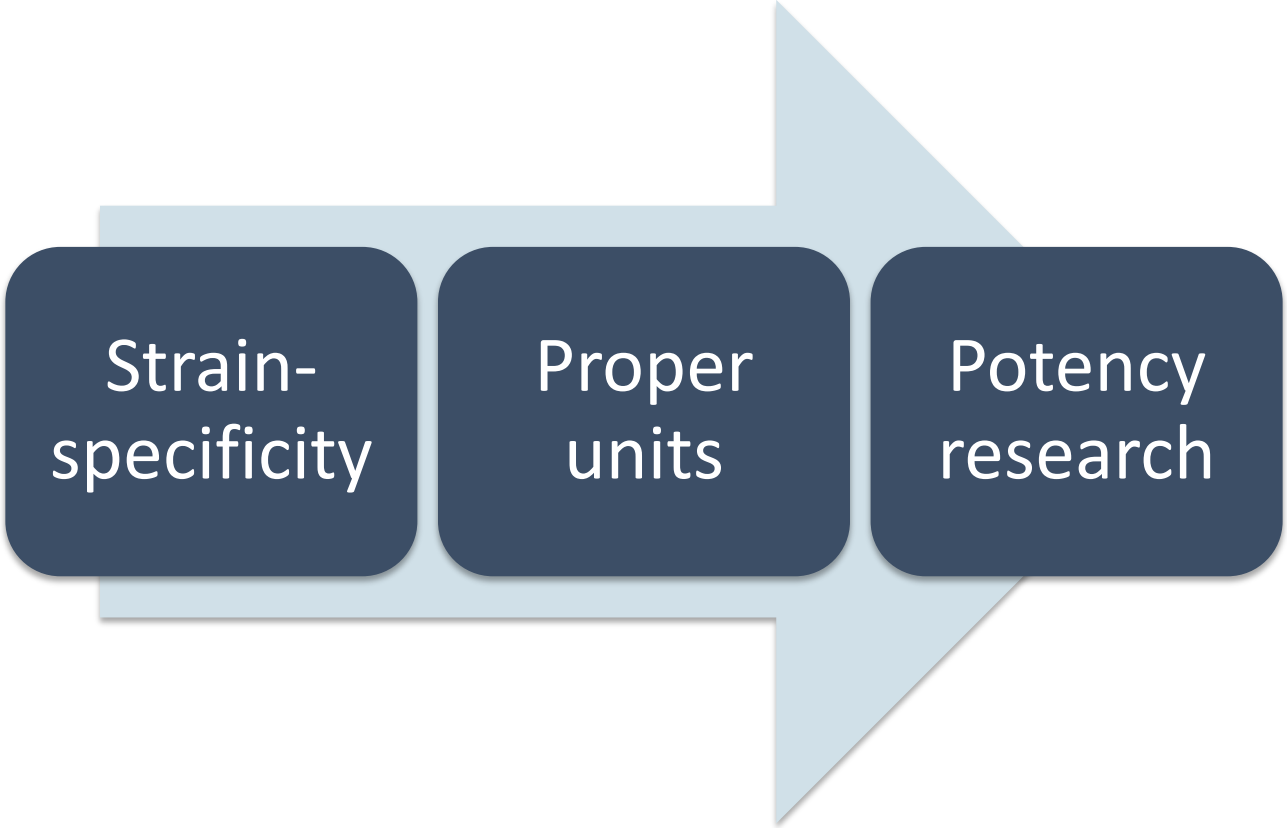
Guidelines for Choosing Quality Probiotics



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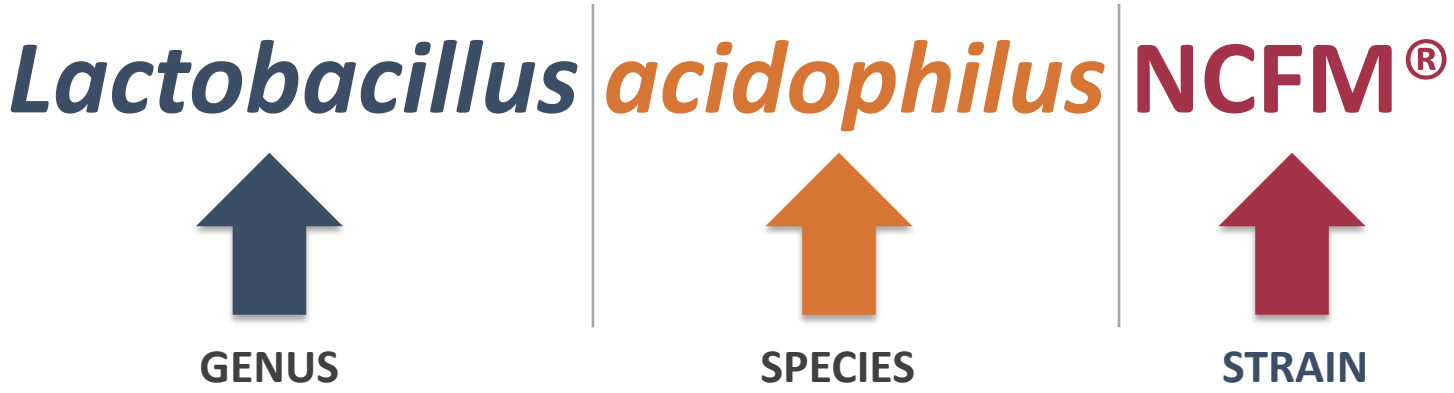
Genus, Species, & Strain

Bacteria are categorized by genus, species, and strain. Each probiotic bacteria MUST be identified at this level; each strain carries unique health benefits.



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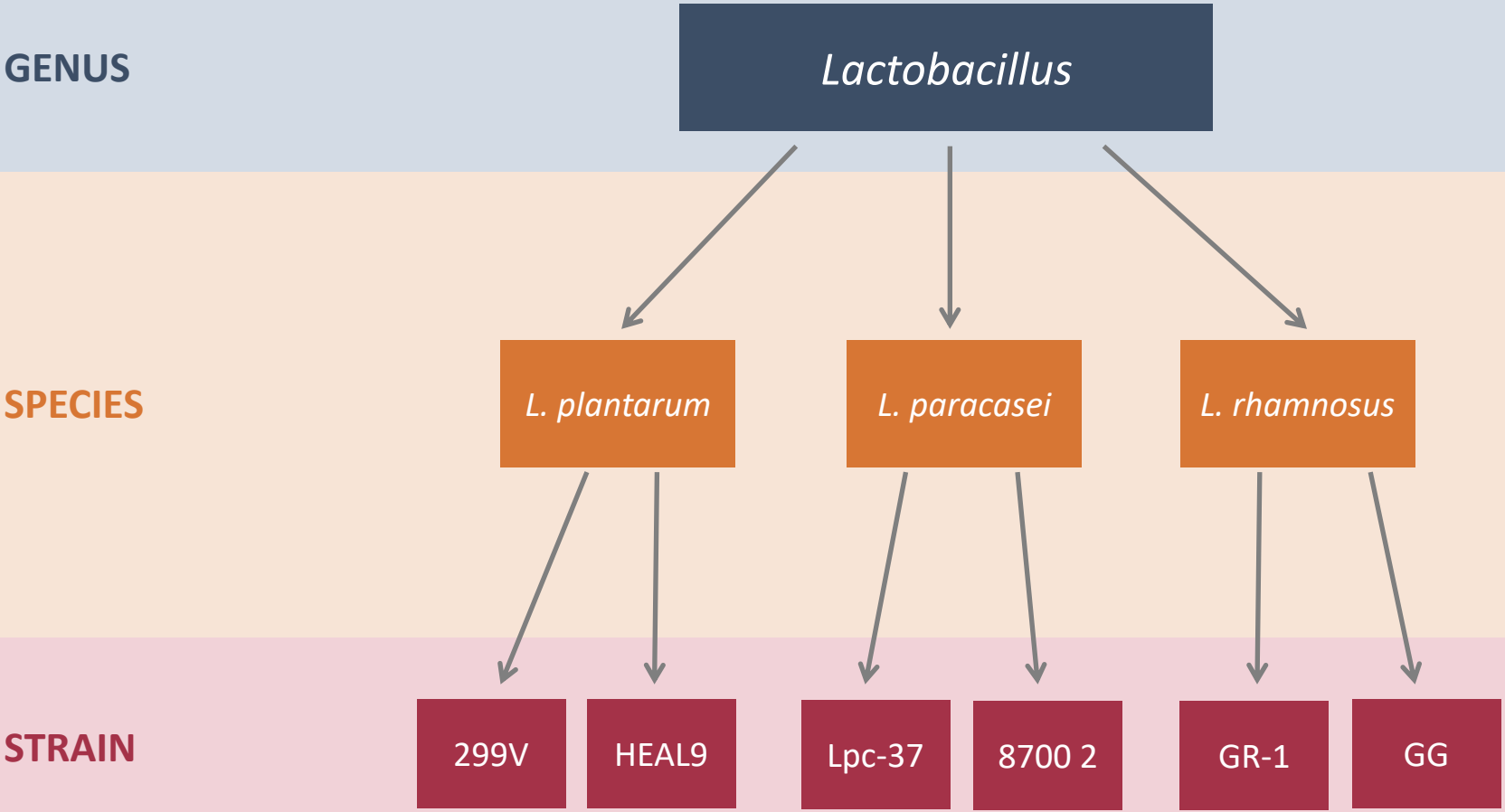

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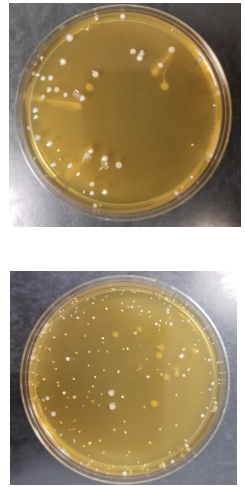
CRN/IPA Guidelines for Probiotic Viability

Council for Responsible Nutrition	International Probiotics Association



- Look for appropriate units on label: colony-forming units (CFUs)
- Ensure amount of CFUs listed is guaranteed up until expiration date, not just at time of manufacture
- Main concern should be the available CFUs **at time of consumption**

https://www.crnusa.org/sites/default/files/pdfs/CRN-IPA-Best-Practices-Guidelines-for-Probiotics.pdf. Accessed May 30, 2018.

