PROBIOTICS, PROBIOTIC TECHNOLOGY

Wilbert Sybesma

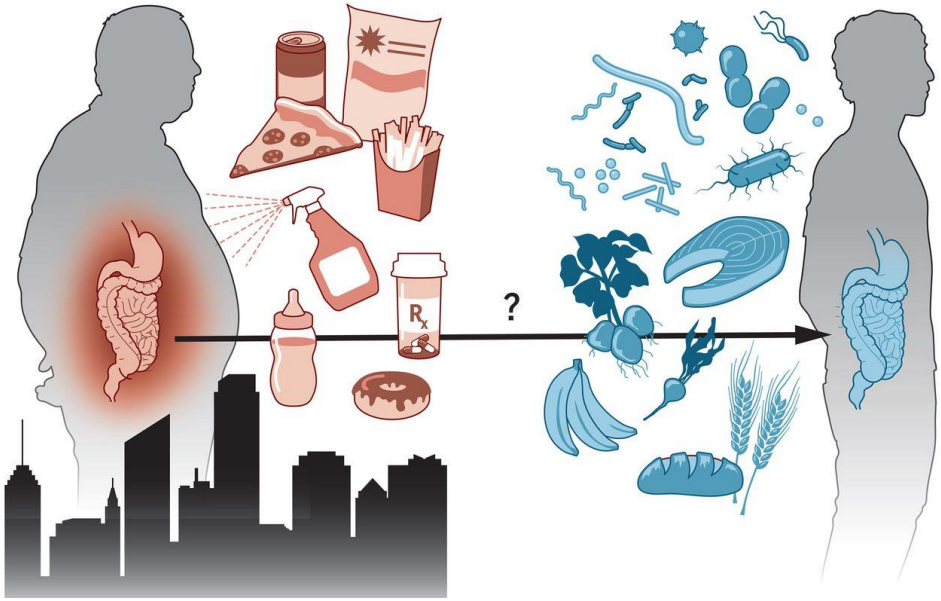
EPFL Food Biotech

WE ARE MADE UP OF MORE BACTERIA THAN THE BODY'S OWN CELLS. MOST BACTERIA ARE FOUND IN TI

In general, we can say that the more diverse the composition of our microbiome, the more resistant we are to bad external influences

By increasing diversity, you increase RESILIENCE

INDUSTRIALIZATION AFFECTS THE HUMAN GUT MICROBIOTA



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Published: 27 July 2017

The theory of disappearing microbiota and the epidemics of chronic diseases

Martin J. Blaser

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HOME › SCIENCE › VOL. 366, NO. 6464 › VULNERABILITY OF THE INDUSTRIALIZED MICROBIOTA

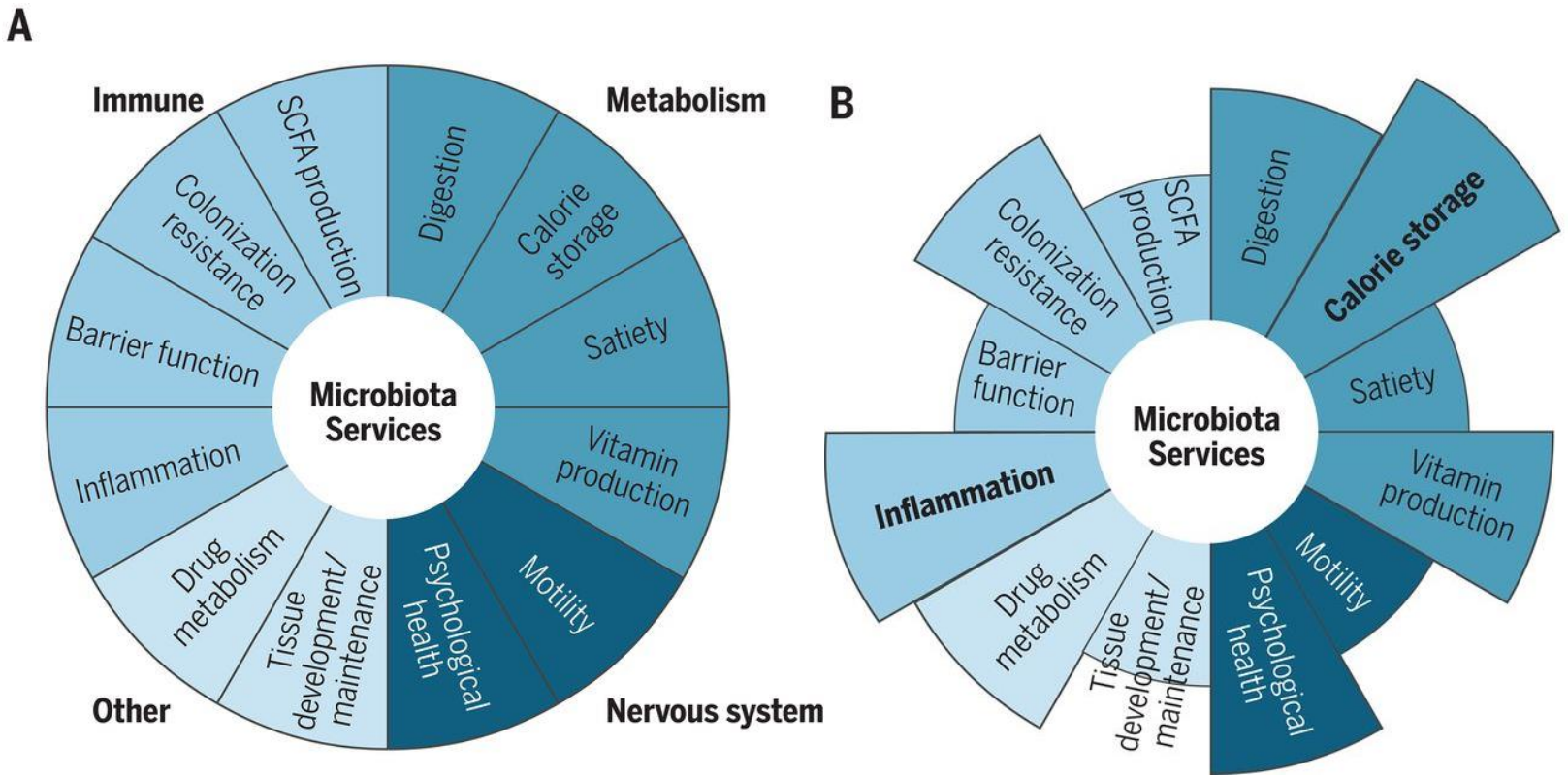
REVIEW

Vulnerability of the industrialized microbiota

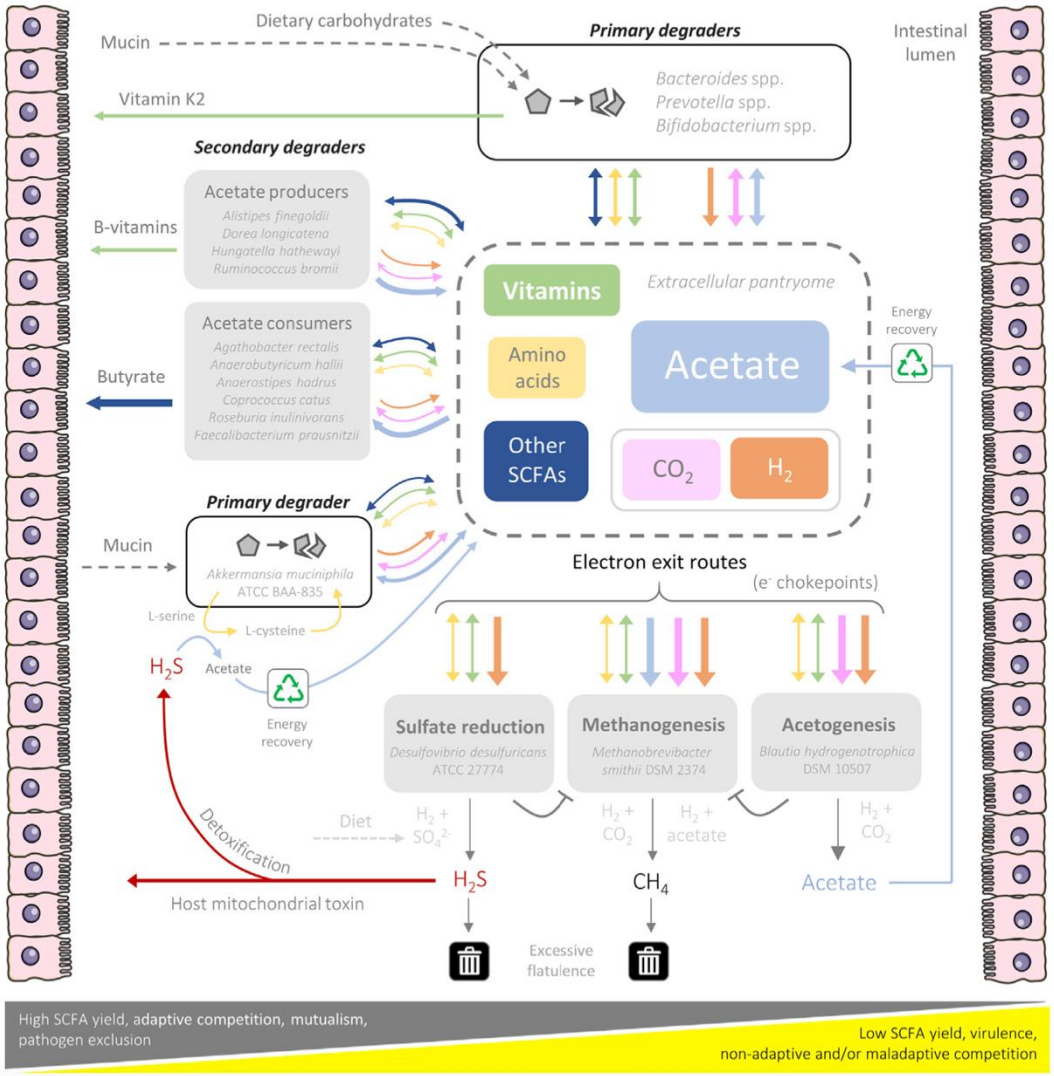
JUSTIN L. SONNENBURG AND ERICA D. SONNENBURG Authors Info & Affiliations

SCIENCE · 25 Oct 2019 · Vol 366, Issue 6464 · DOI: 10.1126/science.aaw9255

WHAT ARE MICROBIOTA SERVICES?



A COMPLEX AND DIVERSE ECOSYSTEM IS BASED ON MANY COLLABORATING MICROORGANISMS



Daisley BA, Koenig D, Engelbrecht K, Doney L, Hards K, Al KF, Reid G, Burton JP. Emerging connections between gut microbiome bioenergetics and chronic metabolic diseases. Cell Rep. 2021 Dec 7;37(10):110087. doi: 10.1016/j.celrep.2021.110087. PMID: 34879270.

A 'pact' between humans and microbes

We ……

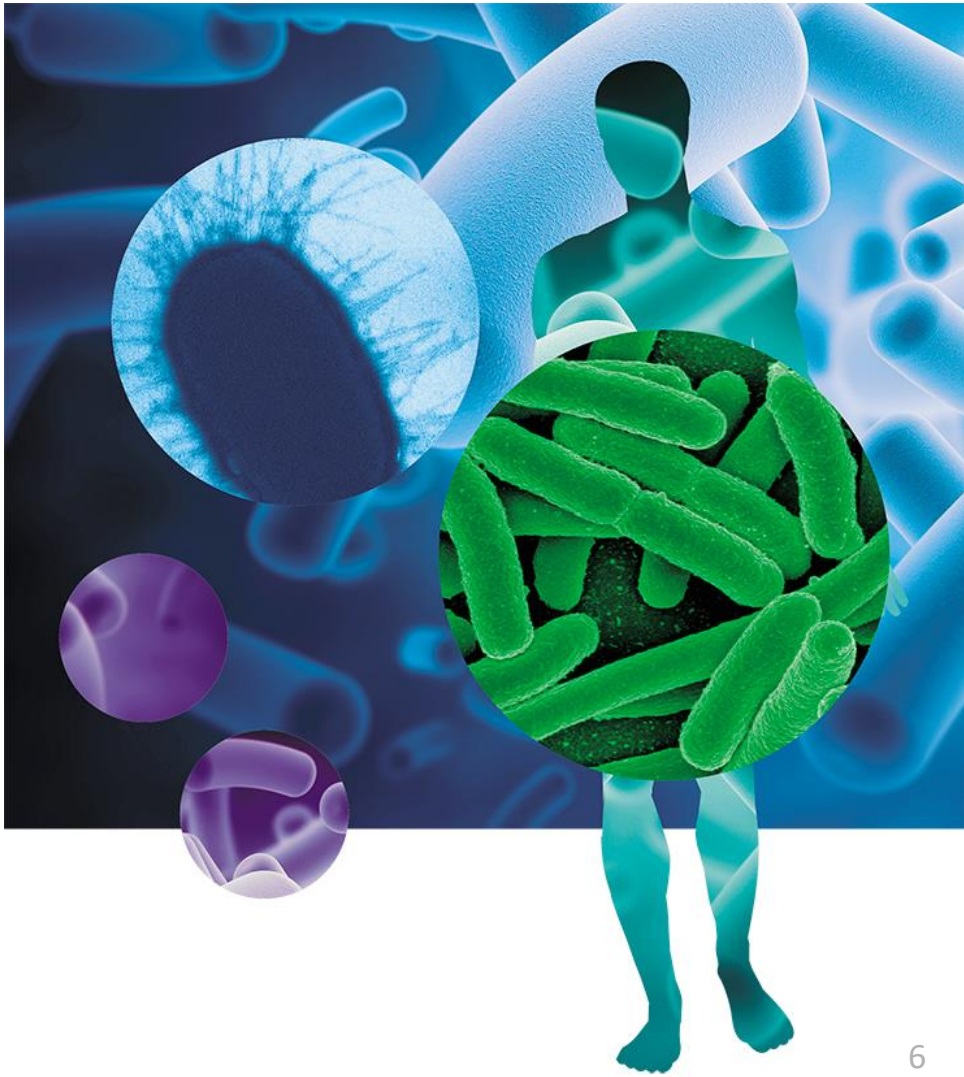
1. Give the bacteria give our body, as a 'home' to live in

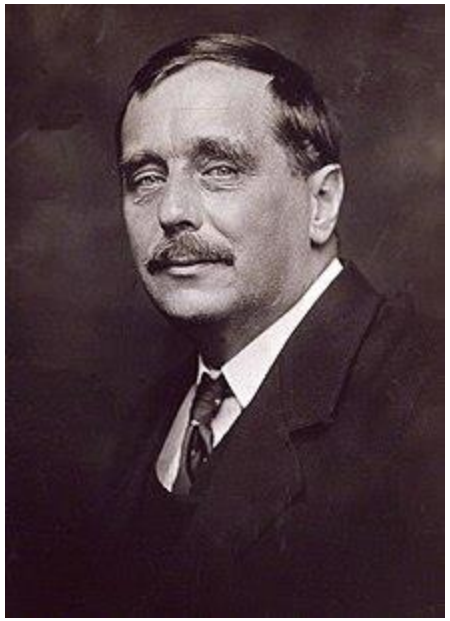
2. Feed the bacteria

They. ……

3. Protect us from bad bacteria

4. Strengthen our immune system.



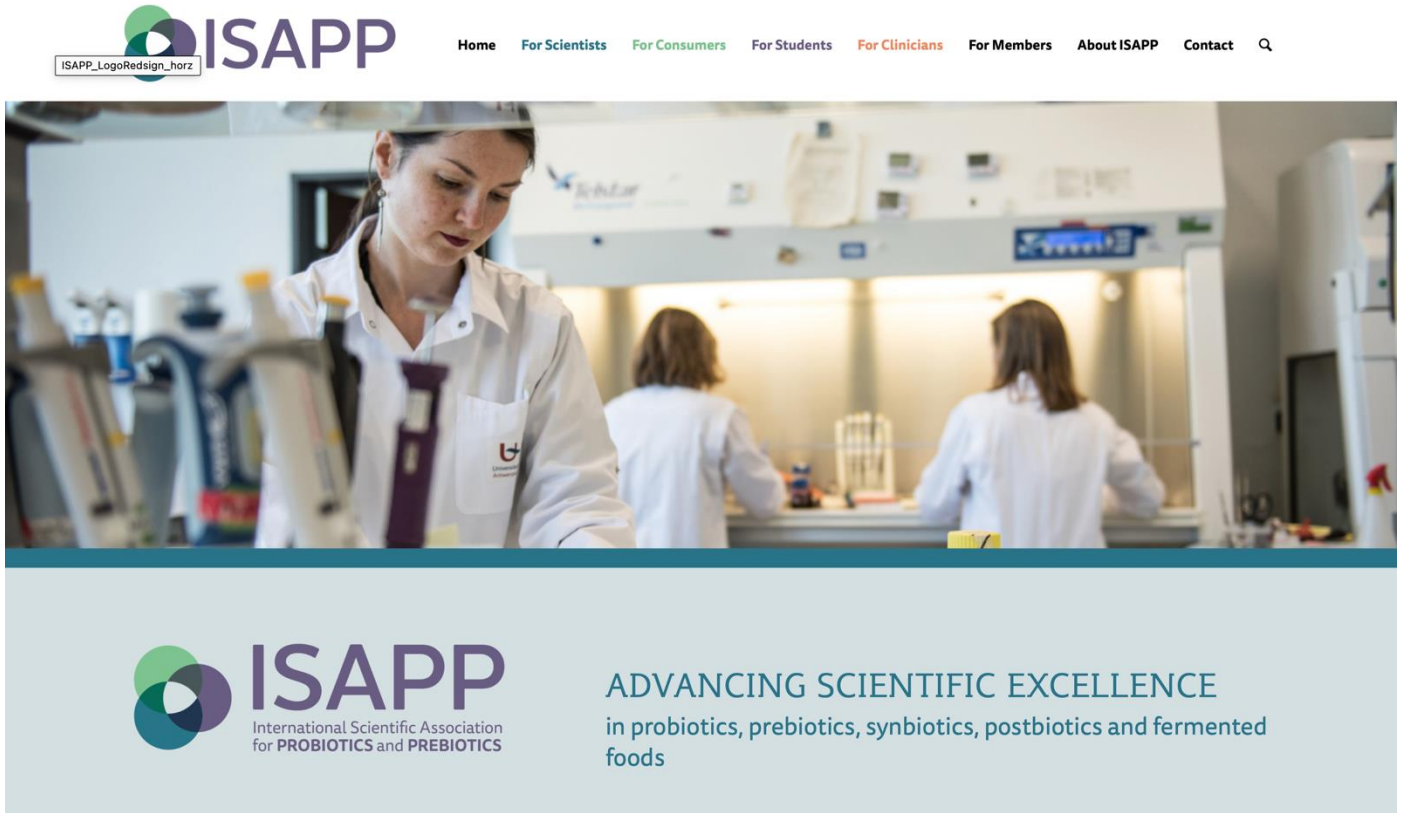


Herbert George Wells
(21 September 1866 – 13 August 1946)

INTERESTING SHORT MOVIES PROBIOTICS, PREBIOTICS

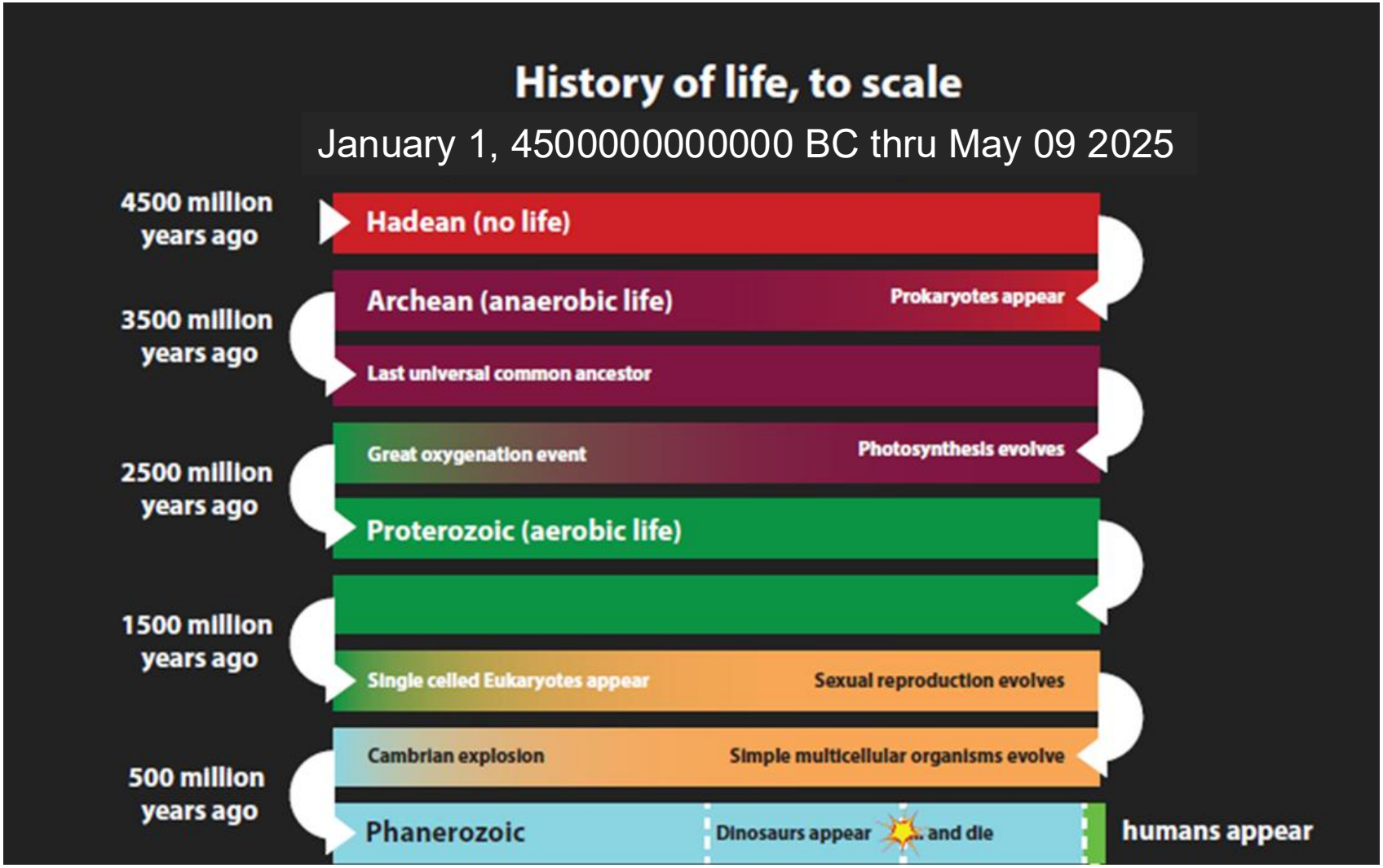
https://isappscience.org/for-consumers/videos/

