THE USE OF PROBIOTICS FOR ALTERING GUT MICROBIOME

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POINT INSTITUTE
OF NUTRACEUTICAL RESEARCH

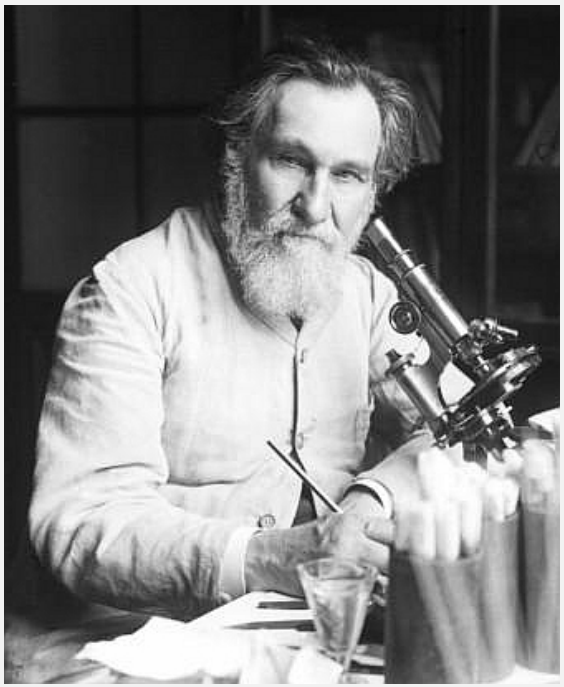
DISCLOSURE OF FINANCIAL RELATIONSHIPS

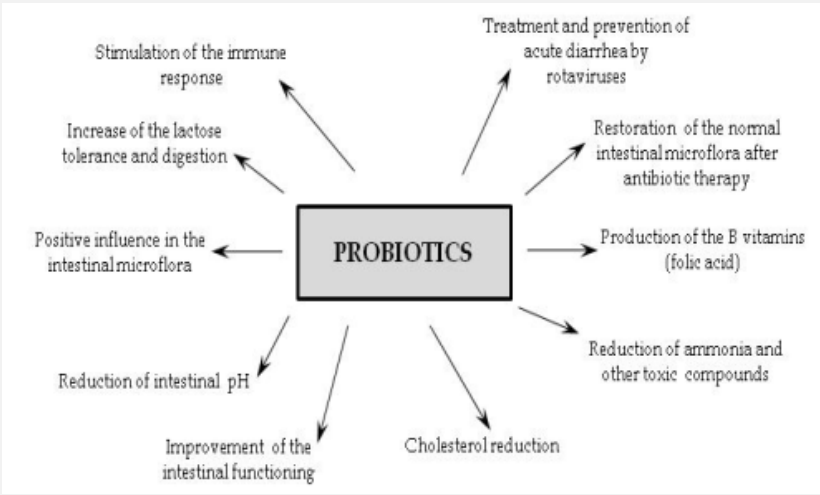
- Ortho Molecular Products
  - Consulting Fees
- Genova
  - Speaker Fees
- Off-Label Usage
  - None

OUR TOPIC IS VERY LARGE
OUR GOAL IS LIMITED

- To give you a foundation to understand clinically-available products that are labeled "Probiotics"
- To understand how these products (and organisms) differ from commensal gut bacteria
- To understand how they are manufactured, labeled, packaged, stored, doses: and why this matters
- To overview the Efficacy data on GI-related outcomes and summarize the future potential of probiotic Therapies.

THE USE OF LIVE CULTURES TO ALTER MICROBIOTA AND AFFECT HUMAN HEALTH IS NOT A NEW CONCEPT





TODAY- PROBIOTICS HAVE GONE "VIRAL"

BEFORE WE START: DEFINING TERMS

- It is important to note that while Probiotics Strains may be the same species as those found in the Human GI tract: Normal Commensal organisms are not Probiotics, and Probiotics are not commensal organisms!
- Important for understanding
  - The regulatory framework of Probiotics- Internationally
  - The therapeutic potential and limitations of Probiotics
  - The differences between Fermented food organisms and "added" probiotics in Functional Foods

IPA GUIDELINES

- Generic Definition (WHO etc.): "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"
- This **Does Not** include:
  - Food-borne bacteria (e.g., dirt of vegetables)
  - or Fermented foods with naturally-occurring or starter cultures
  - **-May include some strains added to fermented foods ***after*** Pasteurization (e.g., strains added to yogurt/Kefir as probiotics)

FERMENTED FOODS: ARE THEY PROBIOTICS?

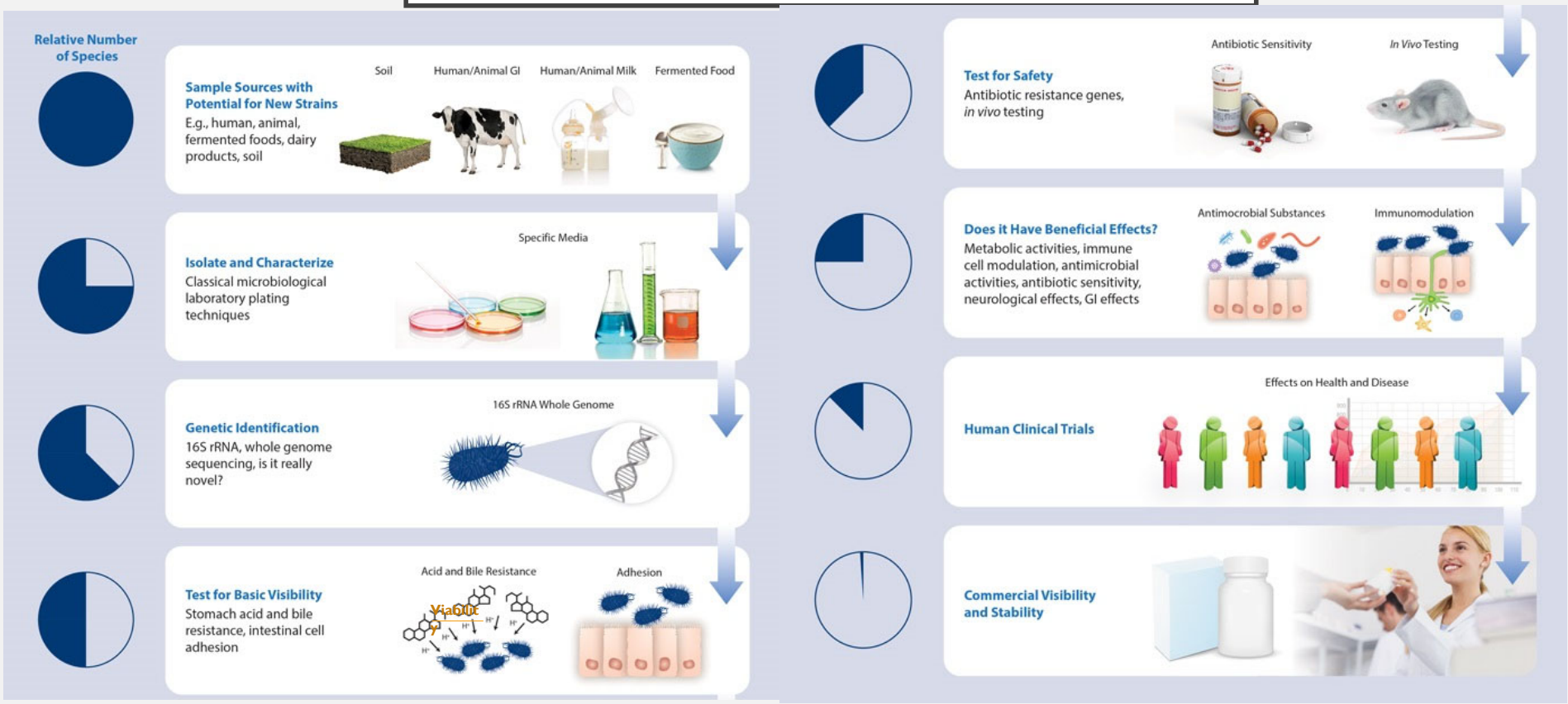
- Simple answer: NO
- Fermented foods contain natural (or added) cultures designed to digest the food during Fermentation (creating organic acids and other byproducts)
- These organisms are often absent from the consumed product (mostly due to storage and packaging)
- Few controlled trials of Fermented foods have been performed to document their Traditional Benefits

SOME COMMON FERMENTED FOODS AND THEIR ORGANISMS

Foods/Beverages	Organisms
TRADITIONAL	
Kimchi *(cabbage)*	*Leuconostoc mesenteroides* and other LAB
Cortido *(cabbage, onions, carrots)*	Not specified
Sourdough	*L. reuteri, S. cerevisiae*
Kvass *(beverage from black or rye bread)*	*Lactobacillus spp.*
Kombucha tea *(black, green, white, pekoe, oolong or Darjeeling)*	Gluconacetobacter and Zygosaccharomyces
Pulque *(beverage from agave plant)*	*Zymomonas mobilis*
Kaffir beer *(beverage from Kaffir maize)*	*Lactobacillus spp.*
Ogi *(cereal)*	*Lactobacillus spp., Saccharomyces spp., Candida spp.*
Igunaq *(fermented walrus)*	Not specified
Miso *(soybeans)*	*Aspergillus oryzae, Zygosaccharomyces, Pediococcus spp.*
Tepa *(Stinkhead fermented fish)*	Not specified
Dosa *(fermented rice batter and lentils)*	*L. plantarum*
Surströmming *(fermented herring, brine)*	*Haloanaerobium praevalens, Haloanaerobium alcaliphilum*
Crème fraîche *(soured dessert cream)*	*L. cremoris, L. lactis*
Fermented sausage	Lactobacillus, Pediococcus, or Micrococcus
Traditional preparation of cod liver oil	Not specified
Hákarl *(fermented shark meat, dried)*	Not specified
Kefir	Kefir grains (combination of LAB and yeasts)
Garum *(fish sauce, ancient Roman condiment)*	Fish intestine microbiota
Natto	*Bacillus subtilis var. natto*
COMMERCIAL/NON-TRADITIONAL	
Yogurt	*L. bulgaricus, S. thermophilus* (starter cultures), adjunct cultures of *Bifidobacterium spp., Lactobacillus spp.*, etc. may be added
Kefir	Commercially produced kefir may use kefir grains at large scale, or pure cultures isolated from kefir grains or commercial cultures to keep flavor consistent; adjunct cultures *Bifidobacteria spp., Lactobacillus spp., Lactococcus spp., Streptococcus spp., Saccharomyces spp.*, etc. may be added
Cheese	LAB starter cultures; adjunct cultures (for flavor/textures) *Lactobacillus spp., Propionibacterium spp.* (eye formation in Swiss cheese), *Penicillium spp.*, etc.
Pickled Vegetables	LAB (*Streptococci spp., Leuconostoc spp., Pediococcus spp., Lactobacillus spp.*)
Sauerkraut *(cabbage)*	LAB (*Lactobacillus spp.*)
Soy sauce	*Aspergillus oryzae* or *Aspergillus sojae* molds, other related microbes *S. cerevisiae, Bacillus spp., Lactobacillus spp.*; can be made by fermentation or by acid hydrolysis
Tempeh	*Rhizopus spp.*
Olives	LAB (*Lactobacillus spp.*)
Beer	*Saccharomyces cerevisiae*
Wine	Various yeast organisms particularly *Saccharomyces cerevisiae*; LAB added in a second step to make red wine

FERMENTED FOODS SHOULD BE ENCOURAGED FOR PATIENTS.
(BUT THEY SHOULD NOT BE USED AS A THERAPEUTIC SUBSTITUTE FOR PROBIOTICS)

EXTREMELY FEW STRAINS OF BACTERIA CAN BE CALLED A "PROBIOTIC"



From: Guilliams TG. Functional Strategies for the Management of Gastrointestinal Disorders (Point Institute, 2016)

COMMON SPECIES OF PROBIOTICS (1) (LACTOBACILLI)

Int J Environ Res Public Health. 2014 May; 11(5): 4745–4767.

Species	Recently published health claims with references (strain specific date is noted where available)
L. rhamnosus	Reduction of viral-associated pulmonary damage (L. rhamnosus CRL1505) [37]; prevention and reduction of severity of atopic dermatitis in children (L. rhamnosus GG) [108]; reduction of risk for developing allergic disease (L. rhamnosus GG) [109], (L. rhamnosus HN001 [110]; anti-diabetic potential (various strains from human infant faecal samples) [111]; prevention of necrotizing enterocolitis in newborns (L. rhamnosus GG) [112]; prevention or treatment of bacterial vaginosis (L. rhamnosus GR-1) [113]; aid in weight loss of obese women (L. rhamnosus CGMCC1.3724) [114]; treatment of acute gastroenteritis in children (L. rhamnosus GG) [115]; reduction of risk for rhinovirus infections in preterm infants (L. rhamnosus GG and L. rhamnosus ATCC 53103) [116]; protection of human colonic muscle from lipopolysaccharide-induced damage (L. rhamnosus GG) [117]
L. acidophilus	Treatment of travellers' diarrhoea [39]; reduction of hospital stay of children with acute diarrhoea [118]; antifungal activity (L. acidophilus ATCC-4495) [119]; prevention or treatment of bacterial vaginosis [113]; treatment of C. difficile-associated diarrhoea [119]; reduction of incidence of febrile urinary tract infections in children [120]; reduction of irritable bowel syndrome symptoms [121].
L. plantarum	Prevention of endotoxin production [35]; antifungal activity (L. plantarum NRRL B-4496) [119] reduction of irritable bowel syndrome symptoms [121].
L. casei	Treatment of functional constipation in adults (L. casei Lcr35 and L. casei Shirota) [43]; treatment of C. difficile-associated diarrhoea [122]; restoration of vaginal flora of patient with bacterial vaginosis (L. casei Lcr35) [123]; reduction of irritable bowel syndrome symptoms [121]; reduction of diarrhoea duration of antibiotic-associated diarrhoea in geriatric patients (L. casei Shirota) [124]; immunomodulatory mechanisms (L. casei Shirota) [125]; improvement of rheumatoid arthritis status (L. casei 01) [126]; protection against Salmonella infection (L. casei CRL-431) [127]; prevention of Salmonella-induced synovitis [128]; treatment of intravaginal staphylococcosis (L. casei IMV B-7280) [129].
L. delbrueckii subsp. bulgaricus	Antibiotic resistance of yogurt starter culture [130]; enhancement of systemic immunity in elderly (L. delbrueckii subsp. bulgaricus 8481) [131]; antibacterial action against E. coli [132]; modulation of brain activity [133].
L. brevis	Protective role in bile salt tolerance (L. brevis KB290) [134]; reduction in plague acidogenicity (L. brevis CD2) [135].
L. johnsonii	Impact on adaptive immunity for protection against respiratory insults [136]; reduction of occurrence of gastritis and risk of H. pylori infection (L. johnsonii MH-68) [137]; inhibition of S. sonnei activity (L. johnsonii F0421) [138]; treatment of perennial allergic rhinitis in children together with levocetirizine (L. johnsonii EM1) [139].
L. fermentum	Prevention or treatment of bacterial vaginosis (L. fermentum RC-14) [113]; blockage of adherence of pathogenic microorganisms on vaginal epithelium [140]; antistaphylococcal action (L. fermentum ATCC 11739) [141]; potential for reduction of insulin resistance and hypercholesterolemia (L. fermentum NCIMB 5221) [142].
L. reuteri	Reduction of low-density lipoprotein cholesterol (L. reuteri NCIMB 30242) [71]; treatment of acute gastroenteritis in children [115]; reduction of diarrhoea duration in children (L. reuteri ATCC 55730) [143]; management of infant colic (L. reuteri ATCC 55730 and L. reuteri DSM 17938) [144]; reduction of onset of gastrointestinal disorders in infants (L. reuteri DSM 17938) [145]; reduction of frequency of proven sepsis, feeding intolerance and duration of hospital stay in preterm infants (L. reuteri DSM 17938) [146].

COMMON SPECIES OF PROBIOTICS (1) (BIFIDOBACTERIUM)

Int J Environ Res Public Health. 2014 May; 11(5): 4745–4767.

B. infantis	Reduction of irritable bowel syndrome symptoms [122]; reduction of necrotizing enterocolitis in preterm infants [147,148,149].
B. animalis subsp. lactis	Treatment of functional constipation in adults (B. animalis subsp. lactis DN-173 010) [43]; reduction of incidence of febrile urinary tract infections in children [121]; modulation of brain activity [133]; reduction of necrotizing enterocolitis in preterm infants [147]; reduction of total microbial counts in dental plaque (B. animalis subsp. lactis DN-173 010) [150]; reduction of total cholesterol (B. animalis subsp. lactis MB 202/DSMZ 23733) [151]; reduction of risk of upper respiratory illness (B. animalis subsp. lactis BI-04) [152].
B. bifidum	Reduction of hospital stay of children with acute diarrhoea [118]; reduction of necrotizing enterocolitis in preterm infants [148,149]; reduction of total cholesterol (B. bifidum MB 109/DSMZ 23731) [151].
B. longum	Prevention and treatment of necrotizing enterocolitis in newborns [51]; reduction of radiation induced diarrhoea [52]; reduction of necrotizing enterocolitis with Bifidobacteria cocktail (B. breve, B. infantis, B. bifidum, B. longum) [149]; reduction of irritable bowel syndrome symptoms [122]; treatment of gastrointestinal diseases (B. longum CMCC P0001) [153]; perinatal intervention against onset of allergic sensitization (B. longum CCM 7952) [154].
B. breve	Prevention and treatment of necrotizing enterocolitis in newborns [51]; reduction of necrotizing enterocolitis with Bifidobacteria cocktail (B. breve, B. infantis, B. bifidum, B. longum) [149]; reduction of cholesterol (B. breve MB 113/DSMZ 23732) [151].

COMMON SPECIES OF PROBIOTICS (1) (OTHER)

Int J Environ Res Public Health. 2014 May; 11(5): 4745–4767.