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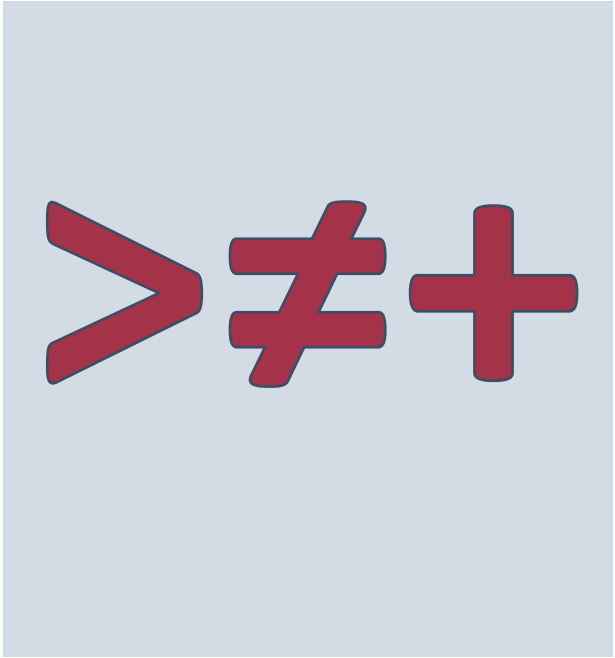
Do greater CFU quantities = greater health benefits?

- Some products MAY work better at higher CFUs than others. This may be due to weak activity of the strain or lack of strain-specific selection
- Effective CFUs (dose selection) should be based upon a strain's (or combination of strains) well-documented clinical health benefits for that specific patient population
- Doses can range from 100 million to over a trillion CFUs per day



https://isappscience.org/infographic-dispelling-myths/. Accessed May 30, 2018.

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Probiotics: 2018 ISAPP Dispelling Myths

Is more better?

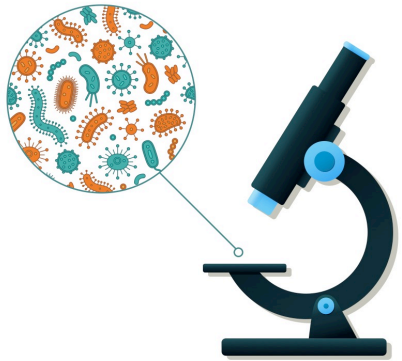
The best dose is one that has been tested in humans and resulted in positive health outcomes. Doses can range from 100 million to over a trillion CFU per day.

Are greater number of strains better?

It depends on the literature. Some studies show benefits of a single-strain probiotic while others show specific combinations of probiotic strains have a positive outcome. More CFU does not guarantee a more beneficial product.

Do probiotics have to alter my microbiota to be effective?

No. As probiotics and their produced substances pass through the gut they interact with immune cells, dietary components in the gut and other microbes.



See also https://isappscience.org/infographic-dispelling-myths/. Accessed May 30, 2018.

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Evidence is Lacking for the Health Benefits of Fermented Foods

- Fermented foods may provide general health benefits but **they are not characterized by strain type or amount of bacteria.** Lack of characterization makes them difficult to study.
- A recent meta-analysis of kombucha concludes that it does not have a defined health benefit[1]
- However, well-defined strains of bacteria have been studied at specific amounts for certain health conditions

Ernst E. *Forschende Komplementarmedizin und Klassische Naturheilkunde.* 2003;10(2):85-87.

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Example of Specific Strains Studied at Specific Doses

Article title: Probiotics reduce symptoms of antibiotic use in a hospital setting: A randomized dose response study.

"equal amounts of ***Lactobacillus acidophilus*** **NCFM®, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium lactis* Bi-07, and *Bifidobacterium lactis* Bl-04**…capsules contained the probiotic combination at either a low-dose: [**4.17 billion colony forming units (CFUs)** or high-dose: **17.00 billion CFUs**] or placebo."

Ouwehand AC et al. *Vaccine.* 2014;32(4):458-463.

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Example of Specific Strains Studied in Specific Population

Article title: Probiotic effects on cold and influenza-like symptom incidence and duration in children.

"…**326 eligible children (3-5 years of age)** were assigned randomly to receive placebo (*N*104), *Lactobacillus acidophilus* NCFM (*N*110), or L. acidophilus in combination with *Bifidobacterium animalis* subsp *lactis* Bi-07 (N112). Children were treated twice daily for 6 months."

"CONCLUSION: **Daily dietary probiotic supplementation for 6 months** was a *safe, effective way to reduce fever, rhinorrhea, and cough incidence and duration and antibiotic prescription incidence*, as well as the number of missed school days attributable to illness, **for children 3 to 5 years of age.**"

Leyer GJ et al. *Pediatrics.* 2009;124(2):e172-e179.

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Population-Specific, Dose and Strain-Specific Study Example

- Randomized, placebo-controlled trial to assess illness reduction
- Population: Healthy, physically active adults (241 males, 224 females, 18-60 years old, who **exercise at least 30 min 3x/week**)
- Dose: *Lactobacillus acidophilus* NCFM and *Bifidobacterium animalis subsp. lactis* Bi-07 (NCFM & Bi-07) **5×10^9 CFU** each per day
- Duration: 150 days (5 months)
- Results:
  - Delayed the onset of upper respiratory tract illness events
  - NCFM + Bi-07 group had a **significantly higher level of physical activity load (Duration x intensity)** (on average, 4 exercise sessions/week)
    - Exercise duration and intensity was significantly increased
- Possible that NCFM and Bi-07 helps reduce illness' negative effects on physical activity

West NP et al. *Clinical Nutrition*. 2014;33(4):581-587.

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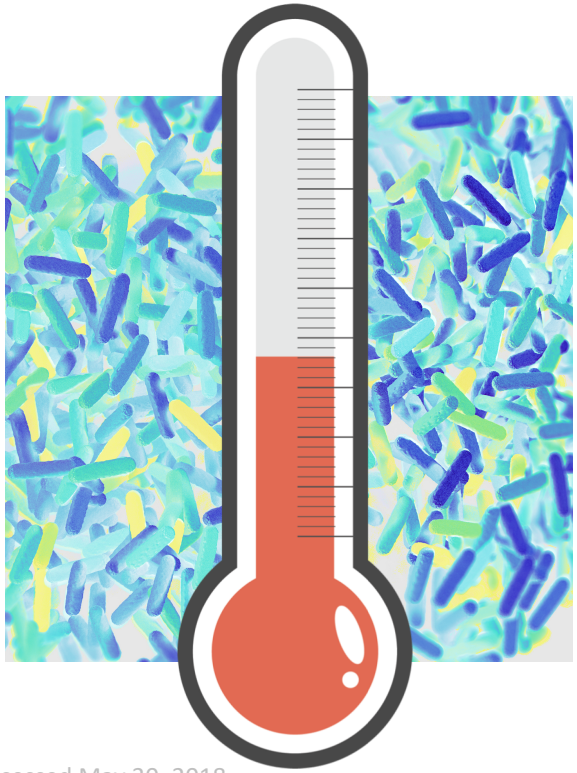
Stability Testing

- Stability testing should be conducted under the same temperature conditions as the recommended storage conditions on the finished product label
- Label should reflect label claim through end of shelf life (up until expiration date)
- As few publications on strain storage conditions exist, stability testing should be conducted to provide data to support proper storage conditions

https://www.crnusa.org/sites/default/files/pdfs/CRN-IPA-Best-Practices-Guidelines-for-Probiotics.pdf. Accessed May 30, 2018.

Storage Conditions

- The storage conditions are determined by the results of the stability tests and/or published literature on the strain
- Each strain may require different storage conditions (refrigeration or room temperature)
- Manufacturers should provide instructions for storage and handling based on individual formulations and packaging and conditions throughout product's shelf life



https://www.crnusa.org/sites/default/files/pdfs/CRN-IPA-Best-Practices-Guidelines-for-Probiotics.pdf. Accessed May 30, 2018.

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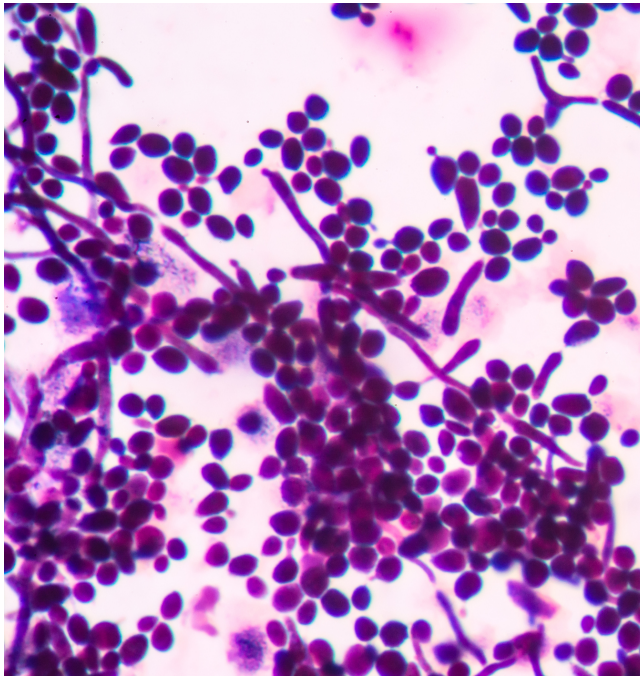
Probiotic Yeast

- *Saccharomyces boulardii* is a very well studied, commonly used probiotic yeast that was found in a meta-analysis to be effective in children and adults in reducing the risk of antibiotic-associated diarrhea[1]
- It has been studied to provide symptom relief for various gastrointestinal conditions (IBS, Crohn's)[1,2]
- Quantification of yeast is not consistent; some manufacturers list by weight (milligrams) and others list by CFU[3]



1. Szajewska H et al. *Aliment Pharmacol Ther.* 2015;42(7):793-801.
2. Kelesidis T et al. *Therap Adv Gastroenterol.* 2012;5(2):111-125.
3. McFarland LV. *World Journal of Gastroenterology.* 2010;16(18):2202-2222.