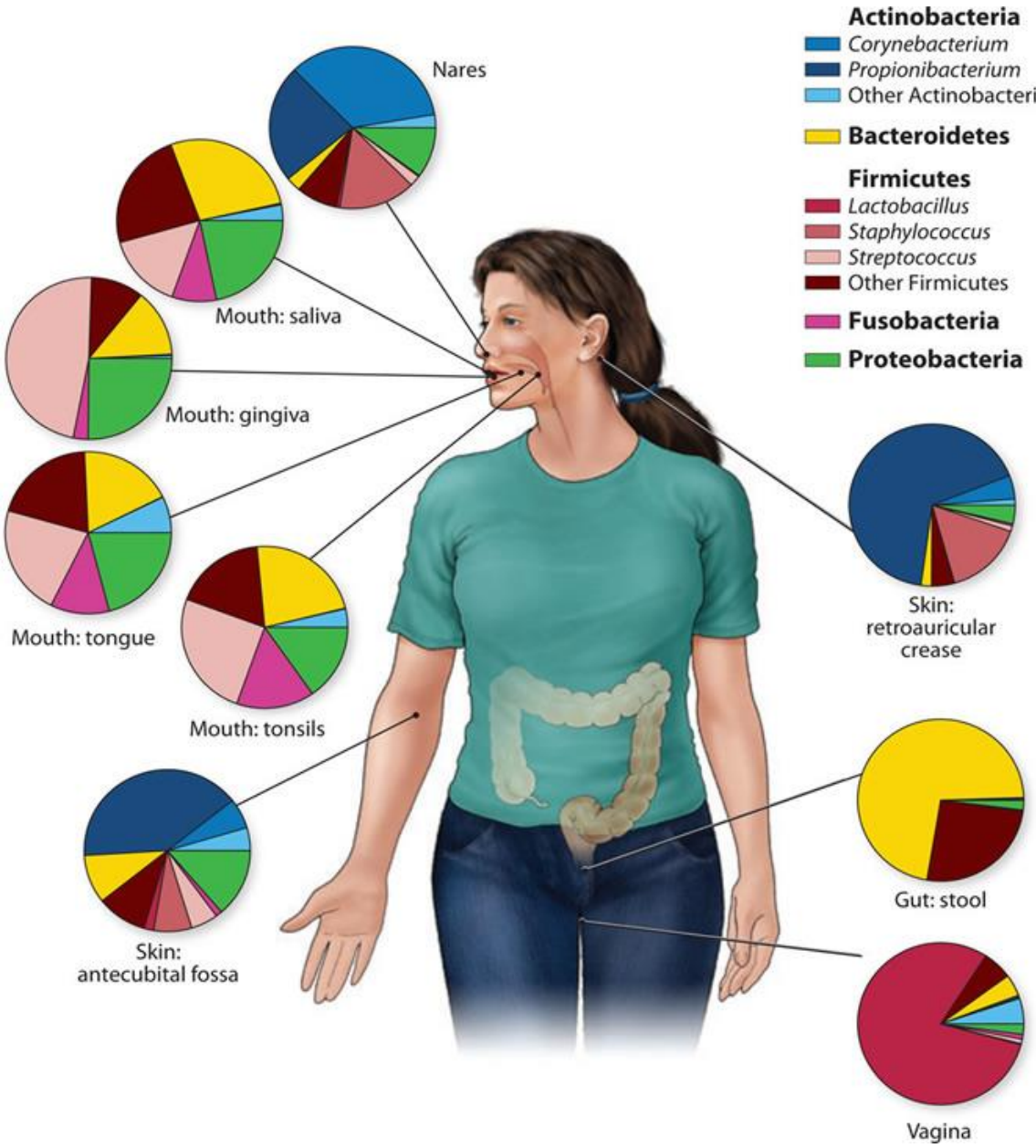
General:
https://isappscience.org/for-consumers/infographics/

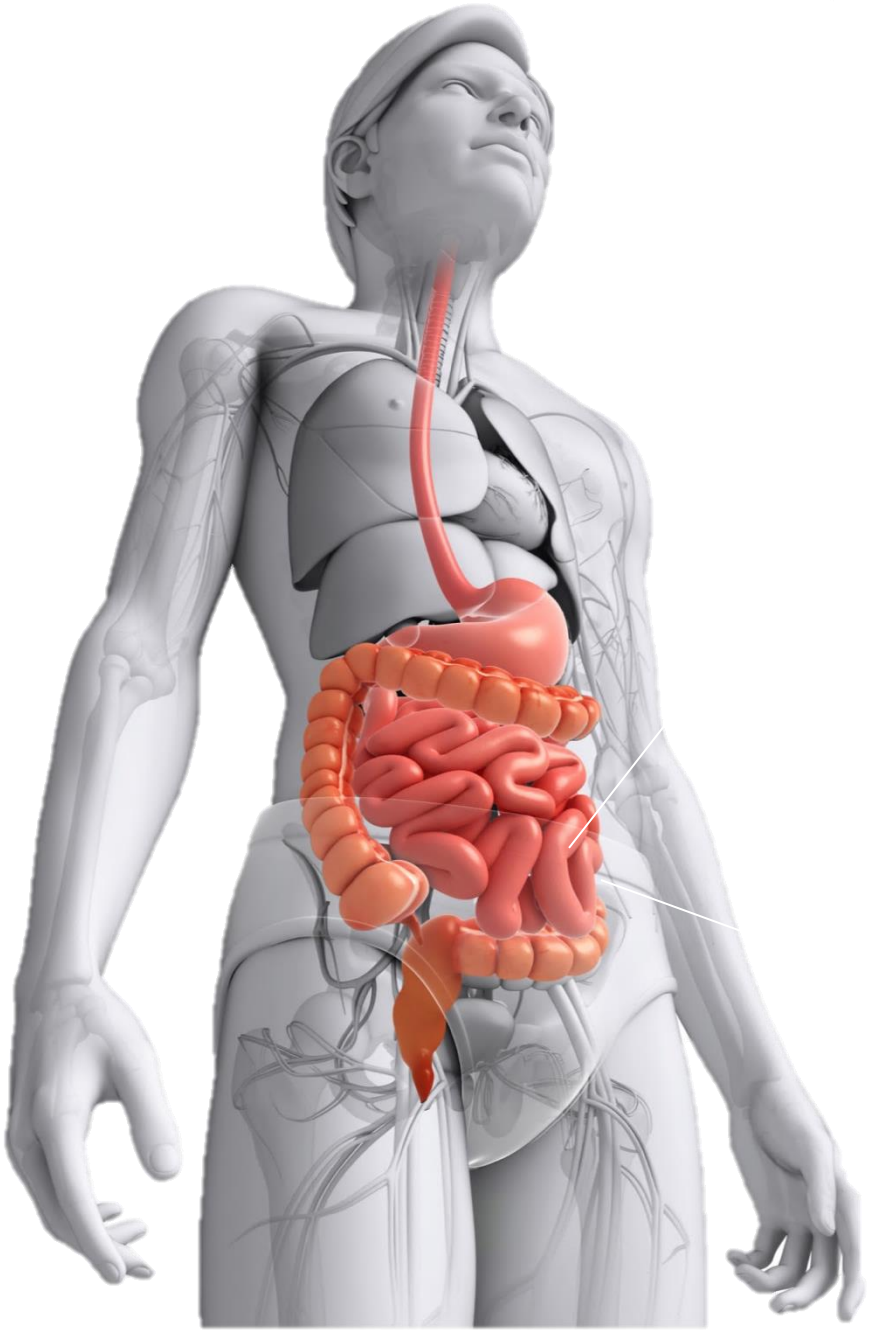


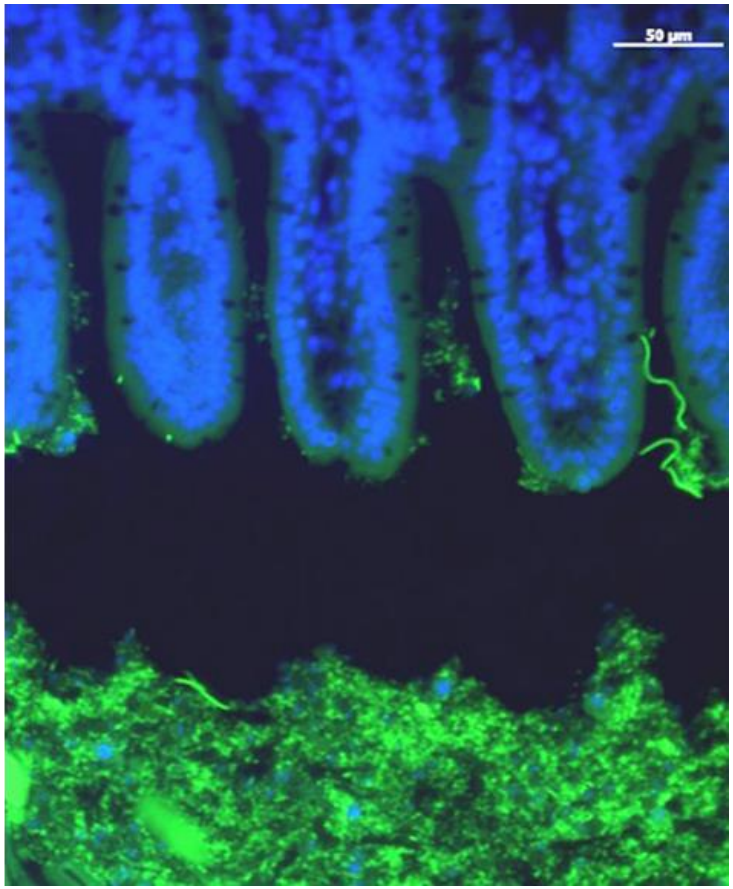
CONTENT

- How it begun
- Probiotics (and prebiotics, synbiotics, postbiotics)
- Probiotic production technology









Microbiome

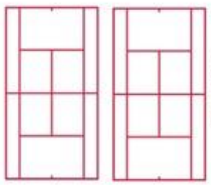
IN NUMBERS

100 Trillion
symbiotic microbes live in and on every person and make up the human microbiota

The human body has more microbes than there are stars in the milky way

95%
of our microbiota is located in the GI tract

150:1
The genes in your microbiome outnumber the genes in our genome by about 150 to one

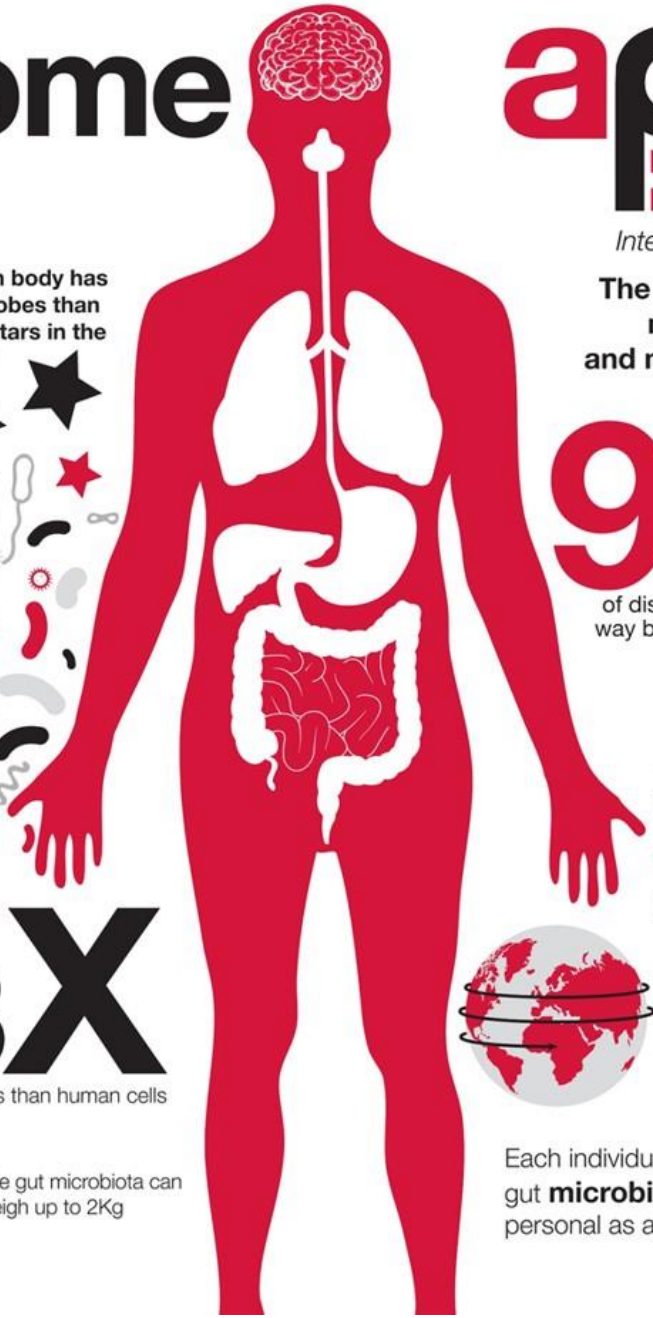


The surface area of the **GI tract** is the same size as 2 tennis courts

You have 1.3X
more microbes than human cells

>10,000
Number of different microbial species that researchers have identified living in and on the human body

2kg
The gut microbiota can weigh up to 2Kg



apc Microbiome Ireland
Interfacing Food & Medicine

The microbiome is more medically accessible and manipulable than the human genome

90%
It is thought that of disease can be linked in some way back to the gut and health of the microbiome

5:1
Viruses:Bacteria
in the gut microbiota

2.5
The number of times your body's microbes would circle the earth if positioned end to end

Each individual has a unique gut **microbiota**, as personal as a fingerprint

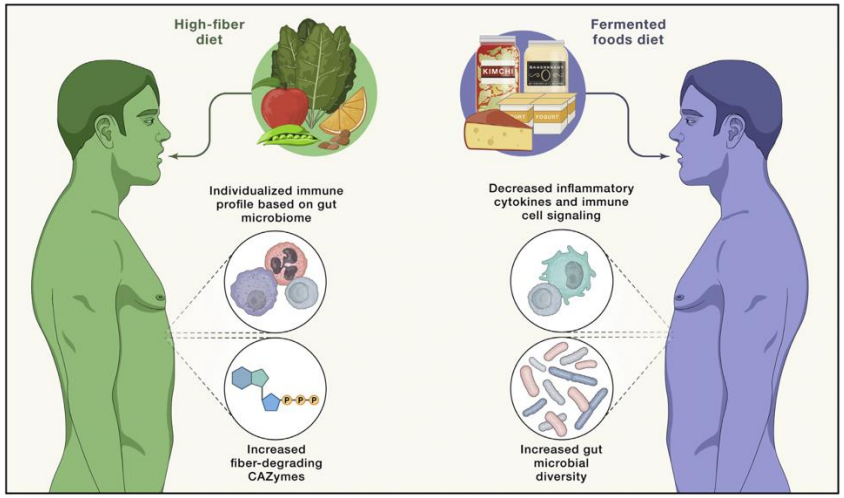
IMAGINE… NO IMMUNE SYSTEM

https://www.time-lapse-footage.com/video-clip-944/rotting-hamburger-decomposition-timelapse
https://www.youtube.com/watch?v=Vl8z-PFGHtk

OPTIONS FOR VALUE CREATION WITH PROBIOTICS AND/OR FERMENTED FOOD

Probiotics Reason Why

FERMENTED FOOD DIET INCREASES MICROBIOME DIVERSITY AND REDUCES INFLAMMATORY PROTEINS



Cell

CellPress

Article

Gut-microbiota-targeted diets modulate human immune status

Hannah C. Wastyk,[2,7] Gabriela K. Fragiadakis,[1,7] Dalia Perelman,[3] Dylan Dahan,[1] Bryan D. Merrill,[1] Feiqiao B. Yu,[5] Madeline Topf,[1] Carlos G. Gonzalez,[4] William Van Treuren,[1] Shuo Han,[1] Jennifer L. Robinson,[3] Joshua E. Elias,[5] Erica D. Sonnenburg,[1,6,*] Christopher D. Gardner,[3,*] and Justin L. Sonnenburg[1,6,8,*]

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https://doi.org/10.1016/j.cell.2021.06.019

Overall microbial diversity increases by eating yogurt, kefir, fermented cottage cheese, kimchi and other fermented vegetables, plant-based brine drinks, and kombucha tea, with larger portions having a more substantial effect.

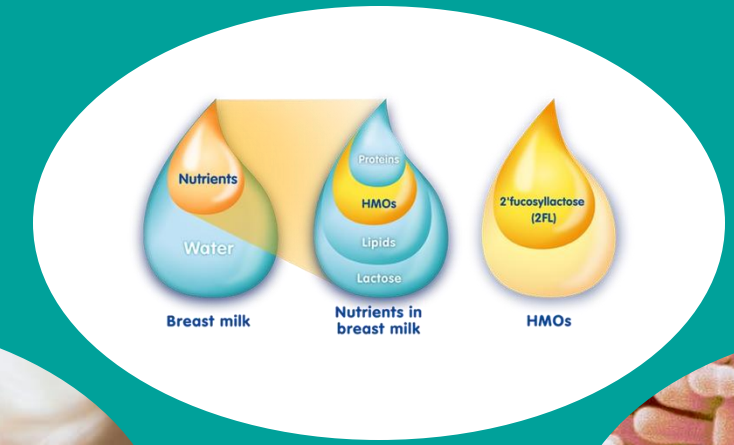
We get good bacteria through

- *Birth canal*
- *Human milk*
- *Our Nutrition*
- *Our environment*

The composition of our microbiome is negatively affected by

- *Too many sugars and too little fiber*
- *Alcohol*
- *Pesticides*
- *Too much fatty meat*
- *Antibiotics / Medication*
- *Too much stress*

Through human milk we get many healthy lactobacillus and bifidus bacteria.
And, part of human milk is made up of fiber to feed these bacteria



‘BIOTICS’ BETTER EXPLAINED

Prebiotics

Non-digestible fiber compounds that stimulate the growth and activity of beneficial gut microorganisms.

Probiotics

Live microorganisms that inhabit the microbiome and confer health benefits when consumed in sufficient amounts.

Synbiotics

A combination of prebiotics and probiotics.

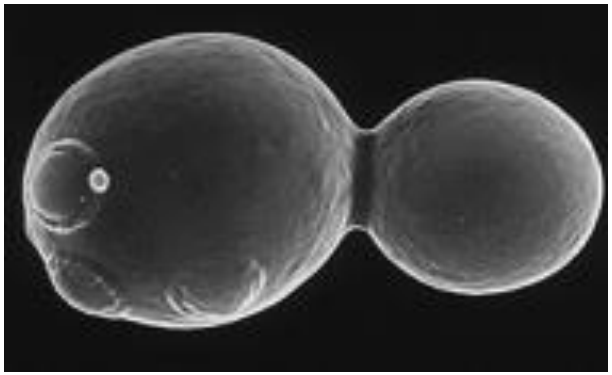
Postbiotics

Products of prebiotic and probiotic activity that mimic some of the same benefits as probiotics, but also offer additional health benefits.

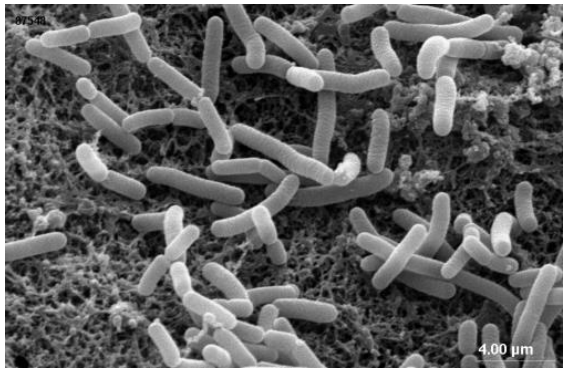
How do we define probiotics today?