Saccharomyces (Yeast)	S. boulardi	Treatment of travellers' diarrhoea [39]; treatment and reduction of diarrhoea duration regardless of cause [7,33,56,57,58]; treatment of irritable bowel syndrome [59]; treatment of moderate ulcerative colitis [60,61]; treatment and reduction of recurrent pseudomembrane colitis infection caused by C. difficile [62]; treatment of acute gastroenteritis in children [115].
Lactococcus	L. lactis subsp. lactis	Treatment of antibiotic-associated diarrhoea [33]; adhesion to vaginal epithelial cells (L. lactis subsp. lactis KLDS4.0325) [65]; nisin production (L. lactis subsp. lactis CV56) [66]; modulation of brain activity [133]; antimicrobial activity against C. difficile [155]; antimicrobial and probiotic properties (L. lactis subsp. lactis ATCC 11454) [156].
Enterococcus	E. durans	Antibiotic and antioxidant activity (E. durans LAB18s) [70], adherence to colonic tissue and anti-inflammatory activity [157].
	E. faecium	Treatment of antibiotic-associated diarrhoea [34]; efficient animal probiotic [73].
Streptococcus	S. thermophilus	Reduction of irritable bowel syndrome symptoms [122]; antibiotic resistance of yogurt starter culture [130]; reduction of necrotizing enterocolitis in preterm infants [147,148].
Pediococcus	P. acidilactici	Pediocin production with antimicrobial and probiotic properties (P. acidilactici UL5) [156]; bacteriocin production [158]; elimination of H. pylori infections (P. acidilactici BA28) [159].
Leuconostoc	L. mesenteroides	Leucoin production, probiotic profile (survival at low pH, in presence of bile salts, in presence of pepsin) (L. mesenteroides B7) [160].
Bacillus	B. coagulans	Treatment of antibiotic-associated diarrhoea [34,42], treatment of bacterial vaginosis (B. coagulans ATCC PTA-11748) [161]; immunological support (B. coagulans GandenBC30) [162]; prevention of caries in children [163].
	B. subtilis	Efficient animal probiotic [74,75]; treatment of diarrhoea and aiding in H. pylori eradication (B. subtilis R0179) [76]; production of nitric oxide [164].
	B. cereus	Efficient animal probiotic (B. cereus NVH75/95) [80].
Escherichia	E. coli Nissle 1917	Treatment of functional constipation in adults [43]; treatment of inflammatory bowel disease [83]; treatment of gastrointestinal disorders [84]; pro-inflammatory potential [165]; prevention of surface ocular diseases [166]; reduction of Salmonella enterica Typhimurium intestinal colonization by iron competition [167].

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Determining the safety of microbial cultures for consumption by humans and animals

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- Has the strain been characterized for the purpose of assigning an unambiguous genus and species name using currently accepted methodology?
- Has the strain genome been sequenced?
- Is the strain genome free of genetic elements encoding virulence factors and/or toxins associated with pathogenicity?
- Is the strain genome free of functional and transferable antibiotic resistance gene DNA?
- Does the strain produce antimicrobial substances?
- Has the strain been genetically modified using rDNA techniques?

For strains to be used in human food:

- Do the expressed product(s) that are encoded by the introduced DNA have a history of safe use in food?
- Was the strain isolated from a food that has a history of safe consumption for which the species, to which the strain belongs, is a substantial and characterizing component (not simply an 'incidental isolate')?
- Has the species, to which the strain belongs, undergone a comprehensive peer-reviewed safety evaluation and been affirmed to be safe for food use by an authoritative group of qualified scientific experts?
- For strains to be used in human food: Do scientific findings published since completion of the comprehensive peer-reviewed safety evaluation cited in question 9a continue to support the conclusion that the species, to which the strain belongs, is safe for use in food?
- Does the strain induce undesirable physiological effects in appropriately designed safety evaluation studies?

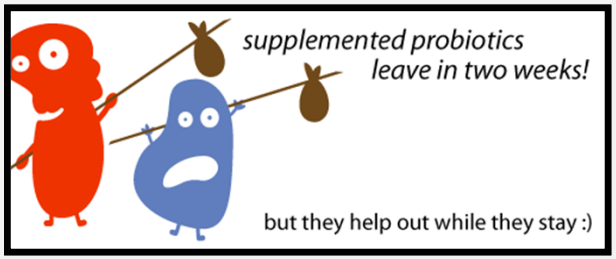
PROBIOTICS ARE NOT COMMENSAL

Photograph by Robert Clark. Wolf and maltese dog provided by Doug Seus's Wasatch Rocky Mountain Wildlife, Utah

- Probiotic strains should be thought of as highly domesticated cousins of a very small fraction of the total gut "Wild-type" microbiota
- They function as part of the temporary or transient microbiota when consumed by humans.

RE-INOCULATE?

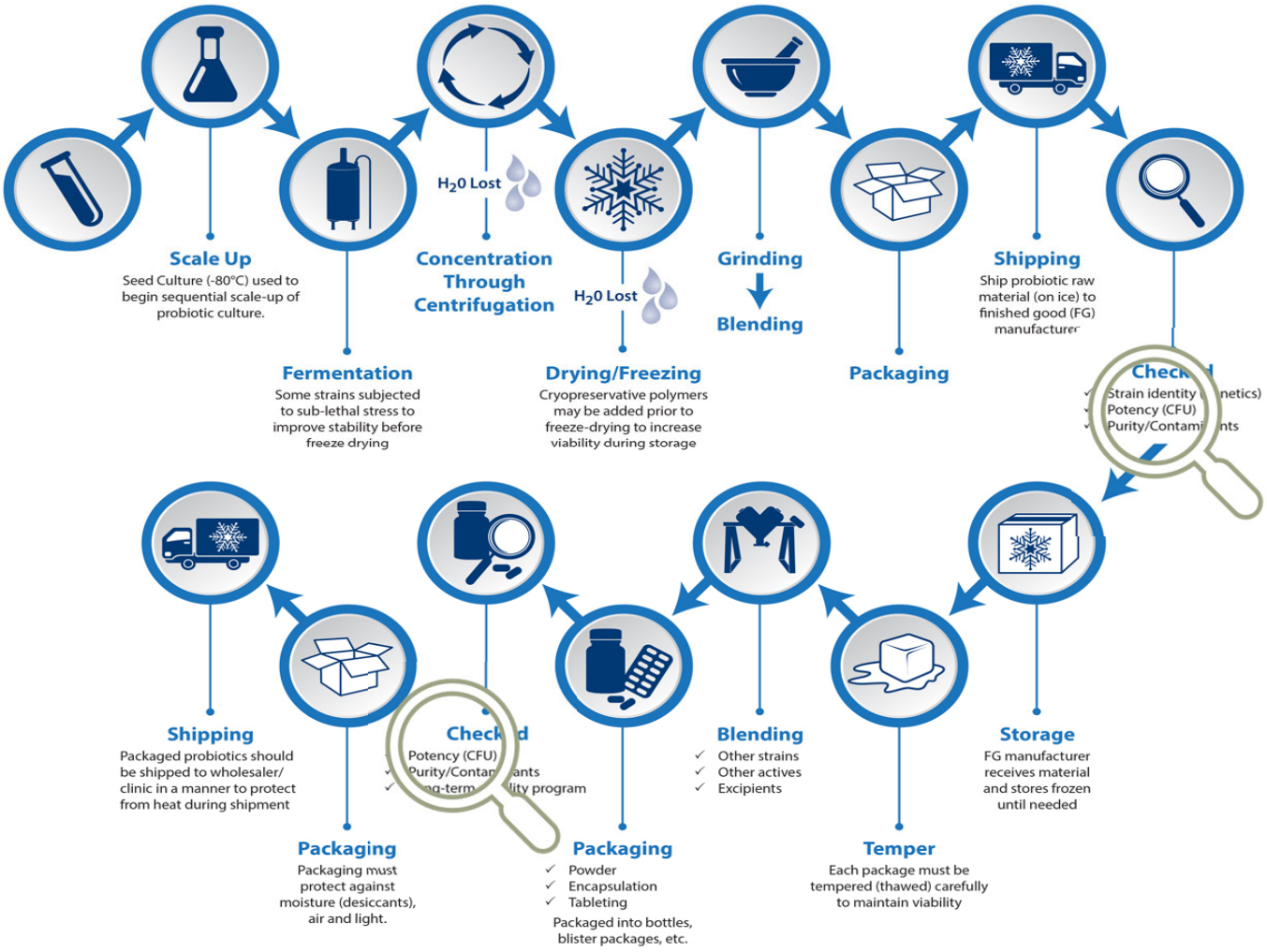
- This "R" of the 4R program is a bit misleading:
  - Commercially prepared probiotics are originally may be derived/isolated from a Human source (though most are not)
  - They are selected for many reasons, one of which is that they can be grown in commercial preparations
  - Commercially prepared probiotics are temporary residents of the gut (Perhaps a month or so) and rarely multiply in the GI
  - They have a transient effect.



DOES THE ORIGINAL SOURCE MATTER?

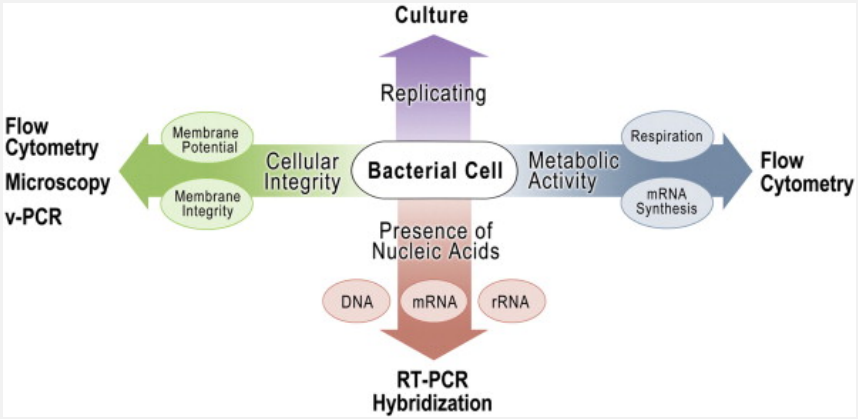
- While many marketers of Probiotics claim that human-derived strains are required or have proven benefits that differ from those derived from animals, soil, dairy etc.- there is no evidence to suggest this is true
- The lifecycle of many organisms would have permitted isolation from several potential sources- the source used is most often the one best characterized in a culture collection- even if isolated from another source later on.
- Commercial Probiotics are highly domesticated versions- regardless of their original source

Commercial Preparation of Probiotics



MEASURING CELL VIABILITY- WHEN ARE CELLS "ALIVE"

- The current International Standard comes from plating bacteria: CFU (Colony forming Unit). This requires that the organism can be plated at various dilutions and individual colonies counted.
- New technologies are challenging the notion that viable cells will always form colonies from a single cell (fluorescent measure of membrane viability)
- VBNC "viable but not culturable"- should we be using a different way to measure viability?



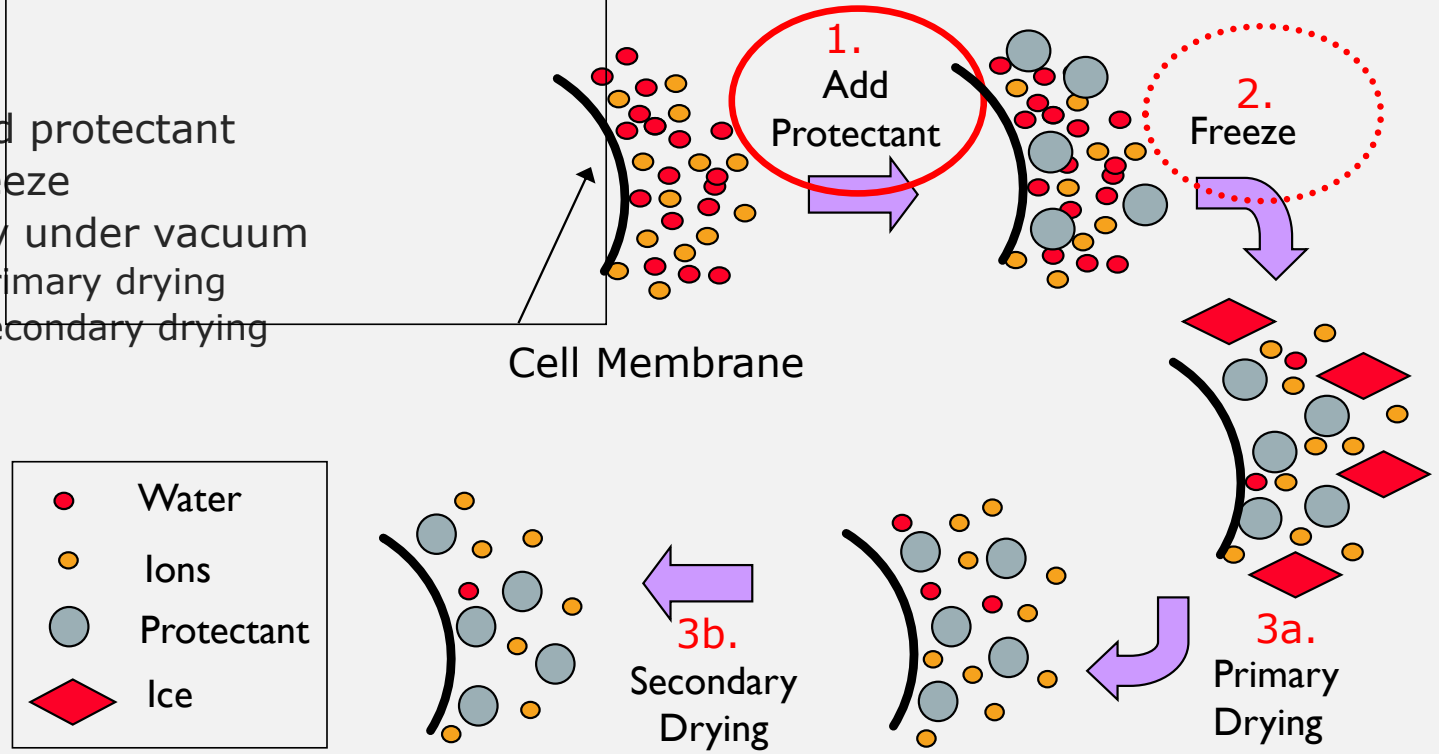
Davis C. Enumeration of probiotic strains: Review of culture-dependent and alternative techniques to quantify viable bacteria. *J Microbiol Methods.* 2014 Aug;103:9-17.

STABILITY- KEY TO DELIVERING LIVE ORGANISMS

- Select appropriate strains (not all "real world" strains work as probiotics)
  - Ability to withstand HCL, Bile, enzymes, etc.
- Growth and Preparation of probiotic to withstand freeze-drying.
- Manufacturing to protect strains from light, heat, moisture.
- Formulated to meet label claim at room temperature (Overage needed at time of manufacturing)
- Refrigeration of unopened product will extend shelf life- but may compromise opened product (moisture)

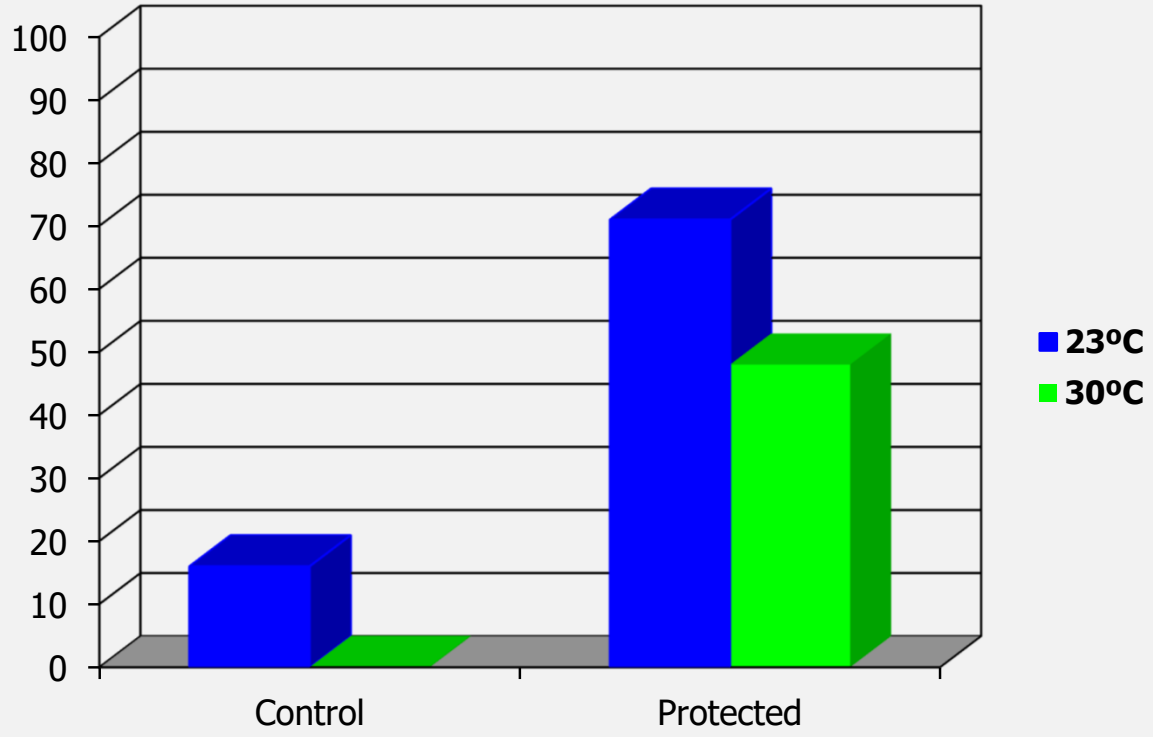
CRYOPROTECTANTS ARE OFTEN ADDED TO PROTECT THE CELL DURING THE FREEZE-DRYING PROCESS

1. Add protectant
2. Freeze
3. Dry under vacuum
   - primary drying
   - secondary drying



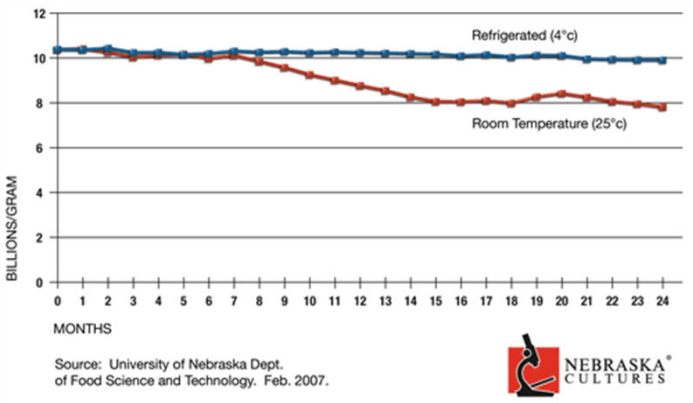
PROTECTANT TECHNOLOGY PROVIDES SUPERIOR PROBIOTIC STABILITY AT RT

18 Month *L. acidophilus* Viability in a low Aw carrier (Aw = 0.05)



REFRIGERATION AND PROBIOTICS

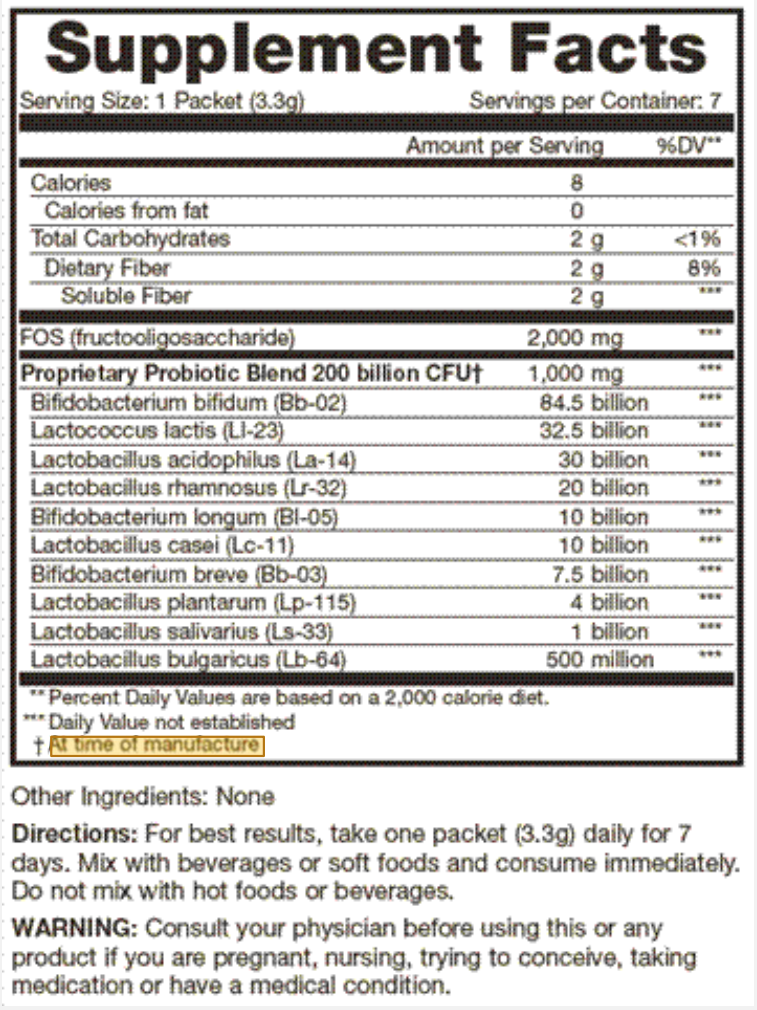
- all probiotics will have a longer shelf-life (when measured by CFU) if they are refrigerated during Pre-use storage
- Products designed for non-refrigeration will have an overage built-in (should be based on historical data)
- Some products will claim enumeration at time of manufacturing only- these should only be used if refrigerated through entire storage
- Refrigerating opened bottles may result in condensation and should not be considered safe from degradation.