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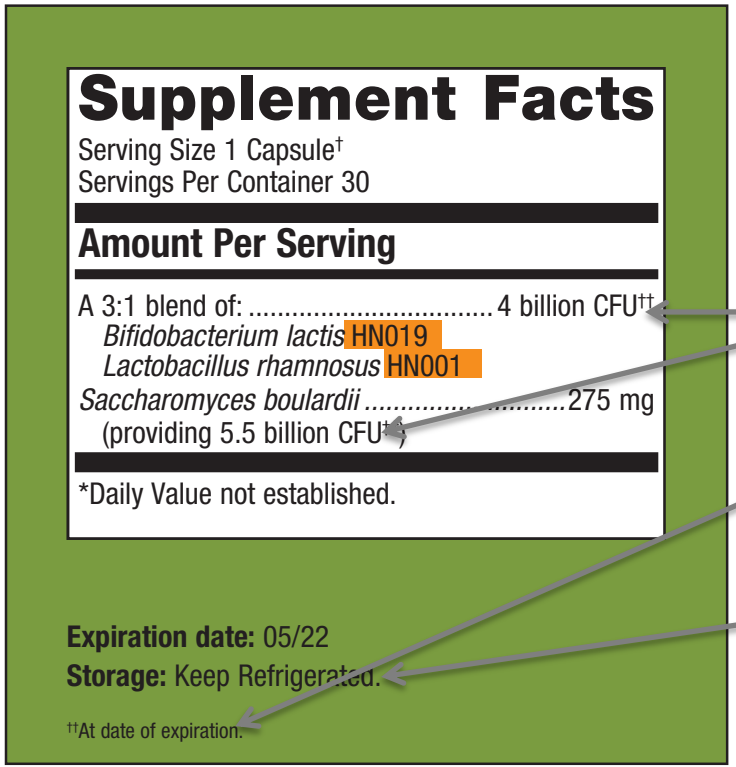
Identifying Marks of a Quality Probiotic



1. Strain specified
2. CFU (Colony Forming Unit) labeled
3. Quantity is guaranteed through expiration
4. Storage conditions are listed

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Summary:
Characteristics of a Quality Probiotic

1. Strain specified
2. CFU (Colony Forming Unit) labeled
3. Quantity is guaranteed through expiration
4. Storage conditions are listed

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Evidence-Based, Strain-Specific Probiotic Health Benefits

Strain(s)	Indications
Lactobacillus acidophilus NCFM *and* *Bifidobacterium lactis* Bi-07	Gastrointestinal, immune health, and recurring intestinal distress support[1]; different doses are suggested for children and adults[2]
Lactobacillus plantarum 299v	Helps support occasional irritation and bowel discomfort; promotes integrity of the gastrointestinal barrier[3]
Bifidobacterium lactis B420	Supports body weight maintenance[4]
Lactobacillus rhamnosus GR-1 *and Lactobacillus reuteri* RC-14	May help maintain healthy vaginal microflora and support urogenital health[5]
Lactobacillus paracasei 8700:2 and Lactobacillus plantarum HEAL9	Helps support healthy nasal, sinus, and respiratory function[6]
*Bifidobacterium lactis (*Bi-07*), Lactobacillus acidophilus* NCFM*, Bifidobacterium lactis (*BI-04*) and Lactobacillus paracasei (*Lpc-37*)*	Provides relief for abdominal discomfort associated with loose stools and occasional diarrhea[7]

1. Ringel-Kulka T et al. *J Clin Gastroenterol.* 2011;45(6):518-525.
2. Larsen N et al. *FEMS Microbiol Ecol.* 2011;75(3):482-496.
3. Ducrotte P et al. *World J Gastroenterol.* 2012; 18(30): 4012–4018.
4. Stenman LK et al. *EBioMedicine.* 2016;13:190-200.
5. Cribby S et al. *Interdiscip Perspect Infect Dis.* 2008;256490.
6. Popova M et al. *J Appl Microbiol.* 2012;113(6):1305-1318.
7. Ouwehand AC et al. *Vaccine.* 2014;32(4):458-463.

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Evidence-Based, Strain-Specific Probiotic Health Benefits

Strain(s)	Indications
Lactobacillus acidophilus NCFM, *Bifidobacterium lactis* Bi-07 and *Fructooligosaccharides* (FOS)	Supports immune health and digestive support. Fructooligosaccharides is a prebiotic shown to promote the growth of beneficial bacteria.[1]
Bifidobacterium lactis HN019, *Lactobacillus rhamnosus* HN001, and *Saccharomyces boulardii*	Provides support for immune health and occasional loose stools. May be ideal for patients who travel often.[3]
Bifidobacterium animalis ssp lactis (BB-12) and *Lactobacillus rhamnosus* GG	Strains are shown to support healthy microbial balance in infants and young children.[4]
Saccharomyces boulardii, Bifidobacterium lactis Bi-07, *Lactobacillus plantarum* Lp-115, *Lactobacillus salivarius* Ls-33, *Lactobacillus* NCFM, *Streptococcus thermophilus* St-21, and *Bifidobacterium lactis* BI-04	Combination of strains provide multidimensional support for both the upper and lower GI tract for digestive and immune health.[2]
Lactobacillus salivarius UCC118	May influence tight junctions between intestinal cells and may beneficially influence immune cell signaling processes.[5,6]

1. Ringel-Kulka T et al. *J Clin Gastroenterol.* 2011;45(6):518-525.
2. Popova M et al. *J Appl Microbiol.* 2012;113(6):1305-1318.
3. Patel R et al. *Clin Infect Dis.* 2015; 60(Suppl 2): S108–S121.
4. Szajewska H et al. *BMC Pediatr.* 2013; 13: 185.
5. O'Callaghan J et al. *Appl Environ Microb.* 2012;78(15):5196-5203.
6. Miyauchi E et al. *Am J Physiol Gastrointest Liver Physiol.* 2012; 303(9):G1029-G1041.

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Lactobacillus salivarius UCC118

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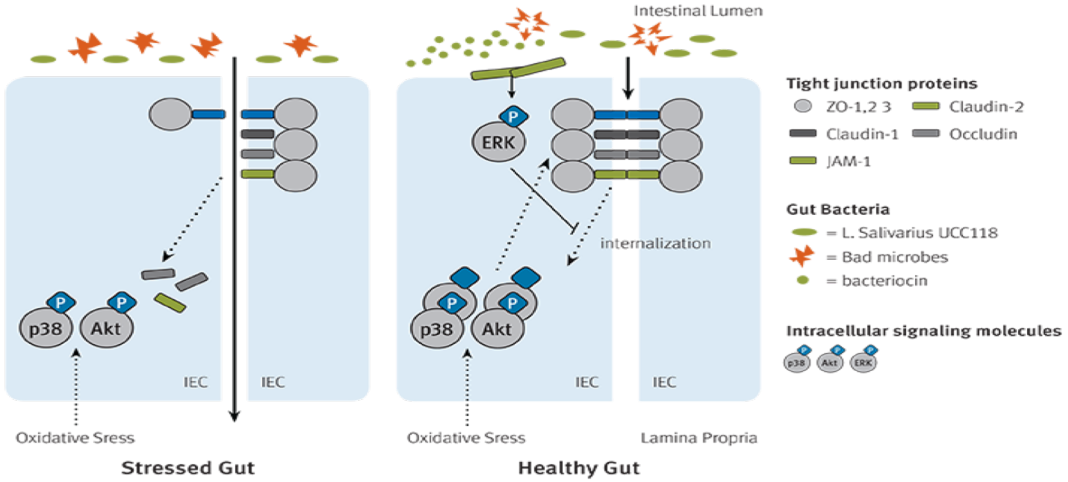
Effect of *L. salivarius* UCC118 on Tight Junction Proteins

Study Design

- Human Intestinal Epithelial Cell Line: CaCo2
- Oxidative stress: Hydrogen Peroxide (H_2O_2) exposure
- Outcome: Localization of tight junction proteins
- Hypothesis: UCC118 will prevent the cellular internalization of tight junction proteins associated with oxidative stress



Miyauchi E et al. *Am J Physiol Gastrointest Liver Physiol.* 2012;303(9):G1029-1041.

