



The Human Intestinal Microbiota and Health Benefits of Probiotics



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CSUN

**MARILYN MAGARAM CENTER
FOR FOOD SCIENCE,
NUTRITION AND DIETETICS**

Outlines

- **Definitions**
- **Structure and Function of the Digestive System**
- **Facts about human intestinal microbiota**
- **Functions of human intestinal microbiota**
- **Healthy and balanced intestinal microbiota**
- **What is a probiotic?**
- **Yogurt Cultures and Probiotics**
- **Purchasing a Probiotic Product**

Definitions

- **Gut:**

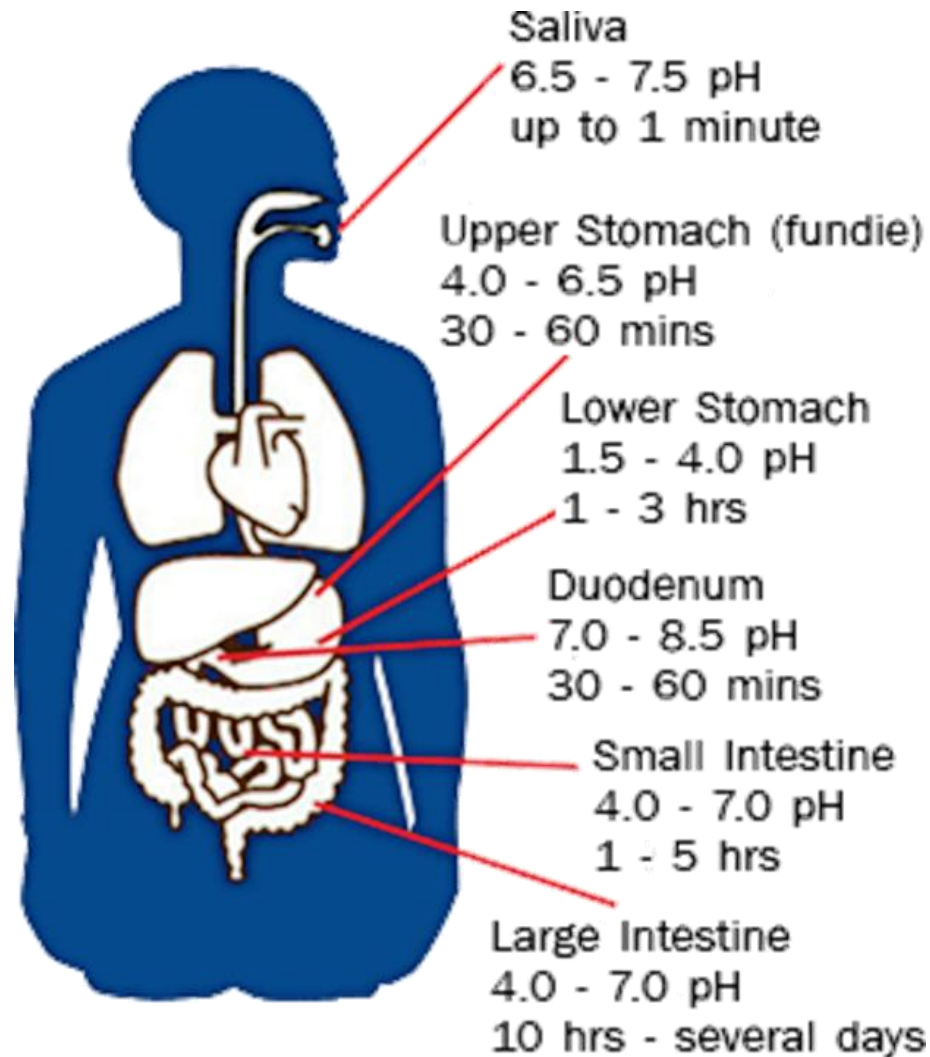
- The lower part of the digestive tract relating to the small and large intestines or bowel.
- The 'tube' runs from mouth to anus. The term '**gut**' usually refers to the stomach and intestines.

- **Intestinal Microbiota:**

- Microorganisms dominate the human gut ecosystem.
- *A generic designation for the different groups of bacteria that reside in the intestines of humans and animals.*

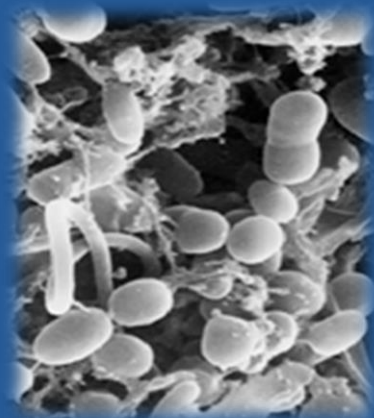


The Human Digestive Tract pH Range Diagram



Interesting facts about our human intestinal microbiota

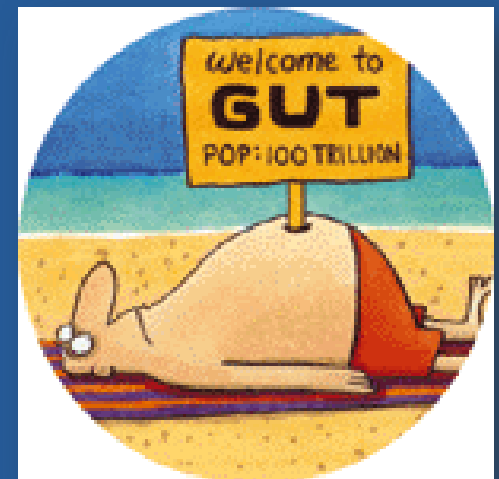
- The GI tract of a normal fetus is sterile.
- Immediately after vaginal delivery, babies may have bacterial strains in the upper GI tract derived from the mothers' feces.
- After birth, environmental, oral and skin bacteria are readily transferred from the mother to the infant through suckling, kissing, and caressing.
- All infants are initially colonized by large numbers of *E. coli* and streptococci.
- Within a few days, bacterial numbers reach 10^8 to 10^{10} /g of feces
- Diverse microbial population develops with the introduction of other foods.
- Bacteria in the gut fulfills useful functions for humans, including digestion of unutilized energy substrates; stimulating cell growth; repressing the growth of harmful microorganisms; training the immune system to respond only to pathogens; and defending against some diseases.
- Majority of the cells in a human body are non-human.



100 trillion microbial cells



10 trillion human cells



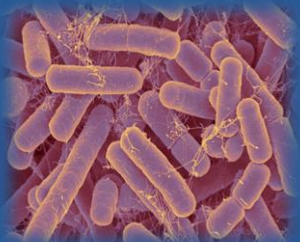
The Human Intestinal Microbiota



Lactobacillus



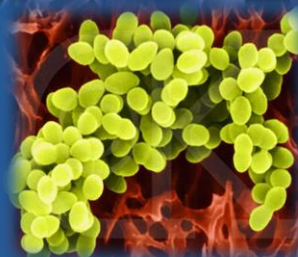
Bifidobacterium



Bacteroides



Escherichia coli



S. aureus

70% of immune system is located in intestinal tract.

- 700 different species of bacteria
- %99.9 strict anaerobes
- ~100 trillion bacteria (~ 3 lbs.)