Solution:
Manufacturer: Make small batches more frequently
Provider/Supplier: Keep inventory fresh
Consumer: Buy one or two months supply and use immediately

LABELING INFO CAN DIFFER

- Packet product containing 200 billion CFU
- At the time of manufacturing (no overage for shelf life)
- Each strain and amount is listed



Supplement Facts

Serving Size: 1 Packet (3.3g) — Servings per Container: 7

	Amount per Serving	%DV**
Calories	8	
Calories from fat	0	
Total Carbohydrates	2 g	<1%
Dietary Fiber	2 g	8%
Soluble Fiber	2 g	***
FOS (fructooligosaccharide)	2,000 mg	***
Proprietary Probiotic Blend 200 billion CFU†	1,000 mg	***
Bifidobacterium bifidum (Bb-02)	84.5 billion	***
Lactococcus lactis (Ll-23)	32.5 billion	***
Lactobacillus acidophilus (La-14)	30 billion	***
Lactobacillus rhamnosus (Lr-32)	20 billion	***
Bifidobacterium longum (Bl-05)	10 billion	***
Lactobacillus casei (Lc-11)	10 billion	***
Bifidobacterium breve (Bb-03)	7.5 billion	***
Lactobacillus plantarum (Lp-115)	4 billion	***
Lactobacillus salivarius (Ls-33)	1 billion	***
Lactobacillus bulgaricus (Lb-64)	500 million	***

** Percent Daily Values are based on a 2,000 calorie diet.
*** Daily Value not established
† At time of manufacture

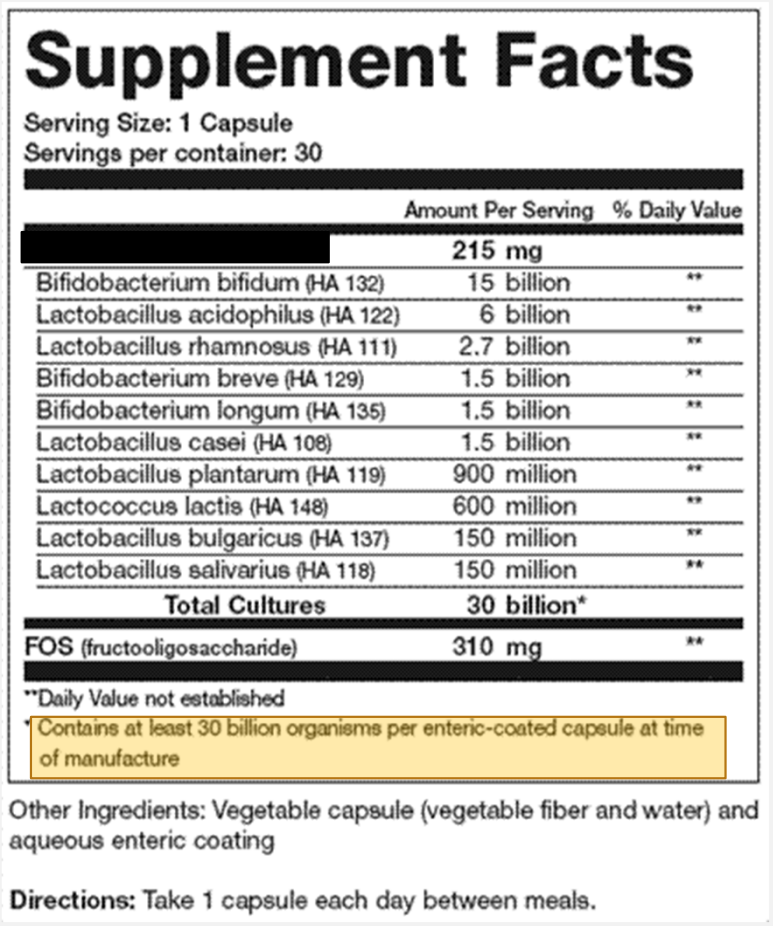
Other Ingredients: None

Directions: For best results, take one packet (3.3g) daily for 7 days. Mix with beverages or soft foods and consume immediately. Do not mix with hot foods or beverages.

WARNING: Consult your physician before using this or any product if you are pregnant, nursing, trying to conceive, taking medication or have a medical condition.

LABELING INFO CAN DIFFER

- Synbiotic- Includes both Probiotics and Prebiotic (FOS)
- Here the term "CFU" is not used- though footnote says "Organisms"
- Amount determined at time of manufacturing- (No overage to reach expiration).



Supplement Facts

Serving Size: 1 Capsule
Servings per container: 30

	Amount Per Serving	% Daily Value
[redacted]	215 mg	
Bifidobacterium bifidum (HA 132)	15 billion	**
Lactobacillus acidophilus (HA 122)	6 billion	**
Lactobacillus rhamnosus (HA 111)	2.7 billion	**
Bifidobacterium breve (HA 129)	1.5 billion	**
Bifidobacterium longum (HA 135)	1.5 billion	**
Lactobacillus casei (HA 108)	1.5 billion	**
Lactobacillus plantarum (HA 119)	900 million	**
Lactococcus lactis (HA 148)	600 million	**
Lactobacillus bulgaricus (HA 137)	150 million	**
Lactobacillus salivarius (HA 118)	150 million	**
Total Cultures	**30 billion***	
FOS (fructooligosaccharide)	310 mg	**

**Daily Value not established

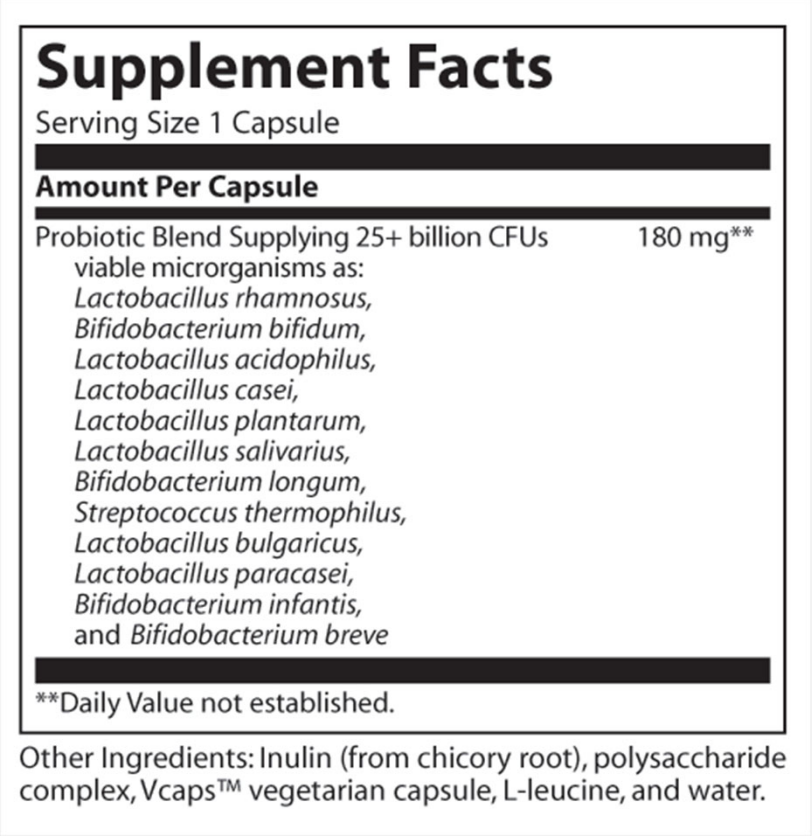
*Contains at least 30 billion organisms per enteric-coated capsule at time of manufacture

Other Ingredients: Vegetable capsule (vegetable fiber and water) and aqueous enteric coating

Directions: Take 1 capsule each day between meals.

LABELING INFO CAN DIFFER

- Synbiotic- Includes both Probiotics and Prebiotic (inulin)
- Probiotic (Proprietary) Blend (should be listed In order of amount)
- No strain numbers



Supplement Facts

Serving Size 1 Capsule

Amount Per Capsule

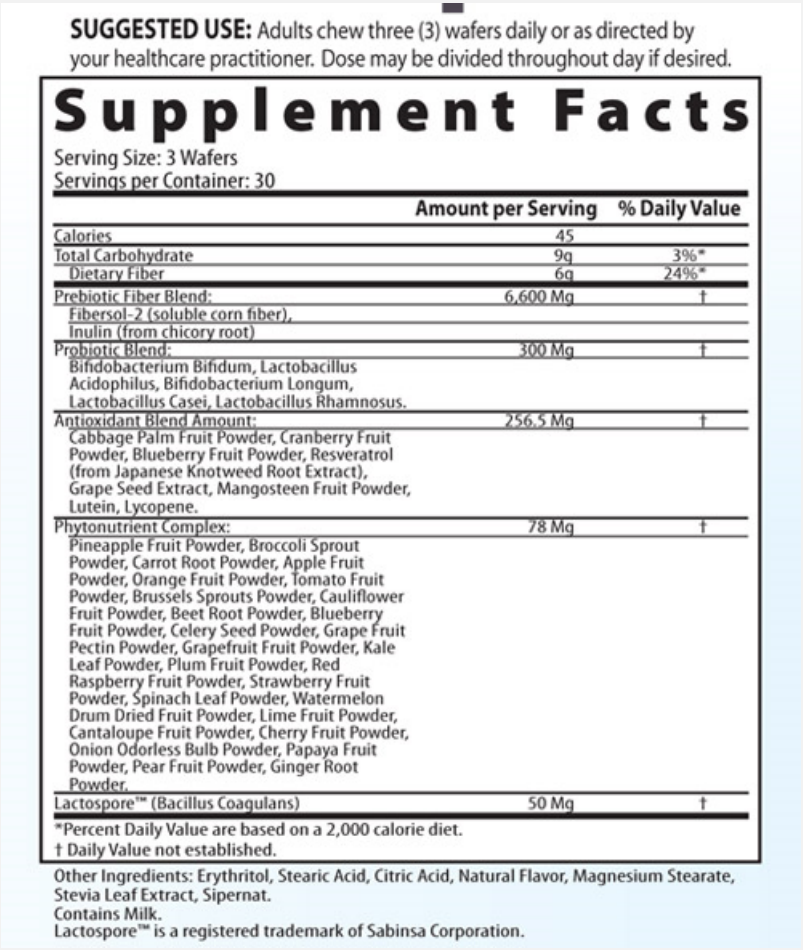
Probiotic Blend Supplying 25+ billion CFUs 180 mg**
viable microrganisms as:
Lactobacillus rhamnosus,
Bifidobacterium bifidum,
Lactobacillus acidophilus,
Lactobacillus casei,
Lactobacillus plantarum,
Lactobacillus salivarius,
Bifidobacterium longum,
Streptococcus thermophilus,
Lactobacillus bulgaricus,
Lactobacillus paracasei,
Bifidobacterium infantis,
and *Bifidobacterium breve*

**Daily Value not established.

Other Ingredients: Inulin (from chicory root), polysaccharide complex, Vcaps™ vegetarian capsule, L-leucine, and water.

LABELING INFO CAN DIFFER

- Synbiotic- Includes both Probiotics and Prebiotic
- Probiotic Blend- in Mg, not CFU
- Note that spore "Probiotic" is not listed with other probiotics
- No strain numbers



SUGGESTED USE: Adults chew three (3) wafers daily or as directed by your healthcare practitioner. Dose may be divided throughout day if desired.

Supplement Facts

Serving Size: 3 Wafers
Servings per Container: 30

	Amount per Serving	% Daily Value
Calories	45	
Total Carbohydrate	9g	3%*
Dietary Fiber	6g	24%*
Prebiotic Fiber Blend:	6,600 Mg	†
Fibersol-2 (soluble corn fiber), Inulin (from chicory root)		
Probiotic Blend:	300 Mg	†
Bifidobacterium Bifidum, Lactobacillus Acidophilus, Bifidobacterium Longum, Lactobacillus Casei, Lactobacillus Rhamnosus.		
Antioxidant Blend Amount:	256.5 Mg	†
Cabbage Palm Fruit Powder, Cranberry Fruit Powder, Blueberry Fruit Powder, Resveratrol (from Japanese Knotweed Root Extract), Grape Seed Extract, Mangosteen Fruit Powder, Lutein, Lycopene.		
Phytonutrient Complex:	78 Mg	†
Pineapple Fruit Powder, Broccoli Sprout Powder, Carrot Root Powder, Apple Fruit Powder, Orange Fruit Powder, Tomato Fruit Powder, Brussels Sprouts Powder, Cauliflower Fruit Powder, Beet Root Powder, Blueberry Fruit Powder, Celery Seed Powder, Grape Fruit Pectin Powder, Grapefruit Fruit Powder, Kale Leaf Powder, Plum Fruit Powder, Red Raspberry Fruit Powder, Strawberry Fruit Powder, Spinach Leaf Powder, Watermelon Drum Dried Fruit Powder, Lime Fruit Powder, Cantaloupe Fruit Powder, Cherry Fruit Powder, Onion Odorless Bulb Powder, Papaya Fruit Powder, Pear Fruit Powder, Ginger Root Powder.		
Lactospore™ (Bacillus Coagulans)	50 Mg	†

*Percent Daily Value are based on a 2,000 calorie diet.
† Daily Value not established.

Other Ingredients: Erythritol, Stearic Acid, Citric Acid, Natural Flavor, Magnesium Stearate, Stevia Leaf Extract, Sipernat.
Contains Milk.
Lactospore™ is a registered trademark of Sabinsa Corporation.

"LIVE MICROORGANISMS THAT, WHEN ADMINISTERED IN ADEQUATE AMOUNTS, CONFER A HEALTH BENEFIT ON THE HOST"

The Controversies are Just Beginning

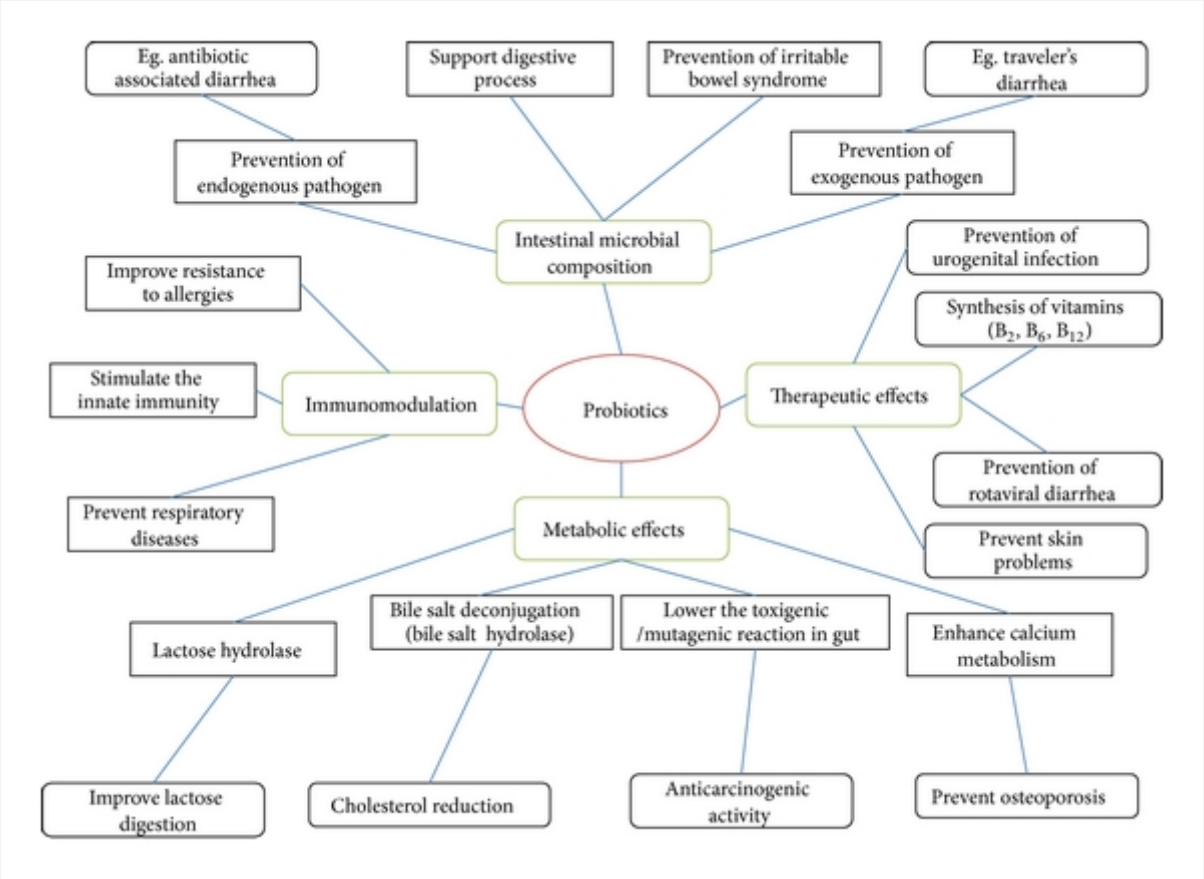
- Ignoring the Debate about the meaning of "Live"…….What does it mean to "confer a health benefit" ? And
- What are "Adequate Amounts" of a probiotic?