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Pretreatment with UCC118 prevents disruption of intestinal epithelial cell tight junctions, in a validated *in vitro* model of human intestinal epithelial cell oxidative stress

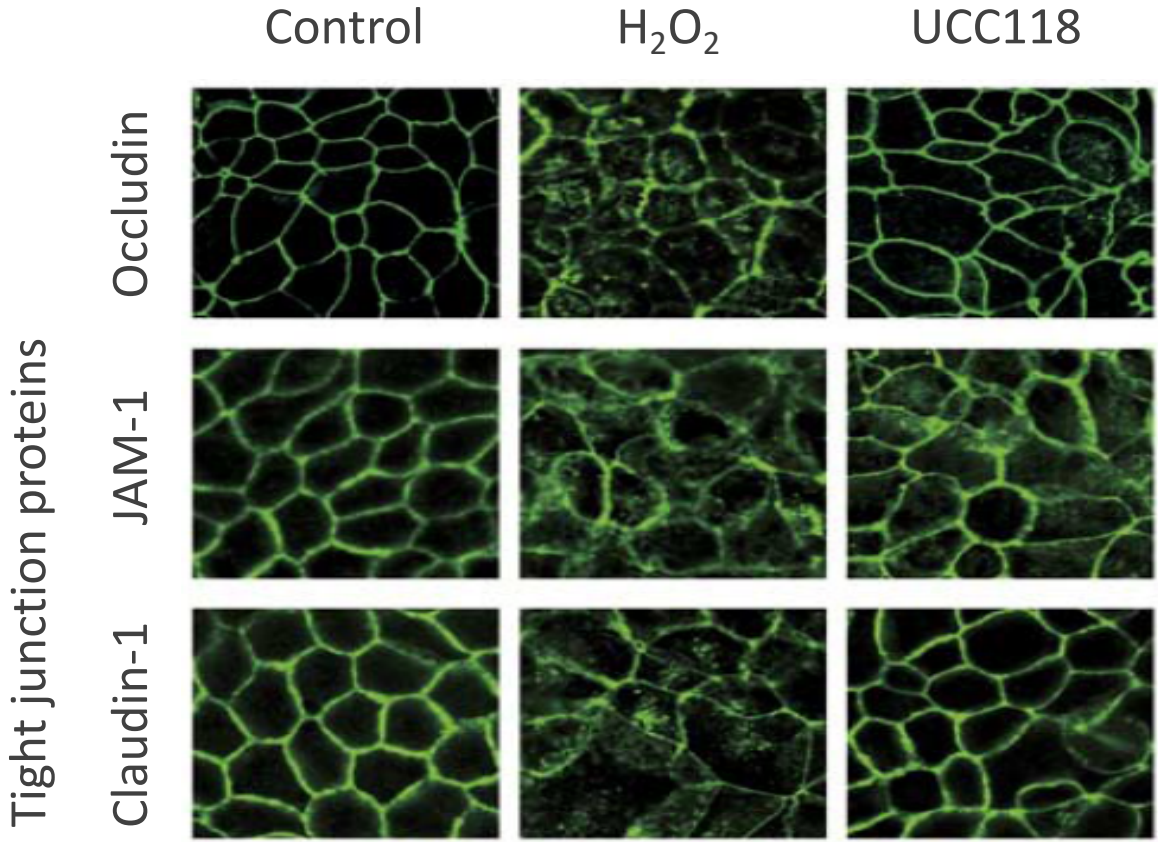

Image selected from Figure 5 in Miyauchi E et al. *Am J Physiol Gastrointest Liver Physiol.* 2012;303(9):G1029-1041.
License 4359481306032 from APS

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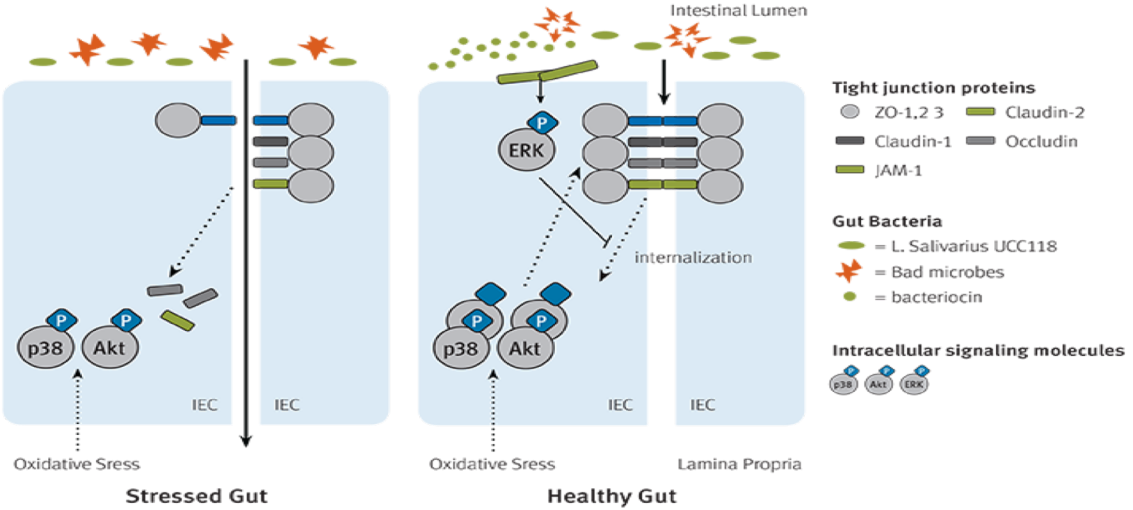
Conclusions

- UCC118 prevented the internalization of tight junction proteins after oxidative stress
- Not all strains of *Lactobacillus salivarius* have this capacity
- UCC118 protects tight junction functionality in intestinal epithelial cells



See also Miyauchi E et al. *Am J Physiol Gastrointest Liver Physiol.* 2012;303(9):G1029-1041.

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In vivo effectiveness of bacteriocin produced by *L. salivarius* UCC118

- To demonstrate the *in vivo* effectiveness of the bacteriocin produced by *L. salivarius* UCC118, researchers at University College Cork conducted an infectious challenge study in an animal model:
  - Pathogen: Listeria (EDGe expressing)
  - Animal model: Mice
  - Treatment Groups:
    - Control
    - UCC118
    - UCC118 – bacteriocin-deficient
  - Detection method: Listeria in liver, whole body imaging
- These types of pathogen challenge studies are not typically conducted in humans for ethical reasons.

Corr SC et al. *Proc Natl Acad Sci U S A*. 2007;104(18):7617-7621.

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UCC118 prevents *Listeria* infection, via a bacteriocin dependent mechanism in mice

See also Corr SC et al. *Proc Natl Acad Sci U S A*. 2007;104(18):7617-7621.

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Conclusions

- *L. salivarius* UCC118 prevented *Listeria* infection in an animal model[1]
- The effect was dependent upon the production of bacteriocin by UCC118[1]
- In addition, *L. salivarius* UCC118 has been shown to reduce certain *Firmicutes* genus members in mice and pig microbiota and *Spirochetes* levels in the mice and pig microbiota[2]
  - This effect was also dependent upon the production of bacteriocin[2]

1. Corr SC et al. *Proc Natl Acad Sci U S A*. 2007;104(18):7617-7621.
2. Riboulet-Bisson E et al. *PLOS ONE*. 2012;7(2):e31113.

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Emerging Data on *L. salivarius* UCC118

- Intestinal colonization
  - Detection in feces[1,2]
  - Detection at surface of ileum and adhesion to colon (*in vivo*)[3]
- Efficacy
  - Pilot scale study in patients with Crohn's disease[4]
  - Pilot quality improvement study in patients with SIBO[5]

1. Collins JK et al. *Microb Ecol Health D.* 2002;14(2):81-89.
2. O'Connor A et al. *P Nutr Soc*. 2010;69(OCE1).
3. Dunne C et al. *Microb Ecol Health D.* 2004;16(2-3):96-104.
4. O'Mahony L et al. Gastroenterology 2000;118(Issue4, Part 1), A853. Abstract presented at Digestive Disease Week and the 101st Annual Meeting of the American Gastroenterological Association, May 21-24, 2000 in San Diego, CA.
5. Cresci G et al. Effects of *Lactobacillus salivarius* UCC118 in reducing symptoms of small intestinal bacterial overgrowth [abstract] (2016). (http://journals.sagepub.com/pb-assets/cmscontent/PEN/CNW16_Monday_Poster_Abstracts_revised.pdf)

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L. salivarius UCC118 in an open label study in patients with Crohn's disease

- 21 consecutive patients with **mildly active** Crohn's disease needing a therapeutic change
- Patients were already taking a stable dose of oral 5-ASA but were not on steroids, had a CDAI of 150-320
- Trial involved probiotic therapy (**1x10^{10} organisms in yogurt/day for 6 weeks**) **instead of steroid therapy**
- Efficacy was quantified in terms of a change in CDAI and steroid avoidance
- Compliance was confirmed by faecal isolation of the probiotic in all subjects

What is CDAI? Crohn's Disease Activity Index is a composite measure of the symptoms of Crohn's.
A score < 150 = remission
150-450 = active disease
> 450 = severe disease

O'Mahony L et al. *Gastroenterology* 2000;118(Issue4, Part 1), A853. Abstract presented at Digestive Disease Week and the 101st Annual Meeting of the American Gastroenterological Association, May 21-24, 2000 in San Diego, CA.

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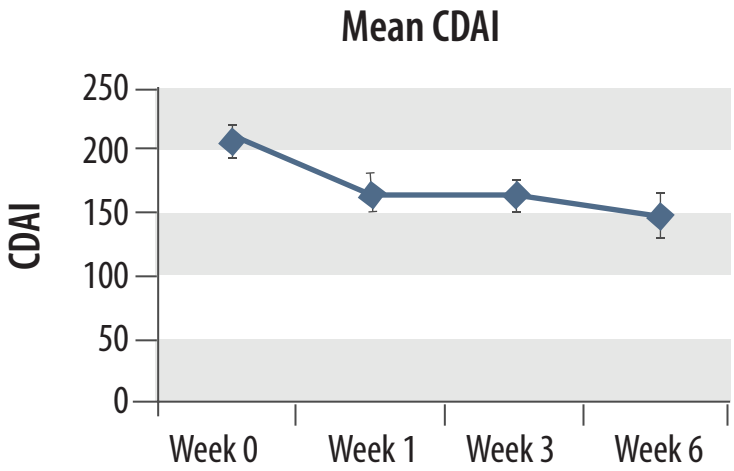
UCC 118 Decreased Crohn's Disease Activity Index (CDAI)

- CDAI scores ranged from 175-250 at study enrollment
- For the 19 patients who completed the trial without steroids,
  - Mean CDAI at week 0 was 208
  - Mean CDAI at 3 weeks was 167
  - Mean CDAI decreased to 146.6 at 6 weeks (p=0.0049, significant)
- At 2 months, 11 of the subjects remained steroid free (did not need steroids to manage their Crohn's)



O'Mahony L et al. *Gastroenterology* 2000;118(Issue4, Part 1), A853. Abstract presented at Digestive Disease Week and the 101st Annual Meeting of the American Gastroenterological Association, May 21-24, 2000 in San Diego, CA.