Beneficial bacteria

- Lactobacilli
- Bifidobacteria

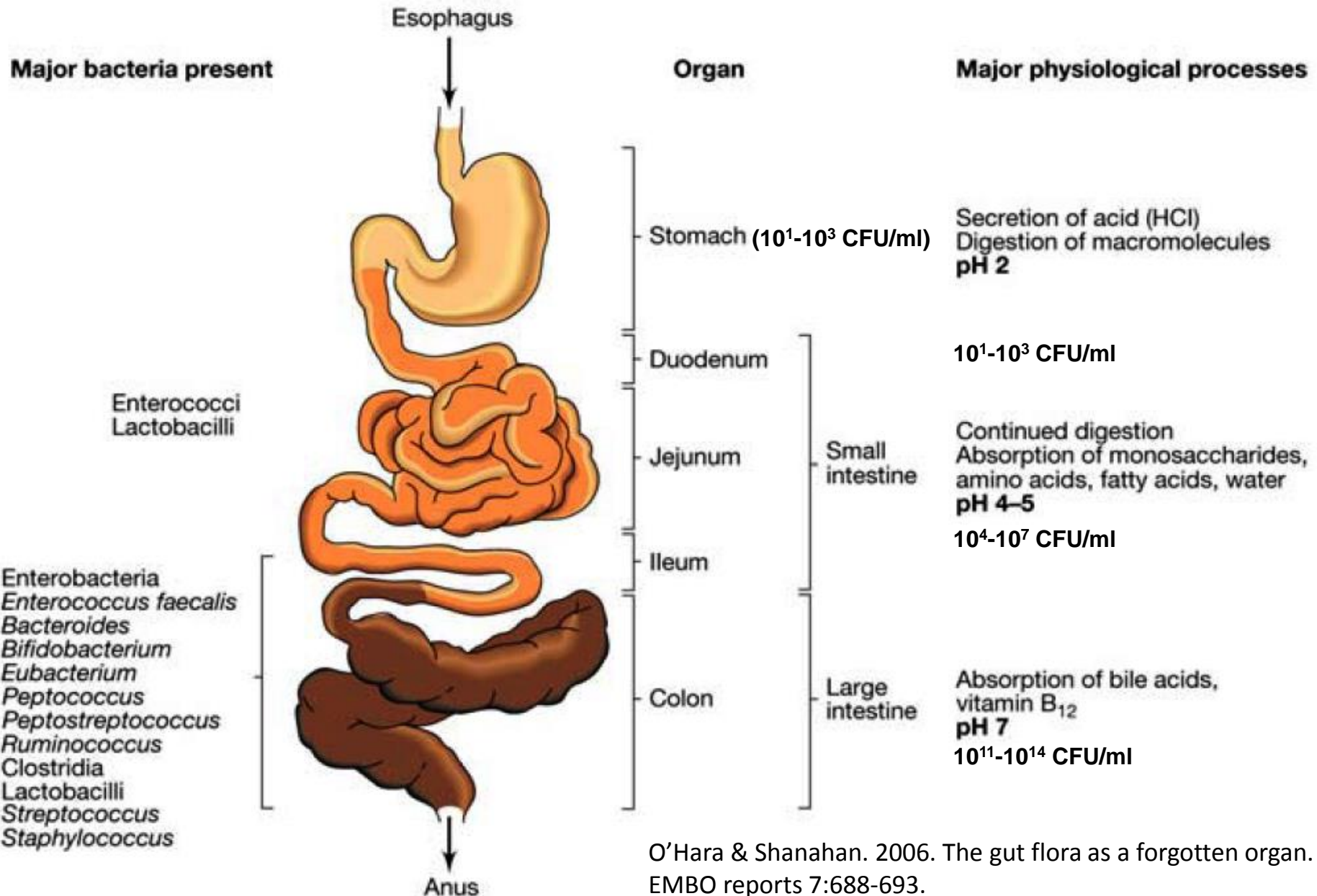
Neutral Bacteria

- Bacteroides*

Harmful bacteria

- *Staphylococcus aureus*
- *E. coli*
- *Clostridium difficile*
- *Salmonella* species
- *Helicobacter pylori*

The Human Intestinal Microbiota



O'Hara & Shanahan. 2006. The gut flora as a forgotten organ. EMBO reports 7:688-693.

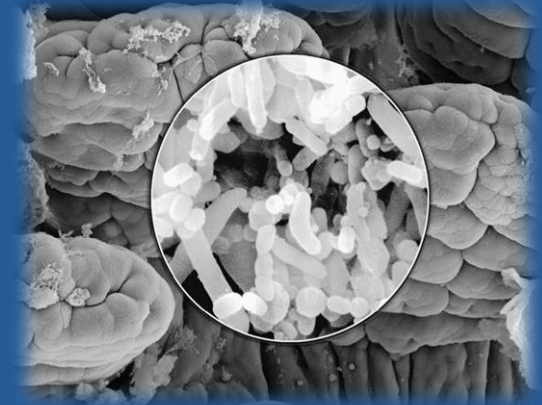
What do we know about intestinal microbiota colonizing our gut?

Large number

- 10^{14} microbial cells in the gut
- 80% not cultured yet

Large diversity

- Found in humans
 - Up to 700 different bacterial phylotypes
 - ~1200 viral types
- Individuals
 - ~130-200 different bacterial phylotypes in a given person
 - Fecal bacteria different from mucosal



Unique to an individual

- An individual's microbiota is a fingerprint. Thus we all have our own “poop-print”.

Large metabolic potential

- The human microbiome contains roughly 100X as many genes as does the human genome.
- Largely stable after weaning through adulthood until old age

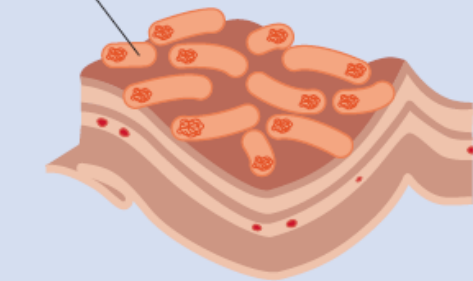
Not static residents, but actively communicate with the host

Main functions of intestinal microbiota

Protective functions

Pathogen displacement
 Nutrient competition
 Receptor competition
 Production of anti-microbial factors e.g., bacteriocins, lactic acids

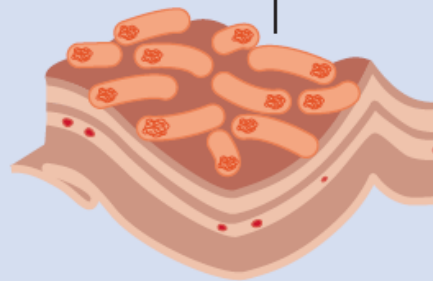
Commensal bacteria



Structural functions

Barrier fortification
 Induction of IgA
 Apical tightening of tight junctions
 Immune system development

IgA



Metabolic functions

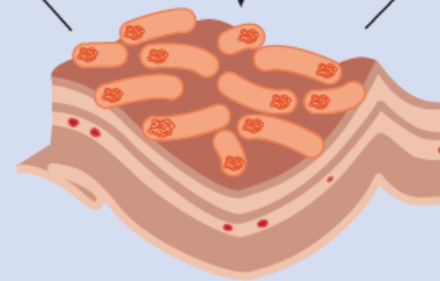
Control IEC differentiation and proliferation
 Metabolize dietary carcinogens
 Synthesize vitamins e.g., biotin, folate

Ferment non-digestible dietary residue and endogenous epithelial-derived mucus
 Ion absorption
 Salvage of energy

Short-chain fatty acids

Mg²⁺
 Ca²⁺
 Fe²⁺

Vitamin K
 Biotin
 Folate



Healthy - balanced intestinal microbiota

Potential for harmful/pathogenic effects

Potential for health promoting functions

Pathogens
(inc. production
of toxins)