PROBIOTICS MUST "CONFER A HEALTH BENEFIT"

- What level of evidence is needed to determine if a particular species or strain of bacteria confers a health benefit?
- What biomarker or outcome should be used?
- Who determines this threshold (FDA, TGA, WHO, EFSA, IPA)?

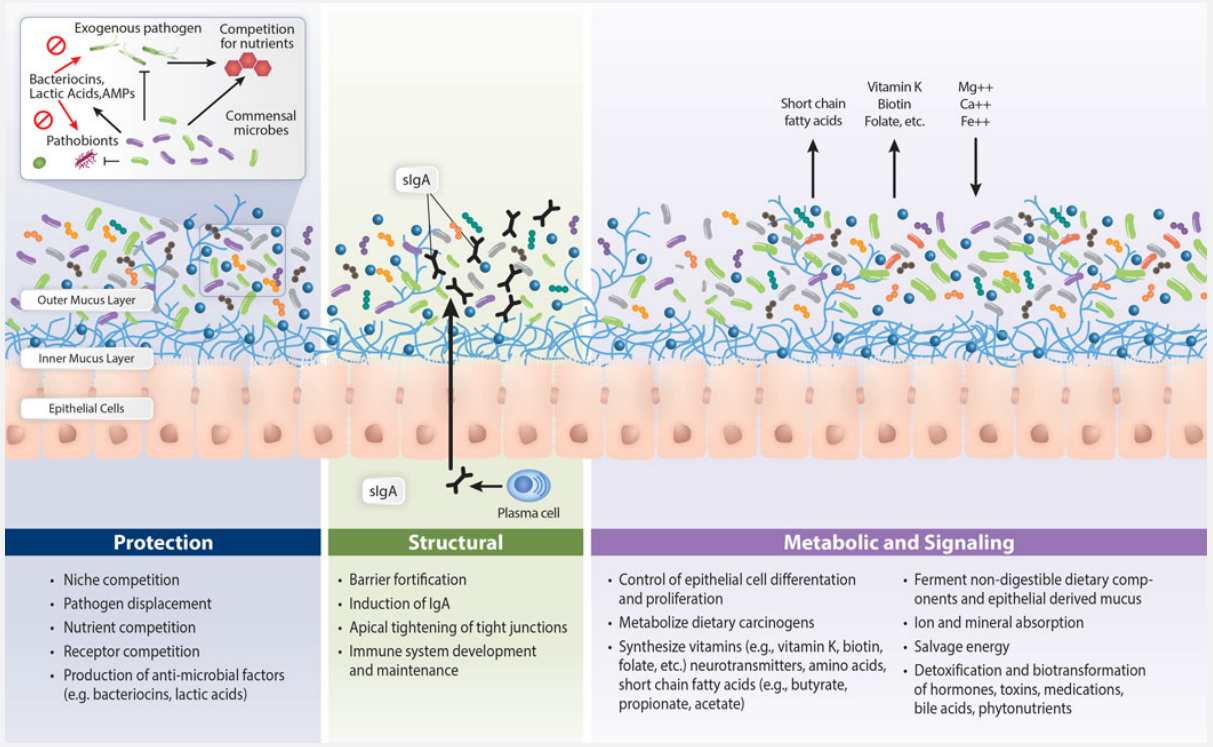
THERAPEUTIC USES OF PROBIOTICS

(ARE THESE ALL EVIDENCE-BASED?)



DO THEY FUNCTION LIKE COMMENSALS?

- Probiotics have profound interaction with the host immune system, allow for alterations in other commensal species, provide metabolic activities and more…



SOME DEAD PROBIOTIC STILL "WORK"

Nutr Res Rev. 2010 Jun;23(1):37-46. doi: 10.1017/S0954422410000090. Epub 2010 Apr 20.

The probiotic paradox: live and dead cells are biological response modifiers.

Adams CA[1].

Int J Mol Sci. 2015 Oct 28;16(10):25881-96. doi: 10.3390/ijms161025881.

Heat Killed Lactobacillus reuteri GMNL-263 Reduces Fibrosis Effects on the Liver and Heart in High Fat Diet-Hamsters via TGF-β Suppression.

Ting WJ[1], Kuo WW[2], Hsieh DJ[3], Yeh YL[4,5], Day CH[6], Chen YH[7], Chen RJ[8], Padma VV[9], Chen YH[10], Huang CY[11,12,13].

Int Immunopharmacol. 2016 Jul;36:39-50. doi: 10.1016/j.intimp.2016.03.033. Epub 2016 Apr 22.

Live and heat-killed probiotic Lactobacillus casei Lbs2 protects from experimental colitis through Toll-like receptor 2-dependent induction of T-regulatory response.

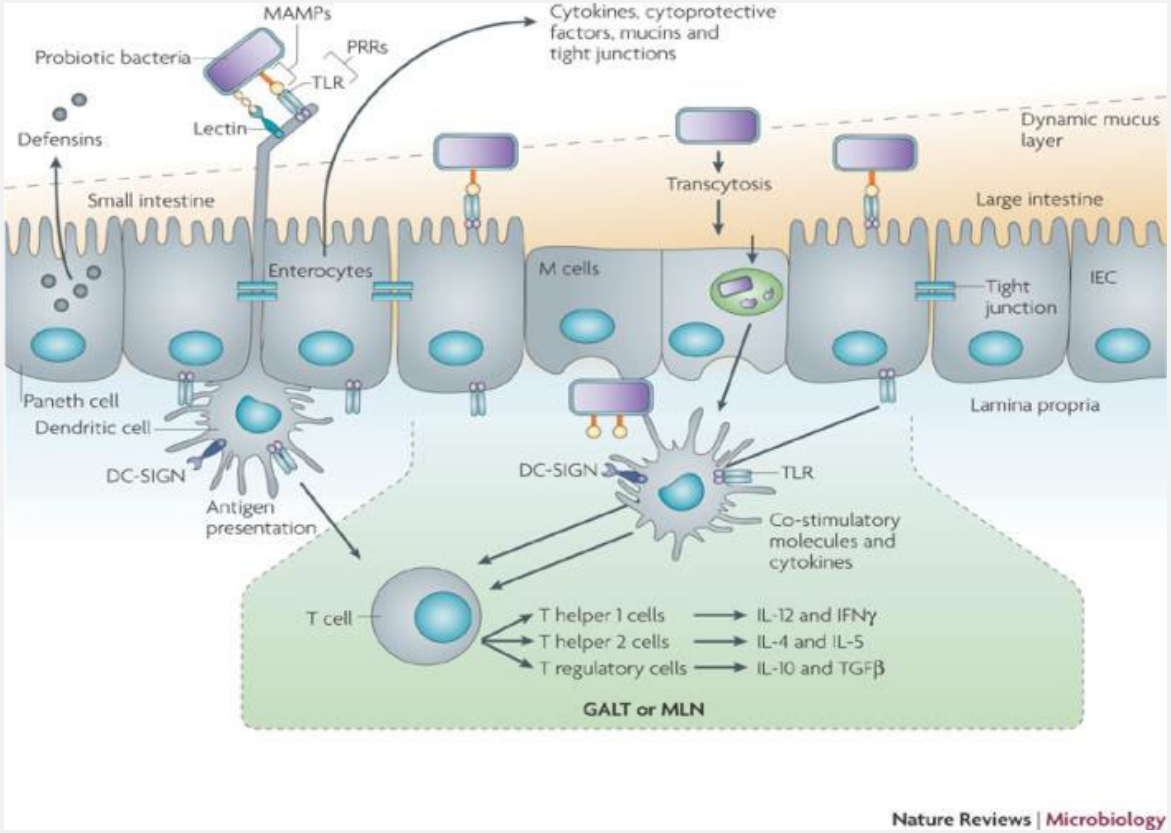
Thakur BK[1], Saha P[1], Banik G[2], Saha DR[1], Grover S[3], Batish VK[3], Das S[4].

Benef Microbes. 2015;6(4):441-9. doi: 10.3920/BM2014.0108. Epub 2015 Feb 12.

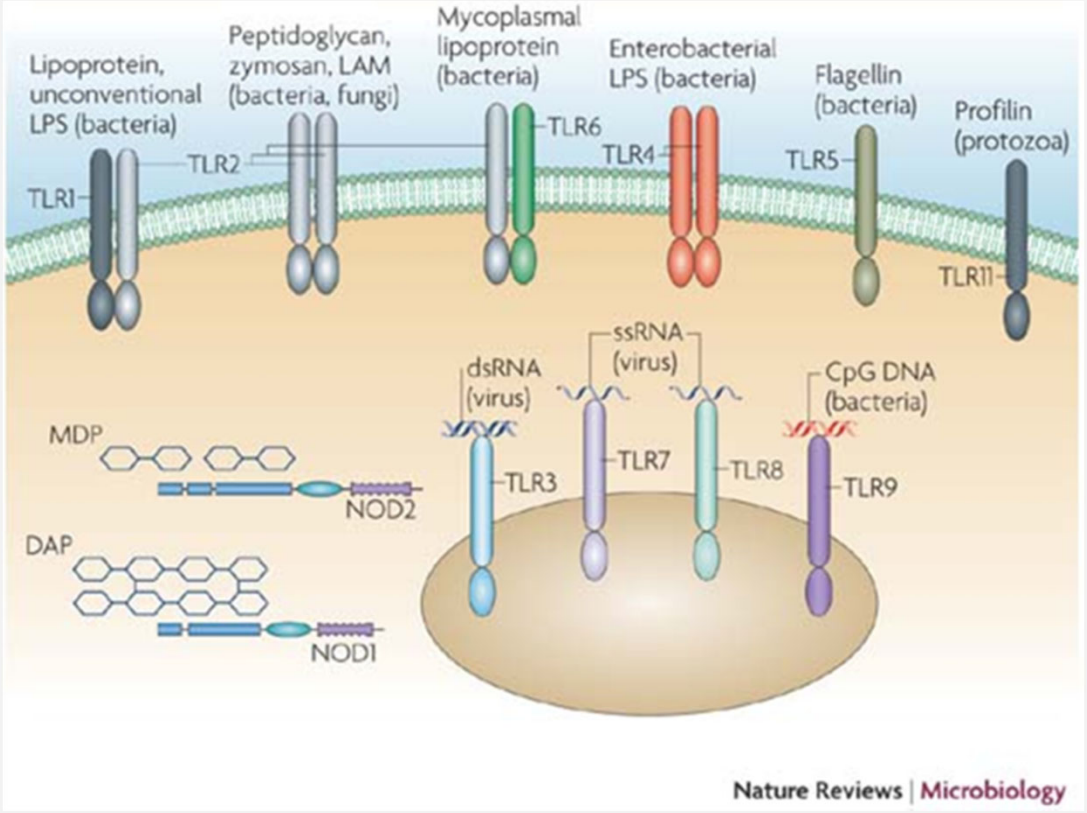
Heat-killed Lactobacillus gasseri can enhance immunity in the elderly in a double-blind, placebo-controlled clinical study.

Miyazawa K[1], Kawase M, Kubota A, Yoda K, Harata G, Hosoda M, He F.

PROBIOTICS AND DENDRITIC CELL SIGNALING

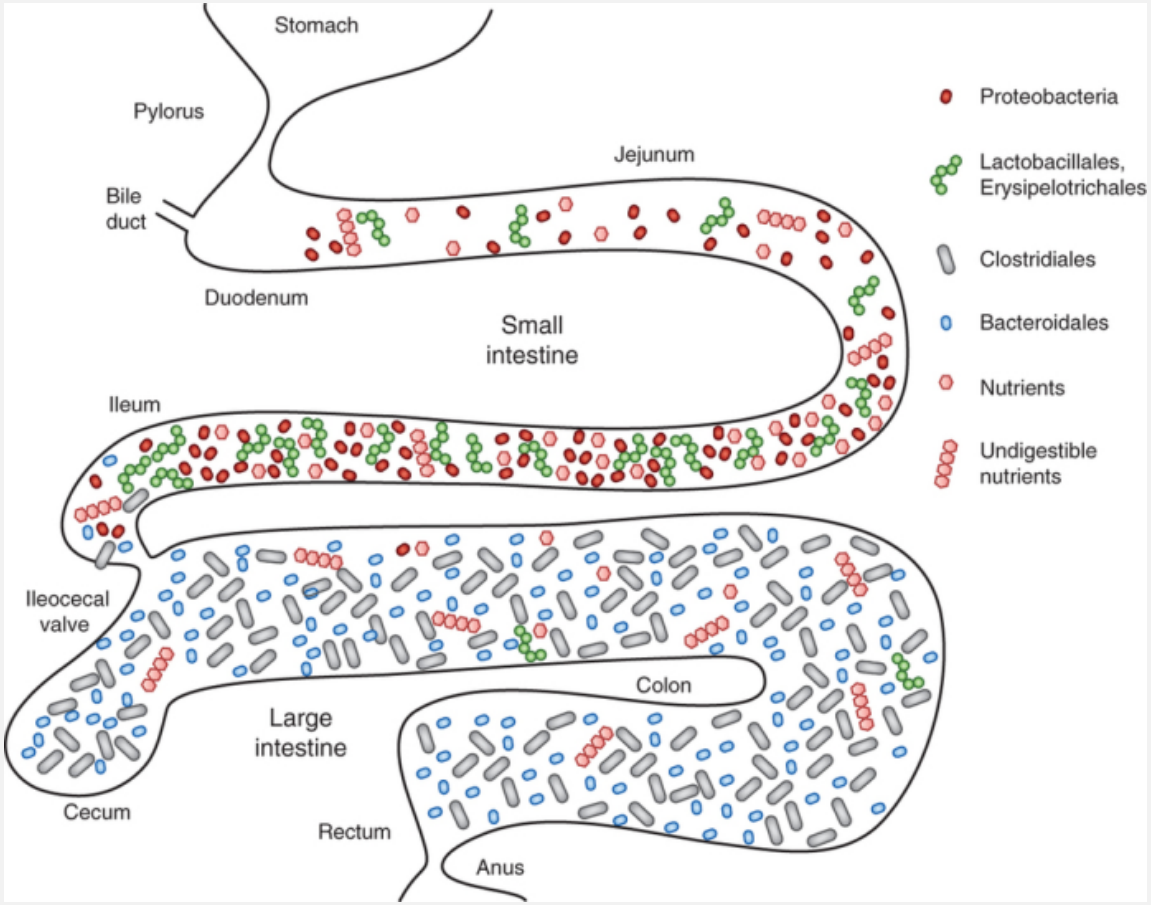


PATTERN RECOGNITION RECEPTORS

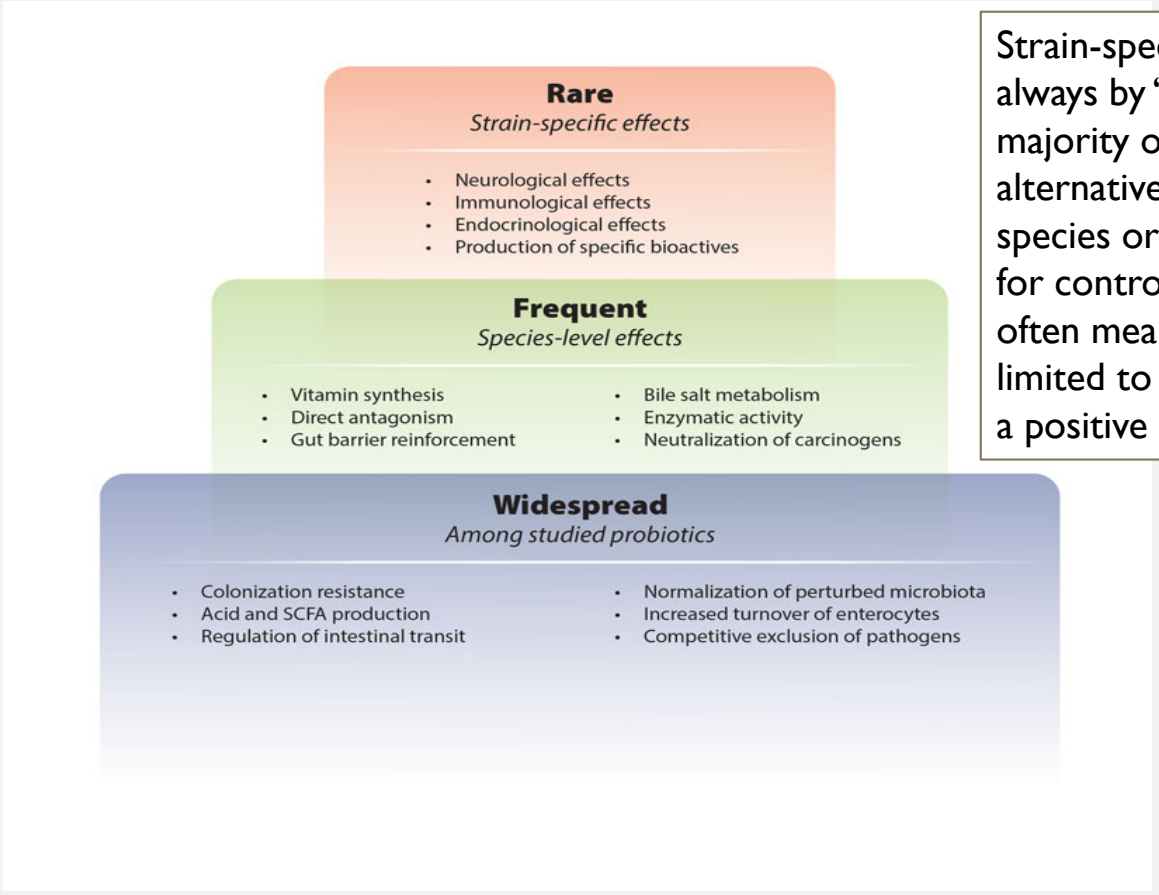


Nucleotide-binding/Oligomerization Domain (NOD)
Toll-like Receptors (TLR)

SELECTING THE "RIGHT" STRAIN



LEVELS OF BENEFIT: STRAIN SPECIFICITY



Strain-specific data are almost always by "default"- as majority of studies do not use alternative strains from same species or even other species for controls. Strain-specific often means our data is limited to this strain and it had a positive effect.