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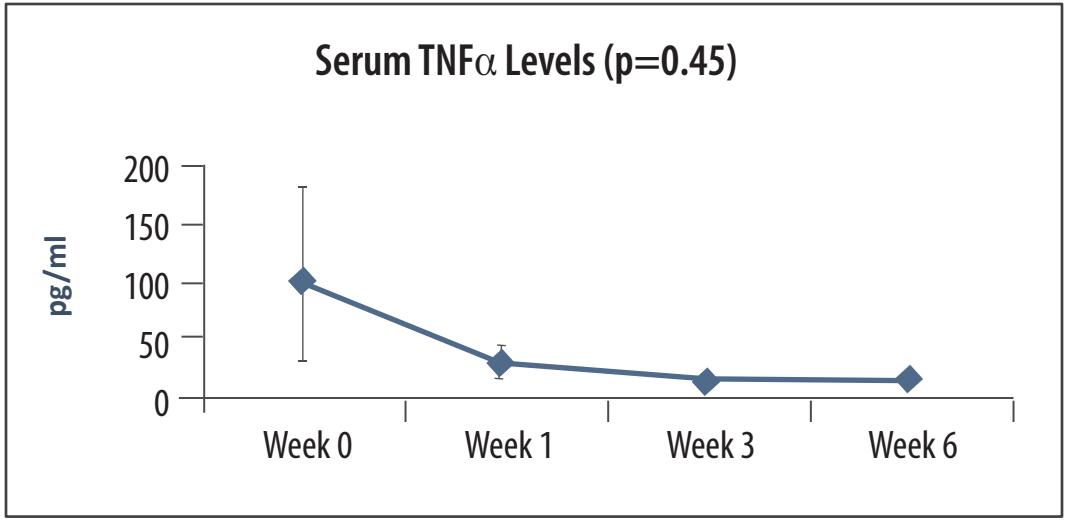
In the same clinical study, UCC118 improved TNF-α response in patients with Crohn's disease

While there was no statistically significant change in TNF-α levels (106.14 pg/ml at week 0, 14.617 pg/ml at week 6; p>0.05),
those patients with a high baseline TNF-α level had significantly reduced levels at week 6.



O'Mahony L et al. *Gastroenterology* 2000;118(Issue4, Part 1), A853. Abstract presented at Digestive Disease Week and the 101st Annual Meeting of the American Gastroenterological Association, May 21-24, 2000 in San Diego, CA.

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Limitation and Strengths of the Study

Strengths	Limitations
• Decrease in disease score and TNF-α levels without steroid therapy is notable • Crohn's flare rarely clears up spontaneously without aggressive treatment	• Open label • No control group

O'Mahony L et al. *Gastroenterology* 2000;118(Issue4, Part 1), A853. Abstract presented at Digestive Disease Week and the 101st Annual Meeting of the American Gastroenterological Association, May 21-24, 2000 in San Diego, CA.

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UCC118 Improved Symptoms in Patients with Small Intestinal Bacterial Overgrowth (SIBO)

Quality Improvement Trial, Cleveland Clinic Center for Gut Rehabilitation and Transplantation, Outpatient Clinic

- ***L. salivarius* UCC118** was swapped out for the usual probiotic and given in addition to standard of care
  - 29 patients with SIBO were given *L. salivarius* UCC118 (10^8 CFU/day) daily for 90 days
  - Patients reported symptoms at 30, 60 and 90 days
  - Patients' comments regarding changes to their SIBO symptoms were recorded

Cresci G et al. Effects of *Lactobacillus salivarius* UCC118 in reducing symptoms of small intestinal bacterial overgrowth [abstract] (2016). (http://journals.sagepub.com/pb-assets/cmscontent/PEN/CNW16_Monday_Poster_Abstracts_revised.pdf)

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Patients in the trial reported an improvement in their symptoms

Select comments from patients regarding their experience taking UCC118 for SIBO symptoms

"Before my diarrhea was so severe I couldn't make it out of the house. Now I can make it to the grocery store."

"I have not had diarrhea since midway through the trial."

"The foul odor of my stool has really gone away since taking the probiotic."

"I can tell my gas symptoms have worsened since I stopped taking the probiotic."

"I was able to get off antibiotics for 3 months."

"My symptoms disappeared after 30 days and I was able to resume my daily activities."

"I have been symptom-free of SIBO for 2 weeks, I am amazed, astounded, and ecstatic!"

"My diarrhea is no longer present and my stool is more consistent."

"My SIBO symptoms improved in first 1-2 weeks and have been the same since."

"I was symptom-free for 18 hours after starting the probiotic—that's the longest relief I've had in years."

Cresci G et al. Effects of *Lactobacillus salivarius* UCC118 in reducing symptoms of small intestinal bacterial overgrowth [abstract] (2016). (http://journals.sagepub.com/pb-assets/cmscontent/PEN/CNW16_Monday_Poster_Abstracts_revised.pdf)

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Limitation and Strengths of the Study

Strengths	Limitations
• Replacement of standard of care probiotic with UCC118 was notable • Investigators reported decreased antibiotic usage and decreased time to antibiotics in addition to patient reported improvement of symptoms	• Quality Improvement trial • No control group

O'Mahony L et al. *Gastroenterology* 2000;118(Issue4, Part 1), A853. Abstract presented at Digestive Disease Week and the 101st Annual Meeting of the American Gastroenterological Association, May 21-24, 2000 in San Diego, CA.

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Additional Data on *Lactobacillus salivarius* UCC118

A randomized controlled human study shows the safety of oral supplementation of UCC118 in pregnant patient populations[1]

In vitro experiments show that UCC118 treatment of human mesenteric lymph node (MLN) cells may lead to IL-10 production.[2] IL-10 is a cytokine that plays an important role for the proper functioning of the immune system.[3]

1. Lindsay KL et al. *Am J Obstet Gynecol.* 2015;212(4):496.e1-11.
2. O'Mahony L et al. *Am J Physiol-Gastr L*. 2006;290(4):G839-G845.
3. Iyer SS et al. *Crit Rev Immunol*. 2012;32(1):23-63.

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Bifidobacterium animalis ssp *lactis* 420

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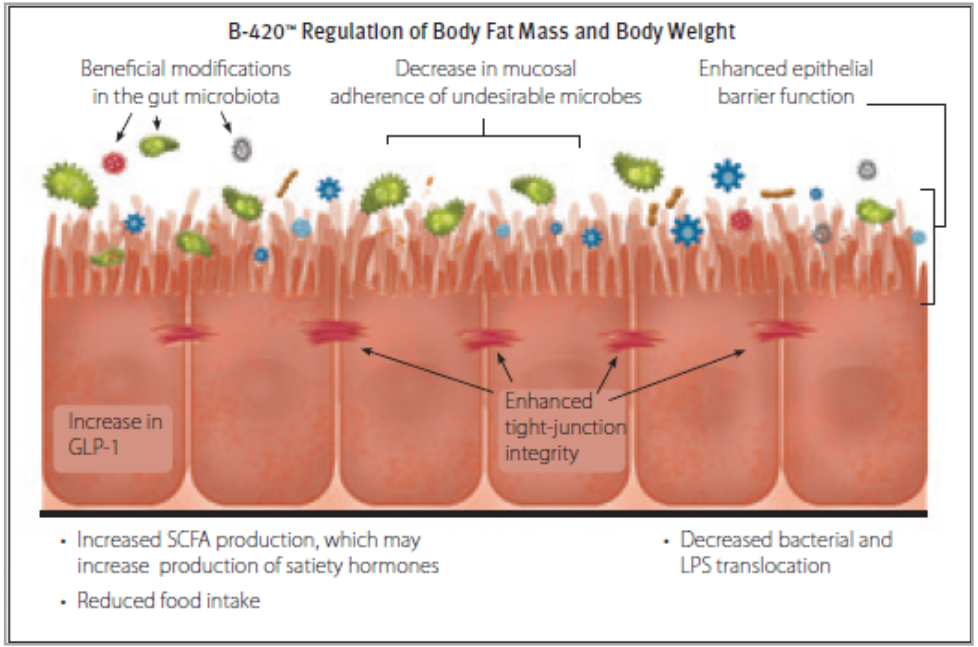
B420 probiotic strain—
Mechanisms of action

All of the actions below are supported by pre-clinical and/or clinical data with B420



Putaala H et al. *Res Microbiol.* 2008;159(9-10):692-698.
Amar J et al. *EMBO Mol Med.* 2011;3(9):559-572.
Lyra A et al. *Gastroenterol Res Pract*. 2012;615051.
Stenman LK et al. *Benef Microbes.* 2014;5(4):437-445.
Stenman LK et al. *Diabetol Metab Syndr.* 2015;12(7):75.
Stenman LK et al. *EBioMedicine*. 2016;13:190-200.

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Summary of mechanisms of action

Bifidobacterium animalis ssp *lactis* 420 has three key potential mechanisms of action that may explain its effects of reducing energy intake in the clinical study

1. Animal studies demonstrate that B420 may increase levels of the anorectic (appetite-reducing) gut peptide GLP-1[1]
2. The clinical study shows that B420 increases total intestinal short-chain fatty acid concentration[2], which may promote the production of GLP-1[3-5]
3. *In vitro* and animal studies demonstrate that B420 may improve gut barrier function[6-9], which may reduce LPS and bacterial translocation from the gut, which may lower LPS signaling in adipose tissue, reducing the signals associated with weight gain[10]

1. Stenman LK et al. *Diabetol Metab Syndr.* 2015;12(7):75.
2. Stenman LK et al. *EBioMedicine*. 2016;13:190-200.
3. Tolhurst G et al. *Diabetes*. 2012;61(2):364-371.
4. Psichas A et al. *Int J Obes (Lond).* 2015;39(3):424-429.
5. Lin HV et al. *PLoS ONE*. 2012;7(4):e35240.
6. Putaala H et al. *Res Microbiol.* 2008;159(9-10):692-698.
7. Lyra A et al. *Gastroenterol Res Pract*. 2012;615051.
8. Amar J et al. *EMBO Mol Med.* 2011;3(9):559-572.
9. Stenman LK et al. *Benef Microbes.* 2014;5(4):437-445.
10. Cox LM et al. *Cell Metabolism*. 2013;17(6):883-894.