"Probiotics are live micro organisms which when administered in adequate amounts confer a health benefit on the host"

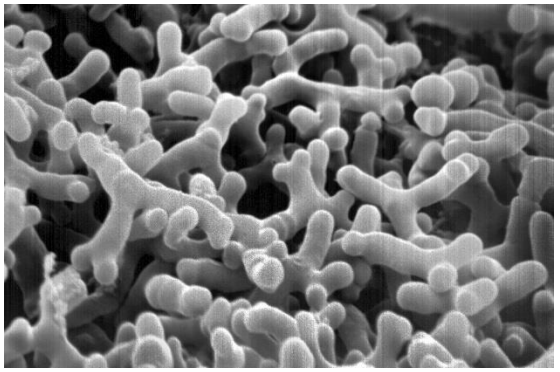
Source: WHO/FAO expert group



Yeast



Lactobacilli

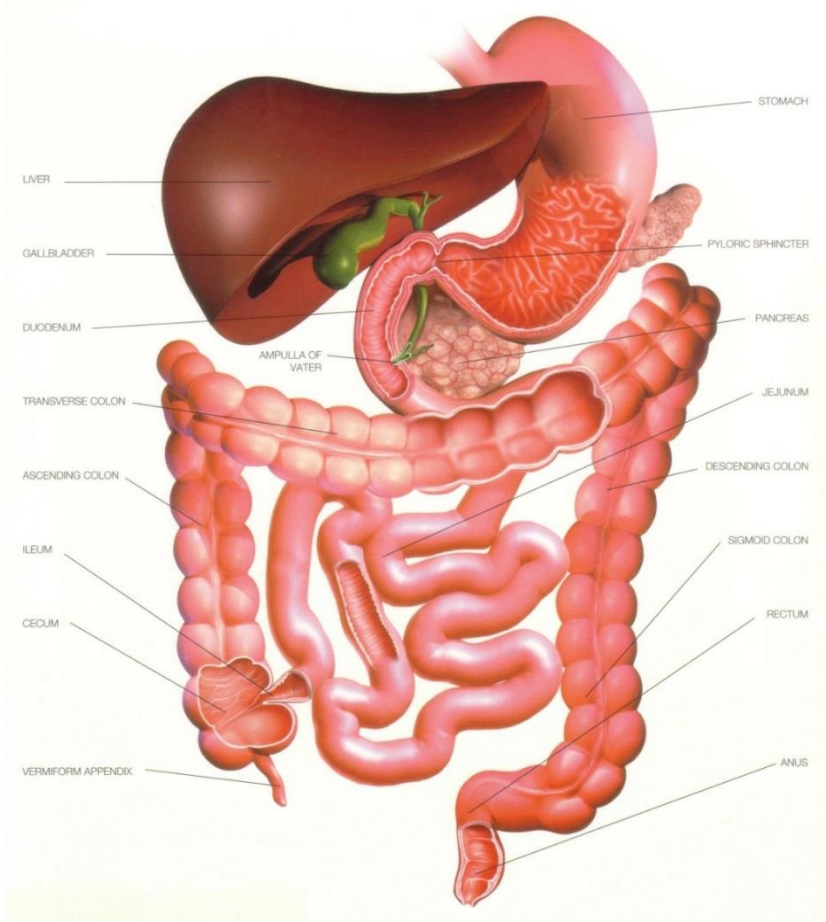


Bifidobacteria

- Typically **Lactobacilli** or **Bifidobacteria**, but also **Enterococci** or **Bacilli** (plus others). Some **yeasts** are also probiotic.

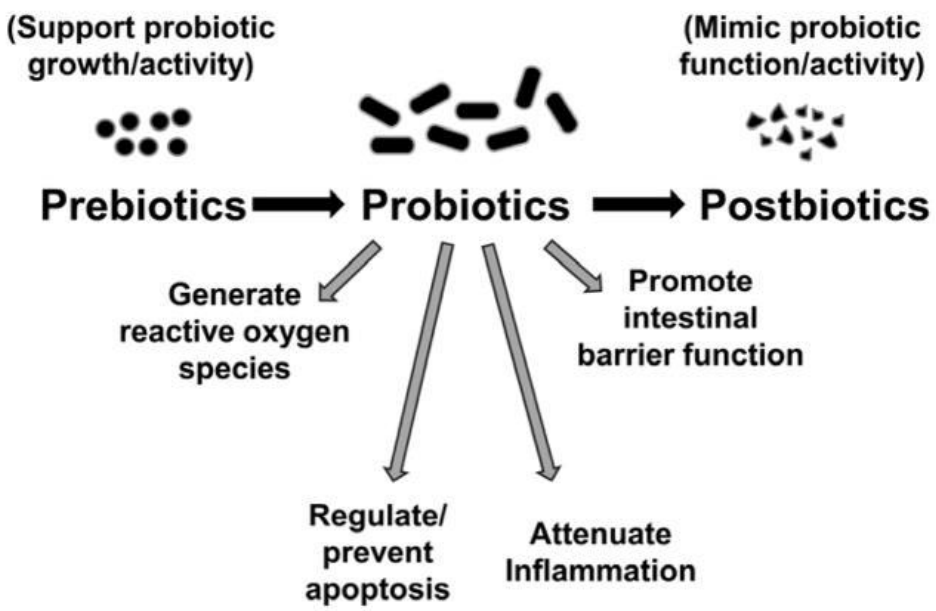
  Note: not all Lactobacilli and Bifidobacteria are probiotic
- They colonize the GIT (gastro intestinal tract) only temporarily

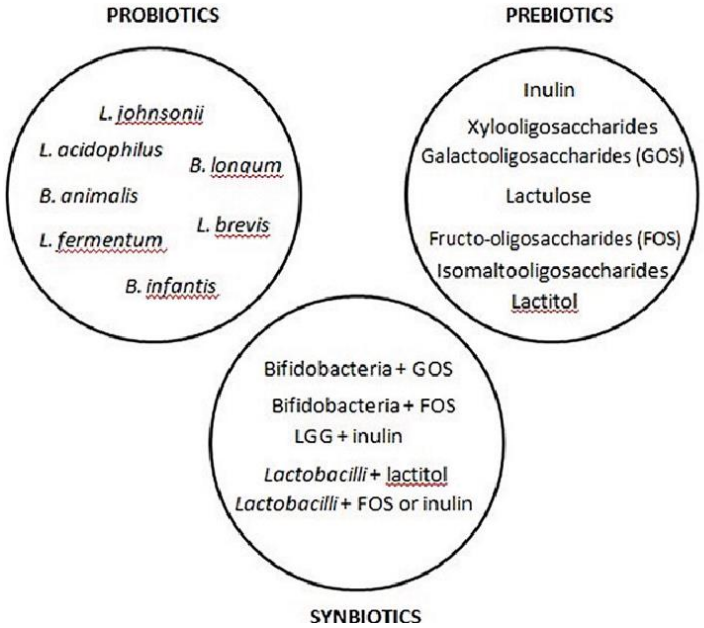
DEFINITIONS
PROBIOTIC, PREBIOTIC, SYMBIOTIC



- Probiotic:
  - Specific exogenous health beneficial bacteria
- Prebiotic:
  - Specific ingredient (oligosaccharides) non digestible by the host but able to support the growth of beneficial endogenous bacteria
- Symbiotic:
  - Combination of a probiotic and a prebiotic
  - Taillor made substrate for the probiotic

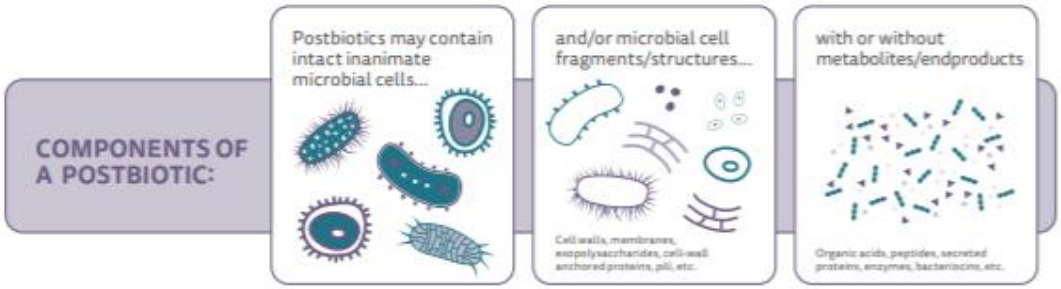
Good source of information: https://isappscience.org/for-consumers/infographics/


(Support probiotic growth/activity)
(Mimic probiotic function/activity)
Prebiotics
Probiotics
Postbiotics
Generate reactive oxygen species
Promote intestinal barrier function
Regulate/ prevent apoptosis
Attenuate Inflammation


PROBIOTICS
L. johnsonii
L. acidophilus
B. longum
B. animalis
L. brevis
L. fermentum
B. infantis
PREBIOTICS
Inulin
Xylooligosaccharides
Galactooligosaccharides (GOS)
Lactulose
Fructo-oligosaccharides (FOS)
Isomaltooligosaccharides
Lactitol
Bifidobacteria + GOS
Bifidobacteria + FOS
LGG + inulin
Lactobacilli + lactitol
Lactobacilli + FOS or inulin
SYNBIOTICS

Postbiotics

A postbiotic is a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.



POSTBIOTIC:

- ✓ Derived from microorganisms, but a postbiotic does not have to be derived from a probiotic
- ✓ A deliberate process to terminate cell viability must be applied. The final postbiotic must contain inactivated microbial cells and/or metabolites or cell components
- ✓ Negligible number of viable cells remaining in final product
- ✓ Evidence of a health benefit in the target host
- ✓ Assessment of safety of the postbiotic preparation for the intended use

NOT POSTBIOTIC:

- ✗ Viruses, including bacteriophages
- ✗ Vaccines
- ✗ Filtrates without cell components
- ✗ Purified microbial components (e.g., proteins, peptides, exopolysaccharides)
- ✗ Purified microbial metabolites (e.g., organic acids)

THE POSTBIOTIC DEFINITION EXPLAINED:

Postbiotic is derived from "biotic", relating to living organisms, and "post", meaning after (life).

Preparation recognizes that the specific formulation, including microbial biomass, matrices, and inactivation methods, may play a role in the beneficial effect.

Inanimate recognizes that the terms 'dead' or 'inactive', may suggest an inert material, rather than a material capable of conferring a health benefit.

Components recognizes that health effects may be mediated by a variety of different cell parts or metabolites.

INTERNATIONAL SCIENTIFIC ASSOCIATION FOR PROBIOTICS AND PREBIOTICS

For more information visit **ISAPPscience.org** or follow us on Twitter **@ISAPPscience**

Salminen, et al. Nat Rev Gastroenterol Hepatol (2021). https://www.nature.com/articles/s41575-021-00440-6

https://isappscience.org/for-consumers/infographics/

Examples of Postbiotics

a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host

https://www.theakkermansiacompany.com/

https://www.lacteol.com/

L-137™
Heat-treated Lactobacillus

https://immuno-lp20.com/

24

L-137®

Contains the heat treated, 80°C 20 min, Lactobacillus plantarum strain L-137, first isolated from Narezushi.
> 10 clinical, in-vivo, in vitro studies

L-137™
Heat-treated Lactobacillus

Immuno-LP20
Self-Affirmed GRAS/NDI

MCLS EUROPE

House Wellness Foods
HK L-137 Evidence Center

House

Effects on anti-periodontal diseases

1. Oral Health Prev Dent. 2016; 14(3):207-14. Daily Intake of Heat-killed Lactobacillus plantarum L-137 Decreases the Probing Depth in Patients Undergoing Supportive Periodontal Therapy.

Anti-cold/flu effects in healthy subjects with high levels of psychological stress

2. J Nutr Sci. 2013; 2: e39. Oral intake of heat-killed Lactobacillus plantarum L-137 decreases the incidence and severity of upper respiratory tract infection in healthy subjects with high levels of psychological stress.

Effects on immune functions and health-related QOL in healthy human subjects

3. J Nutr. 2006; 136(12): 3069-73. Daily intake of heat-killed Lactobacillus plantarum L-137 augments acquired immunity in healthy adults.

Effects on anti-inflammation and lipid metabolism in overweight healthy adults

4. Eur J Nutr (2019). Daily intake of heat-killed Lactobacillus plantarum L-137 improves inflammation and lipid metabolism in overweight healthy adults: a randomized-controlled tri

"*Lactobacillus plantarum* strain L-137"

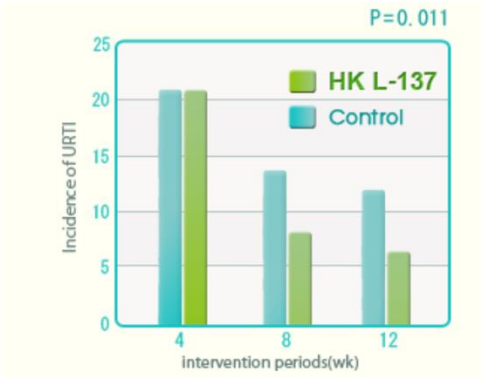
Narezushi is fermented fish pickled with rice

25

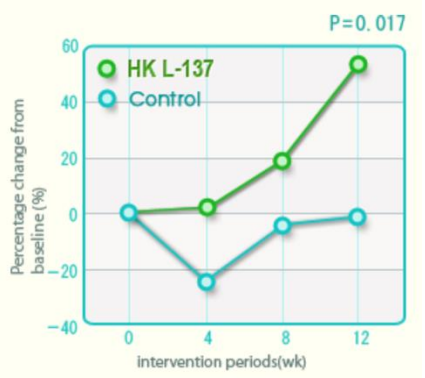
L-137

Heat-treated Lactobacillus

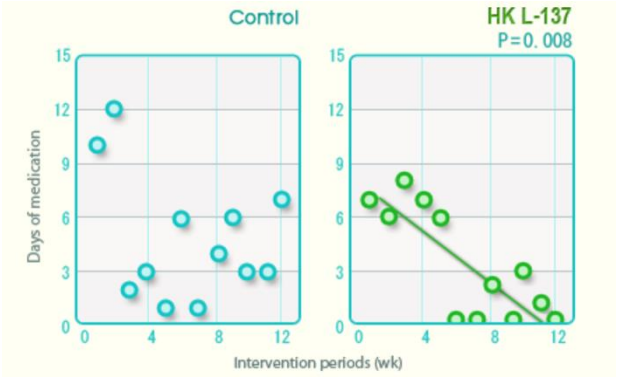
DAILY INTAKE OF HK L-137 AUGMENTS CELLULAR IMMUNITY, THEREBY DECREASING THE INCIDENCE AND SEVERITY OF URTI IN HEALTHY SUBJECTS.



The incidence of URTI in the HK L-137 group was significantly lower than that in the control group (P=0.011).



T cell proliferation was significantly higher in the HK L-137 group than in the control group during the study (P=0.017). (Concanavalin A-induced proliferation).



The days of medication showed a significant negative correlation with duration of HK L-137 intake (P=0.008).

A total of 78 healthy subjects (thirty-three men and forty-five women; mean age 50·6 years) were randomly assigned to receive a tablet containing HK L-137 (10 mg) or a placebo tablet daily for 12 weeks.

Hirose S., et al. (2013). Oral intake of heat-killed Lactobacillus plantarum L-137 decreases the incidence of upper respiratory tract infection in healthy subjects with high levels of psychological stress. *Journal of Nutritional Science, 2*, E39. doi:10.1017/jns.2013.35

L-137, IS APPLIED IN A BROAD RANGE OF FOOD PRODUCTS

FOR SOME OF THESE PRODUCTS, THE QUESTION ARISES HOW HEAT STABLE IS THE ACTIVE INGREDIENT DURING E.G. BAKING AND LONG SHELF LIFE?

Tetra Pak
Postbiotic Flavored Ambient Yoghurt Drink

Note: 5 year shelf Life!

Add lactic acid bacteria while keeping it tasty!

Product name: Lactic acid bacteria for protect and enhance L-137 Supplement

Powder stick packs

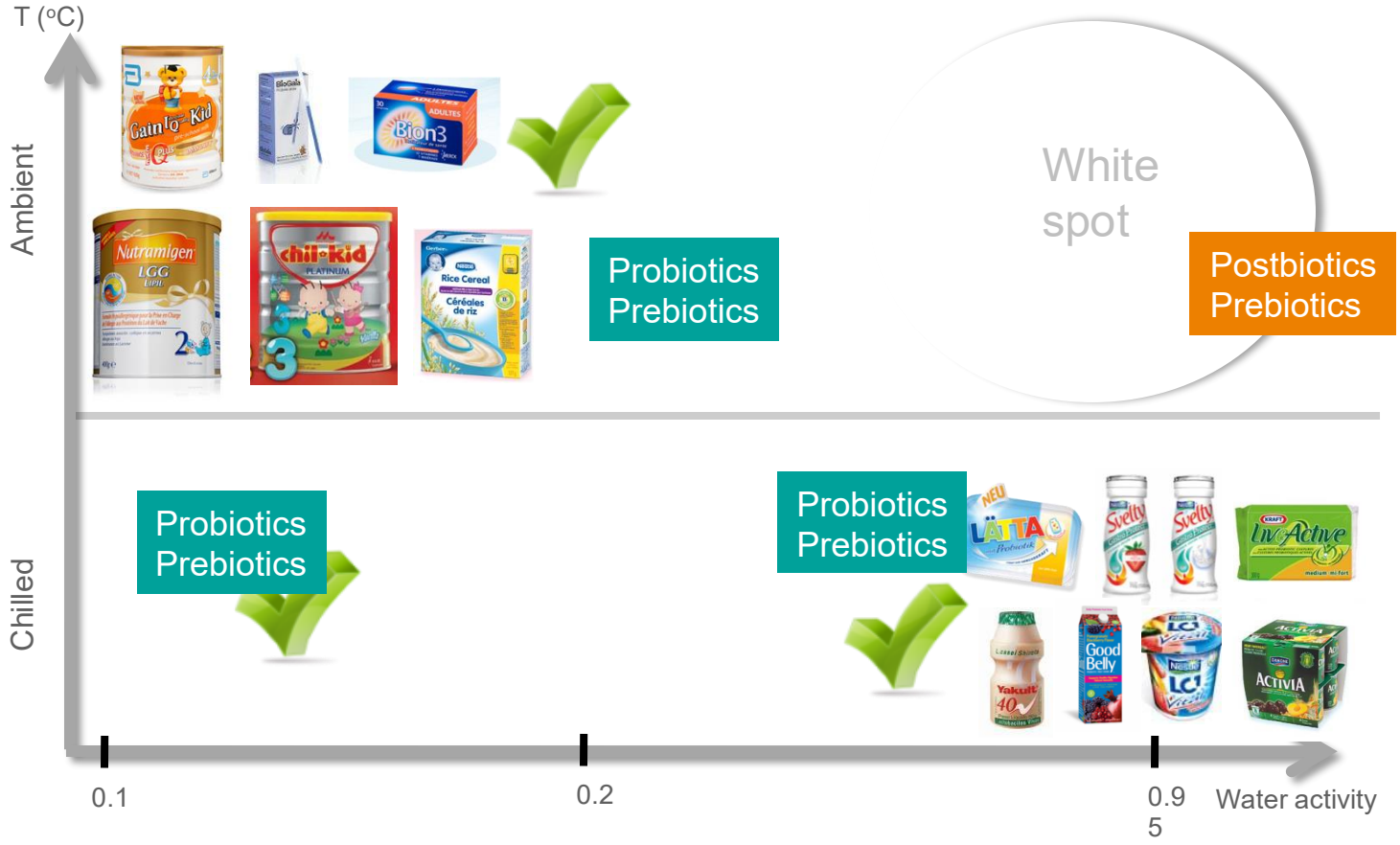
Easy health management

30 sticks

10billion of heat-killed lactobacillus plantarum L-137 in 1 pack

[Dietary supplement] Usage examples

PROBIOTICS DRIED AND AMBIENT VS. LIQUID AND CHILLED



Probiotics, Prebiotics, Synbiotics, Postbiotics and Fermented Foods *DEFINED*

Many terms in the 'biotic' space have emerged. Although probiotics, prebiotics, and synbiotics have more than a 25 year history, other biotic terms are relatively new. These terms are widely used in both scientific literature and popular media, and while they are intended to be descriptive, they often are confusing and misused.

ISAPP has enlisted the help of global experts to provide multiple perspectives in formulating consensus definitions that reflect current science for many of these terms. The goal of the consensus panels (comprising 10 or more global experts) has been to describe clearly what these terms are in order to support their proper use by stakeholders.

In addition to the criteria stipulated below, all substances must be safe for their intended use. For substances required to have a demonstrated health benefit, all product formulations must deliver an efficacious level of substance and must be fully identified.

Probiotic

Consensus definition Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014)

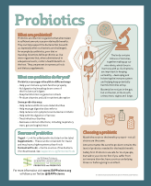
Simple way to conceptualize Live microbes that are good for your health

Live microbes present? Yes

Demonstrated health benefit required? Yes

Examples *Bifidobacterium animalis* subsp. *lactis* XYZ
Genus: *Bifidobacterium*
Species: *animalis*
Subspecies: *lactis*
Strain: *XYZ*

Keep in mind Identity must be confirmed through genome sequencing. Viability must be preserved through the end of shelf life.