COMPREHENSIVE STRAIN APPROACH

16S rDNA sequence relationship of common probiotic strains

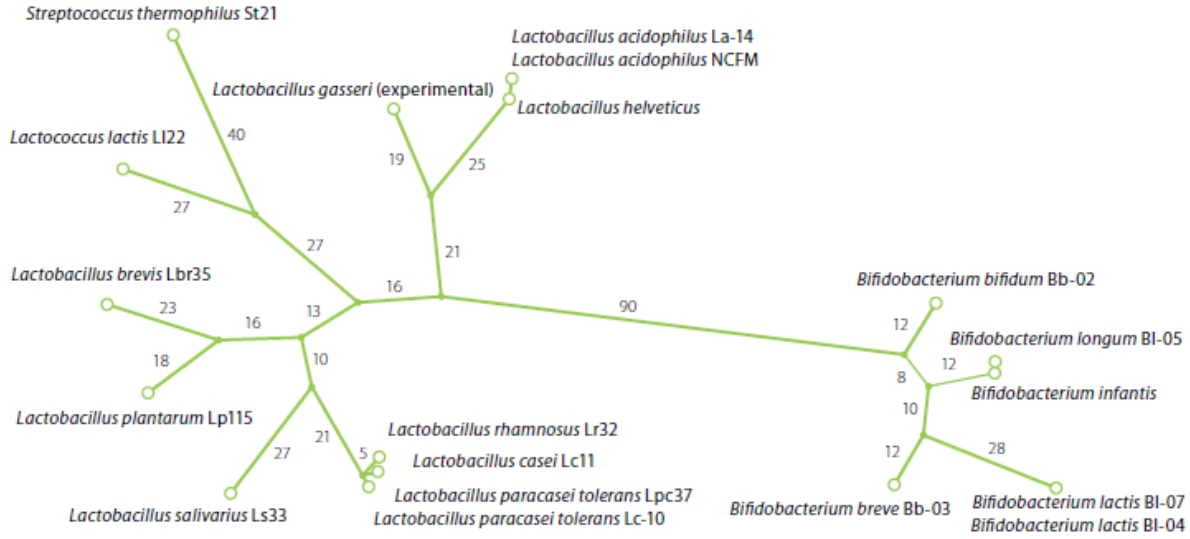


Figure 15 : Phylogenetic relationship between common probiotic strains based on unrooted maximum parsimony clustering of partial 16S rRNA gene sequences (Courtesy of Danisco).

WHAT IS THE RIGHT DOSE?

that takes advantage of the genetic diversity of the available commercial strains will have the best overall benefit in the widest number of patients. Obviously, where evidence is strong for a particular strain or strain combination in subjects similar to the patient seeking therapy, the use of these specific products may be warranted.

Formulary Suggestion

- Modest-Dose Multi-Strain Probiotic-Capsule formula and/or powder [15-40 billion CFU]
- *Saccharomyces boulardii*- only capsule: 5 billion CFU/capsule
- High-Dose Multi-Strain Probiotic- sachet or powder [>100 billion CFU]
- Children's Multi-Strain Probiotic- powder or chewable wafer [1-5 billion CFU/dose]

Therapeutic Rotation of Probiotic Strains

their ability to become "alive" again, this is the best method we have of enumerating the probiotic potential of a product.

Available products range from as little as one billion CFU/dose to as high as 450 billion CFU/dose. For the purpose of non-disease-specific balancing of the GI microflora, daily doses of 15 – 40 billion CFU in adults and one to five billion in children are usually sufficient. Much higher doses (some well over one trillion CFU/day) have been reported to benefit patients with specific clinical conditions (like IBD), but are rarely needed to help most patients without specific dysbiotic abnormalities.[2]

Shelf-life Issues- Do Probiotics Need to be Refrigerated?

Probiotic organisms in foods or supplements are prone to easy degradation and must be manufactured with great care. Responsible companies understand how the manufacturing process (from the time the frozen raw material reaches their facility to the selection of the appropriate shipping method) affects the eventual shelf life and efficacy of their product. Procedures must be put in place to limit exposure to heat, oxygen and moisture

Vaccine 32 (2014) 458–463

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journal homepage: www.elsevier.com/locate/vaccine

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

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Lactobacillus paracasei
Bifidobacterium lactis

ABSTRACT

Probiotics are known to reduce antibiotic associated diarrhea (AAD) and Clostridium difficile associated diarrhea (CDAD) risk in a strain-specific manner. The aim of this study was to determine the dose-response effect of a four strain probiotic combination (HOWARU® Restore) on the incidence of AAD and CDAD and severity of gastrointestinal symptoms in adult in-patients requiring antibiotic therapy. Patients ($n = 503$) were randomized among three study groups: HOWARU® Restore probiotic 1.70×10^{10} CFU (high-dose, $n = 168$), HOWARU® Restore probiotic 4.17×10^{9} CFU (low-dose, $n = 168$), or placebo ($n = 167$). Subjects were stratified by gender, age, and duration of antibiotic treatment. Study products were administered daily up to 7 days after the final antibiotic dose. The primary endpoint of the study was the incidence of AAD. Secondary endpoints included incidence of CDAD, diarrhea duration, stools per day, bloody stools, fever, abdominal cramping, and bloating. A significant dose-response effect on AAD was observed with incidences of 12.5, 19.6, and 24.6% with high-dose, low-dose, and placebo, respectively ($p = 0.02$). CDAD was the same in both probiotic groups (1.8%) but different from the placebo group (4.8%; $p = 0.04$). Incidences of fever, abdominal pain, and bloating were lower with increasing probiotic dose. The number of daily liquid stools and average duration of diarrhea decreased with higher probiotic dosage. The tested four strain probiotic combination appears to lower the risk of AAD, CDAD, and gastrointestinal symptoms in a dose-dependent manner in adult in-patients.

1. Introduction

Antibiotics have provided great medical benefits and have enabled the control of numerous infectious diseases. However, the use of antibiotics may be accompanied by gastrointestinal disturbances; most notably antibiotic-associated diarrhoea (AAD) [1]. The incidence of AAD ranges between 5 and 39% and varies according to individual susceptibility, the patient environment, and the class of antibiotics administered [2,3].

While AAD can be caused by multiple pathogens and has multifactorial etiology, between 10 and 25% of all episodes of AAD, and virtually all those seen in antibiotic-induced pseudo-membranous colitis, are caused by *C. difficile* [4,5]. Incidence rates and proportion of severe cases of *C. difficile*-associated diarrhea (CDAD), and associated mortality, are on the rise [5–7].

Specific probiotic strains have been shown to have various beneficial health effects [8–11]. Recent meta-analyses concluded that probiotics produced relative risk reductions of 44–57% for AAD and 41–71% for CDAD [3,10,12]. Specific probiotics, therefore, seem to be promising as an adjunct to antibiotics to reduce the risk of AAD. Although several studies of specific probiotics against AAD exist, there is currently only one dose-response study comparing 5 and 10×10^{10} CFU; that is limited to elderly [13]. The primary objective of the present study was therefore to investigate the effect of a specific combination of probiotic strains, which has earlier been shown to stabilize the intestinal microbiota [14], on the incidence of AAD at two different doses (4.7×10^{9} and 1.70×10^{10} CFU) in a hospital setting in adults aged 30–70. The secondary objectives were to investigate their influence on the severity and duration of AAD and CDAD.

2. Methods

All research procedures were in strict accordance with a pre-defined protocol and were registered at clinicaltrials.gov

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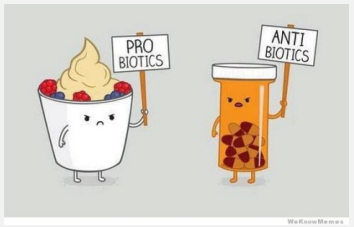
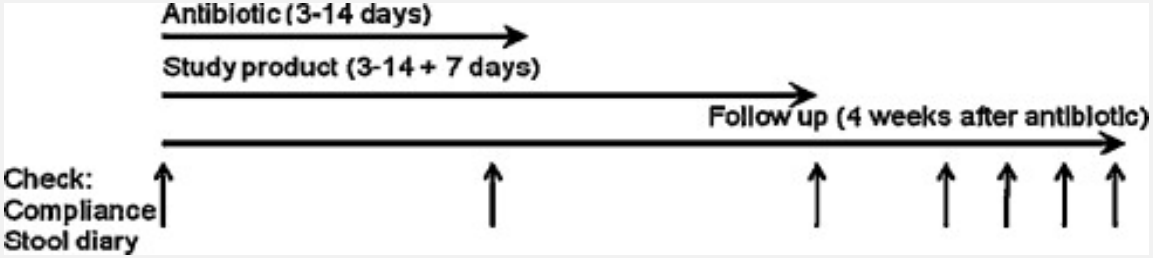
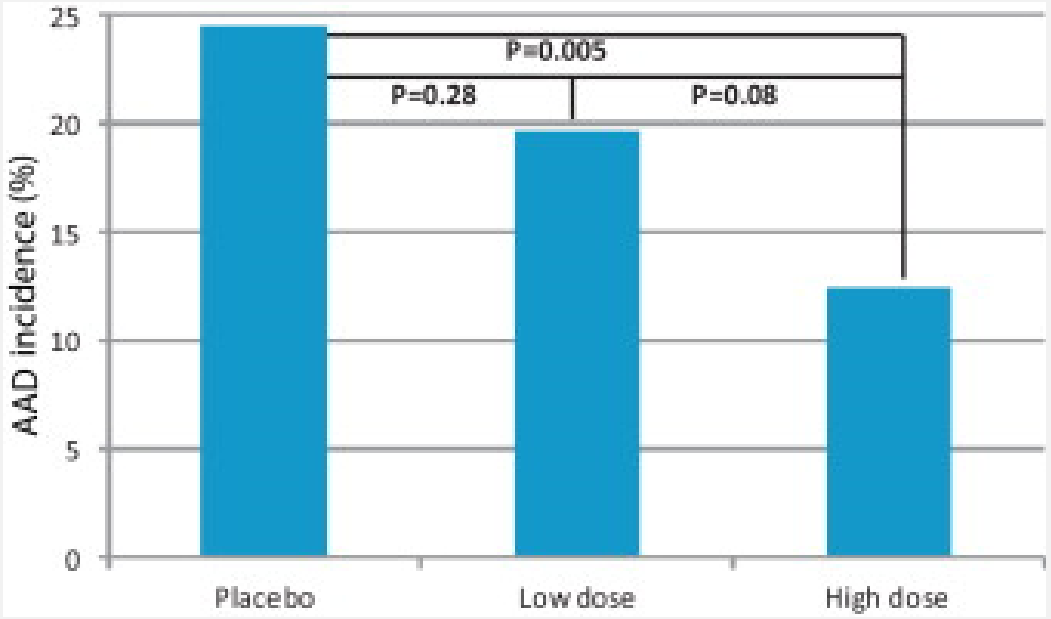
0264-410X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2013.11.053

- Consist of equal amounts of *Lactobacillus acidophilus*, *Lactobacillus paracasei* *Bifidobacterium lactis* (2 strains)
- Low dose: 4.2 Billion
- “High dose”: 17 Billion
- Taken 2 hr after breakfast/antibiotic




Antibiotic (3-14 days)
Study product (3-14 + 7 days)
Follow up (4 weeks after antibiotic)
Check:
Compliance
Stool diary


AAD incidence (%)
25
20
15
10
5
0
P=0.005
P=0.28
P=0.08
Placebo
Low dose
High dose

50 VS 100 BILLION FOR AAD AND C.DIFF

COLON/SMALL BOWEL

Dose–Response Efficacy of a Proprietary Probiotic Formula of *Lactobacillus acidophilus* CL1285 and *Lactobacillus casei* LBC80R for Antibiotic-Associated Diarrhea and *Clostridium difficile*-Associated Diarrhea Prophylaxis in Adult Patients

Xing Wang Gao, MD[1], Mohamed Mubasher, PhD[2], Chong Yu Fang, MD[1], Cheryl Reifer, PhD[2] and Larry E. Miller, PhD[2]

OBJECTIVES: **Standard therapies for antibiotic-associated diarrhea (AAD) and *Clostridium difficile*-associated diarrhea (CDAD) have limited efficacy. Probiotic prophylaxis is a promising alternative for reduction of AAD and CDAD incidence.**

METHODS: **In this single-center, randomized, double-blind, placebo-controlled dose-ranging study, we randomized 255 adult inpatients to one of three groups: two probiotic capsules per day (Pro-2, *n*=86), one probiotic capsule and one placebo capsule per day (Pro-1, *n*=85), or two placebo capsules per day (*n*=84). Each probiotic capsule contained 50 billion c.f.u. of live organisms (*Lactobacillus acidophilus* CL1285® + *Lactobacillus casei LBC80R*® Bio-K+ CL1285). Probiotic prophylaxis began within 36 h of initial antibiotic administration, continued for 5 days after the last antibiotic dose, and patients were followed for an additional 21 days.**

RESULTS: **Pro-2 (15.5%) had a lower AAD incidence vs. Pro-1 (28.2%). Each probiotic group had a lower AAD incidence vs. placebo (44.1%). In patients who acquired AAD, Pro-2 (2.8 days) and Pro-1 (4.1 days) had shorter symptom duration vs. placebo (6.4 days). Similarly, Pro-2 (1.2%) had a lower CDAD incidence vs. Pro-1 (9.4%). Each treatment group had a lower CDAD incidence vs. placebo (23.8%). Gastrointestinal symptoms were less common in the treatment groups vs. placebo and in Pro-2 vs. Pro-1.**

CONCLUSIONS: **The proprietary probiotic blend used in this study was well tolerated and effective for reducing risk of AAD and, in particular, CDAD in hospitalized patients on antibiotics. A dose-ranging effect was shown with 100 billion c.f.u., yielding superior outcomes and fewer gastrointestinal events compared to 50 billion c.f.u. (ClinicalTrials.gov number NCT00958308).**

Am J Gastroenterol 2010; 105:1636–1641; doi:10.1038/ajg.2010.11; published online 9 February 2010

SIGNIFICANTLY LESS AAD INCIDENCE AND C.DIFF DIARRHEA WITH 100 BILLION CFU

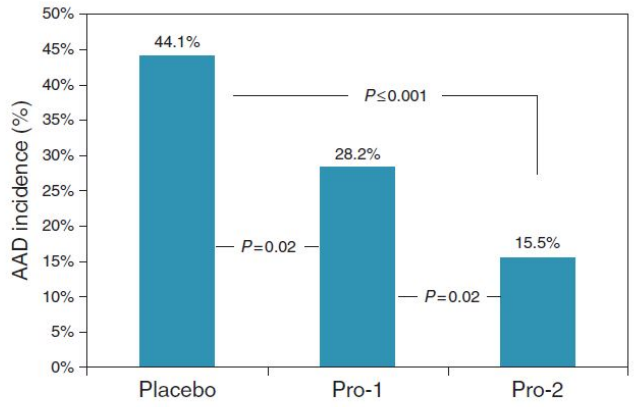


Figure 2. Antibiotic-associated diarrhea incidence by study group. AAD, antibiotic-associated diarrhea; Pro-1, one capsule of probiotics; Pro-2, two capsules of probiotics.

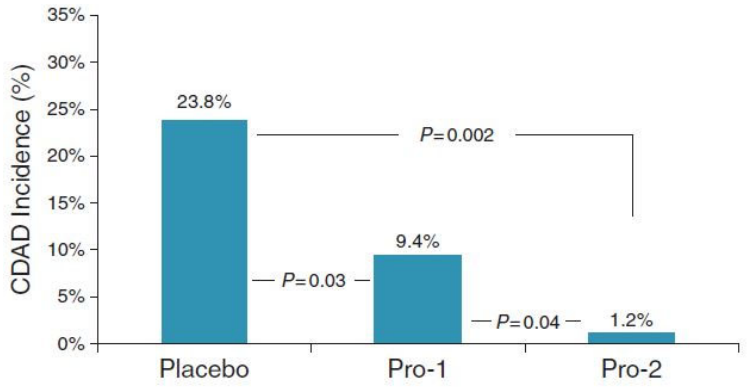


Figure 3. *Clostridium difficile*-associated diarrhea incidence by study group. CDAD, *C. difficile*-associated diarrhea; Pro-1, one capsule of probiotics; Pro-2, two capsules of probiotics.

CAN THE DOSE OF PROBIOTIC CHANGE THE IMMUNOLOGICAL SIGNAL?

OPEN ACCESS Freely available online

PLoS one

Dose-Dependent Immunomodulation of Human Dendritic Cells by the Probiotic *Lactobacillus rhamnosus* Lcr35

Bertrand Evrard[1,2*9], Sophie Coudeyras[3,9], Annie Dosgilbert[1], Nicolas Charbonnel[3], Josette Alamé[2], Arlette Tridon[1,2¶], Christiane Forestier[3¶]

1 CHU Clermont-Ferrand, Laboratoire d'Immunologie, Clermont-Ferrand, France, **2** Clermont Université, Université d'Auvergne, UFR Médecine-Pharmacie, EA4233, Laboratoire d'Immunologie, Clermont-Ferrand, France, **3** Clermont Université, Université d'Auvergne, UFR Pharmacie, Laboratoire de Bactériologie, Clermont-Ferrand, France

Abstract

The response of the immune system to probiotics remains controversial. Some strains modulate the cytokine production of dendritic cells (DCs) *in vitro* and induce a regulatory response, while others induce conversely a pro-inflammatory response. These strain-dependent effects are thought to be linked to specific interactions between bacteria and pattern recognition receptors. We investigated the effects of a well characterized probiotic strain, *Lactobacillus rhamnosus* Lcr35, on human monocyte-derived immature DCs, using a wide range of bacterial concentrations (multiplicity of infection, MOI, from 0.01 to 100). DNA microarray and qRT-PCR analysis showed that the probiotic induced a large-scale change in gene expression (nearly 1,700 modulated genes, with 3-fold changes), but only with high doses (MOI, 100). The upregulated genes were mainly involved in immune response and identified a molecular signature of inflammation according to the model of Torri. Flow cytometry analysis also revealed a dose-dependent maturation of the DC membrane phenotype, until DCs reached a semi-mature state, with an upregulation of the membrane expression of CD86, CD83, HLA-DR and TLR4, associated with a down-regulation of DC-SIGN, MR and CD14. Measurement of the DC-secreted cytokines showed that Lcr35 induced a strong dose-dependent increase of the pro-Th1/Th17 cytokine levels (TNFα, IL-1β, IL-12p70, IL-12p40 and IL-23), but only a low increase in IL-10 concentration. The probiotic *L. rhamnosus* Lcr35 therefore induce a dose-dependent immunomodulation of human DCs leading, at high doses, to the semi-maturation of the cells and to a strong pro-inflammatory effect. These results contribute to a fuller understanding of the mechanism of action of this probiotic, and thus of its potential clinical indications in the treatment of either infectious or IgE-dependent allergic diseases.