- Ps. aeruginosa*
- Proteus*
- Staphylococcus*
- Clostridium*
- Veillonella*

Production of
potential
carcinogens

Intestinal
putrefaction

- Enterococcus*
- E. coli*
- Streptococcus*
- Bacteroides*

Inhibition of
exogenous
and/or harmful
bacteria

Stimulation of
immune functions

Aids digestion
and/or absorption
of food /minerals

Synthesis of
vitamins

2

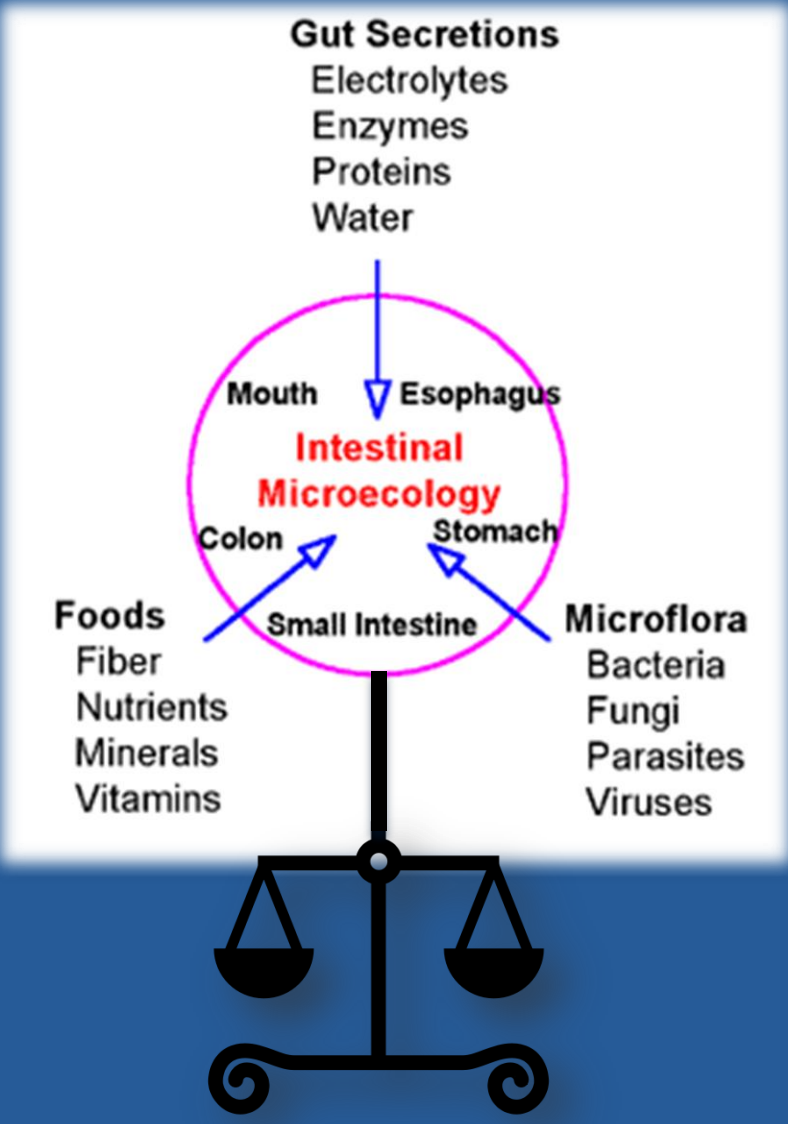
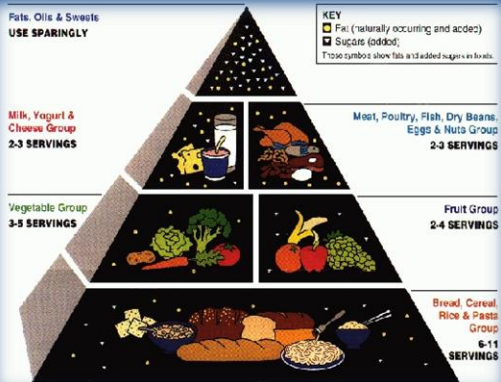
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8

11

Number/g faeces
Log 10 scale

Healthy - balanced intestinal microbiota



Harmful bacteria

- Causes gastrointestinal infections
 - diarrhea
 - ulcer
 - others
- Create unsuitable conditions for beneficial bacteria
- Encourage the growth of pathogenic microorganisms
- Produce toxic substances
 - NH_4^+
 - Nitrosamines
 - Indole
 - H_2S
 - Phenol
 - *p*-cresols
- Generate intestinal gas
 - CH_4
 - H_2
 - CO_2

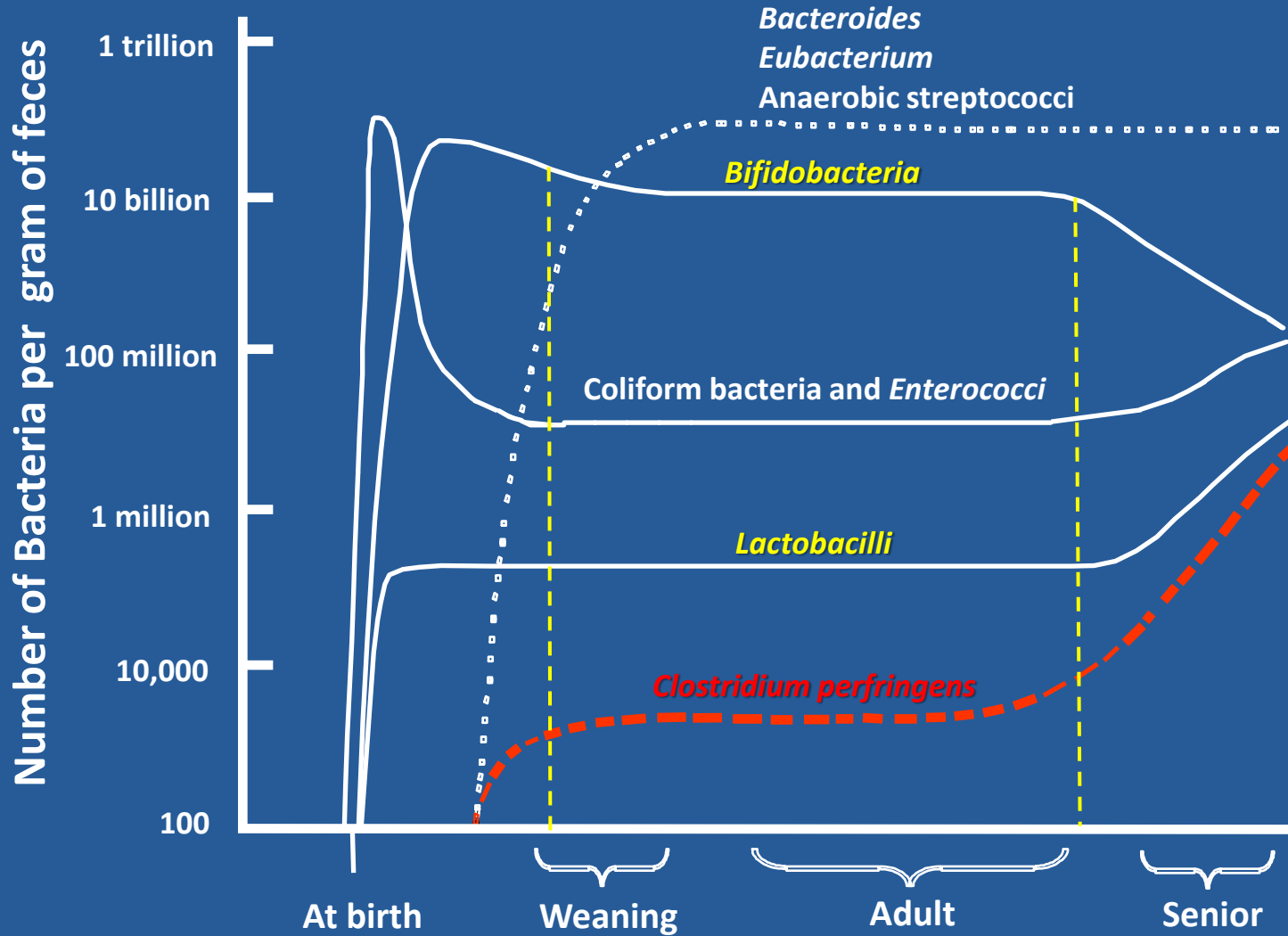


Beneficial bacteria

- **Stimulate bowel motility & reduce constipation and diarrhea**
- **Compete with harmful bacteria for nutrients & attachments sites**
- **Produce antimicrobial substances**
 - Lactic, acetic, propionic and butyric acids (short chain fatty acids)
 - H_2O_2
 - Bacteriocins
 - Lactoperoxidase enzyme system
- **Support immune system**
- **Maintain and restore the barrier function**
- **Improve digestion and bioavailability of nutrients**
- **Decompose harmful substances (NH_4^+ , nitrosamines, indole, H_2S , phenol)**
- **Produce certain vitamins (K and some B vitamins)**

