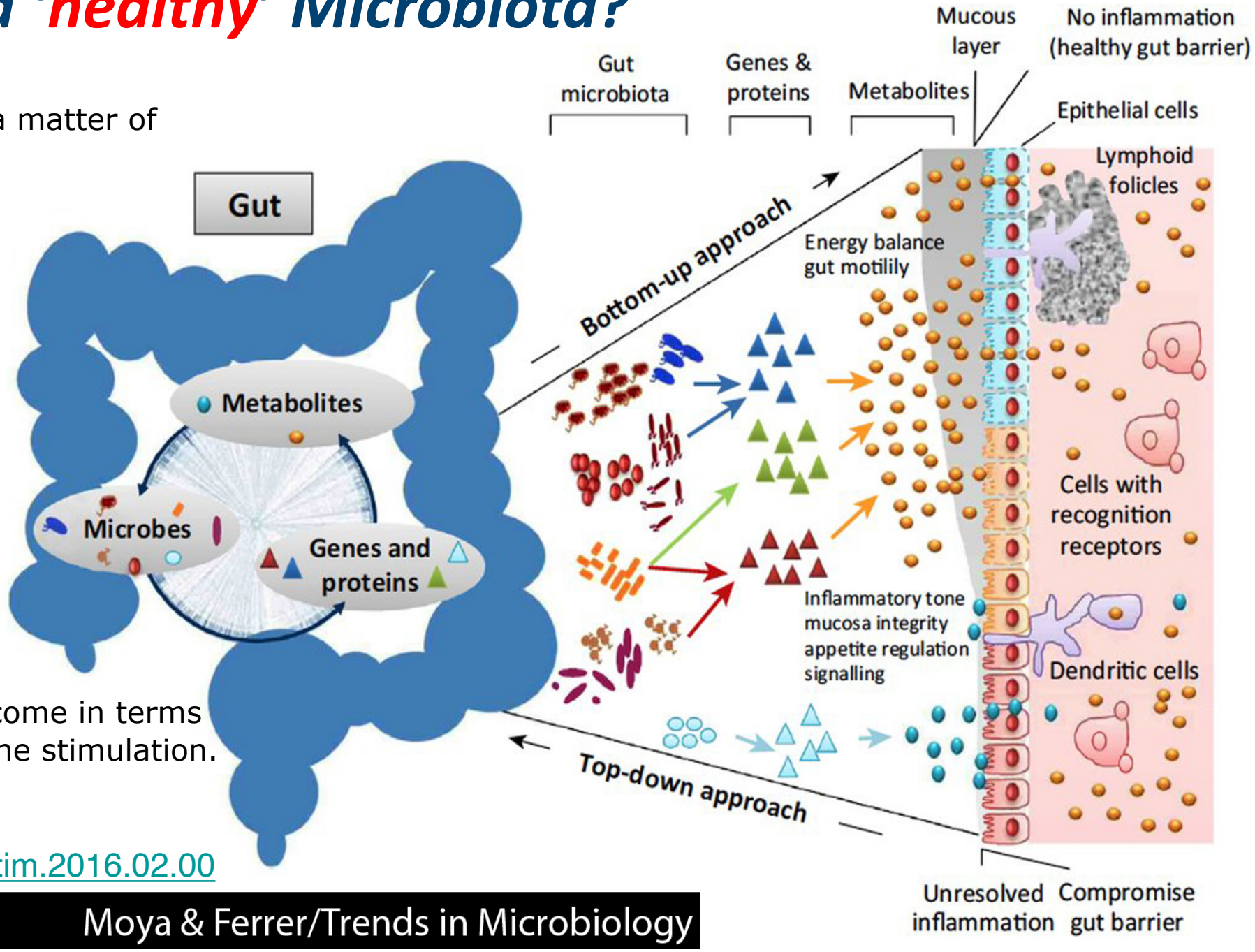
What is a 'healthy' Microbiota?

We do know now it is a matter of
- Bacteria
- Metabolites
- Genetics
- Environment
  - Diet
  - Drugs
  - Stress
  - Smoking
  - Age
  - …

We also know that more than one combination of species can have the same outcome in terms of metabolism or immune stimulation.



DOI:https://doi.org/10.1016/j.tim.2016.02.00

Moya & Ferrer/Trends in Microbiology

A 'healthy' Microbiota may be a « balanced » microbiota

An INCREASING number of criteria are accepted as 'indicators' of a 'healthy' microbiota:

- Besides the ***Balance*** between bad and good bacteria
- With sufficient ***diversity…***
- With sufficient ***resilience…***

This balance can be disturbed at many occasions, by many causes.

Balance of many parameters

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The disturbance of the "balance" is a real risk …

Most people will suffer from intestine-related disease/discomforts at some point in their lives:

• Infectious bowel disease	33%
• Food intolerances (lactose)	15%
• Irritable bowel syndrome (IBS)	10%
• Antibiotics-associated diarrhea (AAD)	5%
• Food allergy (peanut)	2 %
• Diverticulitis	0,74%
• Inflammatory bowel diseases (IBD)	0,4%
• Celiac disease	0,2%
• Colorectal cancer (CRC)	0,06%
• Other GI malignancies	0,01%
• Short bowel syndrome	0,004%

The « absence of symptoms » is maybe an indicator of a « healthy » microbiota.