Citation: Evrard B, Coudeyras S, Dosgilbert A, Charbonnel N, Alamé J, et al. (2011) Dose-Dependent Immunomodulation of Human Dendritic Cells by the Probiotic *Lactobacillus rhamnosus* Lcr35. PLoS ONE 6(4): e18735. doi:10.1371/journal.pone.0018735

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Competing Interests: The authors have declared that no competing interests exist.

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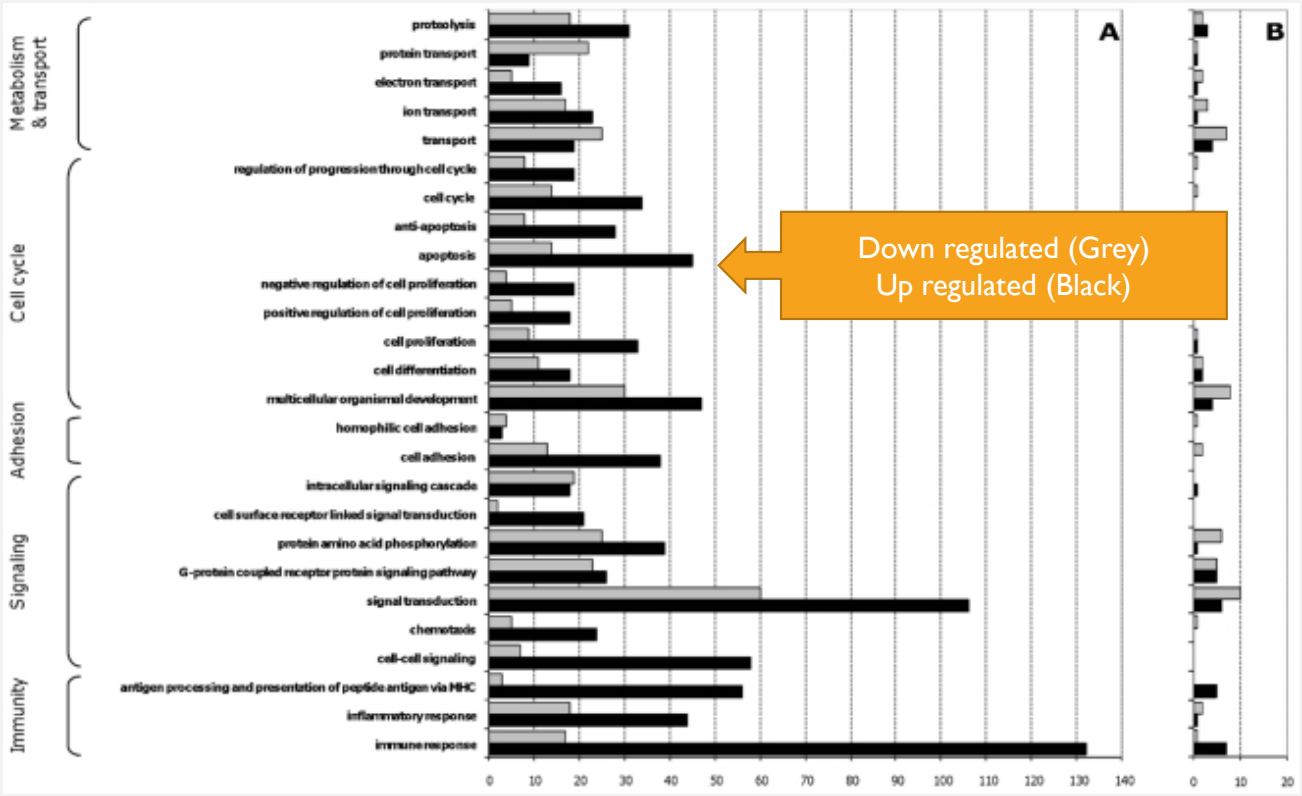
9 These authors contributed equally to this work.

¶ These authors also contributed equally to this work.

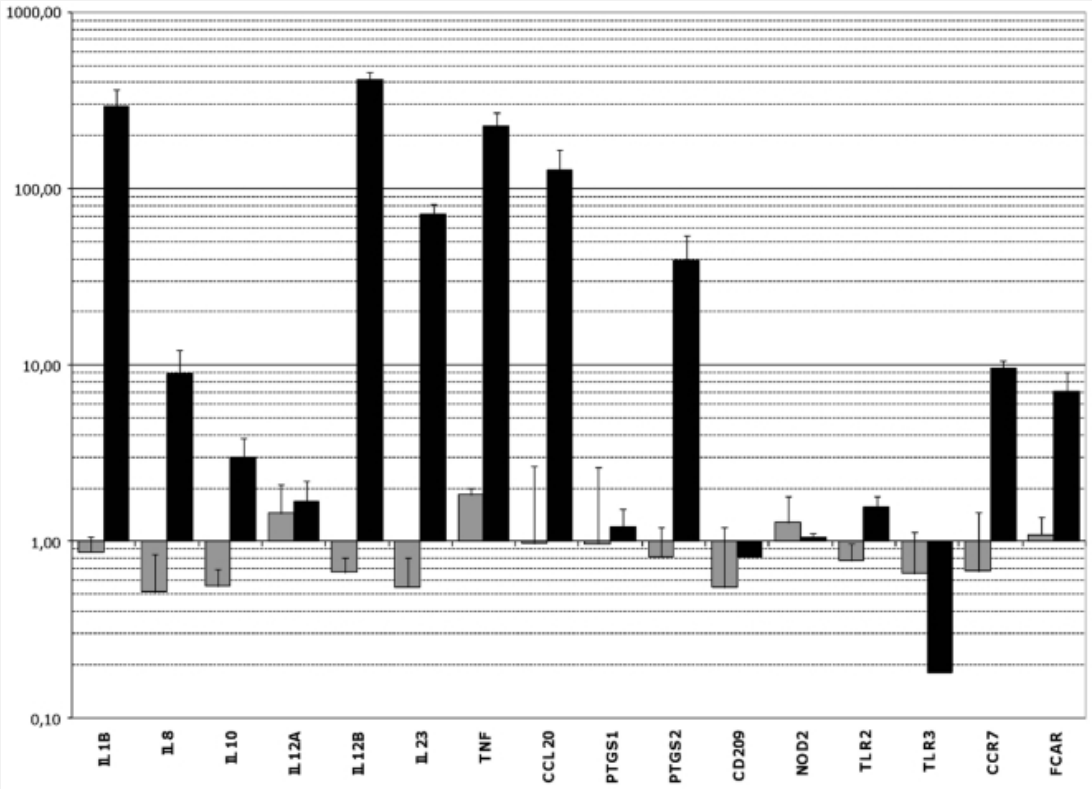
CHANGES IN DENDRITIC CELL GENE EXPRESSION

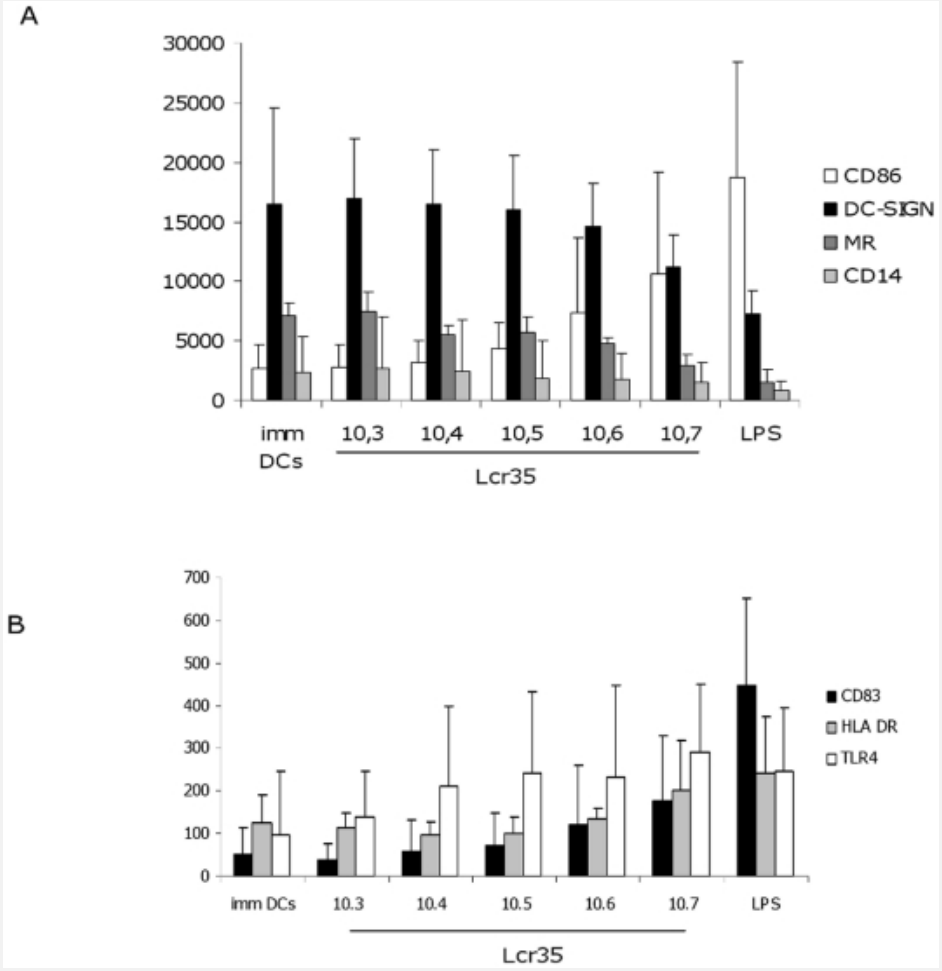
MOI of 10

MOI of 0.01



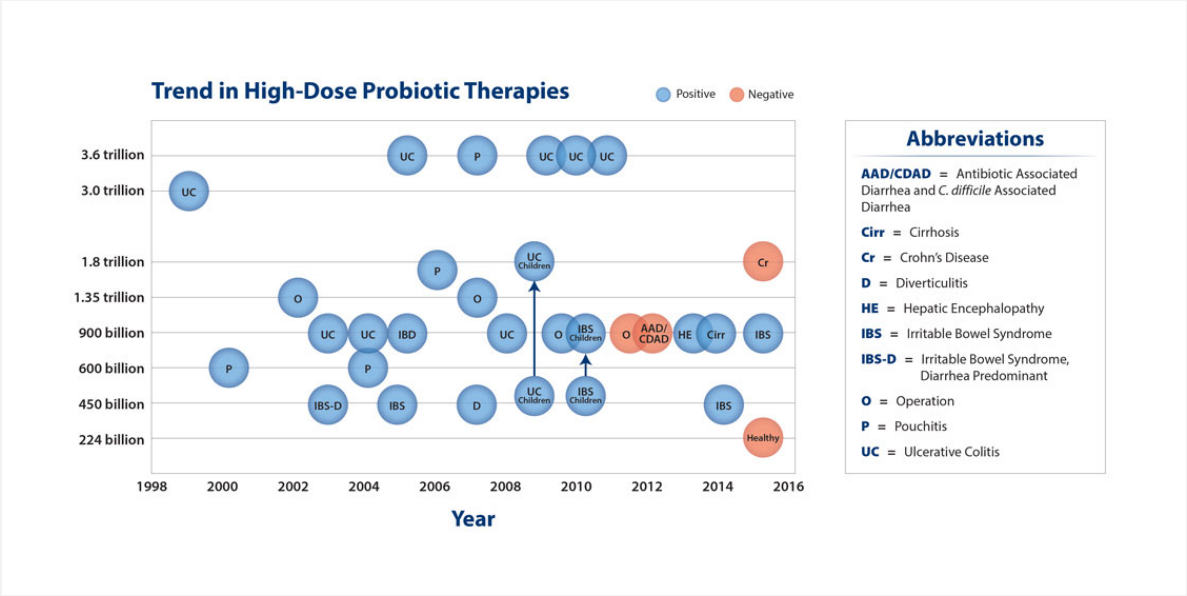
QRT-PCR ANALYSIS OF TARGET GENES EXPRESSION IN DCS AFTER CONTACT WITH THE PROBIOTIC LCR35.





- **Maturation of human mo-DCs after exposure to a range of *L. rhamnosus* Lcr35 concentrations.**
- **A: Markers of high density surface molecules**
- **B: Markers of low density surface molecules**

HIGH DOSE PROBIOTICS- HUMAN STUDIES FOR GI-RELATED OUTCOMES



TAKE WITH FOOD OR WITHOUT?

Beneficial Microbes, December 2011, 2(4): 295-300

The impact of meals on a probiotic during transit through a model of the human upper gastrointestinal tract

T.A. Tompkins[1], I. Mainville[2] and Y. Arcand[2]

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Abstract

Commercial literature on various probiotic products suggests that they can be taken before meals, during meals or after meals or even without meals. This has led to serious confusion for the industry and the consumer. The objective of our study was to examine the impact of the time of administration with respect to mealtime and the impact of the buffering capacity of the food on the survival of probiotic microbes during gastrointestinal transit. We used an *in vitro* Digestive System (IViDiS) model of the upper gastrointestinal tract to examine the survival of a commercial multi-strain probiotic, ProtecFlor®. This product, in a capsule form, contains four different microbes: two lactobacilli (*Lactobacillus helveticus* R0052 and *Lactobacillus rhamnosus* R0011), *Bifidobacterium longum* R0175 and *Saccharomyces cerevisiae boulardii*. Enumeration during and after transit of the stomach and duodenal models showed that survival of all the bacteria in the product was best when given with a meal or 30 minutes before a meal (cooked oatmeal with milk). Probiotics given 30 minutes after the meal did not survive in high numbers. Survival in milk with 1% milk fat and oatmeal-milk gruel were significantly better than apple juice or spring water. *S. boulardii* was not affected by time of meal or the buffering capacity of the meal. The protein content of the meal was probably not as important for the survival of the bacteria as the fat content. We conclude that ideally, non-enteric coated bacterial probiotic products should be taken with or just prior to a meal containing some fats.

Keywords: lactobacilli, *L. helveticus*, *Bifidobacterium*, intestinal model, survival

1. Introduction

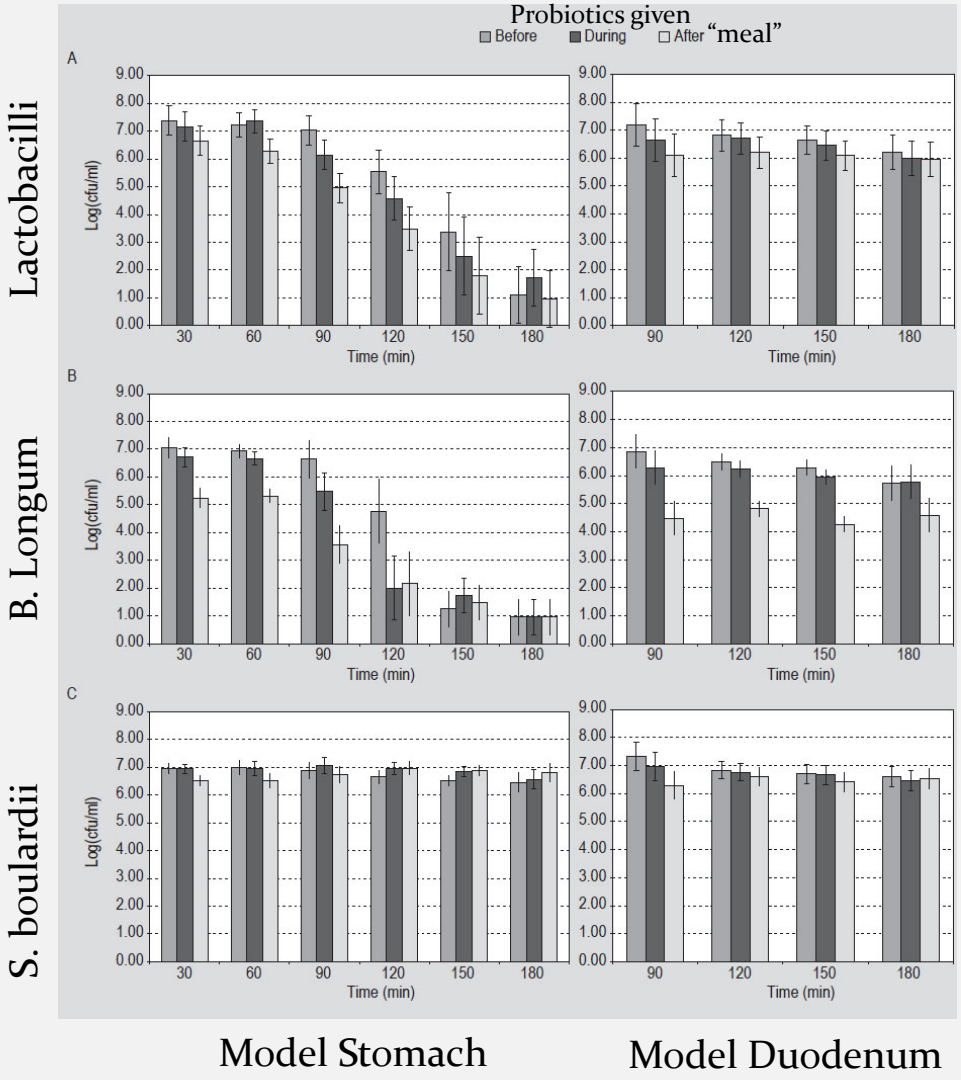
Commercial probiotic preparations come in several formats. Traditionally, in the food industry, probiotics have been delivered as part of a whole fermented dairy product such as a yogurt, kefir or sweet acidophilus milk. In the nutritional supplement industries, they are often delivered in capsules or powders. According to FAO guidelines (FAO, 2002) it is considered essential that the probiotic microbes, no matter how they are delivered, survive the passage through the upper intestinal tract and arrive at their site of action. There are a few intestinal models such as the simulator of the human intestinal microbial ecosystem (SHIME) or the TNO upper intestinal model (TIM-1) which attempt to reproduce the gastrointestinal tract. These models have been used to examine the survival of primarily simple mono-strain lactobacilli-based probiotics often they do not use commercially available probiotics, but rather only laboratory-grown versions (Alander *et al.*, 1999; Blanquet *et al.*, 2004; Kontula *et al.*, 1998). A single bioreactor simulating the upper gastrointestinal tract has been used to demonstrate that milk protein as a food matrix was important for the survival of commercial probiotics (Ritter *et al.*, 2009; Sarmeri *et al.*, 2008). This model showed good correlation with piglet studies. Pitino *et al.* (2010) recently examined the survival of six commercial strains of *Lactobacillus rhamnosus* in their simulator. Other studies have examined the survival of probiotics by enumerating the microorganisms in human faecal samples (Ibrigidi *et al.*, 2003; Dommels *et al.*, 2009; Firmesse *et al.*, 2008; Tsai *et al.*, 2008), but since some probiotics are highly adhesive and selectively partition into the intestinal mucosa (Alenka

ISSN 1876-2033 print, ISSN 1876-2891 online, DOI 10.3920/BM2011.0022

- Enumeration only possible in "model" GI tract
- HPMC-Veggie Cap
- 5 billion CFU (1:1:1:o.4) of
  - *L. helveticus*
  - *L. rhamnosus*
  - *B. longum*
  - *S. boulardii*
- Benef Microbes. 2011 Dec 1;2(4):295-303

STUDY DESIGN

- **Effect of time**
- Two capsules were added to the stomach vessel of the *in vitro* Digestive System (IViDiS) model 30 minutes before, during, or 30 minutes after a breakfast meal consisting of a portion of oatmeal (32 g of oat flakes – cooked with 175 ml of water) and 250 ml of milk (1% milk fat (MF); When the probiotics were given 30 minutes before the breakfast meal, the capsules were added with 125 ml spring water. Each situation was repeated four times.
- **Effect of food/beverage**
- Two capsules were added, with 500 ml of either milk (1% MF), oatmeal-milk gruel (same as above), 500 ml apple juice (pH 3.5 and contained no preservatives) or 500 ml spring water to the IViDiS. Each situation was repeated four times.