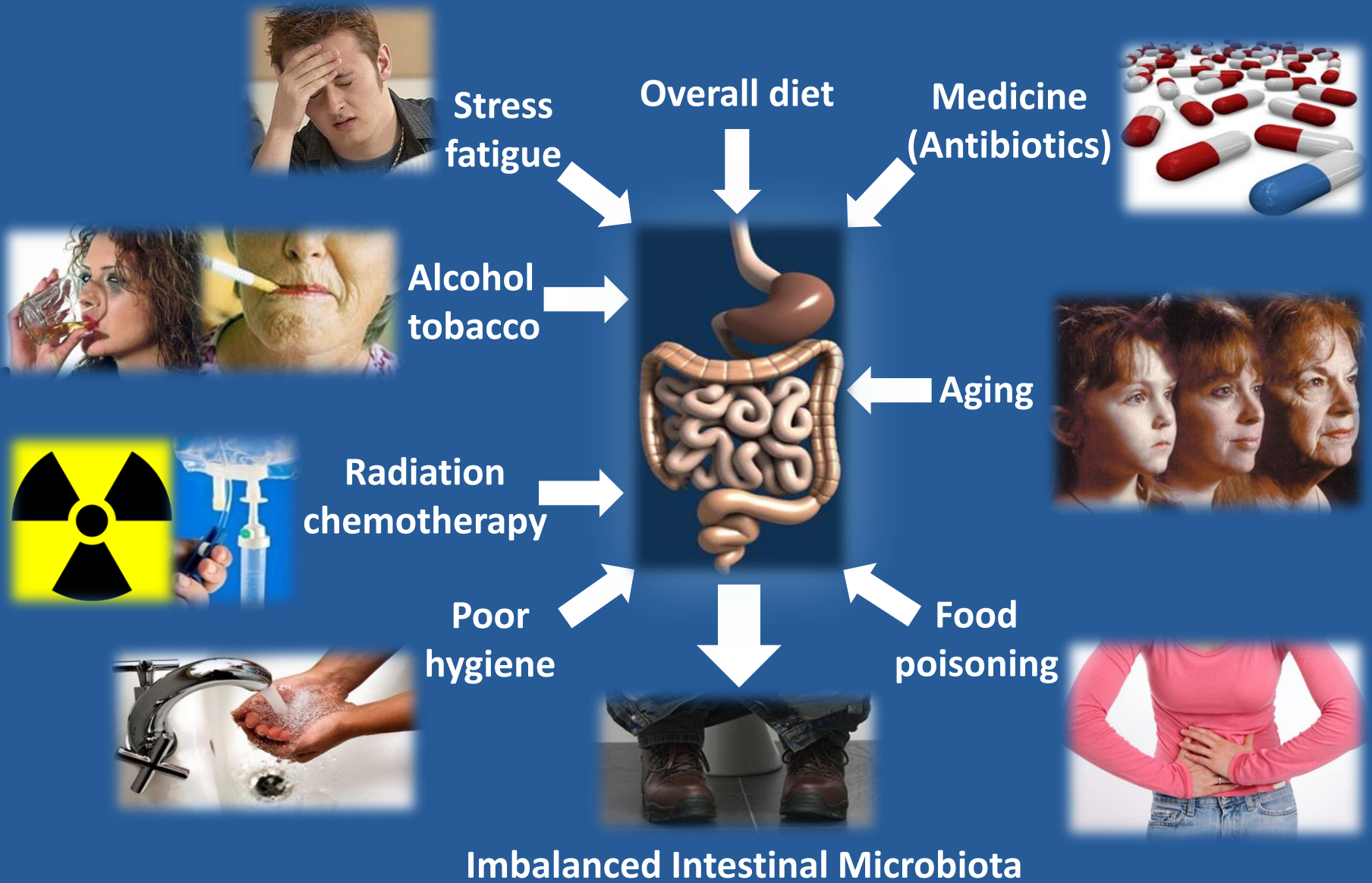


Intestinal Microbiota and Aging



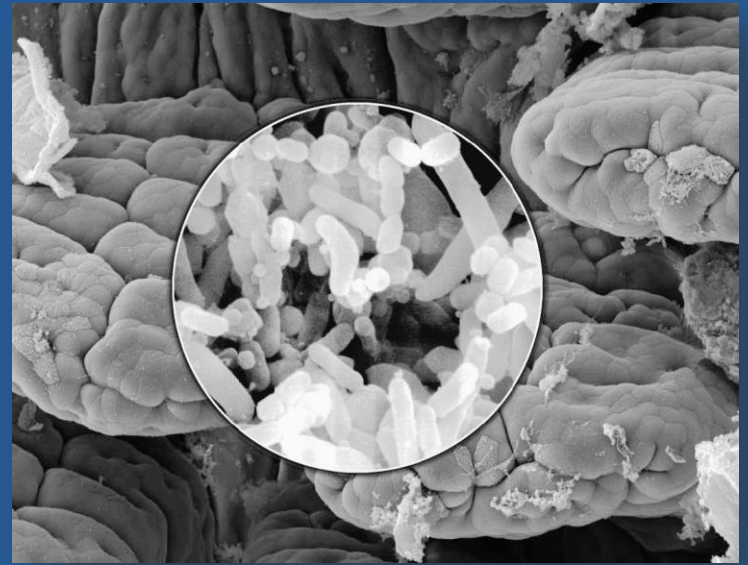
(Mitsuoka, 1992)

Factors affecting intestinal microbiota



Other factors affecting intestinal microbiota

- Bactericidal chemicals in drinking water
- Oral Contraceptives
- Corticosteroids
- Sugar in the diet
- Caesarean birth
- Lack of breastfeeding
- Pesticides in food
- Heavy metals
- *Helicobacter pylori*
- Gastrointestinal pH
- Bowel transit time



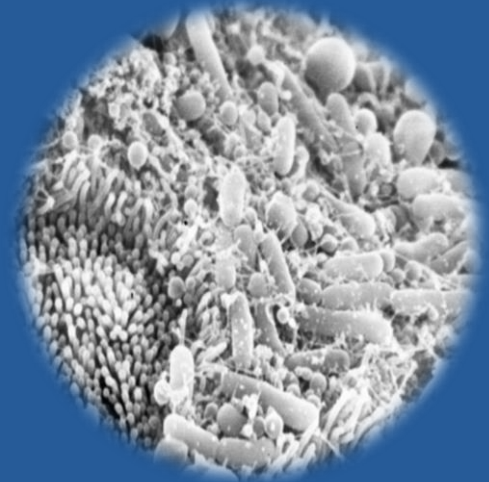
Imbalanced intestinal flora can lead to:

- **Gastrointestinal disorders**
 - Constipation & diarrhea
 - Irritable bowel syndrome
 - Inflammatory bowel disease
- **Urinary tract infections**
- **Abdominal inflammations**



What don't we know about intestinal microbiota colonizing the human gut?