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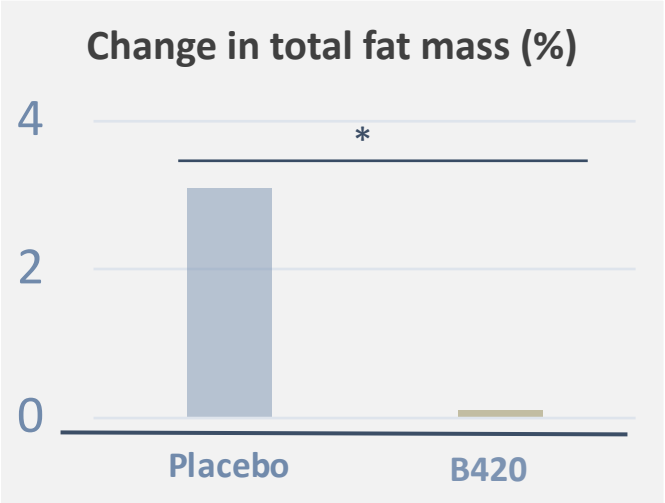
B420 clinical data[1] —
Body fat

Bifidobacterium animalis ssp. *lactis* 420 helps control body fat

*In a 6-month clinical study, of overweight individuals, those taking *Bifidobacterium animalis* ssp. *lactis* 420 showed reduced body fat mass compared to placebo group



1. Stenman LK et al. *EBioMedicine*. 2016;13:190-200.

* Indicates placebo vs. B420 groups significantly different at 6 months ($p<0.05$; per protocol post-hoc analysis)

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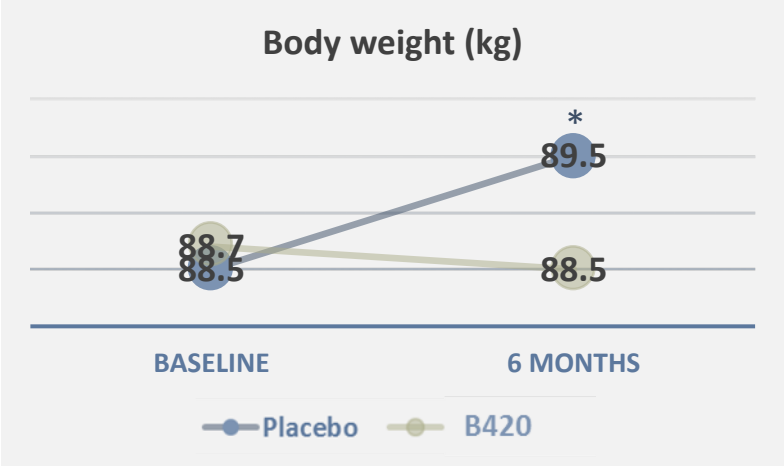
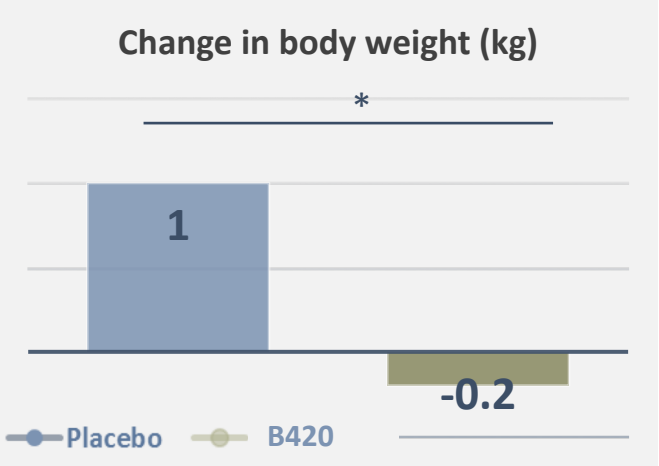
B420 clinical data[1] —
Body weight

- ***Bifidobacterium animalis* ssp. *lactis* 420 helps control body weight and body weight regulation**
- Preliminary evidence shows that *Bifidobacterium animalis* ssp. *lactis* 420 may **help contribute to long-term weight maintenance**





1. Stenman LK et al. *EBioMedicine*. 2016;13:190-200.

* Indicates placebo vs. B420 groups significantly different at 6 months ($p<0.05$; per protocol post-hoc analysis)

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Multiple Strains of Probiotics in One Formula

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Probiotic Efficacy: Multiple Strain or Single Strain?

- A recent analysis concluded that it is **unknown** whether a multiple strain formula or a single probiotic strain is more effective[1]
- Why? **Lack of studies** directly evaluating a multiple strain combination versus a single strain
- Example: Multiple probiotics have been evaluated in people with Inflammatory Bowel Disease [IBD]; however, none have been evaluated against each other in the same study. These probiotics include the following:
  - 8 species blend of *Lactobacillus plantarum, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis,* and *Streptococcus salivarius subsp. thermophilus*
    - not identified in reference by strains. Note: Clinical studies need to specify the specific strains because if there are different strains, there could be different results (for example, *L. plantarum strain* MF1298 did not mitigate irritable bowel syndrome [IBS], while *L. plantarum* strain 299v was found to provide relief for IBS) [1]
  - *Lactobacillus rhamnosus* GG [1]
  - *Lactobacillus salivarius* UCC118[2]
  - *Escherichia coli* Nissle 1917[3,4]

1. Korada SK et al. *CPD*. 2019;24(35):4150-4153.
2. O'Mahony L et al. Gastroenterology 2000;118(Issue4, Part 1):A853. Abstract presented at Digestive Disease Week and the 101st Annual Meeting of the American Gastroenterological Association, May 21-24, 2000 in San Diego, CA.
3. Kruis W et al. *Gut.* 2004;53(11):1617–1623.
4. Rembacken BJ et al. *Lancet*. 1999;354(9179):635–639.

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Efficacy of Multiple Strain V. Single Strain Is Unknown

- Demonstrated efficacy of multiple strains v. single strains depends on available literature
- Indications from some *in vitro* or animal studies, not human studies, might suggest multiple strains work together effectively; [1] however, clinical studies are needed to know efficacy in humans **(some clinical studies compared multiple strains to single strain and used different doses)**[1]
- Multiple probiotics have been shown to be effective for certain conditions but have not been evaluated in the same study. Examples (Note: none of the following were compared in the same study):[2]
  - **Vaginal health**: Reviewers stated that *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 persist more in the vagina and that *Lactobacillus rhamnosus* GG was less efficient at preventing infections of the urinary tract although these strains were evaluated in different studies[2]
  - **Preventing diarrhea**:
    - Single strains (such as *L. rhamnosus* GG for children's acute gastroenteritis[3,4] or the probiotic yeast *S. boulardii* for traveler's diarrhea[5,6]) as well as multiple strain combinations (*Lactobacillus acidophilus* NCFM, *Lactobacillus paracasei* Lpc-37, *Bifidobacterium lactis* Bi-07, and *Bifidobacterium lactis* Bl-04 for antibiotic-associated diarrhea[7]) have shown efficacy
    - Difficult to conclude which strains are better due to lack of comparison within same study

1. Chapman CMC et al. *Eur J Nutr*. 2011;50(1):1-17.
2. Korada SK et al. *CPD*. 2019;24(35):4150-4153.
3. Ouwehand AC. *Benef Microbes.* 2017;8(2):143-151.
4. Szajewska H et al. *Aliment Pharm Ther*. 2013;38(5):467-476.
5. Kollaritsch H et al. *Travel Med Int.* 1989;7(1):9-18.
6. Kollaritsch H et al. *Fortschr Med.* 1993;111(9):152-156.
7. Ouwehand AC et al. *Vaccine.* 2014;32(4):458-463.

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Safety Evaluations of Multiple Strain Probiotics Are Just as Important as Safety Evaluation of Single Strains

- Safety outcomes are inconsistently reported in clinical studies on probiotics[1]
- Safety needs to be evaluated at the strain-specific level
  - Pathogenic *Escherichia coli* O157:H7 apparently causes diarrhea while probiotic *Escherichia coli* Nissle 1917 reportedly reduces diarrhea[2]
  - *Bacillus anthracis* is well known as a species for its ability to spread the disease anthrax through spores in the air; on the other hand, *Bacillus coagulans* strains (GBI-30, 6086) have established generally recognized as safe (GRAS) status[3] in the US and are ubiquitous on the market
- Safety concerns exist for probiotic use in certain populations:[1]
  - Critically ill
  - Severely immunocompromised patients
- Potential side effects of a probiotic may include gastrointestinal symptoms such as bloating, gas, diarrhea, abdominal cramps or pain, nausea, constipation, or vomiting

1. Doron S et al. *Clin Infect Dis*. 2015;60(Suppl 2):S129-S134.
2. Korada SK et al. *CPD*. 2019;24(35):4150-4153.
3. GRAS Notice (GRN) No. 660. Available at https://www.fda.gov/media/100025/download. Accessed August 13, 2019.

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Clinical Trial NCT04044144

Title: Prospective Tolerability of a Probiotic Dietary Supplement

Summary: The dietary supplement contains eight probiotic strains to support general gastrointestinal and immune health. Each of the strains has been previously studied in human subjects. However, the present eight strain combination has not been evaluated in human subjects. This prospective study will evaluate the tolerability of the eight strain formula in healthy adults.

Sponsor: Metagenics, Inc.