- Survival is only marginally better when consumed 30 minutes before the meal.
- Bifido more susceptible
- S. boulardii less vulnerable

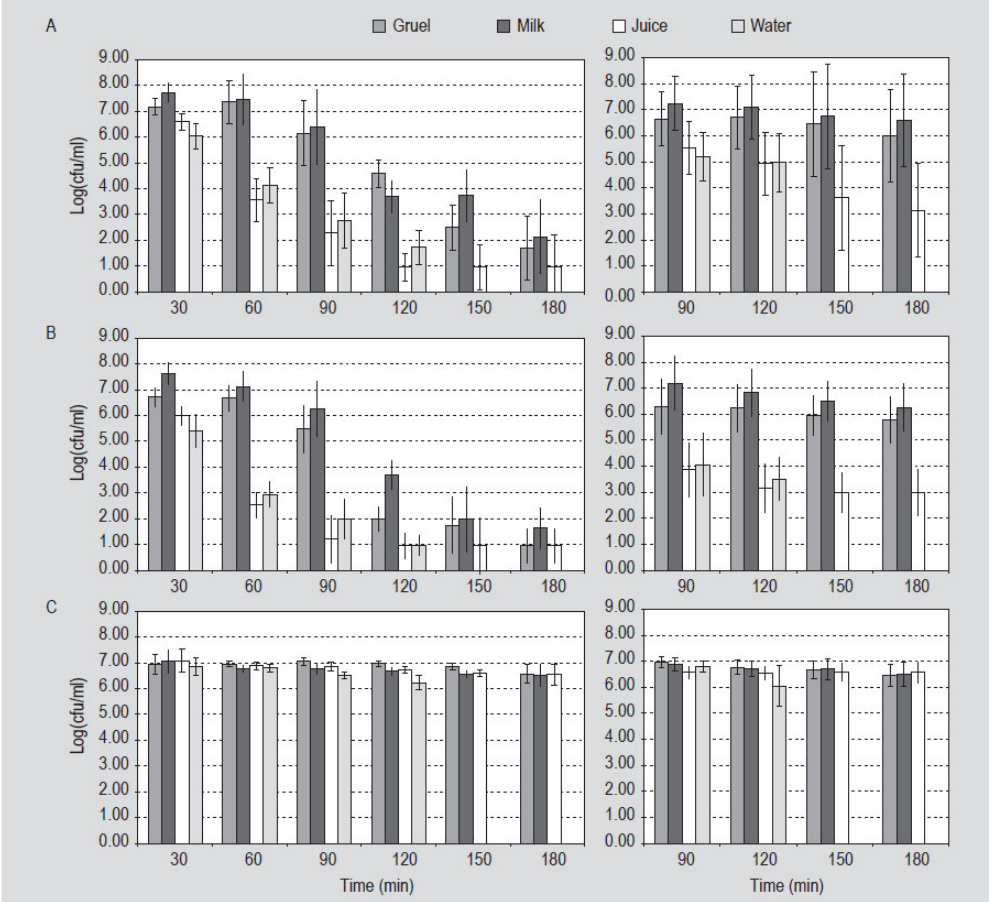


Figure 4. The survival of probiotic microorganisms as a function of time (min) through the stomach (left graph) and duodenal (right graph) vessels of the IViDiS model with oatmeal-milk gruel, 1% MF milk, apple juice or spring water. (A) Lactobacilli survival; (B) *Bifidobacterium longum* survival; (C) *Saccharomyces cerevisiae boulardii* survival.

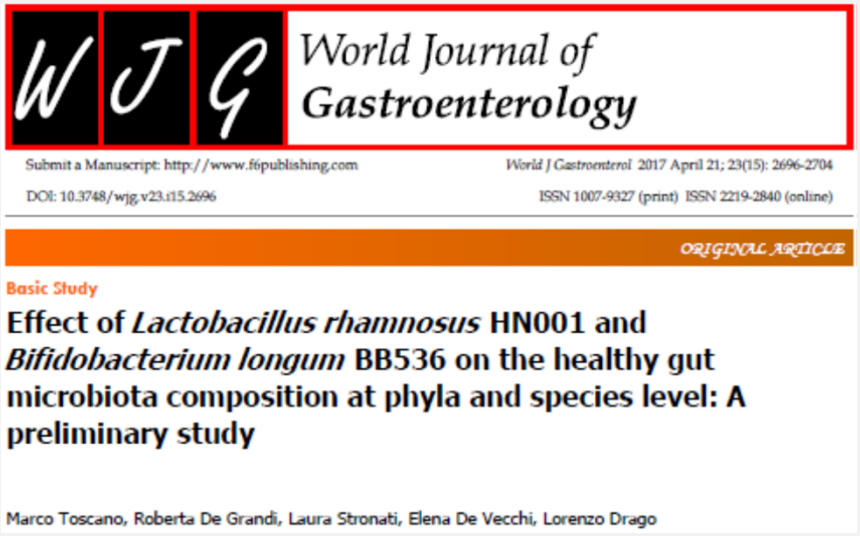
- Milk, or buffering liquid/food preserves viability over water or juice alone.

WHAT THIS STUDY MAY SUGGEST:

- Probiotics may survive slightly better when consumed 30 min before a meal (compared to 30 minutes after)
- This difference is much less significant than non-compliance
- Milk (or perhaps fat or buffering) appears to protect probiotics when consuming probiotics on an empty stomach- whereas water and apple juice are less protective.
- Taking probiotics regularly may offset these dose-to-dose differences.

DO PROBIOTICS CHANGE MEASURES OF GUT MICROBIOME (TEST)

- Surprisingly few studies have looked into this
- Most labs tell us that the common dose of a probiotic strain cannot be detected on stool analysis (mostly plated analysis)
- DNA approaches have not been systematically studied



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ORIGINAL ARTICLE

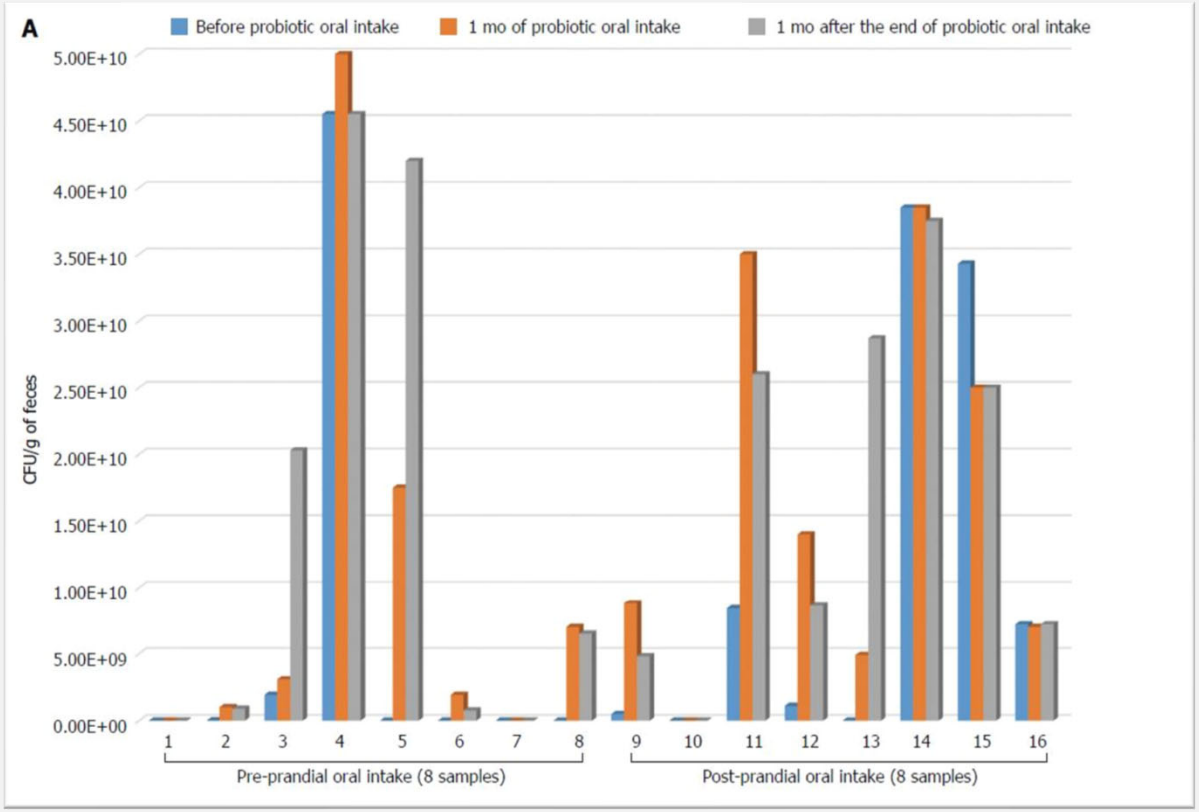
Basic Study

Effect of *Lactobacillus rhamnosus* HN001 and *Bifidobacterium longum* BB536 on the healthy gut microbiota composition at phyla and species level: A preliminary study

Marco Toscano, Roberta De Grandi, Laura Stronati, Elena De Vecchi, Lorenzo Drago

- 20 healthy subjects
- 4 billion cfu B. Longum + 1 billion cFU L. Rhamnosus
- 10 consumed probiotics (Sache) 30 min before Breakfast
- 10 consumed Probiotics 30 minutes after breakfast
- One month continuous use
- Stool samples tested using plating and quantitative PCR for species presence and overall species diversity

BIFIDOBACTERIUM LONGUM QUANTIFICATION IN STOOL



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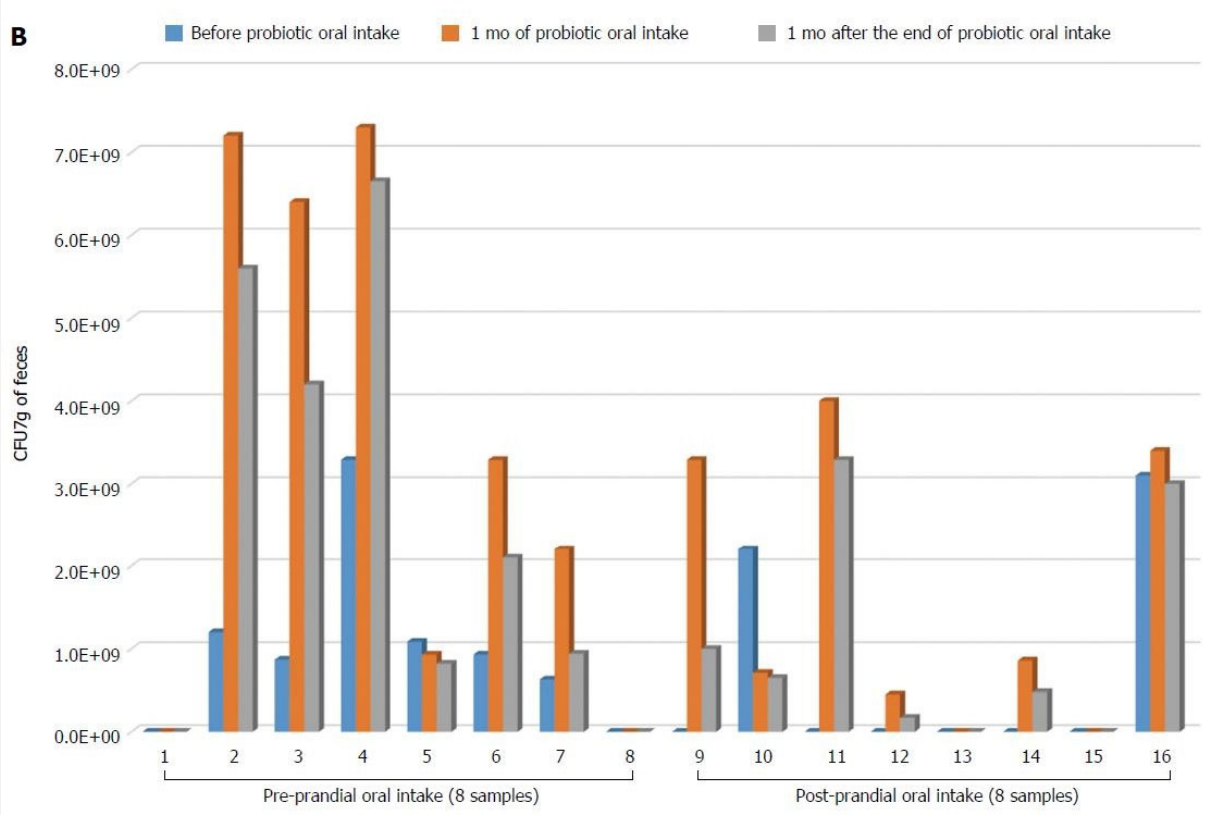
LACTOBACILLUS RHAMNOSUS QUANTIFICATION IN STOOL



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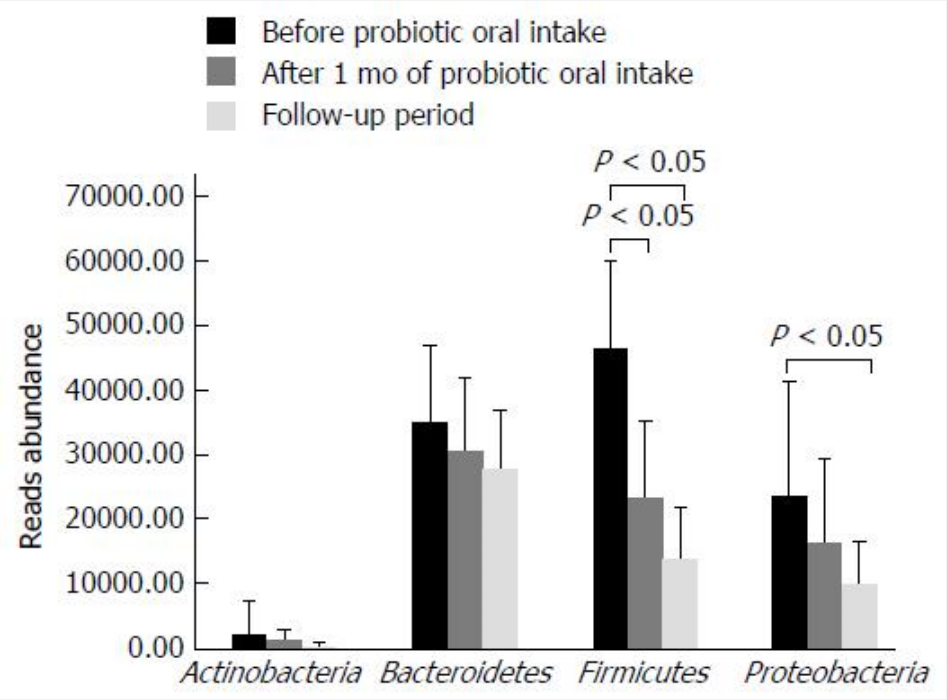
PHYLUM-LEVEL CHANGES OVER TIME



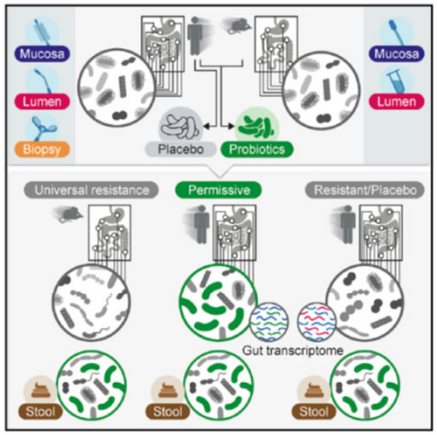
There were no statistical differences in measures of microbiota diversity in these subjects taking probiotics

Article

Cell

Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features

Graphical Abstract



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In Brief

Probiotics transiently colonize the human gut mucosa in highly individualized patterns, thereby differentially impacting the indigenous microbiome and host gene-expression profile, a trait which is predictable by baseline host and microbiome features, but not by stool shedding.