- What the ideal composition is
- How the composition is determined
 - Host genotype likely has a significant impact on initially selecting which bacterial groups colonize the intestinal microbiota
 - Diet then affects metabolic activity of those groups selected by host genotype
- How differences in the composition may impact disease
- How alteration of the populations or activities of the microbiota may influence health and disease



Methods for studying microbial composition in the gut

- **Selective cultivation**

- *Poor discrimination and shows only the 'tip of the iceberg'*
- Many oxygen sensitive organisms

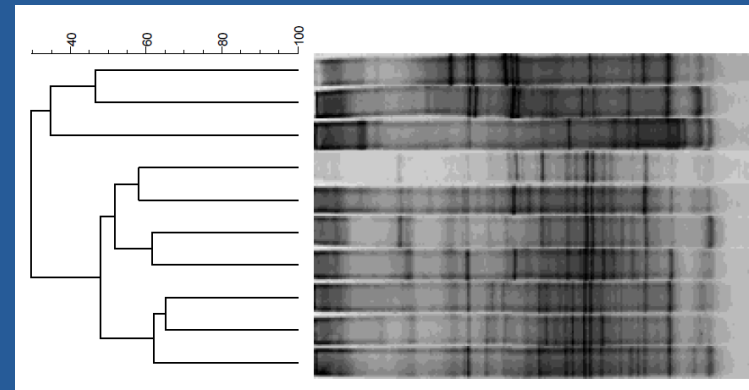
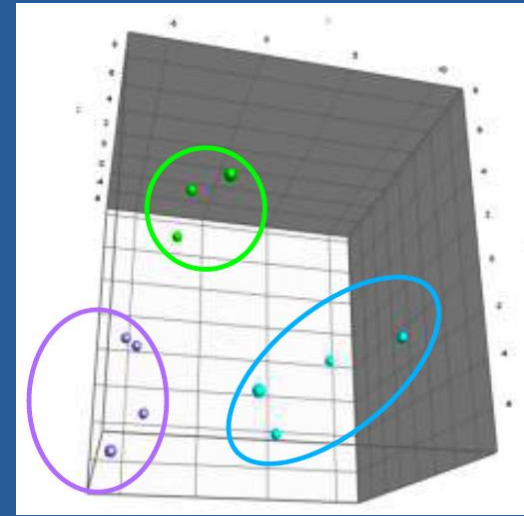
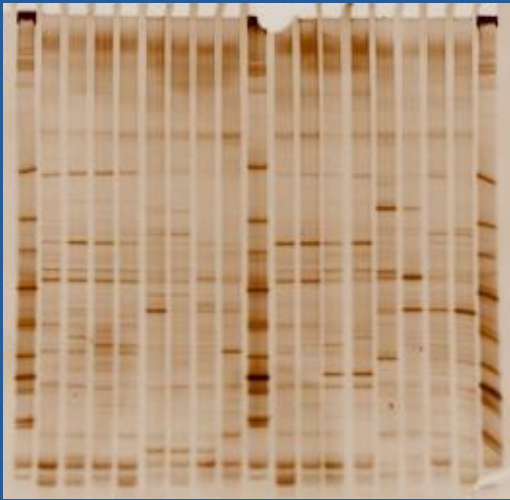
- **16S PCR-based methods**

- *DGGE (Denaturing Gradient Gel Electrophoresis)*
- *T-RFLP (Terminal Restriction Fragment Length Polymorphism)*

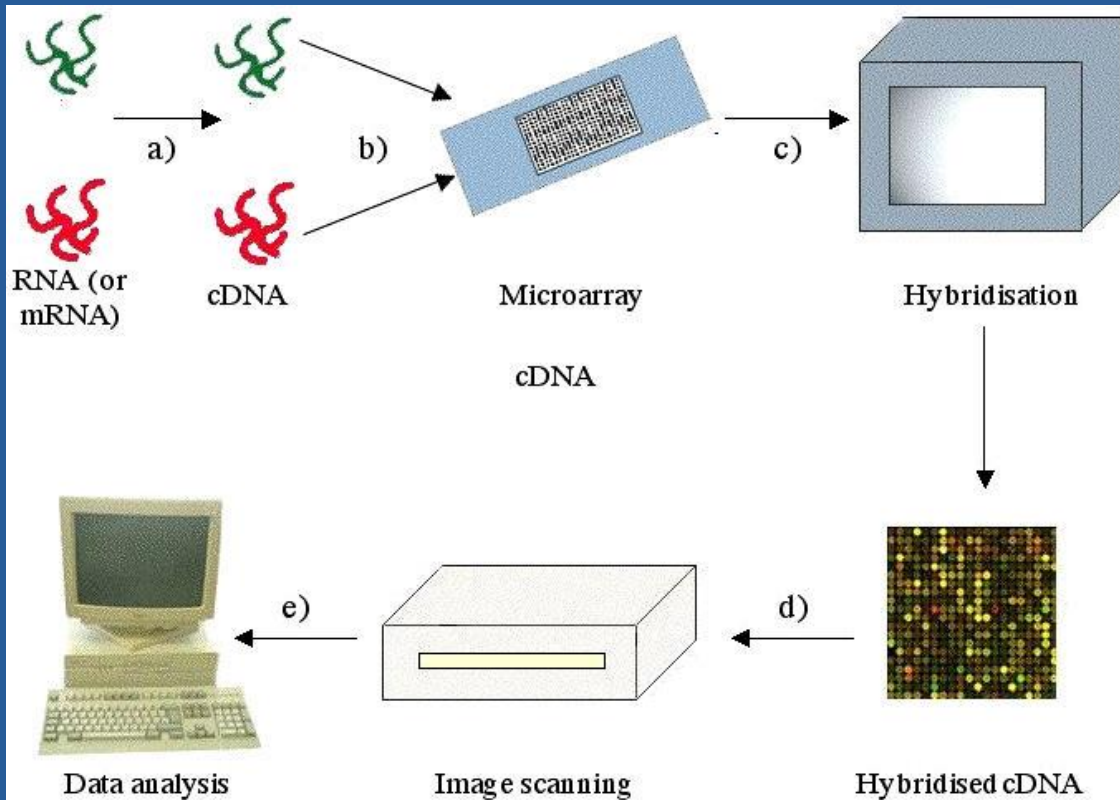
- **Genomic-based approaches**

- Microarrays (e.g. 16S based)
- Metagenomic sequencing

Example: Analysis of composition of intestinal microbiota by DGGE of PCR-amplified ribosomal rRNA genes



Example: Microarray analysis



- Typically 16S gene based (phylogenetic array)
- However, could also target other genes or their expression
- Data handling and analysis is essential

Intestinal Flora Analysis System- “YIF SCAN”

- **YIF SCAN** allows quick, simple, and accurate analysis without cultivating intestinal flora.
- Selectively quantifies bacteria based on the unique gene sequences (RNA and DNA) of individual intestinal flora.
- It does not require a high level of skill. Applicable to bacteria existing in high numbers or low numbers.



*The image shows an automatic nucleic-acid extraction device, which is for extracting RNA or DNA from bacteria contained in fecal material.

Probiotics: Modern definition



FAO

The word '**probiotic**' is Greek, meaning
Pro (for) + **biotic** (bios) (life) = For Life



WHO

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

Source: FAO/WHO (2001) Expert Consultation,
<http://www.fao.org/es/ESN/Probio/probio.htm>

History of Probiotics

- Traditional soured milk & fermented dairy products - consumed for centuries.
- Persian version of the Old Testament (Genesis 18:8) states “Abraham owed his longevity to the consumption of sour milk.”
- **2000 B.C.** Ancient Assyrians called **yogurt** as "**lebeny**“ meant “**life**“. Interestingly, the modern word '**probiotic**' can literally be translated to “**for life**“, today.
- **76 B.C.** The Roman historian Plinius recommended the administration of fermented milk products for treating gastroenteritis .
- **11th century.** The use of **yogurt** (yoghurt, yoghurt, yogourt) by mediaeval Turks is recorded in the books *Diwan Lughat al-Turk* by **Mahmud Kashgari** and *Kutadgu Bilig* by **Yusuf Has Hajib**. In both texts the word "**yoghurt**" is mentioned in different sections and its use by nomadic Turks is described.
- **1905** - Bulgarian student of medicine in Geneva, **Stamen Grigorov** (1878–1945) first **examined the microflora of the Bulgarian yoghurt**. In 1905, he described it as consisting of a spherical and a rod- like lactic acid bacteria.
- **1907** - The rod-like bacterium was called *Lactobacillus delbrueckii* sp. *bulgaricus* by Elie Metchnikoff.