Prebiotic

Consensus definition A substrate that is selectively utilized by host microorganisms conferring a health benefit on the host (Gibson et al., 2017)

Simple way to conceptualize Food for beneficial microbes within the host

Live microbes present? No

Demonstrated health benefit required? Yes

Examples Inulin, galactooligosaccharides, fructooligosaccharides

Keep in mind New prebiotics emerging: Polyphenols, oligosaccharides based on xylose, maltose and other sugars

Synbiotic

Consensus definition A mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host (Swanson et al., 2020)

Simple way to conceptualize Complementary synbiotic is a mixture of probiotic + prebiotic

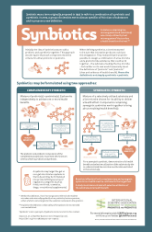
Synergistic synbiotic contains a live microbe and a substrate that it can use for growth

Live microbes present? Yes

Demonstrated health benefit required? Yes

Example Complementary synbiotic: inulin + *Bifidobacterium animalis* subsp. *lactis* XYZ

Keep in mind A health benefit must be shown for a synbiotic as combined, not just the probiotic alone and the prebiotic alone.



Postbiotic

Consensus definition Preparation of inanimate microorganisms and/or their components that confers a health benefit on the host (Salminen et al., 2021)

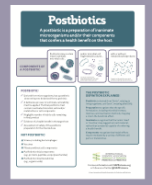
Simple way to conceptualize Non-viable microbes and/or cell components with or without metabolites

Live microbes present? No

Demonstrated health benefit required? Yes

Examples Some infant formulas, some bacterial lysates to prevent recurrent respiratory tract infections, yeast fermentates used in animal feeds

Keep in mind Purified metabolites do not qualify as postbiotics



Fermented Foods

Consensus definition Foods made through desired microbial growth and enzymatic conversions of food components (Marco et al., 2020)

Simple way to conceptualize Popular foods that are made through the growth of live microbes

Live microbes present? Sometimes yes, sometimes no. Live microbes not required in final product.

Demonstrated health benefit required? Not required

Examples Yogurt, kefir, sauerkraut, sourdough bread

Keep in mind Microbes present may be defined or undefined; live or dead

For more information visit **ISAPPscience.org**
or follow us on Twitter **@ISAPPscience**

ISAPP
Connecting science, microbes and health

Introduction to probiotics

Why are they healthy and different from other bacteria?

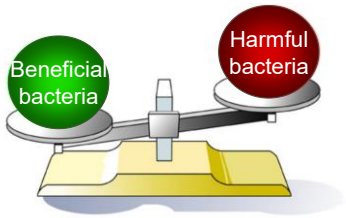
1. Remain active during transfer in the digestive tract; they endure gastric acid, bile acid, and other stressful conditions

2. They are adhesive and can adhere to the surface of the intestine where it inhibits the actions of harmful bacteria

3. Become a natural part of intestinal microbiota, balancing it against disturbances

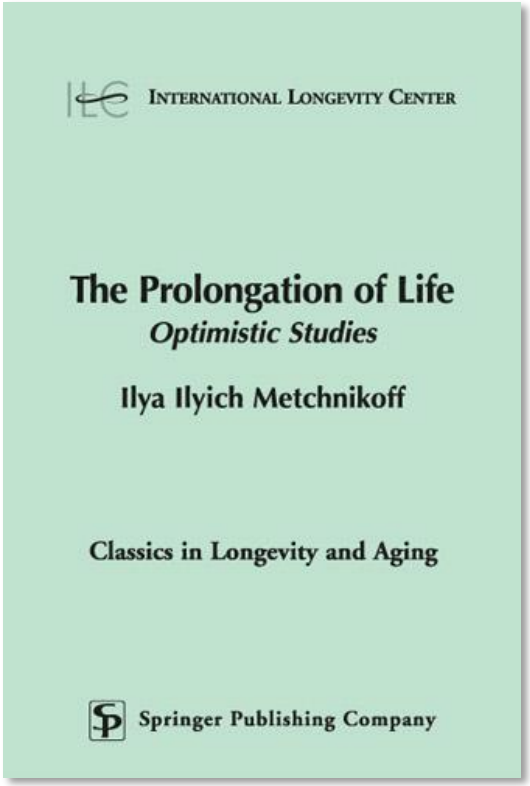
4. Lactobacilli have been shown to bind aflatoxin B, a food borne mycotoxin produced by *Aspergillus niger*

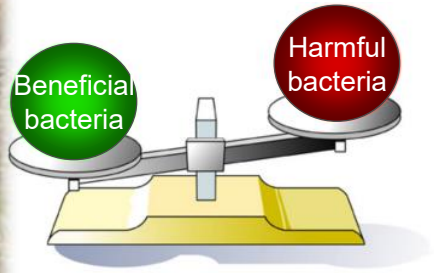
5. ….



The beginning of the probiotic concept

ILYA METCHNIKOFF (1845-1916): "THE PROLONGATION OF LIFE – OPTIMISTIC STUDIES"

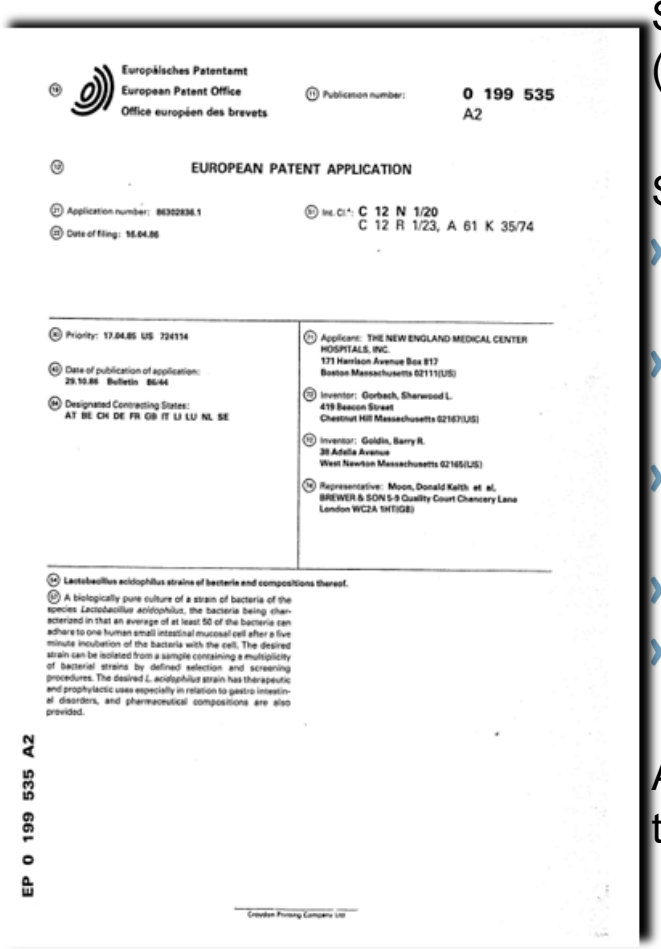




Introduction to probiotics

Minoru Shirota (1899 –1982) was a Japanese scientist. He was the inventor of Yakult, the yogurt-like probiotic drink containing Lactobacillus casei strain shirota.

1985 PATENT DESCRIBING LGG® ISOLATION PROCEDURE - GOLDWIN AND GORBACH



Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 199 535**
A2

EUROPEAN PATENT APPLICATION

(21) Application number: 86302836.1
(22) Date of filing: 16.04.86

(51) Int. Cl.⁴: C 12 N 1/20
C 12 R 1/23, A 61 K 35/74

(30) Priority: 17.04.85 US 724114

(43) Date of publication of application:
29.10.86 Bulletin 86/44

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

(71) Applicant: THE NEW ENGLAND MEDICAL CENTER HOSPITALS, INC.
171 Harrison Avenue Box 817
Boston Massachusetts 02111(US)

(72) Inventor: Gorbach, Sherwood L.
419 Beacon Street
Chestnut Hill Massachusetts 02167(US)

(72) Inventor: Goldin, Barry R.
38 Adella Avenue
West Newton Massachusetts 02165(US)

(74) Representative: Moon, Donald Keith et al,
BREWER & SON 5-9 Quality Court Chancery Lane
London WC2A 1HT(GB)

(54) Lactobacillus acidophilus strains of bacteria and compositions thereof.

(57) A biologically pure culture of a strain of bacteria of the species *Lactobacillus acidophilus*, the bacteria being characterized in that an average of at least 50 of the bacteria can adhere to one human small intestinal mucosal cell after a five minute incubation of the bacteria with the cell. The desired strain can be isolated from a sample containing a multiplicity of bacterial strains by defined selection and screening procedures. The desired *L. acidophilus* strain has therapeutic and prophylactic uses especially in relation to gastro intestinal disorders, and pharmaceutical compositions are also provided.

EP 0 199 535 A2

Croydon Printing Company Ltd

Selection a human stool sample with high levels of lactobacilli (average is 10^9 gram^{-1})

Screen:

- Tolerant for acidic conditions
  (growth between pH 3.0 and pH 3.5)
- Tolerant for bile acids
  (growth in the presence of 1.5 g/l oxgall bile)
- *Ability to attach to small intestinal mucosal cells (> 100 bacteria per cell)*
- Lactic acid production (4 mmol per 24 h of 10^{10} cells)
- Hardy *in vitro* growth (doubling time of 45 min at 37 ºC)

Administration: 10^9 – 10^{10} bacteria to 200 ml of UHT milk for treatment of

- antibiotic-associated diarrhoea
- IBD
- constipation

Claim for colonization of the intestine by this “natural organism”

PROBIOTICS: HOW COULD THEY WORK?

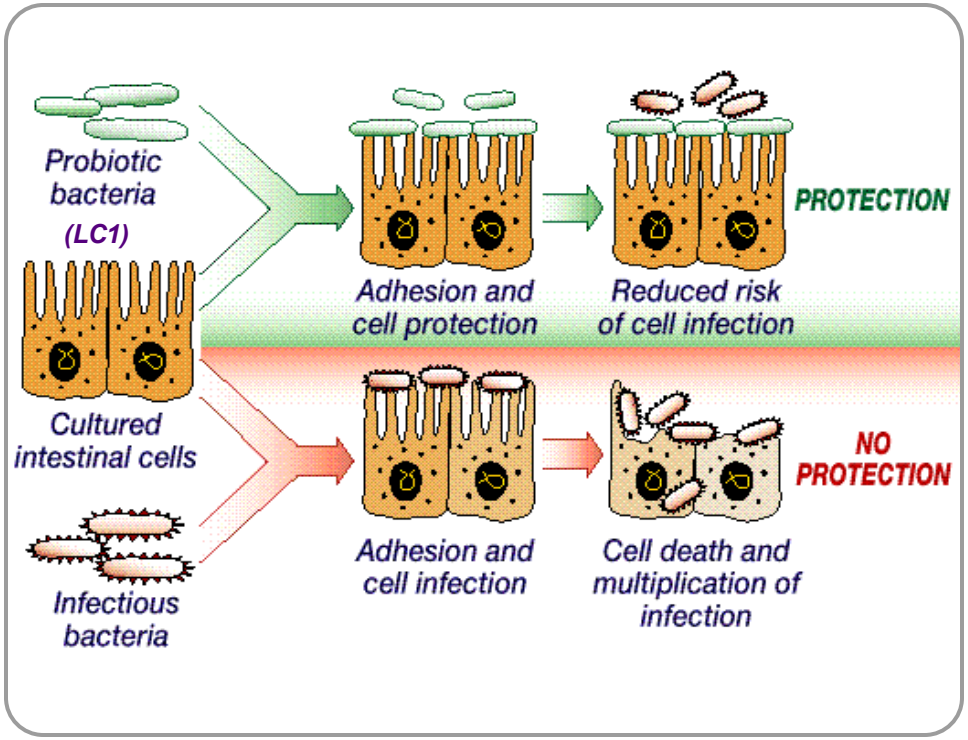
1. Inhibition of pathogens
   - reduction of luminal pH
   - competition for nutrients
   - secretion of anti-microbial compounds (bacteriocins)
   - induction of the production of antimicrobial compounds by the host (defensins)

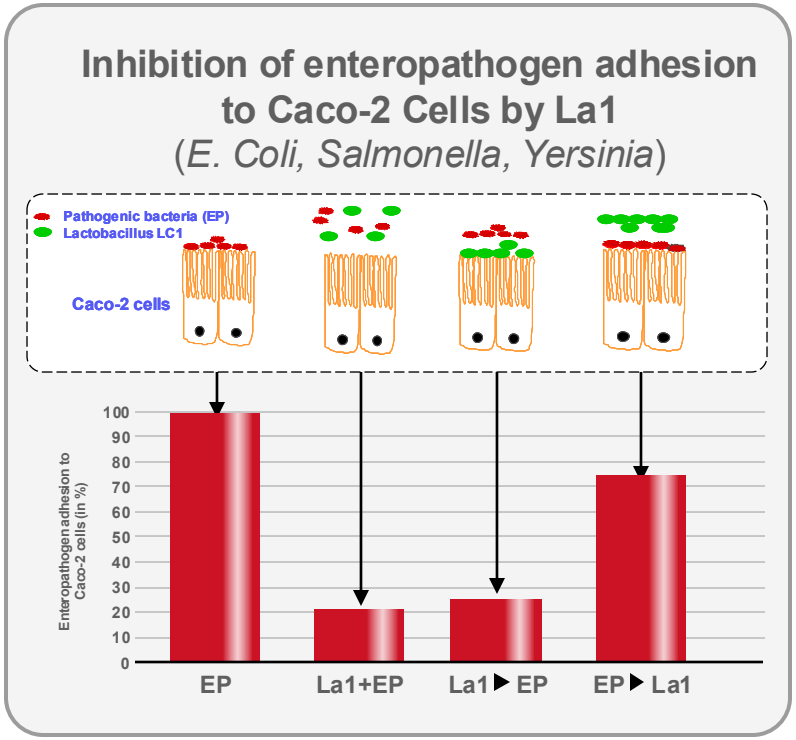
2. Improvement of the epithelial barrier function
   prevention of bacterial adhesion and evasion of epithelial cells

3. Modulation of host immune responses

⇨ Required is adhesion to and/or colonization of the epithelial cell surface

ANTI-PATHOGEN ACTIVITY OF PROBIOTICS COMPETITIVE DISPLACEMENT OF PATHOGENS IN THE GUT



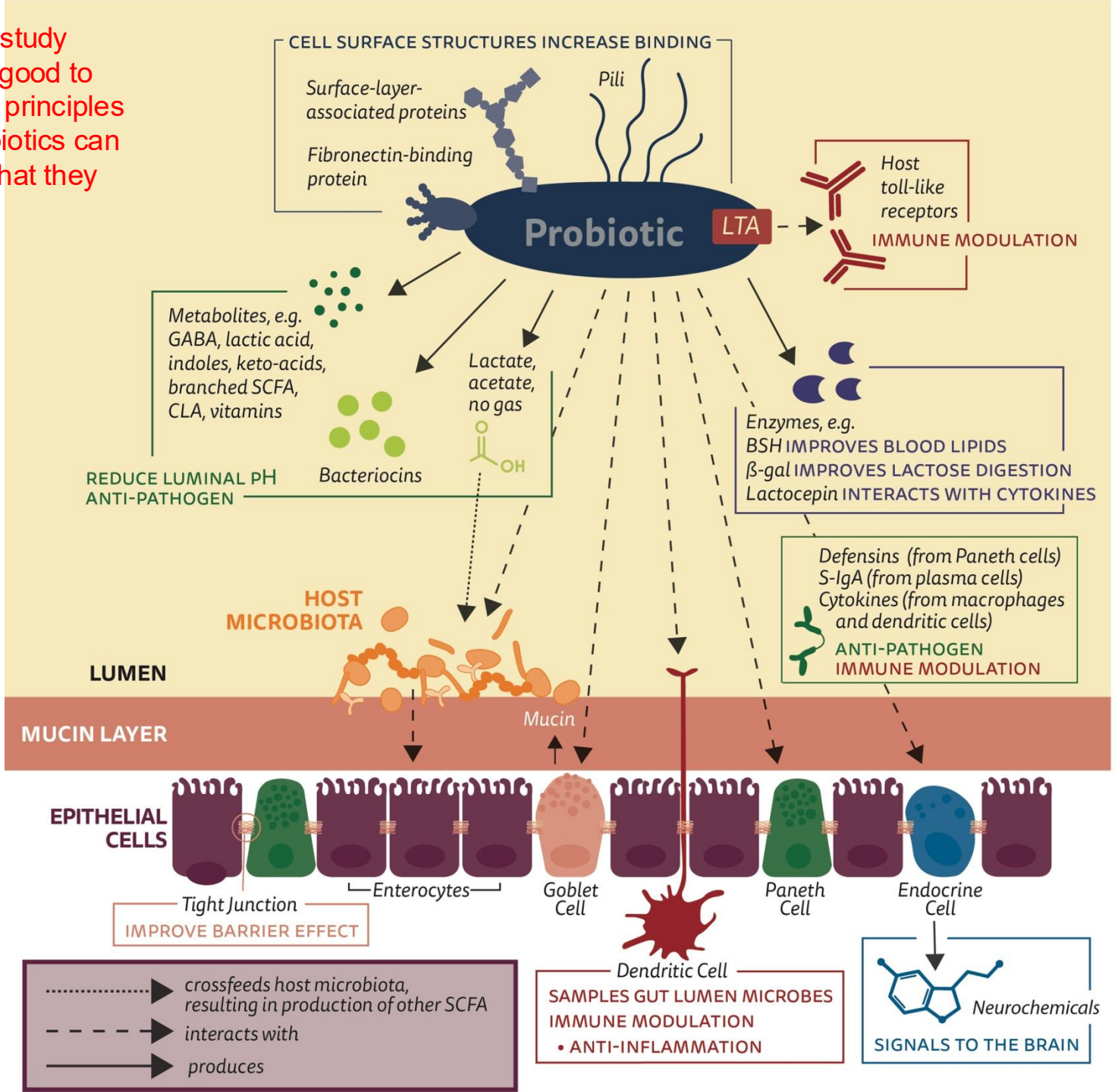


Ref. Nestlé data

PROBIOTICS IN ACTION – MOVIE
L. JOHNSONII AND *E. COLI* O157

Movie is not available on line, but was shown in class

No need to study details, but good to know some principles of how probiotics can work and what they can do

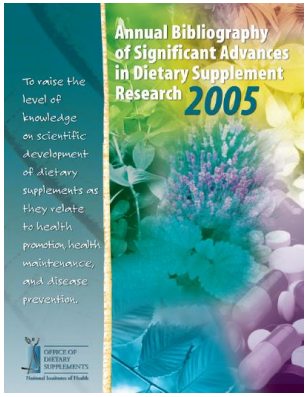


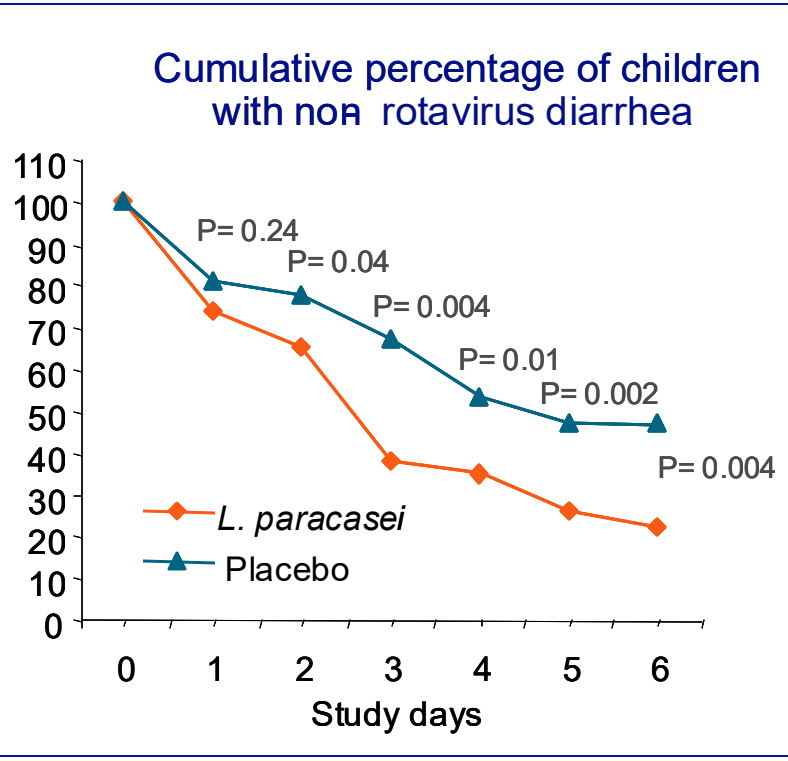
Product example:
GUM powder with *L. paracasei* NCC2461 (ST11)

Study

- 230 infants, 4-24 months
- Diarrhea < 2 days
- 10^{10} *L. paracasei* NCC2461 or placebo for 5 days