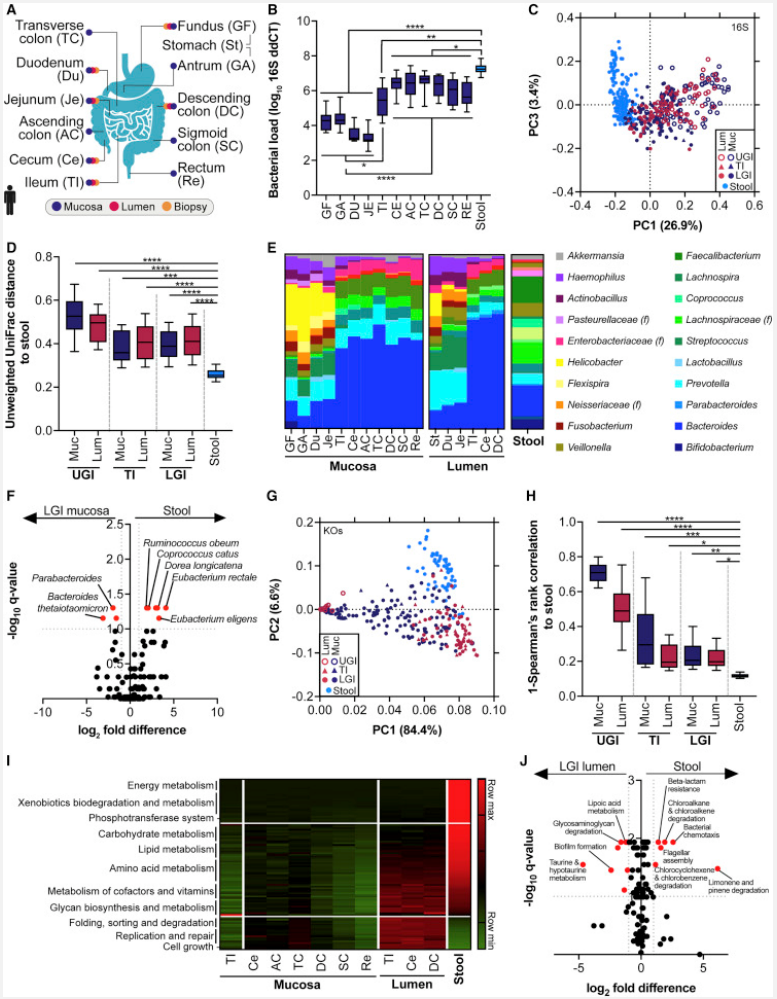
Highlights

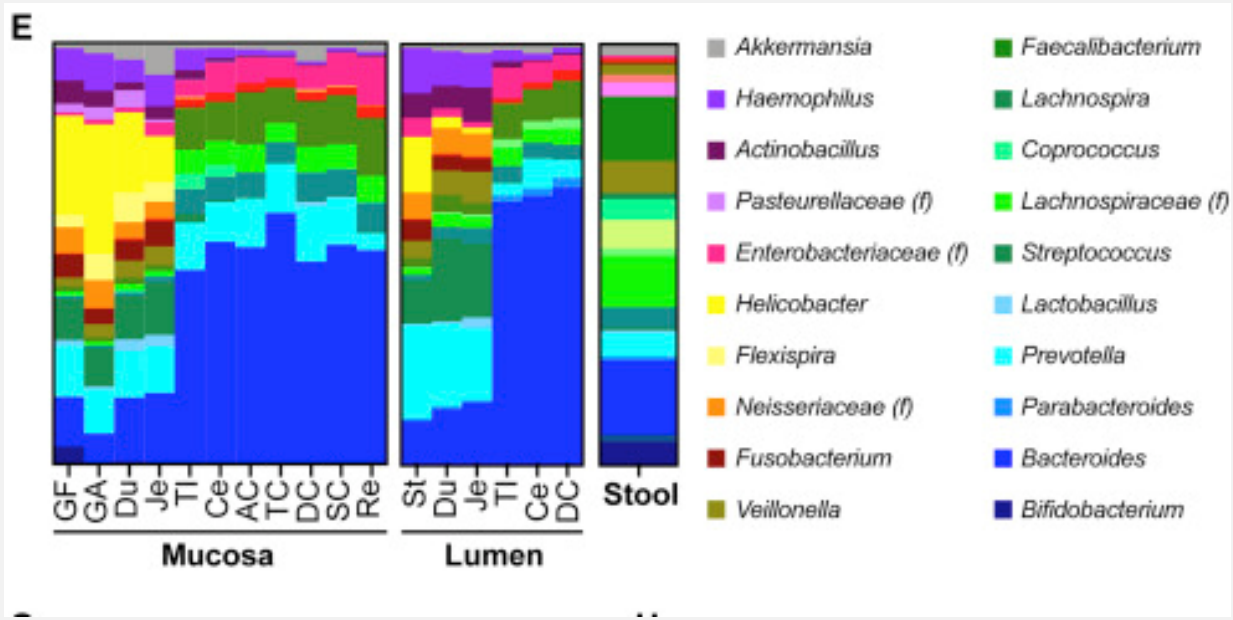
- The murine & human gut mucosal microbiome only partially correlates with stool
- Mice feature an indigenous-microbiome driven colonization resistance to probiotics
- Humans feature a person-specific gut mucosal colonization resistance to probiotics
- Probiotic colonization is predictable by pre-treatment microbiome & host features

Zmora et al., 2018, Cell *174*, 1388–1405
September 6, 2018
https://doi.org/10.1016/j.cell.2018.08.041

CellPress

DIFFERENCES BETWEEN STOOL MICROBIOME AND GI MICROBIOMES





HOST FACTORS AFFECT PROBIOTIC FUNCTION

- They defined subjects that are "Permissive" or "Resistant" to Probiotic Strains based on
  - Pre-Supplementation Commensal Microbiota
    - Species in low abundance in a given region, were more permissive to similar probiotic strains
  - Host immune System Function
  - Digestive Functions
  - Xenobiotic Metabolic Pathways

- Measures of metabolites and Probiotics in the Stool did not predict Metabolic function or colonization elsewhere in the GI tract

THERAPEUTIC USES FOR PROBIOTICS ARE CONSIDERED FOR NEARLY EVERY GI-RELATED (DYSBIOTIC) CONDITION

- Constipation
- diarrhea (traveler's, AAD, CDAD)
- IBS/Sibo
- IBD
- Candida
- H.pylori
- Gerd
- Intestinal Permeability

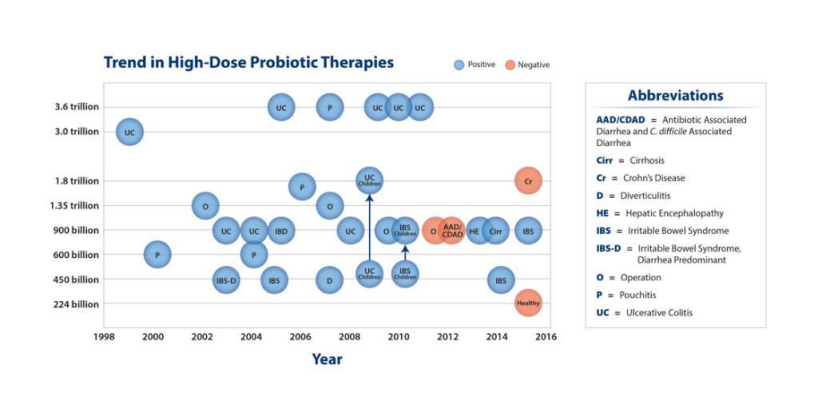
PROBIOTICS FOR ANTIBIOTIC-ASSOCIATED DIARRHEA

- One of the most consistently positive area of clinical benefit- Numerous Published Reviews
- Associated with most strains and most doses
- Data supports higher doses for better outcomes
- Saccharomyces Boulardii- Strong data alone or in combination with Lacto. And bifido Strains

23. Issa I, Moucari R. Probiotics for antibiotic-associated diarrhea: do we have a verdict? *World J Gastroenterol*. 2014 Dec 21;20(47):17788-95.
24. Hempel S et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA*. 2012 May 9; 307(18):1959-69.
25. Berni Canani R, Cucchiara S, Cuomo R, Pace F, Papale F. Saccharomyces boulardii: a summary of the evidence for gastroenterology clinical practice in adults and children. *Eur Rev Med Pharmacol Sci*. 2011 Jul; 15(7):809-22.
26. Pattani R, Palda VA, Hwang SW, Shah PS. Probiotics for the prevention of antibiotic-associated diarrhea and Clostridium difficile infection among hospitalized patients: systematic review and meta-analysis. *Open Med*. 2013 May 28;7(2):e56-67.
27. Lau CS, Chamberlain RS. Probiotics are effective at preventing Clostridium difficile-associated diarrhea: a systematic review and meta-analysis. *Int J Gen Med*. 2016 Feb 22;9:27-37.
28. Allen SJ, Wareham K, Wang D, et al. Lactobacilli and bifidobacteria in the prevention of antibiotic-associated diarrhoea and Clostridium difficile diarrhoea in older inpatients (PLACIDE): a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet*. 2013 Oct 12;382(9900):1249-57.
29. Szajewska H, Kołodziej M. Systematic review with meta-analysis: Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. *Aliment Pharmacol Ther*. 2015 Oct;42(7):793-801.
30. Patro-Golab B, Shamir R, Szajewska H. Yogurt for treating antibiotic-associated diarrhea: Systematic review and meta-analysis. *Nutrition*. 2015 Jun;31(6):796-800.
31. Ouwehand AC, DongLian C, Weijian X, et al. Probiotics reduce symptoms of antibiotic use in a hospital setting: a randomized dose response study. *Vaccine*. 2014 Jan 16;32(4):458-63.
32. Gao XW, Mubasher M, Fang CY, et al. Dose-response efficacy of a proprietary probiotic formula of Lactobacillus acidophilus CL1285 and Lactobacillus casei LBC80R for antibiotic-associated diarrhea and Clostridium difficile-associated diarrhea prophylaxis in adult patients. *Am J Gastroenterol*. 2010 Jul;105(7):1636-41.

PROBIOTICS FOR IBD

- Consistent difference between Crohn's and UC
  - Few studies have shown positive results with Crohn's- Some Limited benefit with S. boulardii and combo strains.
  - Numerous positive studies with UC
    - E.coli Nissle 1917 (not approved in US- but available in elsewhere)
    - High dose blend (VSL#3) numerous positive studies
  - Prebiotics/Fiber can be helpful in some subjects, though others have exacerbating symptoms when using Fermentable fibers



REFERENCE FOR IBD STUDIES

141. Ghouri YA, Richards DM, Rahimi EF, et al. Systematic review of randomized controlled trials of probiotics, prebiotics, and synbiotics in inflammatory bowel disease. *Clin Exp Gastroenterol*. 2014 Dec 9;7:473-87.
142. Lichtenstein L, Avni-Biron I, Ben-Bassat O. Probiotics and prebiotics in Crohn's disease therapies. *Best Pract Res Clin Gastroenterol*. 2016 Feb;30(1):81-8.
143. Lichtenstein L, Avni-Biron I, Ben-Bassat O. The current place of probiotics and prebiotics in the treatment of pouchitis. *Best Pract Res Clin Gastroenterol*. 2016 Feb;30(1):73-80.
144. Derikx LA, Dieleman LA, Hoentjen F. Probiotics and prebiotics in ulcerative colitis. *Best Pract Res Clin Gastroenterol*. 2016 Feb;30(1):55-71.
145. Chibbar R, Dieleman LA. Probiotics in the Management of Ulcerative Colitis. *J Clin Gastroenterol*. 2015 Nov-Dec;49 Suppl 1:S50-5.
146. Guandalini S, Cernat E, Moscoso D. Prebiotics and probiotics in irritable bowel syndrome and inflammatory bowel disease in children. *Benef Microbes*. 2015;6(2):209-17.
147. Plein K, Hotz J. Therapeutic effects of Saccharomyces boulardii on mild residual symptoms in a stable phase of Crohn's disease with special respect to chronic diarrhea--a pilot study. *Z Gastroenterol*. 1993 Feb;31(2):129-34.
148. Guslandi M, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Dig Dis Sci*. 2000 Jul;45(7):1462-4.
149. Steed H, Macfarlane GT, Blackett KL, et al. Clinical trial: the microbiological and immunological effects of synbiotic consumption - a randomized double-blind placebo-controlled study in active Crohn's disease. *Aliment Pharmacol Ther*. 2010 Oct;32(7):872-83.
150. Fujimori S., Tatsuguchi A., Gudis K., Kishida T., Mitsui K., Ehara A., et al: High dose probiotic and prebiotic cotherapy for remission induction of active Crohn's disease. *J Gastroenterol Hepatol* 2007; 22: pp. 1199-1204.
151. Fedorak RN, Feagan BG, Hotte N, et al. The probiotic VSL#3 has anti-inflammatory effects and could reduce endoscopic recurrence after surgery for Crohn's disease. *Clin Gastroenterol Hepatol*. 2015 May;13(5):928-35.e2.
152. Kruis, W., Schutz, E. et al. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther*. 1997; 11(5):853-858.
153. Rembacken, B.J., Snelling, A.M. et al. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet*. 1999; 354(9179):635-639.
154. Kruis, W., Fric, P. et al. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut*. 2004; 53(11):1617-1623.
155. https://www.regulations.gov/document?D=FDA-2012-S-1178-0014.
156. Mardini HE, Grigorian AY. Probiotic mix VSL#3 is effective adjunctive therapy for mild to moderately active ulcerative colitis: a meta-analysis. *Inflamm Bowel Dis*. 2014 Sep;20(9):1562-7.
157. Guslandi M, Giollo P, Testoni PA. A pilot trial of Saccharomyces boulardii in ulcerative colitis. *Eur J Gastroenterol Hepatol*. 2003 Jun;15(6):697-8.
158. Kato K, Mizuno S, Umesaki Y, et al. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther*. 2004 Nov 15;20(10):1133-41.
159. Oliva S, Di Nardo G, Ferrari F, et al. Randomised clinical trial: the effectiveness of Lactobacillus reuteri ATCC 55730 rectal enema in children with active distal ulcerative colitis. *Aliment Pharmacol Ther*. 2012 Feb;35(3):327-34.
160. Wildt S, Nordgaard I, Hansen U, et al. A randomised double-blind placebo-controlled trial with Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp. lactis BB-12 for maintenance of remission in ulcerative colitis. *J Crohns Colitis*. 2011 Apr;5(2):115-21.

PROBIOTICS FOR IBS: LOTS OF CONFUSION

- Over 50 clinical trials have been performed
- Over 10 systematic reviews published
- No consensus on benefit, strains, doses!
- Likely due to complexity of diagnosis and the differing etiologies lumped together with Rome criteria, presence of sibo, Gut-Brain issues etc.
- What we know:
  - Probiotics are Safe in most subjects with IBS
  - The same strain (or combo) will not work in all subjects with an ibs diagnosis
  - Symptom improvements may diminish over time (esp. in IBS-M)
  - Positive outcomes have been seen at both low and high dose

89. Quigley EM. Probiotics in Irritable Bowel Syndrome: The Science and the Evidence. *J Clin Gastroenterol*. 2015 Nov-Dec;49 Suppl 1:S60-4.
90. Mazurak N, Broelz E, Storr M, Enck P. Probiotic Therapy of the Irritable Bowel Syndrome: Why Is the Evidence Still Poor and What Can Be Done About It? *J Neurogastroenterol Motil*. 2015 Oct 1;21(4):471-85.
91. Zhang Y, Li L, Guo C, et al. Effects of probiotic type, dose and treatment duration on irritable bowel syndrome diagnosed by Rome III criteria: a meta-analysis. *BMC Gastroenterol*. 2016 Jun 13;16(1):62.

92. McKenzie YA, Thompson J, Gulia P, et al. British Dietetic Association systematic review of systematic reviews and evidence-based practice guidelines for the use of probiotics in the management of irritable bowel syndrome in adults (2016 update). *J Hum Nutr Diet*. 2016 Jun 6. doi: 10.1111/jhn.12386. [Epub ahead of print]
93. Didari T, Mozaffari S, Nikfar S, Abdollahi M. Effectiveness of probiotics in irritable bowel syndrome: Updated systematic review with meta-analysis. *World J Gastroenterol*. 2015 Mar 14;21(10):3072-84.

PROBIOTICS FOR CANDIDA

- Oral probiotics have been used to reduce candida overgrowth in the GI as well as mouth and vagina with some success
- Direct application has also been successful for candida vulvovaginal candidiasis (not supplement in U.S.)

108. Martinez RC, Franceschini SA, et al. Improved treatment of vulvovaginal candidiasis with fluconazole plus probiotic Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14. *Lett Appl Microbiol*. 2009 Mar;48(3):269-74.
109. Bohbot JM, Cardot JM. Vaginal impact of the oral administration of total freeze-dried culture of LCR 35 in healthy women. *Infect Dis Obstet Gynecol*. 2012;2012:503648.

103. Li D, Li Q, Liu C, Lin M, et al. Efficacy and safety of probiotics in the treatment of Candida-associated stomatitis. *Mycoses*. 2014 Mar;57(3):141-6.
104. Kraft-Bodi E, Jørgensen MR, Keller MK, et al. Effect of Probiotic Bacteria on Oral Candida in Frail Elderly. *J Dent Res*. 2015 Sep;94(9 Suppl):181S-6S.
105. Hatakka K, Ahola AJ, Yli-Knuuttila H, et al. Probiotics reduce the prevalence of oral candida in the elderly--a randomized controlled trial. *J Dent Res*. 2007 Feb;86(2):125-30.
106. Reid G, Burton J, Hammond JA, Bruce AW. Nucleic acid-based diagnosis of bacterial vaginosis and improved management using probiotic lactobacilli. *J Med Food*. 2004 Summer;7(2):223-8.
107. Reid G, Charbonneau D, Erb J, et al. Oral use of Lactobacillus rhamnosus GR-1 and L. fermentum RC-14 significantly alters vaginal flora: randomized, placebo-controlled trial in 64 healthy women. *FEMS Immunol Med Microbiol*. 2003 Mar 20;35(2):131-4.

PROBIOTICS: POSSIBLE CONCERNS

- Safety (Rare concerns usually in gravely sick/immunocompromised individuals)
- D-Lactate Issues (little documentation)
- Histamine (Anecdotal reports, but no published Data)
- Probiotic Use during antibiotic use is encouraged, will not diminish effect of antibiotic, may limit viability of probiotic- consider including S. boulardii with mixed strain product.

GENETICALLY-ENGINEERED PROBIOTICS

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ScienceDirect

Current Opinion in Microbiology

CRISPR-based engineering of next-generation lactic acid bacteria

Claudio Hidalgo-Cantabrana, Sarah O'Flaherty and Rodolphe Barrangou

The advent of CRISPR-based technologies has opened new avenues for the development of next-generation food microorganisms and probiotics with enhanced functionalities. Building off two decades of functional genomics studies unraveling the genetic basis for food fermentations and host–probiotic interactions, CRISPR technologies offer a wide range of opportunities to engineer commercially-relevant *Lactobacillus* and *Bifidobacteria*. Endogenous CRISPR–Cas systems can be repurposed to enhance gene expression or provide new features to improve host colonization and promote human health. Alternatively, engineered CRISPR–Cas systems can be harnessed to genetically modify probiotics and enhance their therapeutic potential to deliver vaccines or modulate the host immune response.

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Current Opinion in Microbiology 2017, 37:79–87

are widespread in lactic acid bacteria (LAB), bifidobacteria, and many members of the human microbiome [7–9], as they confer a selective advantage against phages and plasmids. Type II CRISPR–Cas systems in particular are the most extensively studied to date, due to the genome editing capability of the programmable, precise, portable and efficient Cas9 signature nuclease.

The Cas9 endonuclease can drive DNA binding and cleavage through an engineered single guide RNA (sgRNA) sequence [10]. This Cas9:sgRNA system has led to a wide variety of applications in human, plant, animal and microbe engineering, with the main focus on genome editing [11,12•]. While using CRISPR for genome editing in eukaryotic systems has occurred at lightning speed due to the opportunity to cure human, animal and plant disease, relatively few studies have focused on bacterial genome editing, resulting in an arguably underutilized yet prodigious technology. Therefore, industrial microbes such as starter cultures and probiotic strains are a desirable target to harness CRISPR–Cas systems for

REGULATORY FUTURE OF NEW "PROBIOTICS"

- New Bacteria isolated from human GI tract are often deemed Biologics
- Few new strains have gone Through NDIN process for Dietary supplement use
- Medical Foods….


Who Knows What is Around the Corner Next.
Thank You