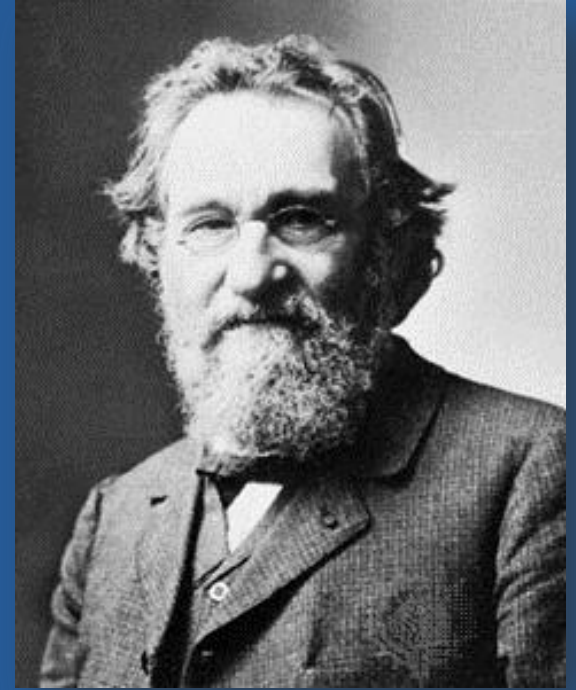
History of Probiotics

- **1907 - Elie Metchnikoff** - the grandfather of modern probiotics claimed that the intake of yogurt containing lactobacilli results in a reduction of toxin-producing bacteria in the gut and this increases the longevity of the host.
- **1917 - Orla Jensen** proved that the production of yoghurt except *Lactobacillus bulgaricus* participate coccus called *Streptococcus thermophilus*.
- **1930 - Dr. Minoru Shirota** successfully isolated and cultured a lactic acid bacterium from a healthy individual's faeces surviving in the gut.
- **1965** - the term '**probiotics**' was **1st** used by **Lilly and Stillwell** to describe '*substances secreted by one organism which stimulate the growth of another*'.
- **1974** - Parker was the first to use the term **probiotic** in the sense that it is used today "organisms and substances which contribute to intestinal microbial balance".
- **1989** - Fuller attempted to improve Parker's definition of probiotic with the following distinction: "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance."

Probiotics: The 1st definition

“The dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes.”

Elie Metchnikoff, 1907



*Elie Metchnikoff
1845 - 1916*

Probiotic Criteria

- A probiotic (generally bacteria or yeast):
 - a microorganism not harmful to human and animal
 - remains viable during processing and shelf life
 - must survive digestion and remain viable in the gut
 - able to bring about a response in the gut
 - associated with health benefits.



Safety of Probiotics

Probiotics in general

- Lactic acid bacteria - history of use in food
- No evidence of pathogenicity
- No problems with transfer of antibiotic resistance

“The benefits of probiotic bacteria such as *Lactobacillus* & *Bifidobacterium* outweigh any potential danger of sepsis” Hammerman et al (2006). BMJ.

Yakult and *Lactobacillus casei* Shirota

- Strain isolated in 1930.
- Consumed > 70 years
- FOSHU (Food for Specified Health Uses)
- >25 million bottles/day drunk in 32 countries
- Safe use in clinical trials



Safety of Probiotics

Organism	Infection potential
<i>Lactobacillus</i>	Mainly nonpathogens; a few opportunistics reported in AIDS patients
<i>Lactococcus</i>	Mainly nonpathogens
<i>Streptococcus</i>	Opportunistics; only <i>S. thermophilus</i> is used in dairy products.
<i>Enterococcus</i>	Opportunistics; some strains exhibit antibiotic resistance.
<i>Bacillus</i>	Only <i>B. subtilis</i> , GRAS status, is reported in probiotics use.
<i>Bifidobacterium</i>	Mainly nonpathogens; some strains are isolated from human infection.
<i>Propionibacterium</i>	Dairy propionibacterial group is a potential candidate for probiotics.
<i>Saccharomyces</i>	Mainly nonpathogens; some strains are isolated from human infection.

Source: Adapted from Donohue and Salminen, 1996.

So why are 'probiotics' so good?

- **Keeping your gut healthy**

- The **probiotics** within your gut help reduce and neutralize some of the more harmful substances of digestion. They also help to discourage the growth of potentially harmful bacteria.

- **Helping your gut to function properly**

- Probiotics help stimulate your digestive process, aid the absorption of nutrients and digest food. Good bacteria help keep your gut moving and your bowels functioning regularly.

- **Supporting your body's natural defenses**

- Did you know that 70% of your immune system is located within your gut? Probiotics help support your immune system in recognizing and dealing with harmful microbes.

Probiotic Health Benefits

- **Gastrointestinal Health**

- Antibiotic-associated diarrhea
- Functional bowel disorders
- Constipation/regularity
- Inflammatory bowel diseases
- Irritable bowel syndrome
- Rotavirus diarrhea in infants
- Traveler's diarrhea
- Other diarrheal infections

- **Immune Health**

- Development of immune system (e.g., stimulating natural killer cell activity)
- Respiratory tract infections
- Atopic diseases