Study selected as one of the best interventional studies by NIH



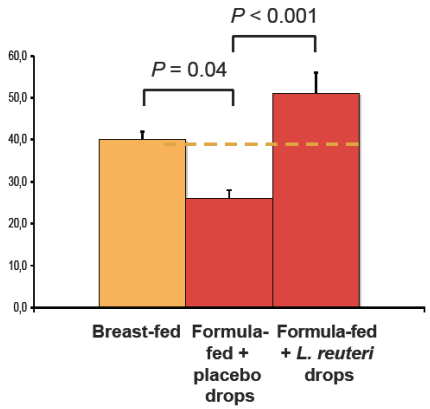


Sarker et al. 2005

Product example:
Infant formulae with *L. reuteri*

VON ERNÄHRUNGSEXPERTEN ENTWICKELT
LR
reuteri

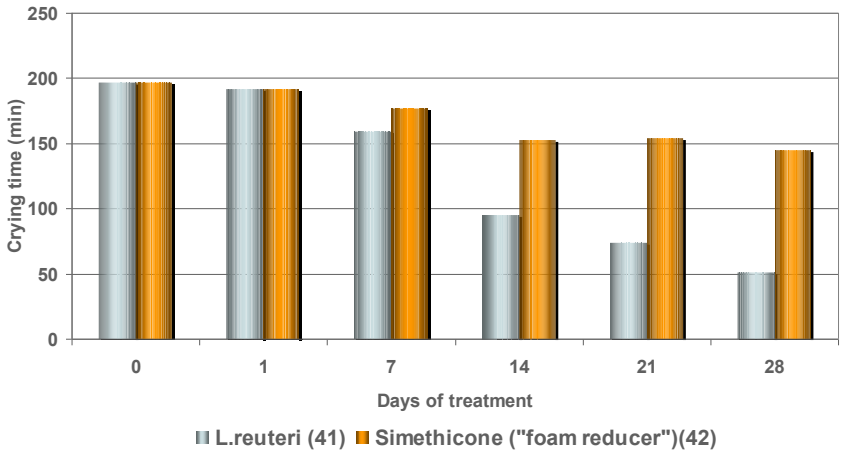
Gastric emptying rate



Indrio et al. J Pediatr 2008

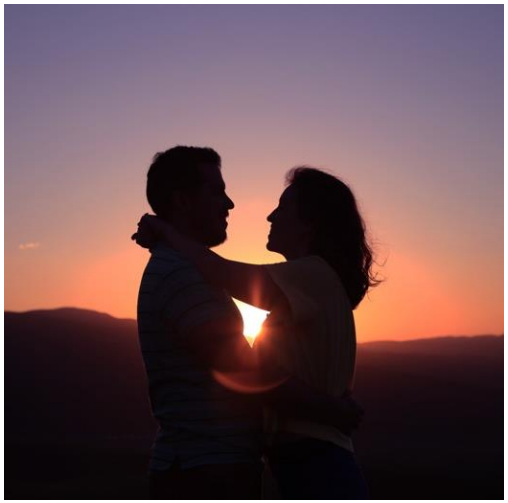
- *L. reuteri* improves gastric motility and GI transit in preterm and term infants
- *L. reuteri* reduces daily crying time in colicky infants
- *L. reuteri* reduces pain intensity in children suffering from recurrent abdominal pain

Crying time



Savino F. et al., Pediatrics 2007

"The Gut Brain Axis"
Connection between brain and gut



1. In love

2. Feeling full after eating

3. Stress at work

- In a mice studies, the composition of the microbiome impacts responses to stress
- *Administering certain probiotics (lactobacillus reuteri) can eliminate the effects of stress.*

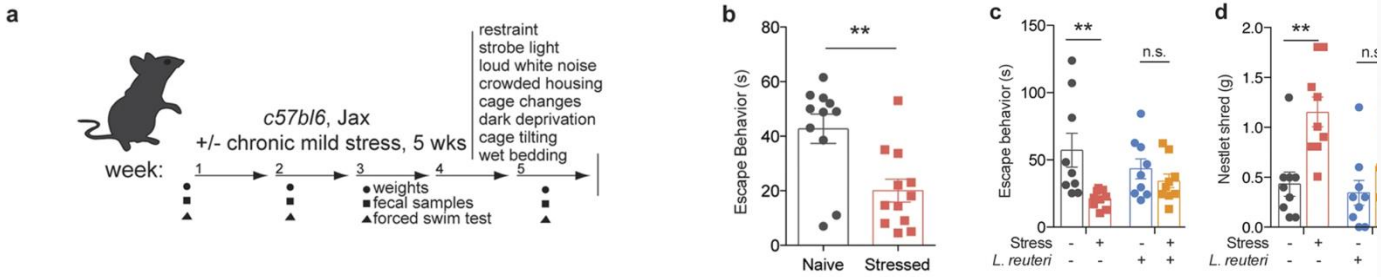
SCIENTIFIC REPORTS

OPEN

Microbiota alteration is associated with the development of stress-induced despair behavior

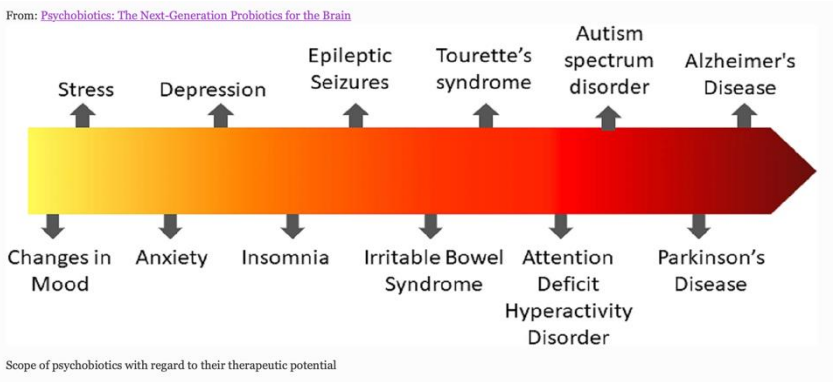
Received: 11 October 2016
Accepted: 31 January 2017
Published: 07 March 2017

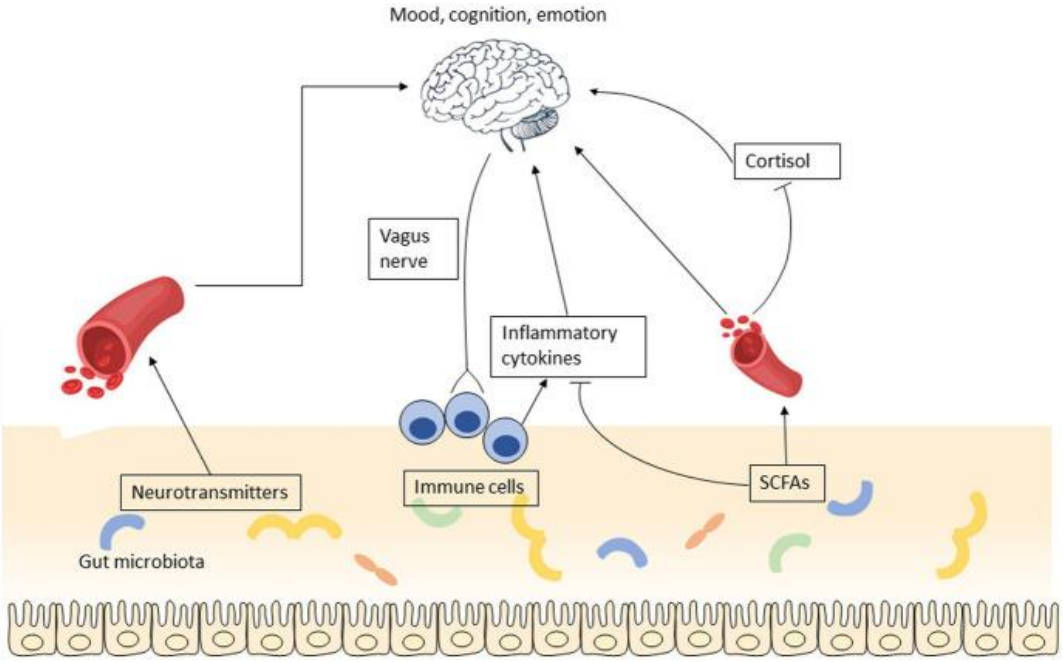
Ioana A. Marin[1,2,3], Jennifer E. Goertz[1,2], Tiantian Ren[4], Stephen S. Rich[5], Suna Onengut-Gumuscu[5], Emily Farber[5], Martin Wu[4], Christopher C. Overall[1,2], Jonathan Kipnis[1,2,3,*] & Alban Gaultier[1,2,3,*]



Mechanisms about how our gut bacteria are connected to our mental health

- Synthesis of neurotransmitters,
- Production of short-chain fatty acids (acetic acid, butyric acid, propionic acid)
- Regulating and limiting inflammation.





Yang Y, Zhou B, Zhang S, Si L, Liu X, Li F. Prebiotics for depression: how does the gut microbiota play a role? Front Nutr. 2023 Jul 6;10:1206468. doi: 10.3389/fnut.2023.1206468. PMID: 37485386; PMCID: PMC10358272.

Certain probiotics produce – or remove- substances that act on the Vagus Nerve

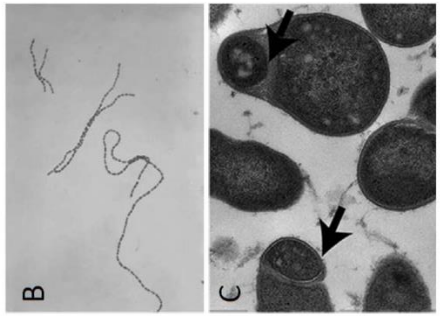
The butyrate producing bacteria Coprococcus

Accepted manuscript

The butyrate-producing and sporeforming bacterial genus *Coprococcus* as a potential biomarker for neurological disorders

Published online by Cambridge University Press: **30 August 2023**

Fleur Notting, Walter Pirovano, Wilbert Sybesma and Remco Kort



Contents lists available at ScienceDirect

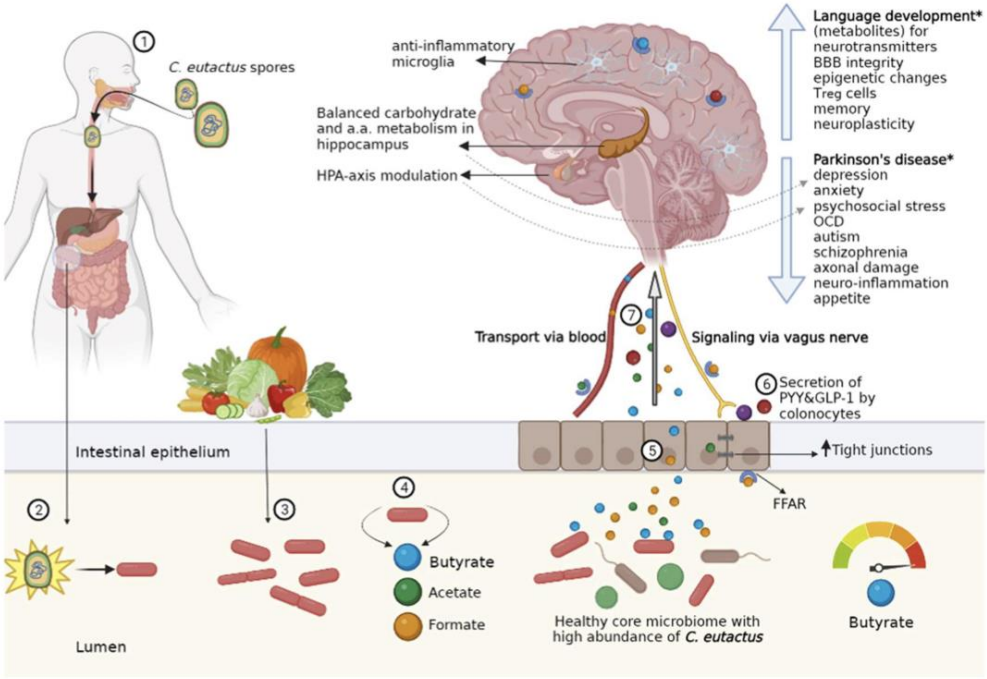
Neurobiology of Stress

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

Probiotic consumption relieved human stress and anxiety symptoms possibly via modulating the neuroactive potential of the gut microbiota

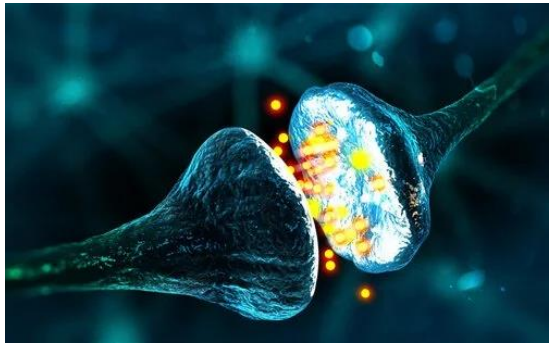
Teng Ma[a,1], Hao Jin[a,1], Lai-Yu Kwok[a], Zhihong Sun[a], Min-Tze Liong[b], Heping Zhang[a,*]

[a] Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, 010018, China
[b] School of Industrial Technology, Universiti Sains Malaysia, 11800, Penang, Malaysia



GABA is produced by gut microbes and plays important role in managing anxiety, stress and fear.

GABA reduces a nerve cell's ability to receive, generate, or send chemical messages to other nerve cells. GABA is known to have a calming effect.



Bifidobacterium adolescentis **as a key member of the human gut microbiota in the production of GABA**

SCIENTIFIC REPORTS
nature research

Sabrina Duranti[1,7], Lorena Ruiz[2,3,7], Gabriele Andrea Lugli[1,7], Héctor Tames[2,3], Christian Milani[1,4], Leonardo Mancabelli[1], Walter Mancino[1], Giulia Longhi[5], Luca Carnevali[6], Andrea Sgoifo[4,6], Abelardo Margolles[2,3], Marco Ventura[1,4], Patricia Ruas-Madiedo[2,3] &

nutrients MDPI

Article

Bifidobacterium longum **subsp. *longum* Reduces Perceived Psychological Stress in Healthy Adults: An Exploratory Clinical Trial**

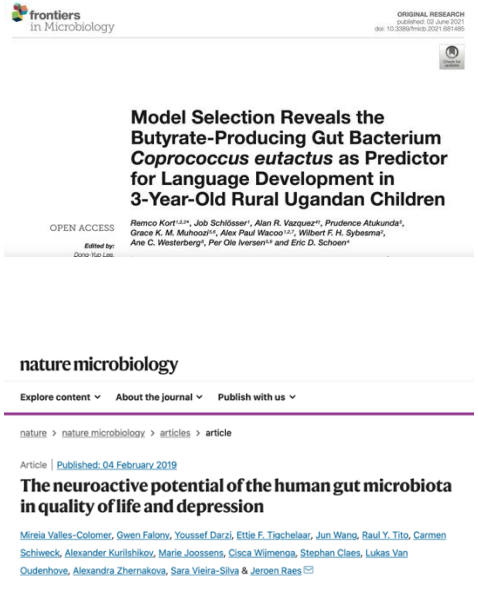
Marcus Boehme [1,*], Noëla Rémond-Derbez [1], Clara Lerond [1], Luca Lavalle [2], Sonia Keddani [1], Myriam Steinmann [1], Andreas Rytz [2], Boushra Dalile [3,4], Kristin Verbeke [3], Lukas Van Oudenhove [3,4], Pascal Steiner [1], Bernard Berger [1], Maria Vicario [1], Gabriela Bergonzelli [1], Sara Colombo Mottaz [2] and Julie Hudry [1]

Duranti S, Ruiz L, Lugli GA, Tames H, Milani C, Mancabelli L, Mancino W, Longhi G, Carnevali L, Sgoifo A, Margolles A, Ventura M, Ruas-Madiedo P, Turroni F. Bifidobacterium adolescentis as a key member of the human gut microbiota in the production of GABA. Sci Rep. 2020 Aug 24;10(1):14112. doi: 10.1038/s41598-020-70986-z. PMID: 32839473; PMCID: PMC7445748.

Two more examples linked to the Gut-Brain Axis bacterium Coprococcus

1. Language development 2. Depression

In a study that looked at the composition of the gut microbiome in young Ugandan children, an association was seen between the presence of the intestinal bacerie Coprococcus eutactus and the development of language skills.



frontiers in Microbiology

ORIGINAL RESEARCH

Model Selection Reveals the Butyrate-Producing Gut Bacterium *Coprococcus eutactus* as Predictor for Language Development in 3-Year-Old Rural Ugandan Children

OPEN ACCESS

nature microbiology

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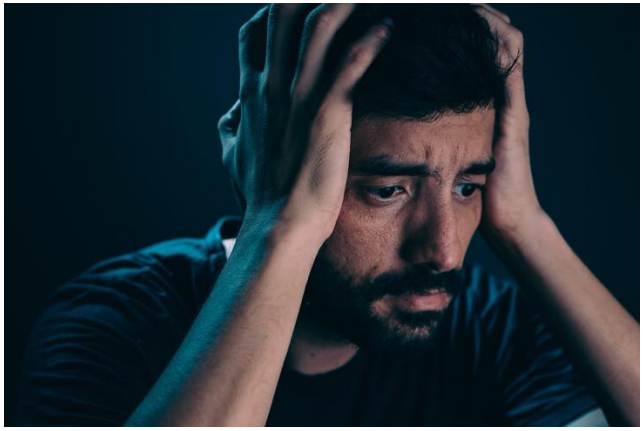
Article | Published: 04 February 2019

The neuroactive potential of the human gut microbiota in quality of life and depression

Mireia Valles-Colomer, Gwen Falony, Youssef Darzi, Ettje F. Tigchelaar, Jun Wang, Raul Y. Tito, Carmen Schiweck, Alexander Kurilshikov, Marie Joossens, Cisca Wijmenga, Stephan Claes, Lukas Van Oudenhove, Alexandra Zhernakova, Sara Vieira-Silva & Jeroen Raes



Coprococcus bacteria were consistently associated with higher quality of life indicators. Together with Dialister, Coprococcus spp. were also depleted in depression, even after correcting for the confounding effects of antidepressants.

Several mood enhancing probiotics are entering the market

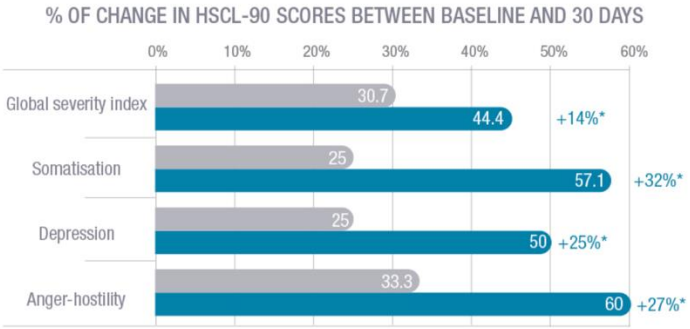
Examples

LALLEMAND

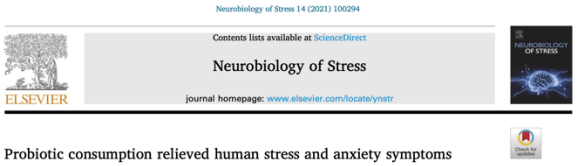
CEREBIOME® significantly improves psychological signs related to occasional stress

Placebo
CEREBIOME®

*$p<0.05$



CEREBIOME® helps alleviate the physical and psychological symptoms of stress and feelings of anxiety.
Since the 2000s, CEREBIOME® (*Lactobacillus helveticus* Rosell®-52 + *Bifidobacterium longum* Rosell®-175) has been documented on the reduction of psychological stress in humans by five clinical studies and nine animal studies.



Neurobiology of Stress 14 (2021) 100294

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Probiotic consumption relieved human stress and anxiety symptoms possibly via modulating the neuroactive potential of the gut microbiota

Teng Ma [a,1], Hao Jin [a,1], Lai-Yu Kwok [a], Zhihong Sun [a], Min-Tze Liong [b], Heping Zhang [a,*]

[a] Key Laboratory of Dairy Biotechnology and Engineering, Ministry of Education, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, 010018, China
[b] School of Industrial Technology, Universiti Sains Malaysia, 11800, Penang, Malaysia

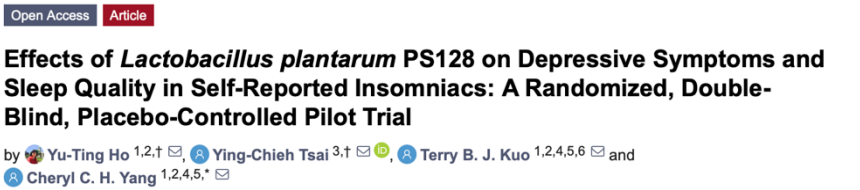
BENED BIOMEDICAL

PS128

a psychobiotic for nervous system balance

new clinical study:
ADHD symptoms improved

- Regulates dopamine (motor control, motivation) and serotonin (mood, sleep)
- Reduces cortisol (stress) levels throughout the body
- Boosts amounts of key SCFAs (short-chain fatty acids) and
- Antioxidant and anti-inflammatory (reduces TNF-α and IL-6, increases IL-10)
- Improves athletic endurance and post-workout physical recovery
- Promotes healthy digestion



Open Access Article

Effects of *Lactobacillus plantarum* PS128 on Depressive Symptoms and Sleep Quality in Self-Reported Insomniacs: A Randomized, Double-Blind, Placebo-Controlled Pilot Trial

by Yu-Ting Ho [1,2,†], Ying-Chieh Tsai [3,†], Terry B. J. Kuo [1,2,4,5,6] and Cheryl C. H. Yang [1,2,4,5,*]

Stress can also be reduced by eating fermented foods and fiber

Hereby also the composition of the gut microbiome changes

Molecular Psychiatry www.nature.com/mp

ARTICLE OPEN

Feed your microbes to deal with stress: a psychobiotic diet impacts microbial stability and perceived stress in a healthy adult population

Kirsten Berding[1✉], Thomaz F. S. Bastiaanssen[1], Gerard M. Moloney[1,2], Serena Boscaini[1], Conall R. Strain[1,3], Andrea Anesi[4], Caitriona Long-Smith[1], Fulvio Mattivi[4,5], Catherine Stanton[1,3,6], Gerard Clarke[1,6], Timothy G. Dinan[1,6] and John F. Cryan[1,2✉]

scientific reports

OPEN

Anxiolytic effects of a galacto-oligosaccharides prebiotic in healthy females (18–25 years) with corresponding changes in gut bacterial composition

Nicola Johnstone[1✉], Chiara Miles[2], Olivia Burn[1], Bartholomeus van den Bogert[2,3], Arjen Nauta[4], Kathryn Hart[3], Paul Sowden[3,4], Philip W. J. Burnet[2] & Kathrin Cohen Kadosh[1✉]

The Journal of Nutrition

Available online 22 February 2023

In Press, Corrected Proof What's this?