Collaborator: National University of Natural Medicine

Recruitment Status: Completed

https://clinicaltrials.gov/ct2/show/NCT04044144?term=NCT04044144&rank=1 . Accessed September 23, 2019.

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Selection Criteria Used for Selecting Multiple Strains in a New Probiotic Complex Currently in the Tolerability Study

- **Safety:** Generally Recognized as Safe (GRAS)
- **Availability:** Probiotic manufacturers compete; sometimes third-party manufacturers provide combinations at an increased cost
- **Stability:** Different probiotic strains have different robustness/survival ability, and they may have different cryoprotectant formulations in their freeze-dried form, which affects their stability; additionally, data on each strain in the same packaging configuration (bottle type, desiccant type) is required to predict long-term stability and end-of-shelf-life viability (CFU at expiration)
- **Density:** Probiotics have different CFU/gram, which affect the ability to put a certain amount in a capsule
- **Clinical efficacy:** Some probiotics have demonstrated stronger efficacy in studies than others; since efficacy is dose-dependent, the right combination of clinically effective dose and density of the probiotic must be taken into account to put into the capsule

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Multiple Probiotic Strain Complex in Study NCT04044144

Genus	Species	Strain	Billion CFU per Capsule	Clinical Efficacy of Dose in Capsule	Reference
Bifidobacterium	*lactis*	Bi-07	12.5	12.5 billion CFU/day of *B. lactis* Bi-07 and 12.5 billion CFU of *L acidophilus* NCFM showed a significant reduction in the number of pain days when taken post-colonoscopy by healthy individuals	D'Souza B et al. *ANZ J Surg*. 2017;87(9):E65-E69.
Lactobacillus	*acidophilus*	NCFM	12.5		
Bifidobacterium	*lactis*	Bl-04	20	20 billion CFU/day taken during two vaccine administrations over 3 weeks showed an increased early response to vaccination by a significant increase in serum IgG	Paineau D et al. *FEMS Immunol Med Microbiol.* 2008;53(1):107-113.
Lactobacillus	*plantarum*	Lp-115	20	20 billion CFU/day during two vaccine administrations over 3 weeks showed an increased early response to vaccination by a nonsignificant increase in serum IgG.	Paineau D et al. *FEMS Immunol & Med Microbiol.* 2008;53(1):107-113.
Lactobacillus	*rhamnosus*	GG	20	20 billion CFU/day led to increased allergen-specific IgA salivary levels in individuals with seasonal allergies	Piirainen L et al. *Ann Allergy Asthma Immunol*. 2008;100(4):338-342.
Lactobacillus	*rhamnosus*	HN001	5	5 billion CFU/day improved measures of immune function (natural killer cell activity and cellular phagocytosis) in elderly participants	Gill HS et al. *Br J Biomed Sci.* 2001;58:94-96.
Bifidobacterium	*lactis*	HN019	5	5 billion CFU/day improved measures of immune function (natural killer cell activity and cellular phagocytosis) in elderly participants	Gill HS et al. *J Clin Immunol.* 2001;21(4):8.
Lactobacillus	*paracasei*	Lpc-37	10	10 billion CFU/day is currently being tested in two RCTs for the following outcomes: 1. Stress response in healthy adults 2. Episodic memory and other cognitive functions in healthy elderly	1. NCT03494725 2. NCT03601559 Searchable at www.clinicaltrials.gov

Study Design and Outcomes

	NCT04044144[1]
Study design	Single arm, open-label
Population	Healthy (*prescreened*) adults n=10
Intervention	105 billion CFU/day probiotic combo x 10 days
Primary outcome	Frequency of *new* adverse events (AEs), serious adverse events (SAEs)
Secondary outcomes	*New* abnormal values on a complete blood count (CBC) and comprehensive metabolic panel (CMP)
Exploratory outcomes	Stool analysis 1) Microbiota including *Lactobacillus, Bifidobacterium, Clostridium*, *Akkermansia muciniphila,* and others 2) Short-chain fatty acids (SCFAs)

Study design and outcomes similar to previous safety and tolerability study done on a probiotic, which was also a single arm, open-label study with monitoring of adverse events and blood cytokines.[2]

1. https://clinicaltrials.gov/ct2/show/NCT04044144?term=NCT04044144&rank=1 . Accessed September 23, 2019.

2. Hibberd PL et al. PLoS One. 2014;9(12).

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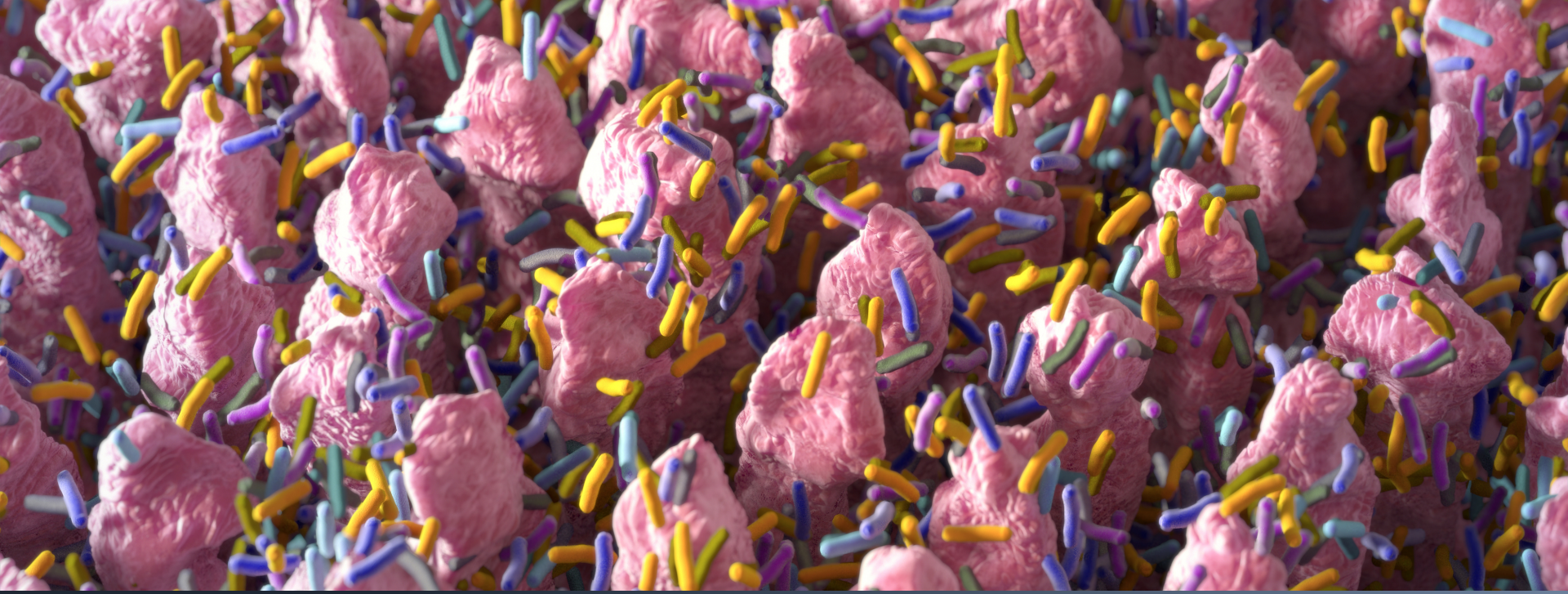

Emerging Microbiome-Related Research

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Fecal Microbial Transplants

- Fecal Microbial Transplants (FMT) transfers feces:
  - Earliest record of usage in 4th century China as “yellow soup” for diarrhea and severe food poisoning treatment
  - Chinese and others used it more and in 18th century, it spread to Europe
  - Currently the only known FDA approved FMT treatment is for recurrent infection with *Clostridium difficile*, which often flourishes after severe antibiotic use and infection during colitis

- FMT is being explored for the treatment of:
  - IBD
  - Insulin resistance and Type 2 Diabetes
  - Dyslipidemia
  - Atherosclerosis
  - Hepatic steatosis

Information summarized from de Groot PF et al. *Gut Microbes*. 2017;8(3):253-267.