- **Vaginal Infections**

- **Oral Health**

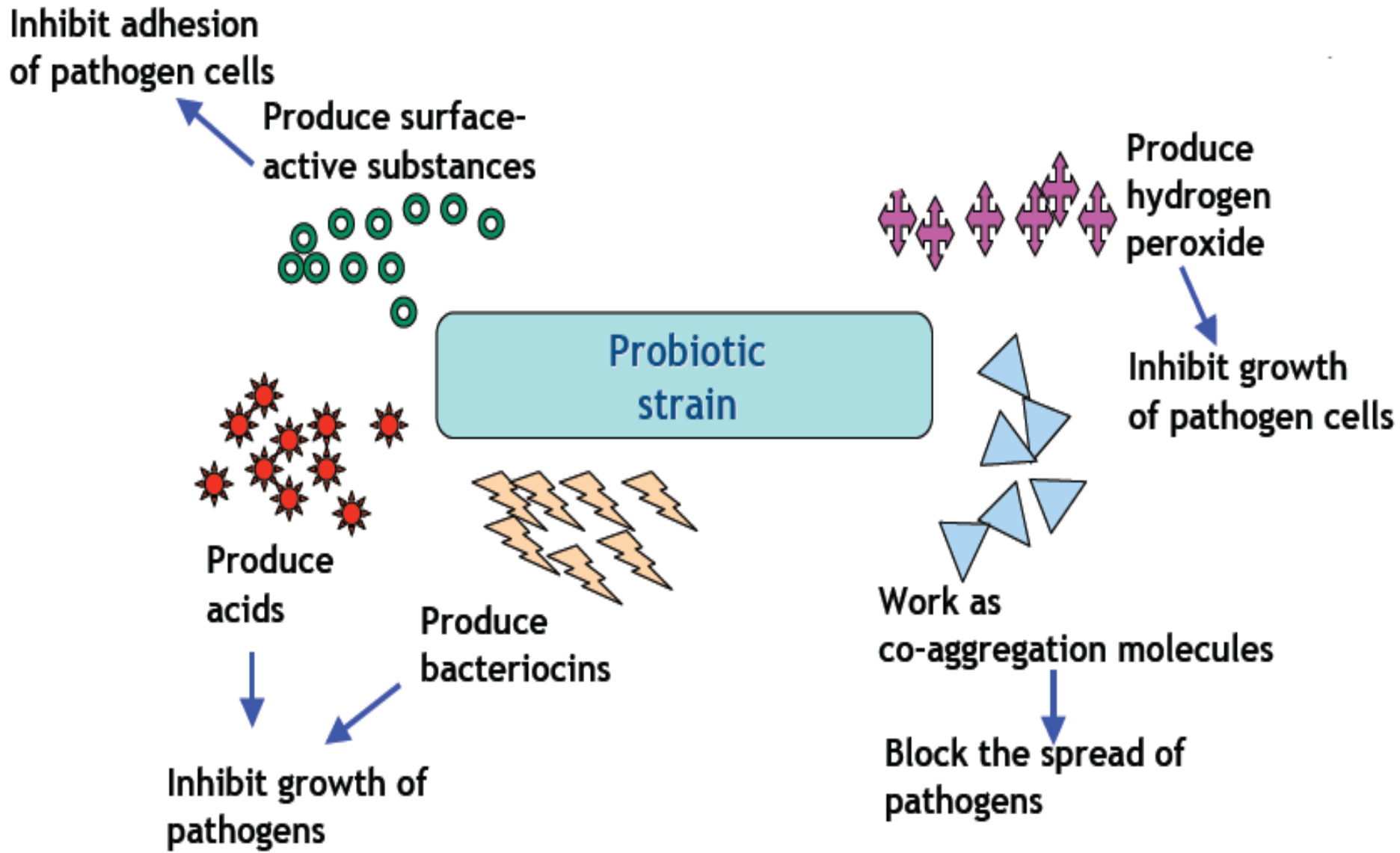
- **Weight Management**

- **Lactose Intolerance**

- **Cholesterol and plasma triglycerides**



Probiotics – Mechanisms of Action in the Gut



Probiotic Product Format Options in USA

- **Food** (e.g., Yakult, Activia™)
- **Supplement** (e.g., Culturelle™, Align™)
- **Medical Food** (VSL#3)
- **Pharmaceutical** (none for human use but IBD, pouchitis, IBS, bacterial vaginosis, antibiotic associated diarrhea are possibilities)
 - Under jurisdiction of Department of Biologics as “Biologic Drugs”

In theory, the same probiotic strain could be used in any of these formats.

Probiotic Market in the USA

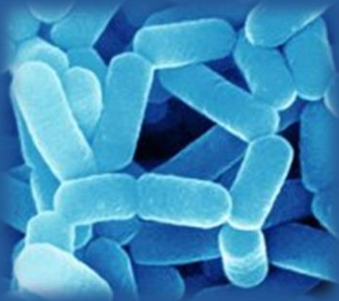
- Most people still do not understand the beneficial effects of probiotics and their contribution to health.
- Mass media awareness
- Health benefits linked with their consumption
- New probiotics products on the marketplace



Most Common Probiotics in the Market

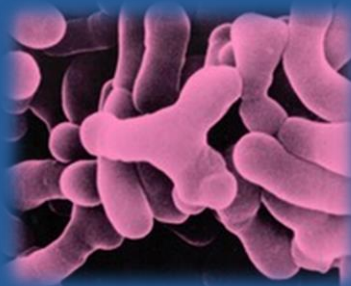
- ***Lactobacillus* sp.**

- *acidophilus*
- *casei*
- *plantarum*
- *rhamnosus*
- *reuteri*

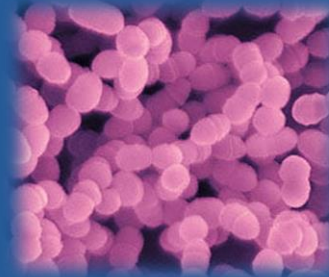


- ***Bifidobacterium* sp.**

- *bifidum*
- *breve*
- *infantis*
- *lactis*
- *longum*



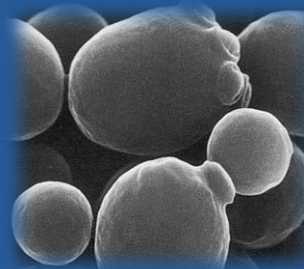
- ***Streptococcus* sp.**



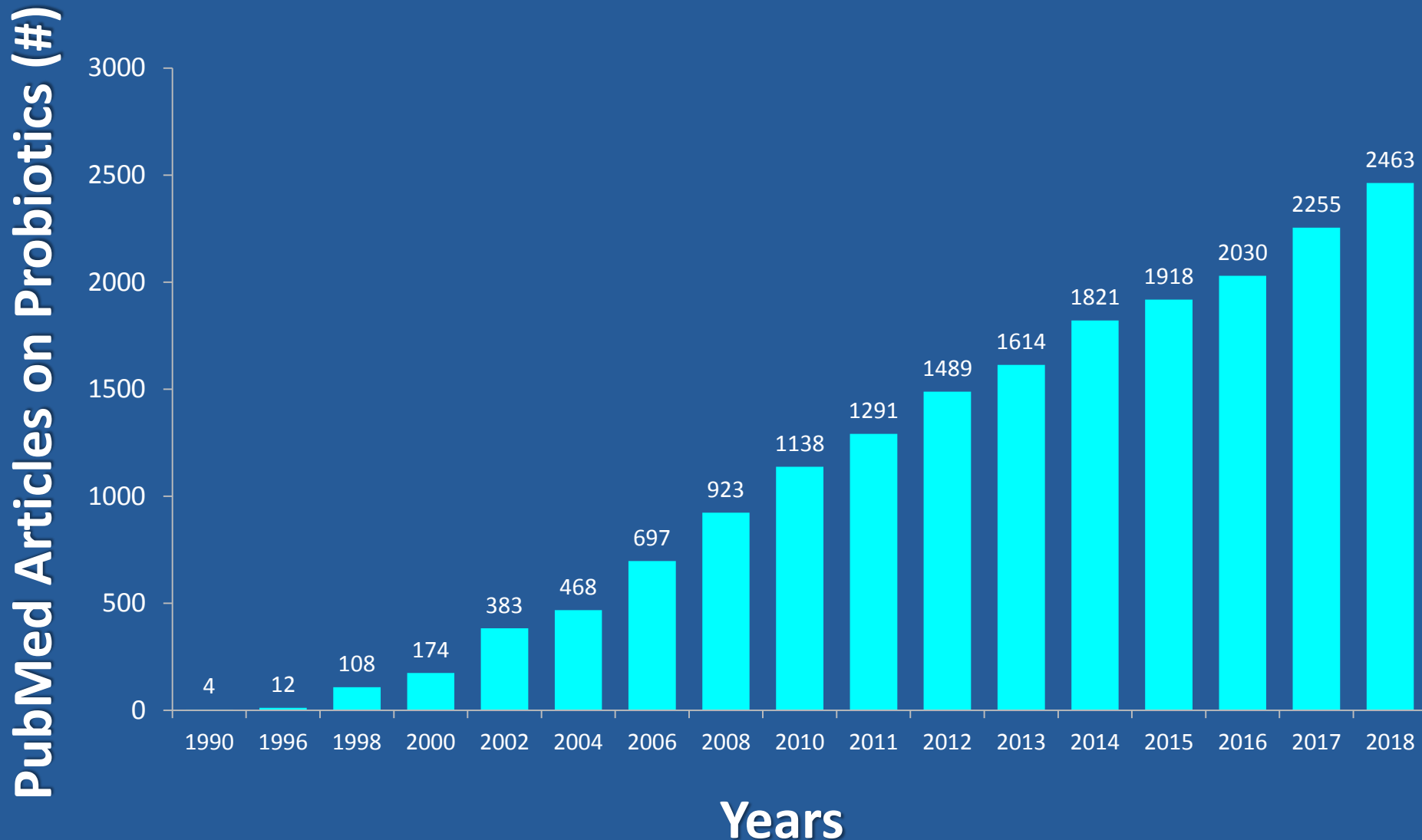
- **VSL#3**



- ***Saccharomyces boulardii***



Scientific interest in probiotics



Source: <http://www.ncbi.nlm.nih.gov/pubmed/> (Accessed on Feb 12, 2019)

Probiotic Foods vs. Supplements

- **By choosing food products containing probiotics**
 - Nutritional benefits of eating that particular food.
 - **Example:** Yogurt is a good source of calcium, riboflavin (Vit. B₂), potassium, and proteins.
- **By choosing a probiotic supplement**
 - missing out the extra nutrients important for good health.