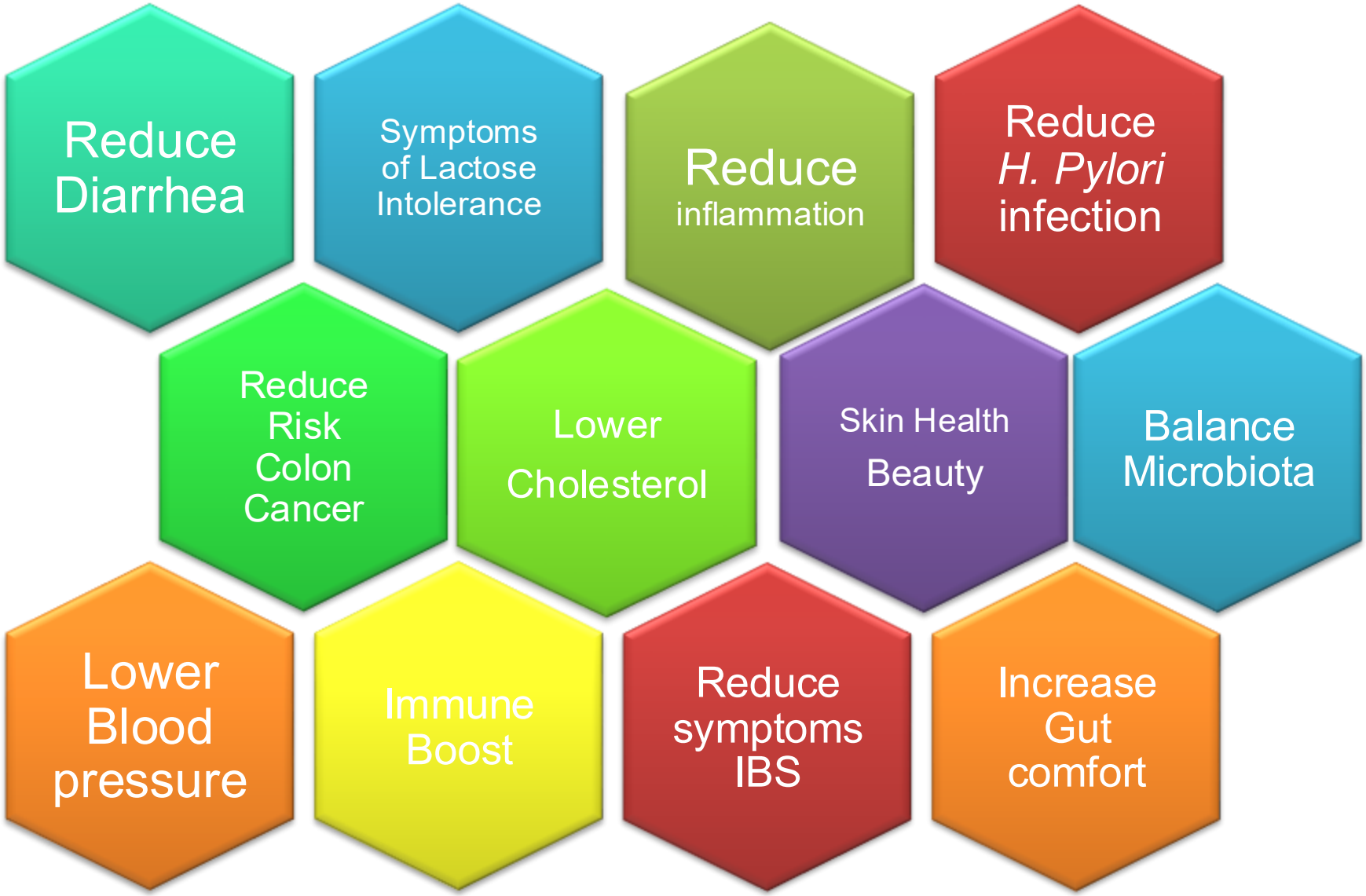
Go to The Journal of Nutrition on ScienceDirect

Nutritional Epidemiology

Positive Health Outcomes Associated with Live Microbe Intake from Foods, Including Fermented Foods, Assessed using the NHANES Database

Colin Hill[1 †], Daniel J. Tancredi[2 †], Christopher J. Cifelli[3], Joanne L. Slavin[4], Jaime Gahche[5], Maria L. Marco[6], Robert Hutkins[7], Victor L. Fulgoni III[8], Daniel Merenstein[9], Mary Ellen Sanders[10]

Probiotic health benefits

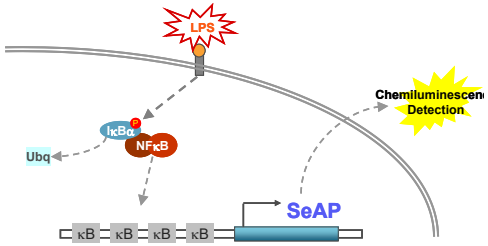


Courtesy Nestlé Research.
Note: These are broad probiotic health benefit areas, not health claims associated with probiotic products.

Development of New Probiotics

Pre-clinical studies
SAFETY - EFFICACY

In vitro
SAFETY – STABILITY - EFFICACY

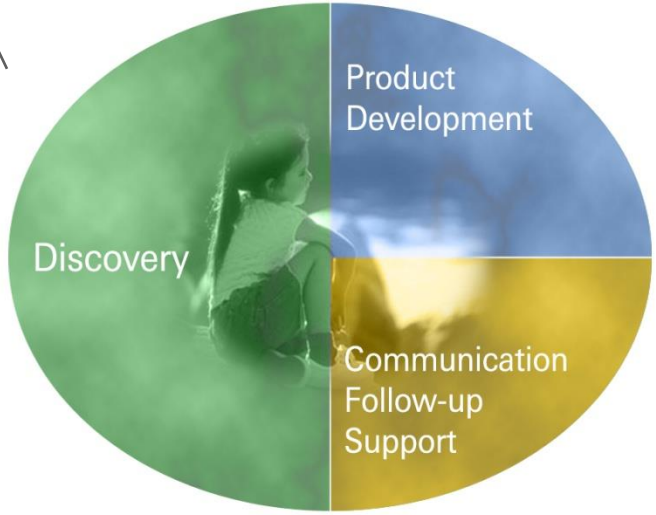


Immunological characterisation
NFkB-driven SeAP reporter assay

Culture Collection
Basic Characterization, IP Status

Human / Clinical Studies
SAFETY - EFFICACY

Nestlé Research Center is certified ISO9001:2008 for "Research on nutrition and food, including preparation of test products and the organization and conducting of clinical trials".

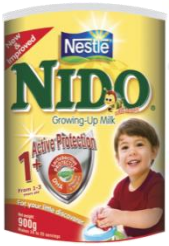


Nestlé Research Center is certified ISO22000:2005 for "Preparation of test products by the Process Lab and the Tasting Corner for sensory evaluation and clinical trials as a result of research on nutrition and food. Maintenance and distribution of industrial strains".

Process & Product
Optimization, Formulation, Scale Up

Documentation & Regulatory Approval

Product Launch



Probiotics:
Many formulations as part of daily life

Shelf-stable dairy, Nutrition

Chilled dairy

Confectionery

Cereals

Pet Care

Beauty & Health care supplements

Non-dairy beverages

Delivery systems

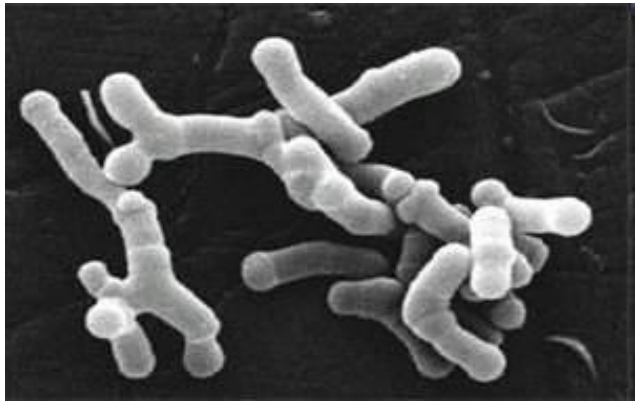
Ice cream

Probiotic Production Technology - Content

1. What
   a. Viability
   b. Yield
   c. Functionality

Definition of Probiotics
"live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"

2. How
   a. Increasing the strain stability
   b. Stabilizing process conditions
      - Fermentation
      - Drying
      - Mode of reconstitution
      - Genetic stability



   c. Understanding the mechanisms of probiotic functionality
3. Scale up
4. Outlook and Conclusions

Probiotic Food Products – Examples from the western world

Frozen products

- Ice cream

Refrigerated products

- Fermented dairy products
- Fruit juices

Shelf-stable products

- Milk powder (infant and children)
- Breakfast cereals, muesli
- Biscuits, bars, candies, chocolate..
- Pet food (esp. cats & dogs)
- Medical food powders
- Supplements

Overview of storage regimes
Dried and ambient vs. Liquid and chilled

T (°C)

Ambient

Chilled

White spot

0.1 0.2 0.95 Water activity

Overview of the manufacturing process options and product formulation

Growth/fermentation

Ingredient

Food grade growth media
In fermenter
Aim: Maximal biomass

- Biomass
  - Drying
    - Mix with end product
  - Encapsulation
    - Drying
      - Mix with end product
  - Sterile addition into end product

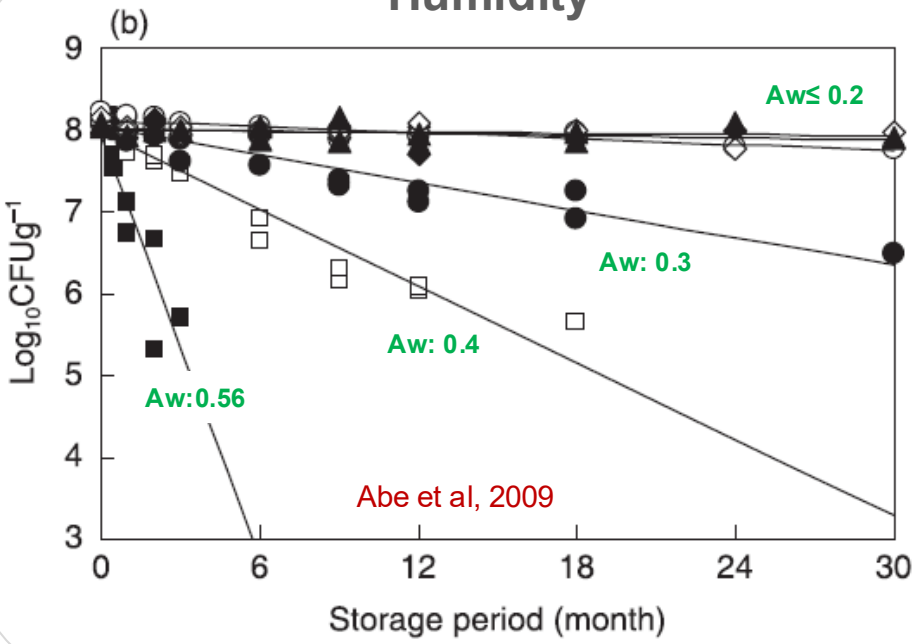
In-Product

In-product fermentation
In container or fermentation tank
Aim: Optimal sensory & biomass

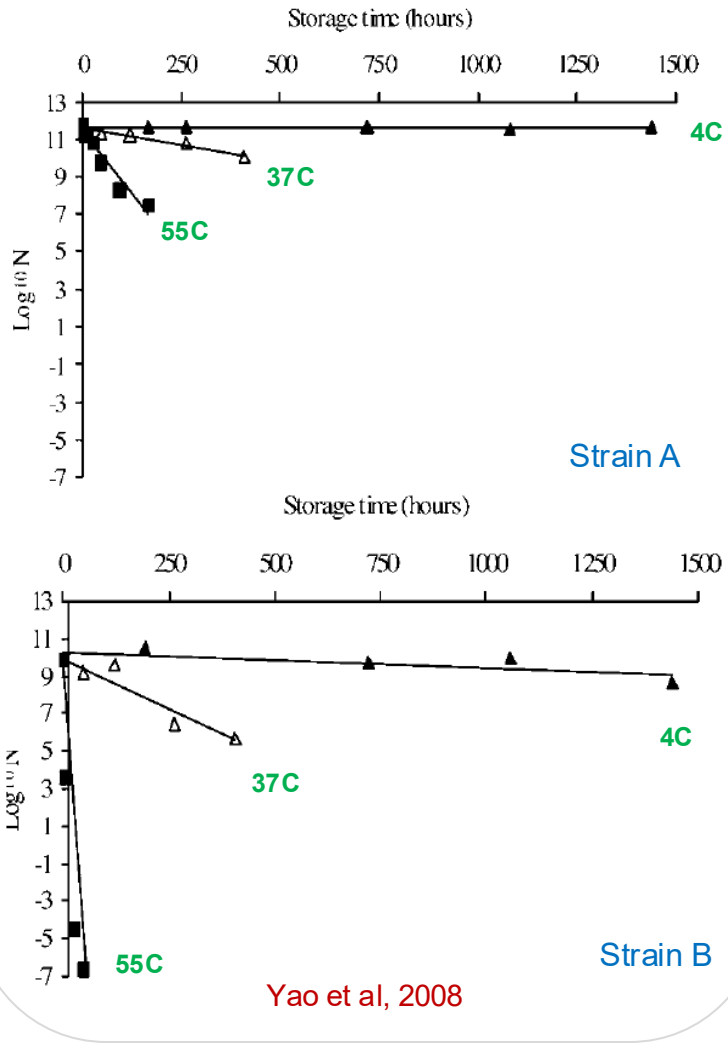
- Fermented milk
  - Drying

Storage of dry products – T and a_w as critical factors

Humidity



Temperature

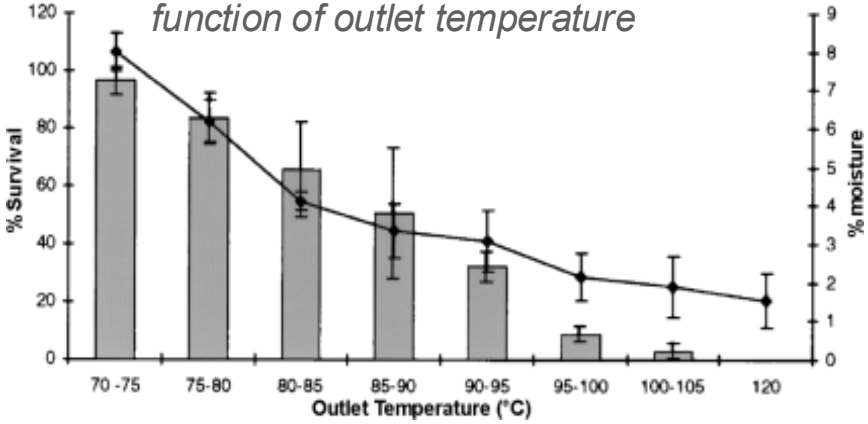


The main challenge is to keep the probiotics viable during processing and storage

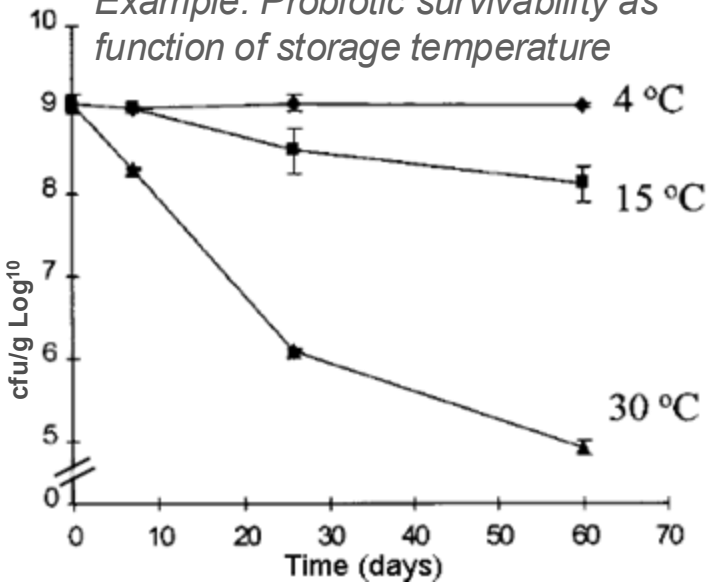
- Production
  - Survivability
  - Extreme temperature changes
  - Osmotic pressure changes
  - Oxygen stress

- Consumption
  - Storage stability
  - Remain viable during gastric transit
  - pH stress

Example: Probiotic survivability as function of outlet temperature



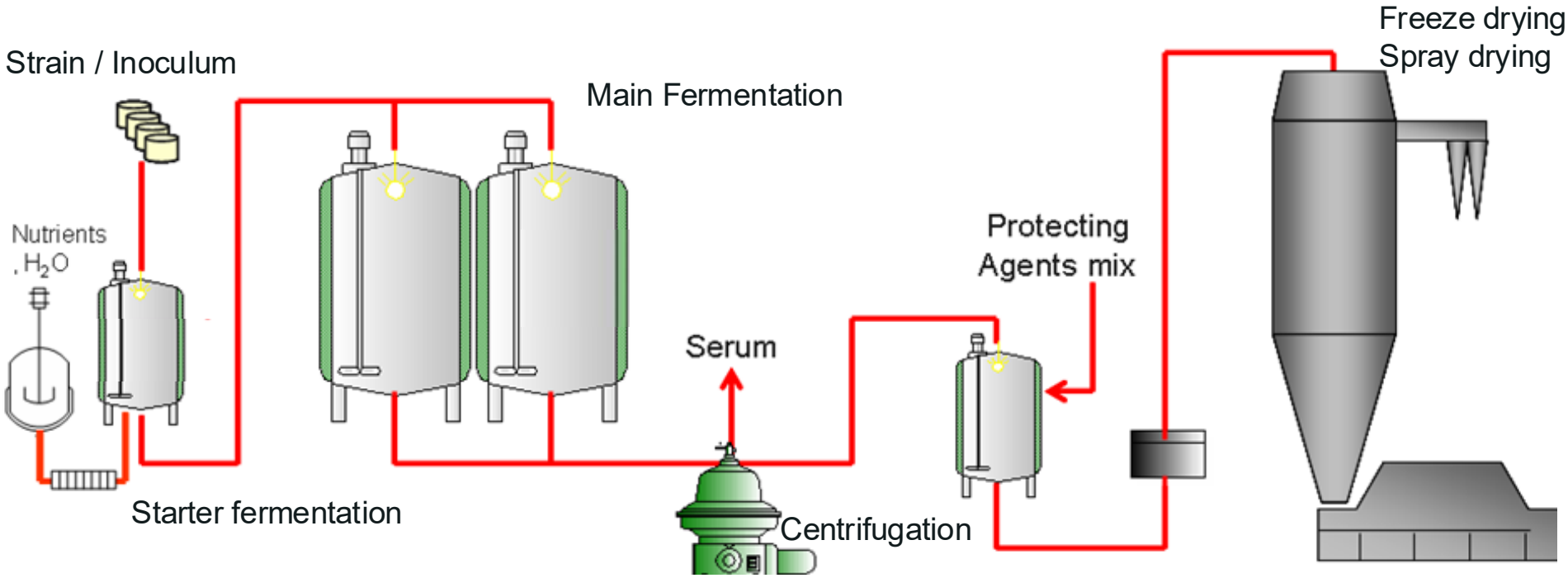
Example: Probiotic survivability as function of storage temperature



I. Jankovic (2010), Ananta *et al.* (2005), R.P. Ross *et al.* (2005), C. Stanton *et al.* (2003), T. Mattila-Sandholm (2002), G.E. Gardiner *et al* (2000).