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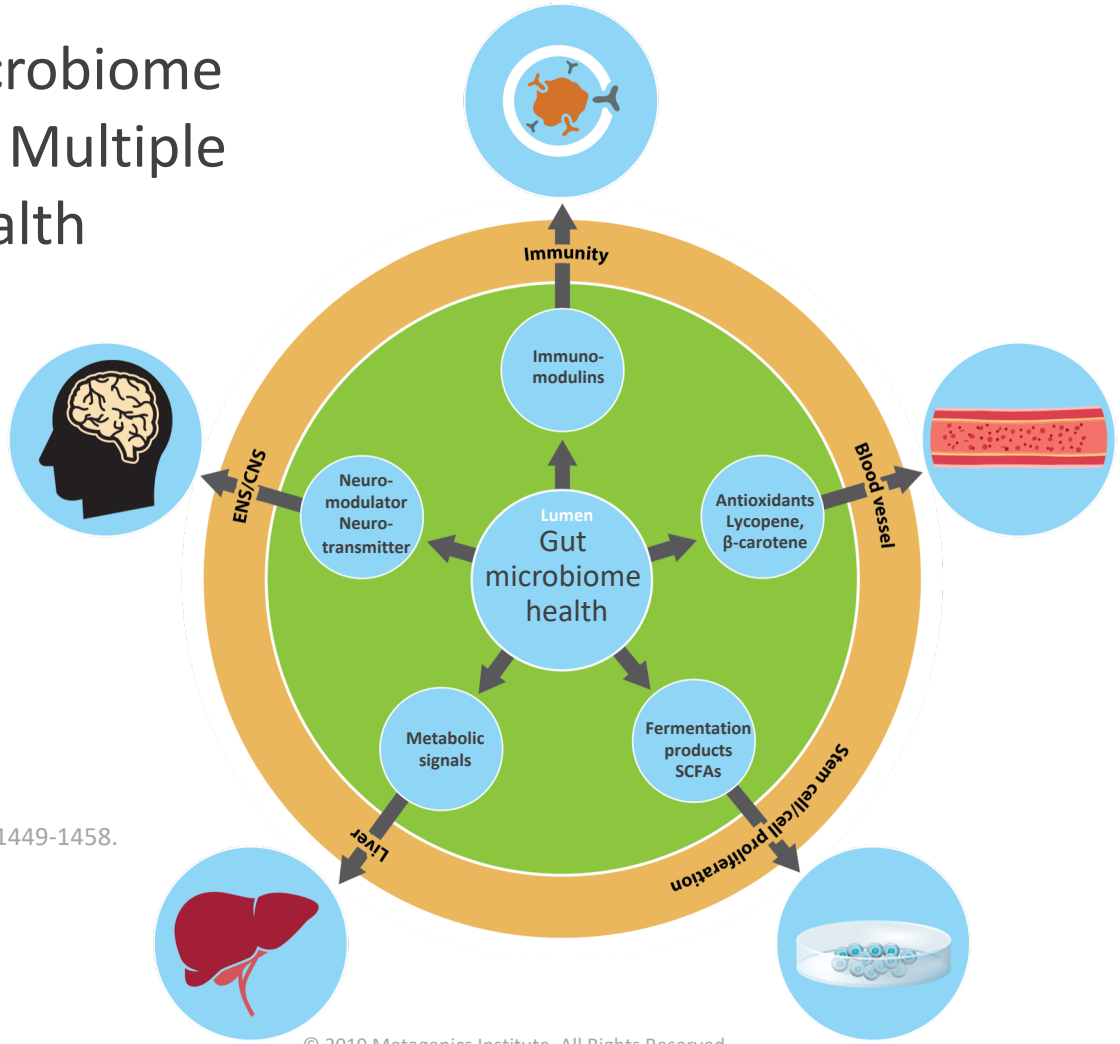
The Gut Microbiome May Impact Multiple Areas of Health



See also Hollister EB et al. *Gastroenterology*. 2014;146(6): 1449-1458.

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Emerging Research: Soil-Based Probiotics (Spore-Forming Bacteria)

- The genus *Bacillus* strains are gaining interest for their enhanced tolerance and survival under the harsh environment of the gastrointestinal tract
- Not all *Bacillus* strains are soil inhabitants; they can be isolated from other sources (air, water, vegetables, human/animal gut)
- Recent research suggests that spore-forming bacteria do carry probiotic attributes
- *Bacillus coagulans* strains (GBI-30, 6086, MTCC 5856, and others) have been the most researched in recent years
- Research is very new and clinical data is very limited

Elshaghabee FMF et al. *Front Microbiol*. 2017;8:1490.

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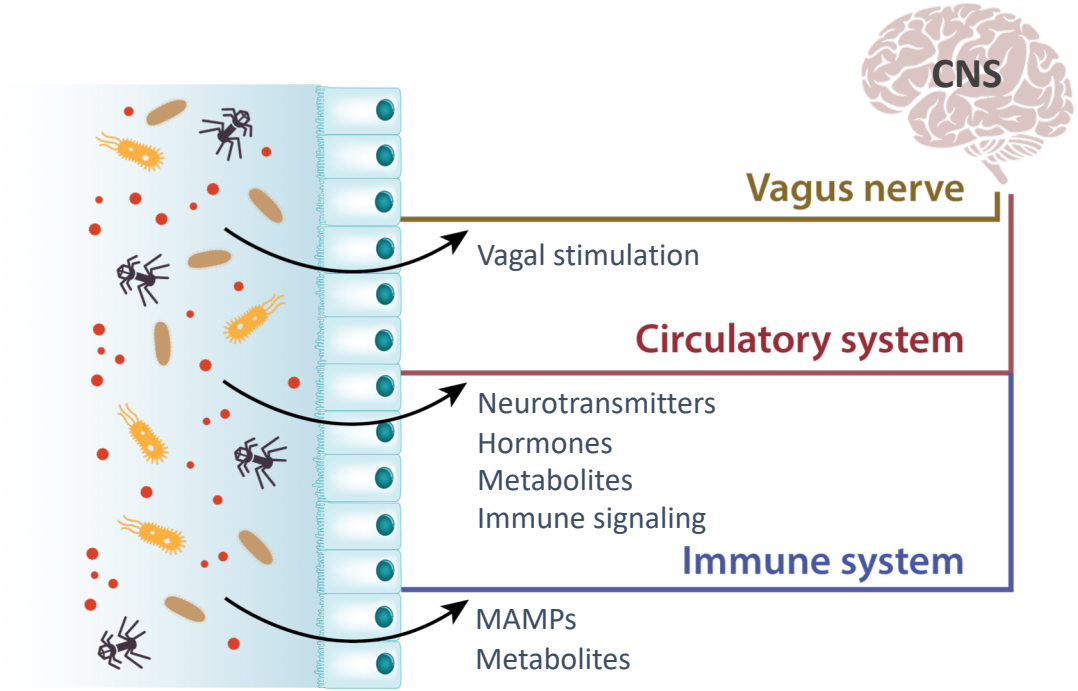
Multiple Pathways for Communication between the Microbiome and the Brain



See also Sampson TR et al. *Cell Host & Microbe*. 2015;17(5):565-576.
MAMP = Microbe-associated molecular patterns

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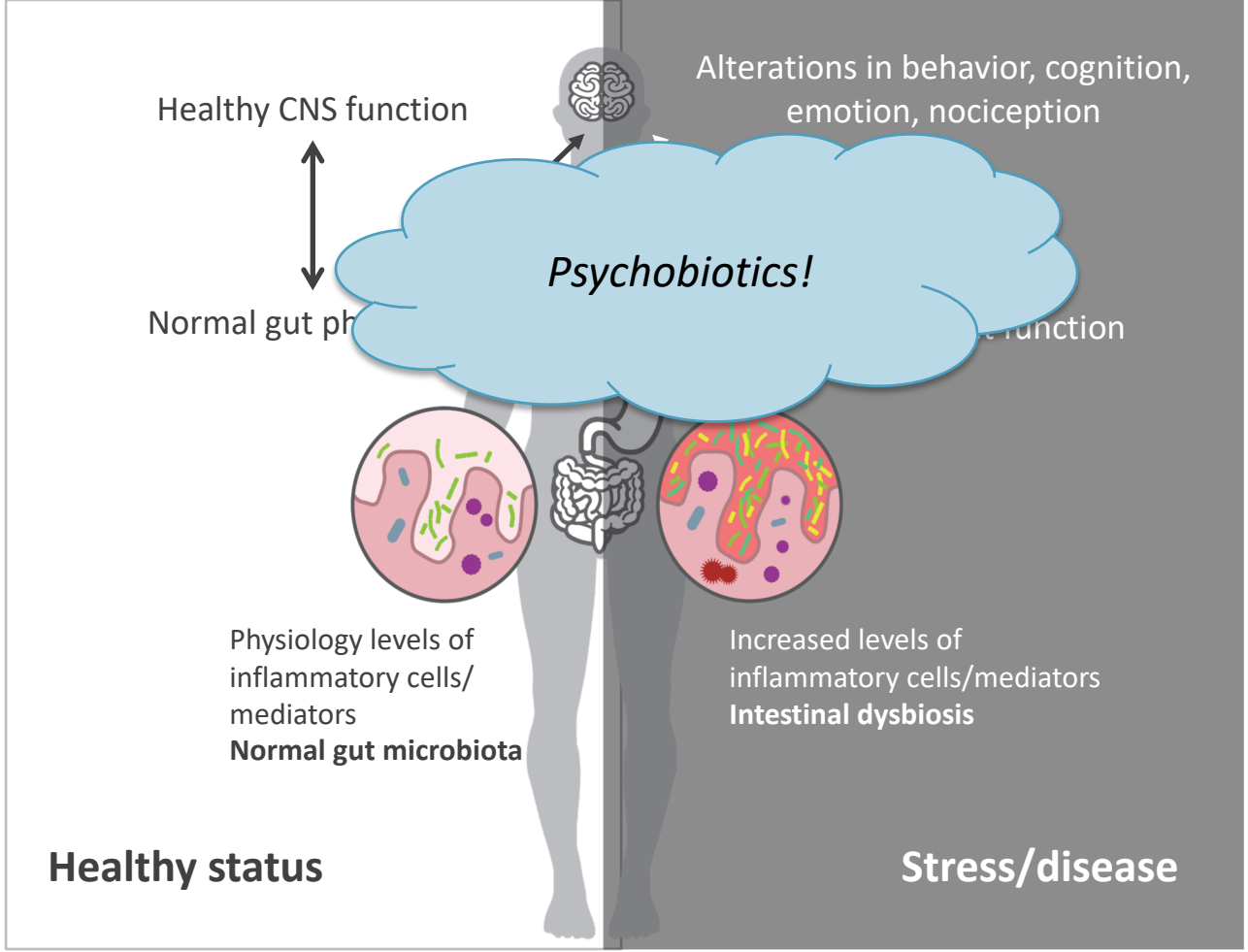
Communication between the brain and gut, including gut microbes, impacts health



See also Grenham S et al. *Front Physiol.* 2011;2:94.

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Key Messages

- Different factors such as diet, drugs, lifestyle and environment can affect the gut microbiome and may result in dysbiosis and loss of diversity
- Microbial dysbiosis and lower diversity are associated with metabolic and gastrointestinal diseases
- Recommending clinically-studied strains of probiotics (that fit proper criteria/guidelines from ISAPP, CRN and IPA for dose (CFU), strain-specification, and quantity guaranteed at expiration) with specific **indications** may help support general health and alleviate certain digestive, respiratory, metabolic and urogenital symptoms
- Continued research of the human microbiome is necessary in healthy and diseased populations to investigate potential diagnostic and therapeutic solutions

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