What should probiotic labels say?

- **Identify probiotics used**

- Genus, species and strain(s) contained

<i>Bifidobacterium</i>	<i>lactis</i>	CNCM I-2494
genus	species	strain

- **What levels will be present at end of shelf life**

- based on studies showing efficacy

- **Statements of nutritional support (S/F statements)**

- Must be truthful and not misleading

- Must be based on human studies

- **Manufacturer, and contact details**

- **Directions for use and storage conditions**

- **Nutrition Facts and Supplement Facts**

Effective Dose for Probiotic Effect

Depends on strain and health effect

- **Large variations in clinical trials**
 - Type of probiotic
 - Daily dose, 10^8 to 10^{11} cfu/day
 - Supplementation frequency
 - Duration of supplementation(days to months)
 - Method of delivery
- **Few dose-response studies**



Yogurt cultures and probiotics

- Double blind prospective study
- 114 healthy young volunteers
- Fed with yogurt daily for 15 days
- **Analysis:**
 - *Lactobacillus bulgaricus*
 - *Streptococcus thermophilus*
- **Results:** Detection of yogurt bacteria was consistently negative.



Most of the standard yogurt bacteria are not probiotics unless supplemented with clinically proven probiotics...

Del Campo et al., 2005.

Some probiotic products label **species** only

Supplement Facts

Serving Size: 1 Capsules
Servings per container: 60

Amount Per Serving	% DV
Proprietary Probiotic Blend	67mg *
Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium lactis, Bifidobacterium longum, Lactobacillus acidophilus , Lactobacillus brevis, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus gasseri, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus salivarius, Lactococcus lactis, Streptococcus thermophilus.	

* Daily Value not established.

Other ingredients: Rice maltodextrin, magnesium stearate.

Supplement Facts

Serving Size: 1 Packet (1500 mg)
Servings Per Container: 60

	Amount Per Serving	% DV
Calories	6	
Total Carbohydrate	1 g	<1%
Sugars	1 g	†
Vitamin C (as ascorbic acid)	75 mg	125%
Vitamin B6 (as pyridoxine)	.4 mg	20%
Vitamin B1 (as thiamine)	.4 mg	27%
Vitamin B2 (as riboflavin)	.4 mg	23%

Probiotic Blend —
Bacillus subtilis (500 million CFU), *Enterococcus faecalis*
 (500 million CFU), *Bacillus coagulans* (500 million CFU).†

†Percent Daily Value (DV) not established.

Other Ingredients: Refined Yeast Powder, Dietary Fiber as Galactomannan,
 Fructo-oligosaccharides (FOS), Beta Cyclodextrin, Erythritol, Lemon Juice Powder, Canola Oil.

Contains an ingredient derived from milk. (12 ppm Casein)

THREELAC is Manufactured in Japan.

Supplement Facts

Serving Size 1 Capsule

Each Capsule Contains	% Daily Value
Pro-B Feminine Probiotic Strains	5 Billion cells †
Lactobacillus rhamnosus GR-1	
Lactobacillus reuteri RC-14	

†Daily Value not established.

OTHER INGREDIENTS: Dextrose anhydrate,
 Potato starch, Microcrystalline cellulose, and
 magnesium stearate.

CAPSULE INGREDIENTS: Gelatin, Titanium
 dioxide

FloraMax

Supplement Facts

Serving Size: 2 capsules
Servings per Container: 30

	Amount per Serving	% Daily Value
Proprietary Blend	200 mg	
Lactobacillus Acidophilus		†
Lactobacillus Salivarius		†
Bifidobacterium Bifidum		†
Bifidobacterium Infantis		†
Bifidobacterium Longum		†
FOS (Fructooligosaccharides)	200 mg	†
L-Glutamine	200 mg	†
N-Acetyl D-Glucosamine	50 mg	†

† Daily value not established

* Percent Daily Values are based on a 2000 calorie diet.

Other Ingredients: rice flour (400 mg per 2 capsules) and
 gelatin capsule (gelatin and water)

Recommended Usage: Take 2 capsules a day.

Contains approximately 6 billion organisms per capsule at
 time of manufacture.

Contains 120 million organisms per capsule at time of
 expiration if properly stored.

Supplement Facts

Serving Size: 1 capsule

Amount per Capsule	% Daily Value	
Vitamin C (as Ascorbic Acid and Ascorbyl Palmitate)	60 mg	100%
Cranberry Solids (from Cranberry Puree)	500 mg	*
FOS	70 mg	*
Probiotic culture	50 mg	*

(*Lactobacillus acidophilus*, *Lactobacillus
 casei rhamnosus*, *Lactobacillus bulgaricus*,
Bifidobacterium longum, *Bifidobacterium bifidum*,
 FOS - 1 billion CFU)

* Daily Value not established for this ingredient

Nutrition Facts: Serv Size 1 mint (0.5g) Calories 0,
 Total Fat 0g (0% DV), Sodium 0g (0% DV), Total Carbohydrate
 0g (0% DV), Sugars 0g, Protein 0g. Not a significant source of
 calories from fat, saturated fat, cholesterol, dietary fiber, sugars,
 vitamin A, vitamin C, calcium and iron. Percent Daily Values (DV)
 are based on a 2,000 calorie diet.

Ingredients: Xylitol, Natural Vanilla, ProBiora3™ (*S. oralis* KJ3™, *S. uberis* KJ2™,
S. ratti JH145™ - 300 Million CFU*), Natural Spearmint, Cellulose.

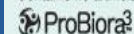
* At time of manufacture.

Directions: Use twice daily. For best results, use after brushing teeth and allow
 the probiotic mint to completely dissolve in the mouth while touching all tooth
 and gum surfaces.

Do not use if safety seals are punctured or broken. Store in a cool, dry place.

Sugar Free • Gluten Free • Dairy Free • Soy Free

Contains no artificial ingredients.



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Conclusions

- Gut microbiota play an important role in human health, especially with regard to immune system development and resistance to pathogens
- Probiotics likely use same mechanisms to impact health as commensals, either directly or indirectly by influencing populations or activities of commensal bacteria
- Accumulation of patient-oriented evidence showing health benefits for probiotics
- Effects are strain-, dose- and matrix-specific
- Marketplace: mixed bag
 - Disconnect between what is studied in published research and what is available commercially, especially in US
 - Some well-validated, properly labeled products

Useful Probiotics Links

- International Scientific Association for Probiotics and Prebiotics (ISAPP): <https://isappsience.org/>
- International Probiotics Association: <http://www.internationalprobiotics.org/>
- US National Institutes of Health, National Center for Complementary and Alternative Medicine: <http://nccam.nih.gov/health/probiotics/>
- Clinical Guide to Probiotic Products Available in USA, http://usprobioticguide.com/?utm_campaign=USA_CHART
- National Dairy Council: <http://www.nationaldairyCouncil.org/NationalDairyCouncil/Health/Digest/dcd76-1Page1.htm>
- **FAO/WHO: The Food and Agricultural Organization and The World Health Organization**
 - Probiotics in Food: <http://www.fao.org/3/a-a0512e.pdf>
 - Guidelines for the Evaluation of Probiotics in Food https://www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf
 - Regulatory and clinical aspects of dairy probiotics: <ftp://ftp.fao.org/es/esn/food/Reid.pdf>

Thank you for your attention!