56

Production of dried –*shelf stable*- probiotics



Yield and viability is influenced by:

- Strain
- Medium composition
- Fermentation Conditions
- Protective/Stabilizing agents
- Down stream processing conditions

Muller et al. Manufacture of probiotic bacteria. In Prebiotics and Probiotics Science and Technology. 2009. Ed Charalampopoulos and Rastall

Alternative drying techniques

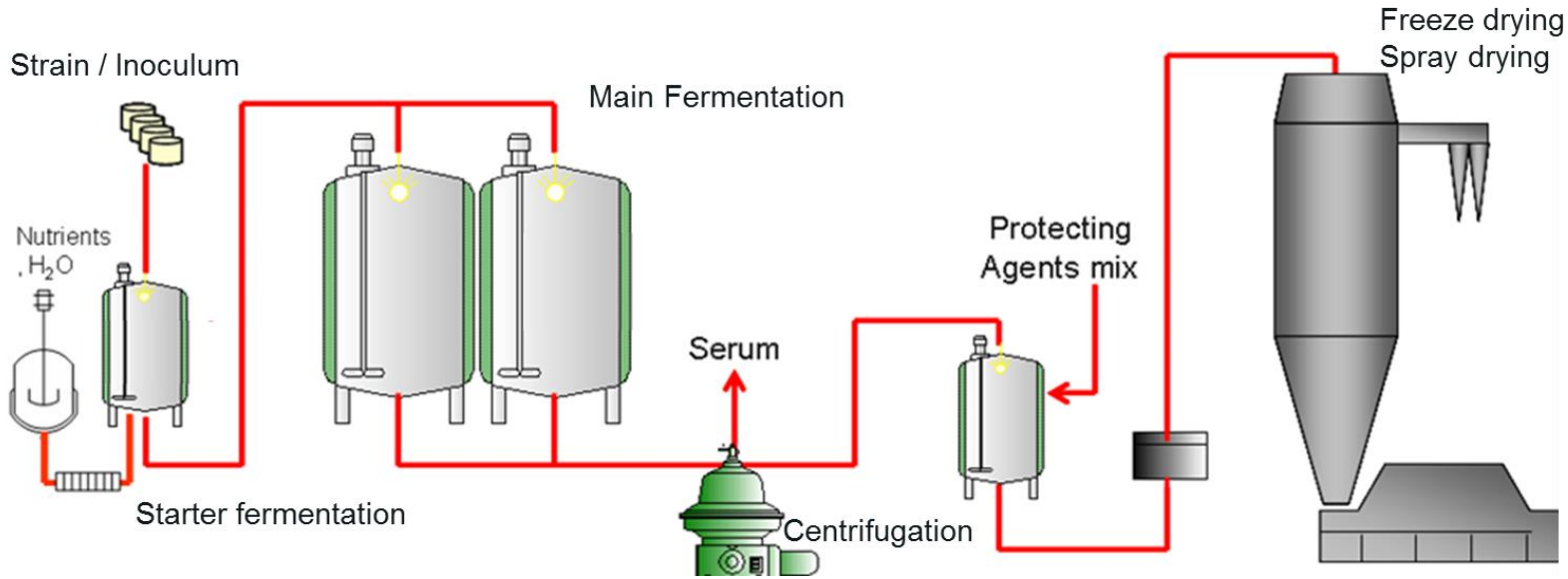
Table 1. Costs of Drying Processes Referenced to That of Freeze Drying[a]

drying processes	fixed costs (%)	manufacturing costs (%)
freeze drying	100.0	100.0
vacuum drying	52.2	51.6
spray drying	12.0	20.0
drum drying	9.3	24.1
fluidized bed drying	8.8	17.9
air drying	5.3	17.9

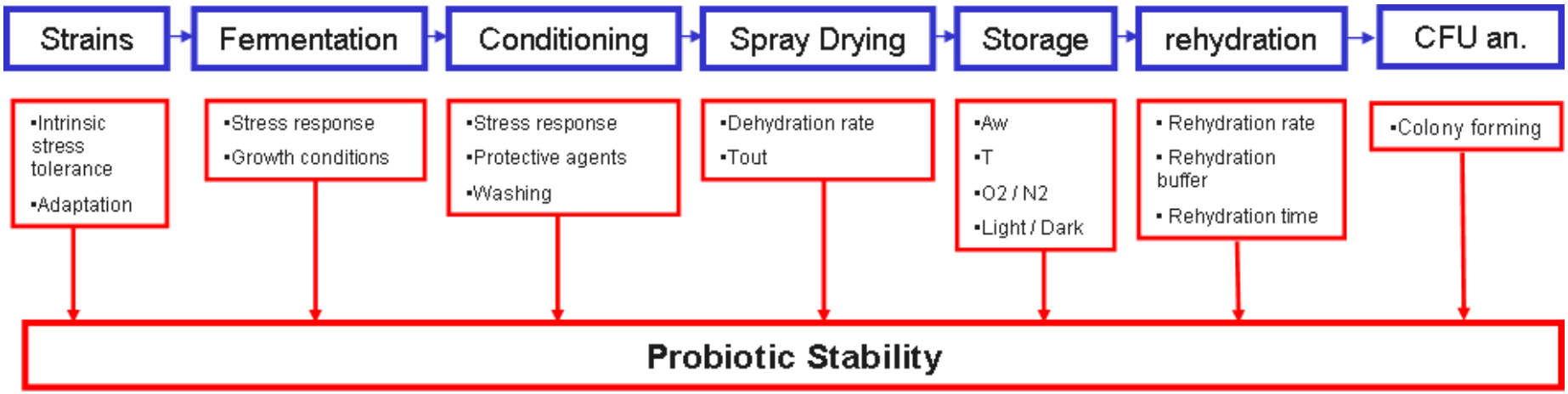
Santivarangkna et al (2007) Biotechnol. Progress

Question:

- What is a potential limitation of this process?
- Do you know a probiotic product concept that does not have this problem?



Probiotic stability is affected by many steps in the production process



Main questions to answer are:

When/where are cells (ir)-reversibly damaged/killed?

→ Drying, storage, rehydration, …?

How/by what are cells (ir)-reversibly damaged/killed?

→ Membrane damage, DNA damage, ribosome damage, protein damage, …? by heat, osmolarity, oxidation, …?

How/what is the cellular response for survival?

→ Membrane repair, DNA repair, ribosome repair, chaperones, anti oxidation, …?

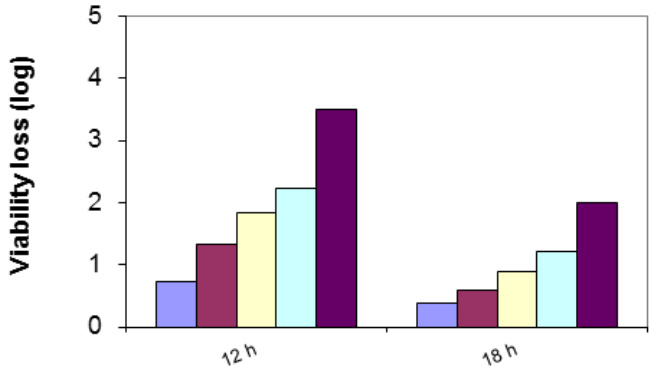
Why do some cells survive better than others?

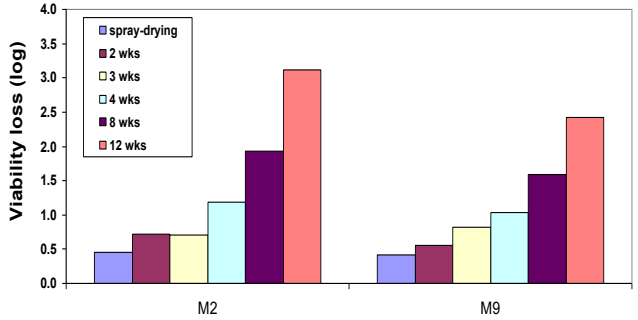
Increased shelf life stability after application of different fermentation conditions

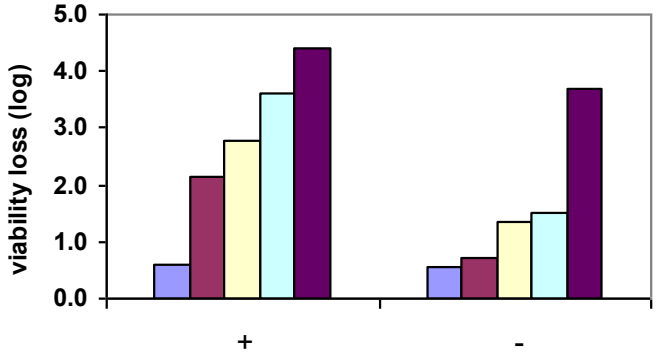
1. Moment of Harvesting / Fermentation Time
→ *Why?*

2. pH controlled fermentation vs. acidified fermentation
→ *Why?*

3. Medium ingredients
→ *Why?*







Bifidus - Accelerated Storage Test
37° C, Aw 0.22

W. Sybesma, A. Dubois unpublished results

Increased shelf life stability after application of different fermentation conditions

1. Moment of Harvesting / Fermentation Time

→ *Cells in stationary phase are more stable than end of exponential phase*

2. pH controlled fermentation vs. acidified fermentation

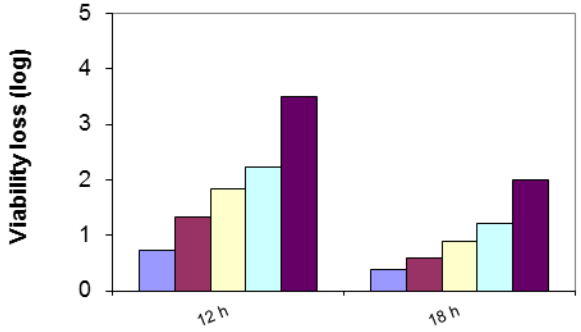
→ *Acidified cultures are more stable than pH controlled cultures*

3. Medium ingredients (e.g. addition of anti oxidants)

→ *Anti- oxidative properties enhancing cell viability*

Bifidus - Accelerated Storage Test
37° C, Aw 0.22

1



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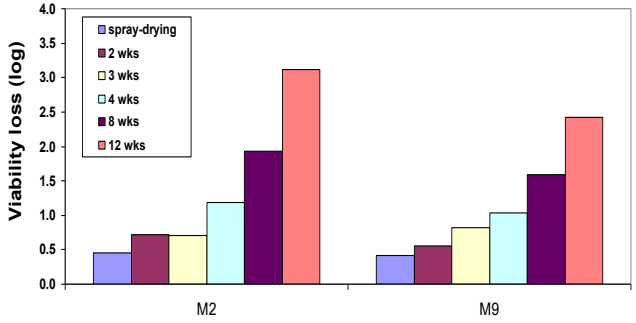

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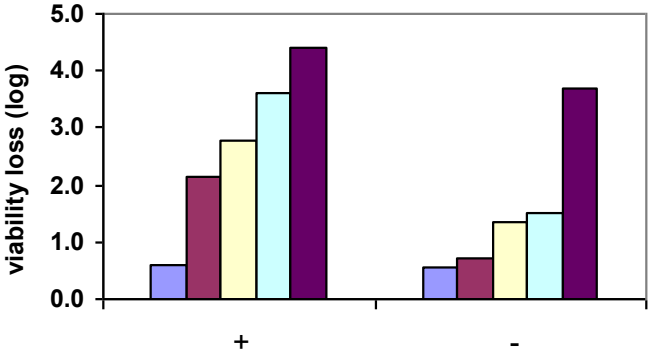
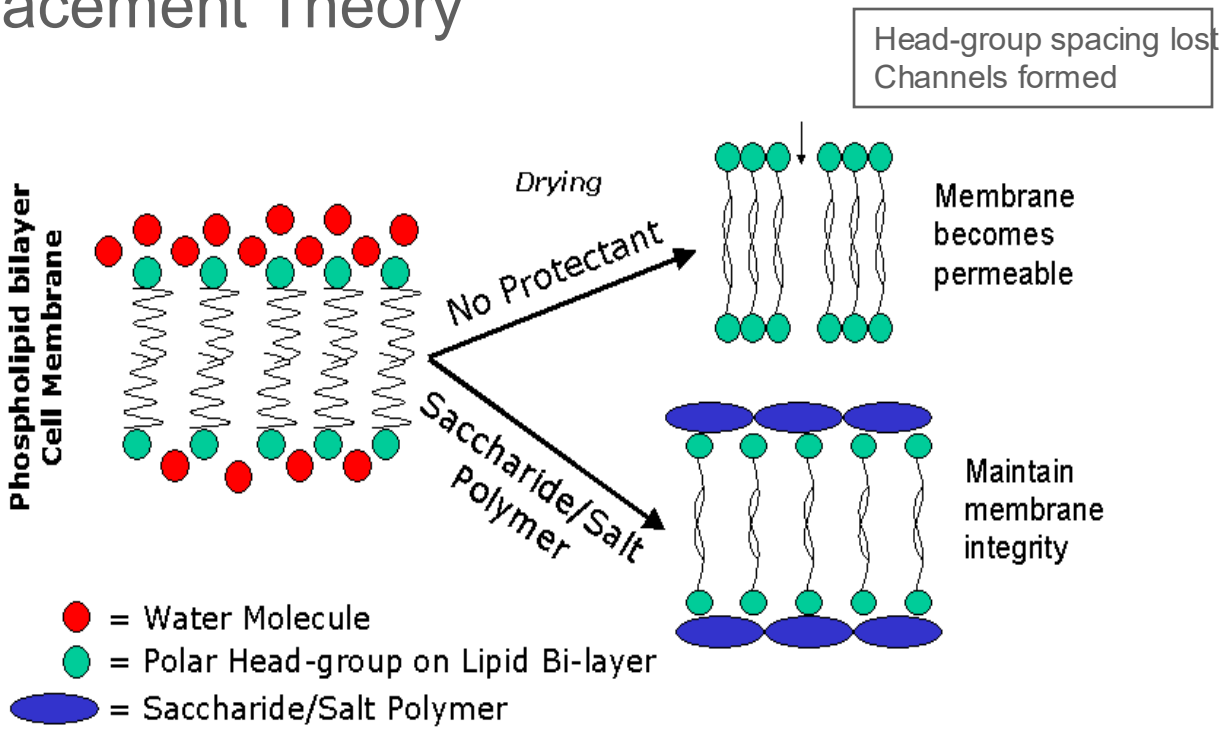


W. Sybesma, A. Dubois unpublished results

Increasing the stability of dried bacteria by using (cryo)protective agents

Water replacement Theory

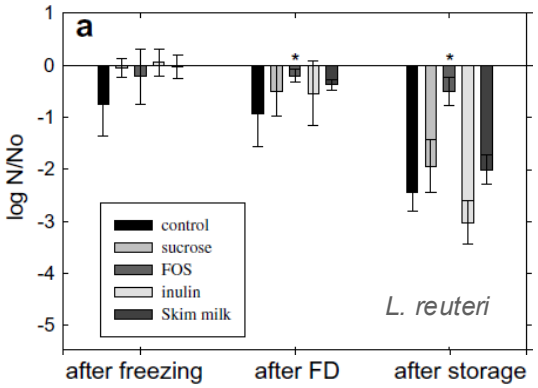


Sucrose and trehalose protect membranes and proteins in vitro from dehydration damage

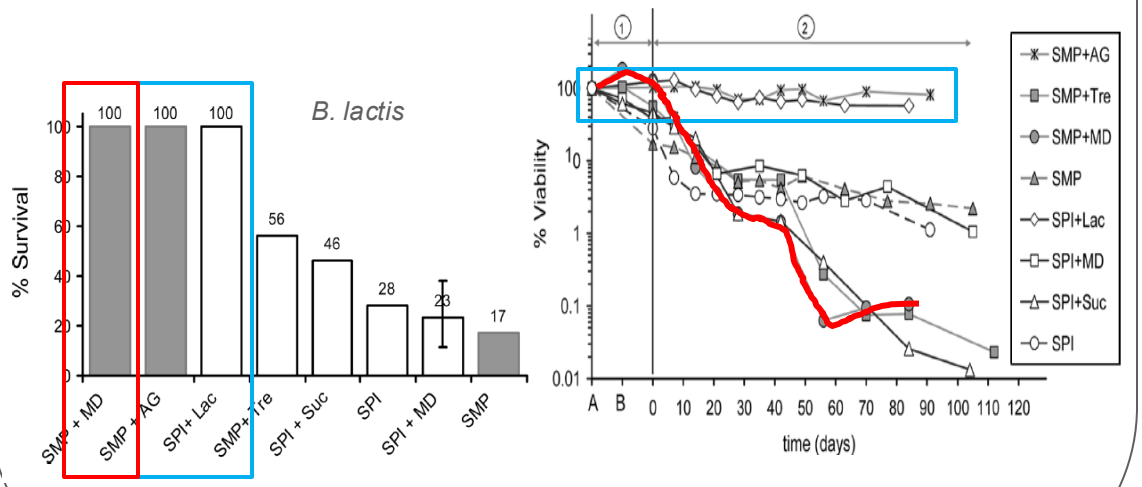
Hianik et al. Bioelecto. and Bioener. 1996 39, 299-302; Viera et al. Biochim. Biophys. Acta 1993 1145,157-167; Anchordoquy et al. Cryobiology 1987 24,324-331.

Encapsulation or protective agent mixes as strategies to minimize viability loss (1/2)

Protective agent/carrier



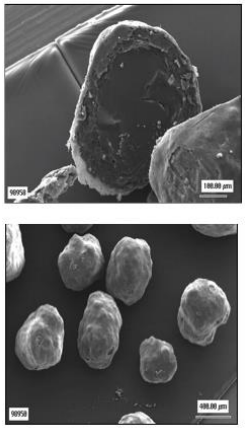
Schwab et al, Cryobiology 55 (2007), 108-114

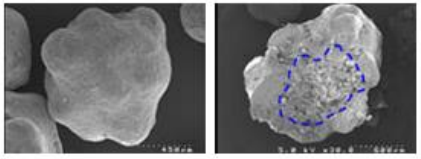


Chavez & Ledeboer, Drying Techol. 25 (2007), 1193-1201

Encapsulation

Microencapsulated
Enterococcus faecium SF68



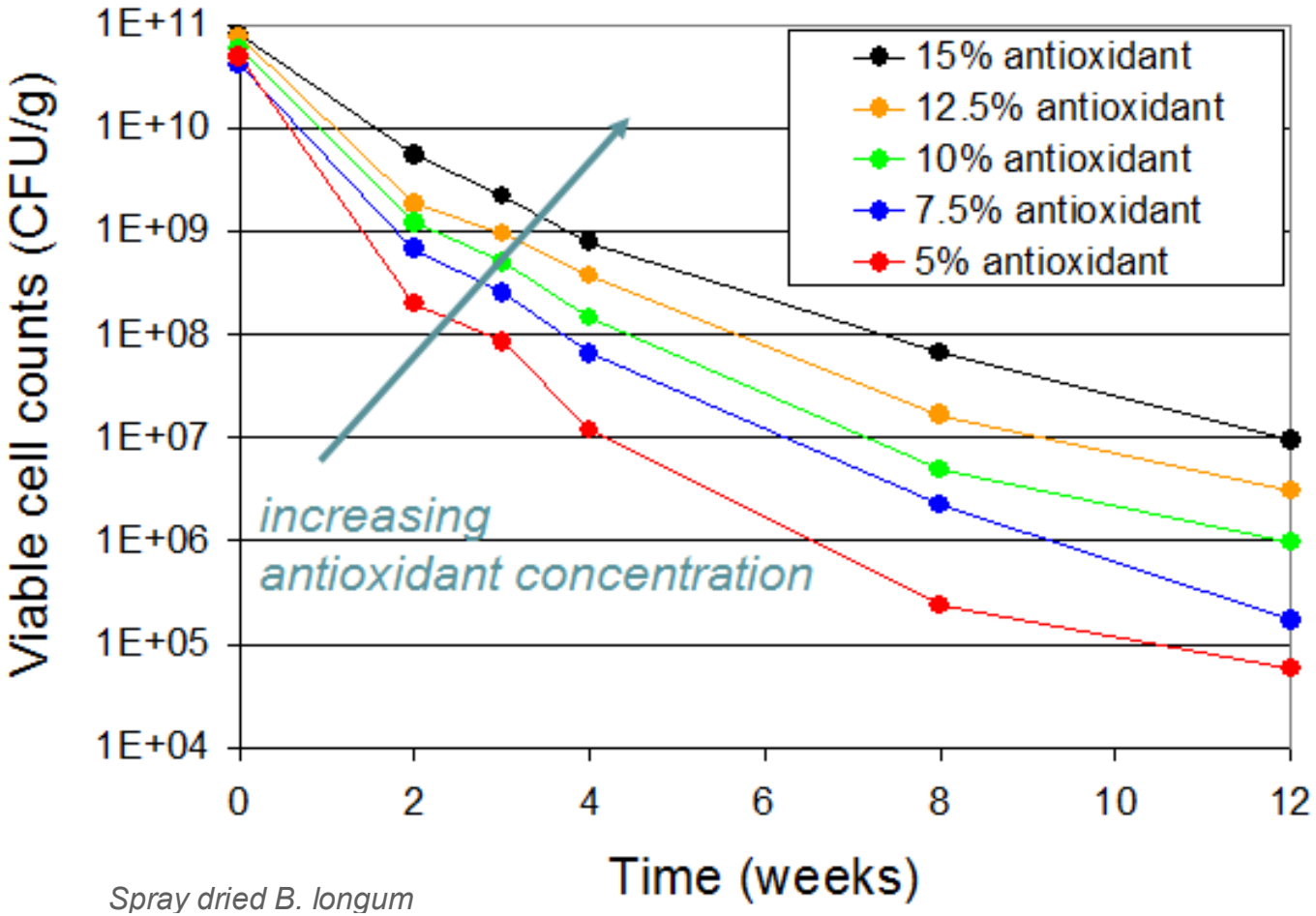


Probiocap outside Probiocap inside

Barry Callebaut, Lal Food

Drying: examples of strategies to minimize viability loss (2/2)

Composition of (cryo)protective agents affects the cell viability

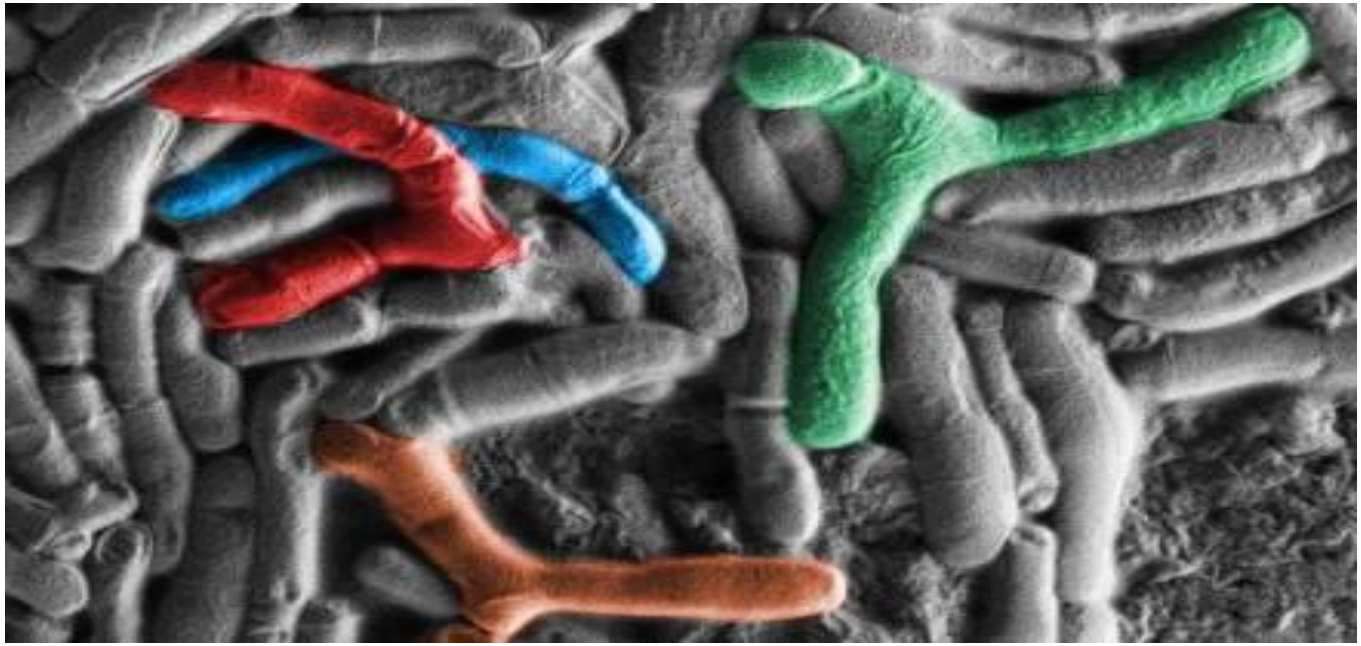


Spray dried B. longum

Accelerated Storage Test

37° C, Aw 0.22

Increasing probiotic yield and stability is important, but ....



.... at the end it is all about functionality and health benefits

>800 published human studies

Immunity
-Immune boost
-Inflammation

Allergy
-Prevention of atopic disease
-Treatment of atopic dermatitis
-Secondary prevention of respiratory allergies

Infections
-*H. pylori*
-Common cold
-Otitis media
-Vaginosis
-Oral health

Diarrhea
-Treatment
-Prevention

Skin health and beauty
-Skin drynes
- Skin sensitivity
-Recovery from UV

Weight management
-Weight gain,
- satiety,
-energy intake

Digestive comfort
-Microbiota balance
-IBS, pain, bloating, flatulence,…
-Quality of life (gut brain axis)
-Lactose intolerance
-Putrafactive fermentation and cancerogenes
-Regulation of gut transit time

Healthy ageing
-CVD
-Improved bioavailability of active ingredients

Performance
-Physical performance
-Cognitive performance

Growth and Development
-Promotion of infants growth
-Improved bioavailability of active ingredients

Probiotics: Do they need to be alive?

it cannot always be ascertained if the claimed functional effects of probiotic preparations are delivered by the biomass, intracellular- or extracellular cell components, and/or media derived bioactive compounds

Different concepts of probiotic production processes and related potential probiotic derived bioactive compound.

Production Process	Potential Probiotic Derived Bioactive Compounds					Products/Examples
	Living probiotic cell	Fermented medium component	Intracellular components	Extracellular components	Killed probiotic cell	
Fermented product as a whole	Yes	Yes	Yes	Yes	Yes	Fermented yogurt drinks with probiotics
Cultivated and down stream processed* without culture medium	Yes	No	Yes	Yes	Yes	Dried culture powders
Cultivated and down stream processed* without cells	No	Yes	No	Yes	No	Spent culture medium, for example, see [64]
Cultivated, killed and downstream processed with or without culture medium	No	Yes	Yes	Yes	Yes	Heat treated lactobacilli, for example, see [65]
Potential mode of action/main active component	Signalling through metabolites or cell components or activity through direct contact	Lactic acid derived from carbohydrates, bioactive peptides derived from milk proteins or raw materials	DNA, cellular proteins or peptides	Bacteriocins, antimicrobial peptides, cell wall components	Activity through direct contact or by cell components	

* For example, frozen, spray dried, freeze dried, encapsulated.

Jankovic, Sybesma et al. Current Opinion in Biotechnology 2010, 21:175–181

Non-viable probiotic microorganisms or probiotic cell components demonstrated to elicit an immune response

For Internal Use Only

BioCare COPENHAGEN

Wanted dead or Alive

What are the differences between live and dead beneficial bacteria

Probiotics are defined as live microorganisms that when administrated in adequate amounts confer a health benefit to the host.

Inactivated ("dead") beneficial bacteria are not considered probiotics. They belong to the group of postbiotics.

Postbiotics are defined as preparations of inanimate microorganisms and/or their components that confers a health benefit on the host.

Dead and live bacteria can have common beneficial effects such as immunomodulatory actions however, there is also differences

What are the main differences between **Probiotics** and **Postbiotics**

Can proliferate and colonize the intestinal microbiome	Cannot proliferate and colonize the intestinal microbiome
Benefits are extensively documented in clinical studies for various health benefits	Benefits are not yet well established and more evidence is needed
Health benefits are strain specific	Different metabolite production by specific strains requires further research
Can excrete bacteriocins that inhibit pathogens	Do not excrete bacteriocins that inhibit pathogens
Stimulate the production of beneficial SCFA	Limited ability to stimulate the production of SCFA
Ability to metabolize harmful molecules (cholesterol), thus benefit host metabolism	Limited ability to metabolize harmful molecules (cholesterol), thus benefit host metabolism
Limited stability	High stability
Risk of transferring antibiotic resistance genes	No risk of transferring antibiotic resistance genes

References: 1. Adams, Nutrition Research Reviews (2010), 23, 37–46; 2. Pique et al., Int. J. Mol. Sci. 2019, 20, 2534; 3. Żółkiewicz et al., Nutrients 2020, 12, 2189

Nutrition Research Reviews (2010), **23**, 37–46
doi:10.1017/S0954422410000090

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

Clifford A. Adams
ANOZENE Nutritional Sciences, Fruithoflaan 103, bus 14, 2600 Berchem, Antwerp, Belgium

International Journal of *Molecular Sciences*
MDPI

Review

Health Benefits of Heat-Killed (Tyndallized) Probiotics: An Overview

Núria Piqué [1,2,*], Mercedes Berlanga [1] and David Miñana-Galbis [1]

1 Microbiology Section, Department of Biology, Healthcare and Environment, Faculty of Pharmacy and Food Sciences, Universitat de Barcelona (UB), Av Joan XXIII, 27-31, 08028 Barcelona, Catalonia, Spain; mberlanga@ub.edu (M.B.); davidminyana@ub.edu (D.M.-G.)
2 Institut de Recerca en Nutrició i Seguretat Alimentària de la UB (INSA-UB), Universitat de Barcelona, 08921 Barcelona, Catalonia, Spain
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Received: 25 April 2019; Accepted: 21 May 2019; Published: 23 May 2019
check for updates

nutrients
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Review

Postbiotics—A Step Beyond Pre- and Probiotics

Jakub Żółkiewicz [1], Aleksandra Marzec [1], Marek Ruszczyński [2] and Wojciech Feleszko [1,*]

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2 Department of Paediatrics, Medical University of Warsaw, Żwirki i Wigury 63A, 02-091 Warsaw, Poland; marek.ruszczynski@gmail.com
* Correspondence: wojciech.feleszko@wum.edu.pl; Tel.: +48-22-317-94-19

Received: 30 June 2020; Accepted: 21 July 2020; Published: 23 July 2020
check for updates

Postbiotics

Nutrients **2020**, *12*, 2189

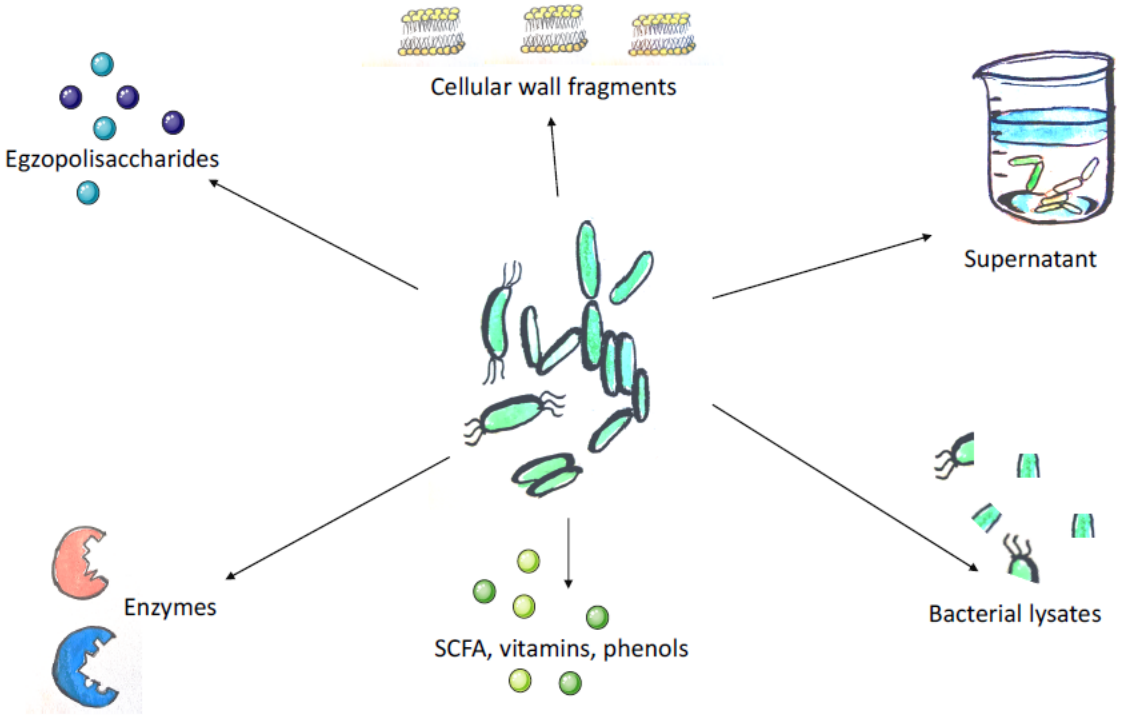
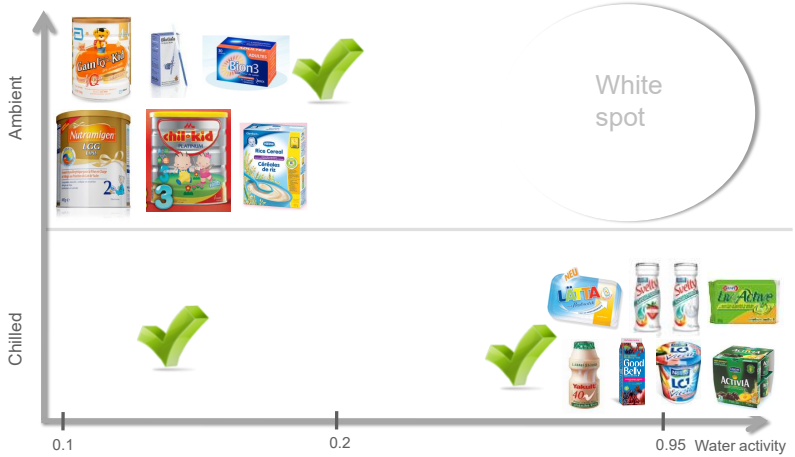


Figure 2. Methods of acquisition of postbiotics. The concept of postbiotics conceals either metabolites or fragments of microorganisms which confer a beneficial effect to the host. The structural heterogeneity of postbiotics implies the abundance of possible techniques used to postbiotics' acquisition. Lysis of bacterial cells may be achieved by chemical and mechanical techniques. These methods include enzymatic extraction, solvent extraction, sonication, and heat. Extraction, dialysis, and chromatography are used to isolate and identify desired molecules. SCFA, short-chain fatty acids.

'paraprobiotics' *-inactivated microbial cells or cell fractions conferring a health benefit to the consumer-* for new product applications

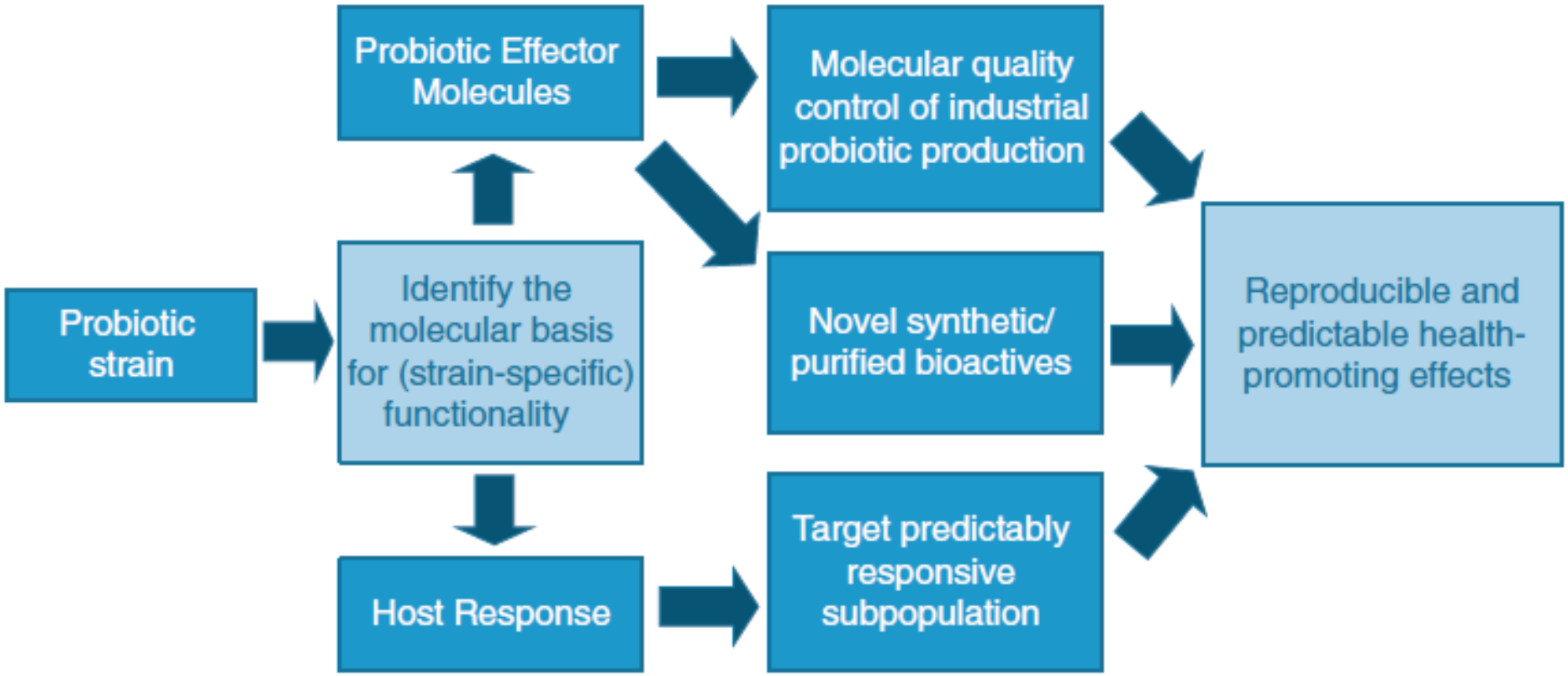
- Extend the use of health promoting bacteria to products in which they are difficult to maintain alive :
  - High moisture & water activity products
  - High acidity products (fruit juices,..)
  - Shelf stable products (cereals, cereal bars..)



- Increased shelf life, less stringent storage conditions, less stringent packaging specifications
- May enable hot usage for consumers
- Safety: Better suited for critically ill or immuno-compromised subjects

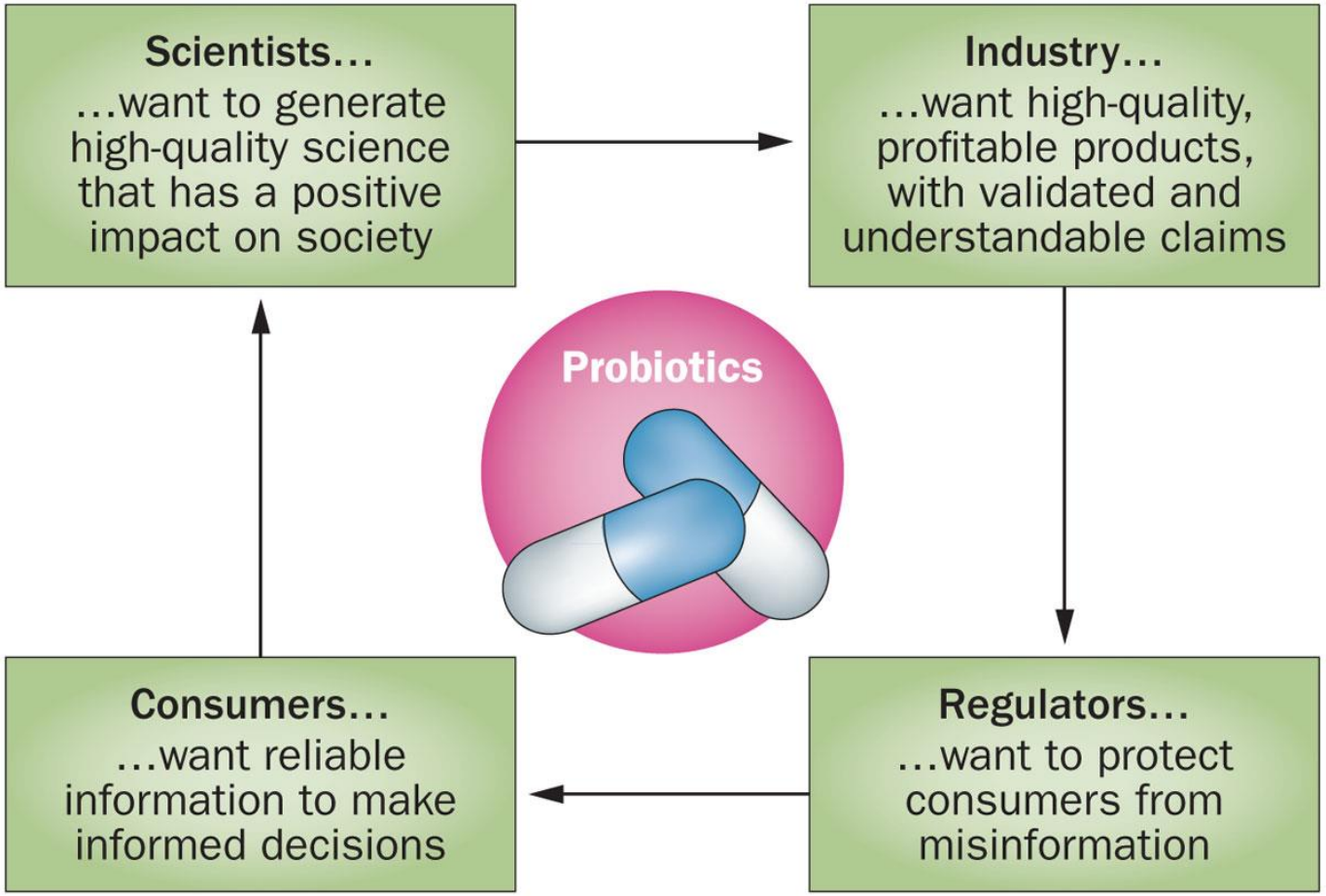
Proposal of paraprobiotic concept
V. Taverniti, S. Guglielmetti. Genes Nutr (2011) 6:261–274

The future: From cfu to understanding mechanism of action and personalized nutrition



Bron , et al. Current Opinion in Microbiology, Volume 16, Issue 3, 2013, 262 - 269

All stakeholders to work together performing their respective duties so that society benefits from the scientific advances of probiotic research



Hill, C. *et al.* (2014) The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic *Nat. Rev. Gastroenterol. Hepatol.* doi:10.1038/nrgastro.2014.66

Movie explaining the microbiome

https://youtu.be/xEo3N9EOpgw