If probiotics can contribute to decrease the number of periods with symptoms they may be considered « beneficial ».

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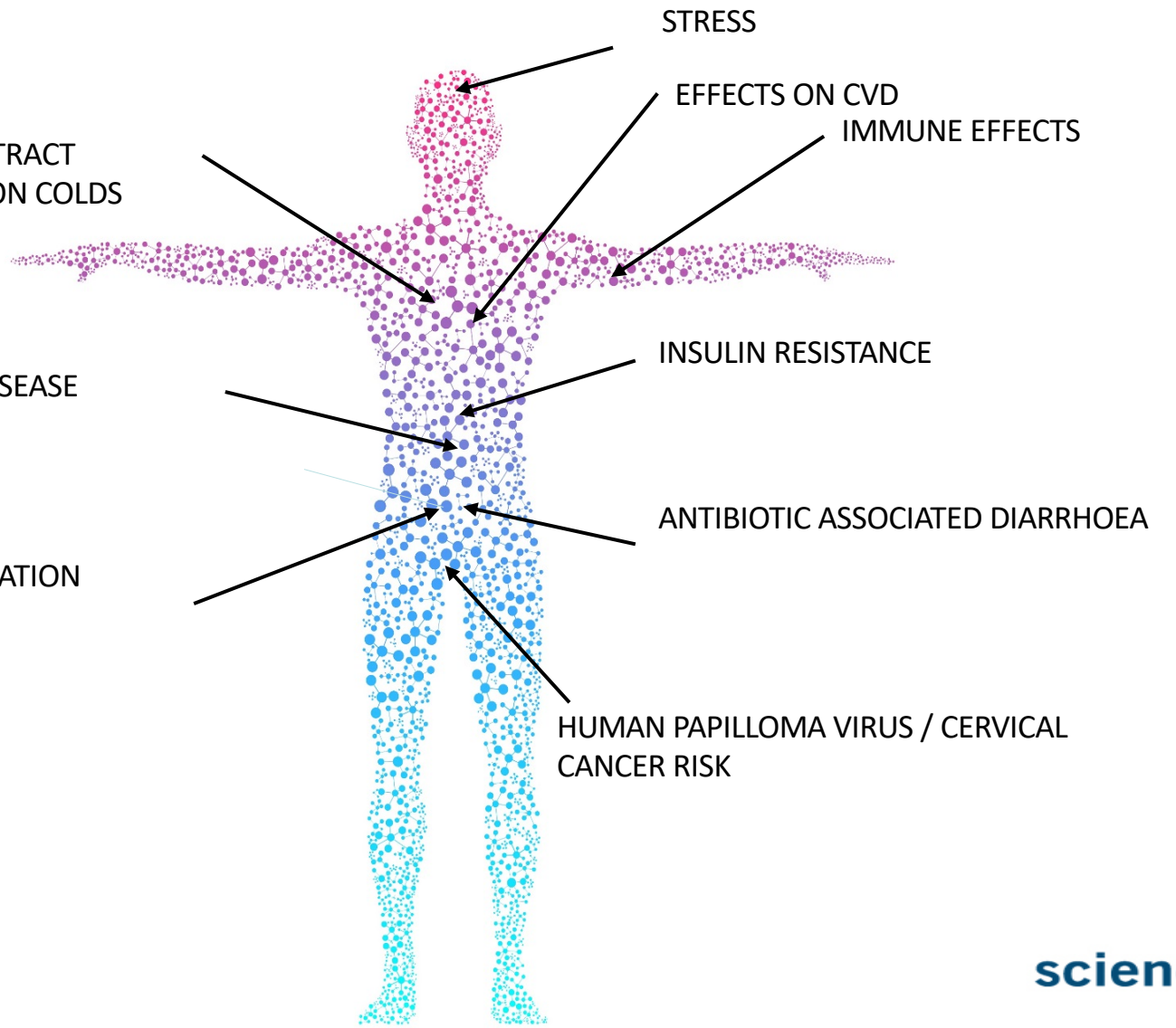
Probiotics: Other Areas of Research



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Conclusion 1

- Probiotics have an enormous potential because of their wide range of interactions with the host, either directly or indirectly through the microbiota.
- Several mechanisms have been studied and described

- The complexity of the ecosystem of the gut, however, the influence of the environment, diet, stress, drugs, and the individual signature of the microbiota, make it difficult to predict the impact of a given probiotic strain

- Therefore, the proof of a probiotic effect can probably only be illustrated through sufficiently powered clinical studies… which will cover this reality of diversity and environmental influences.

- For a probiotic food, these studies need to be performed in a healthy population; for a probiotic drug, these studies need to be performed in a diseased population.

What's it all about?

- Probiotics: what are they ?
- The importance of the microbiota.
- Probiotics: What can they do ?
- Clinical studies as the ultimate proof
  - The immune effects
  - The microbiological effects
  - The metabolic effects
- Conclusions

Effects of probiotics in a healthy population:

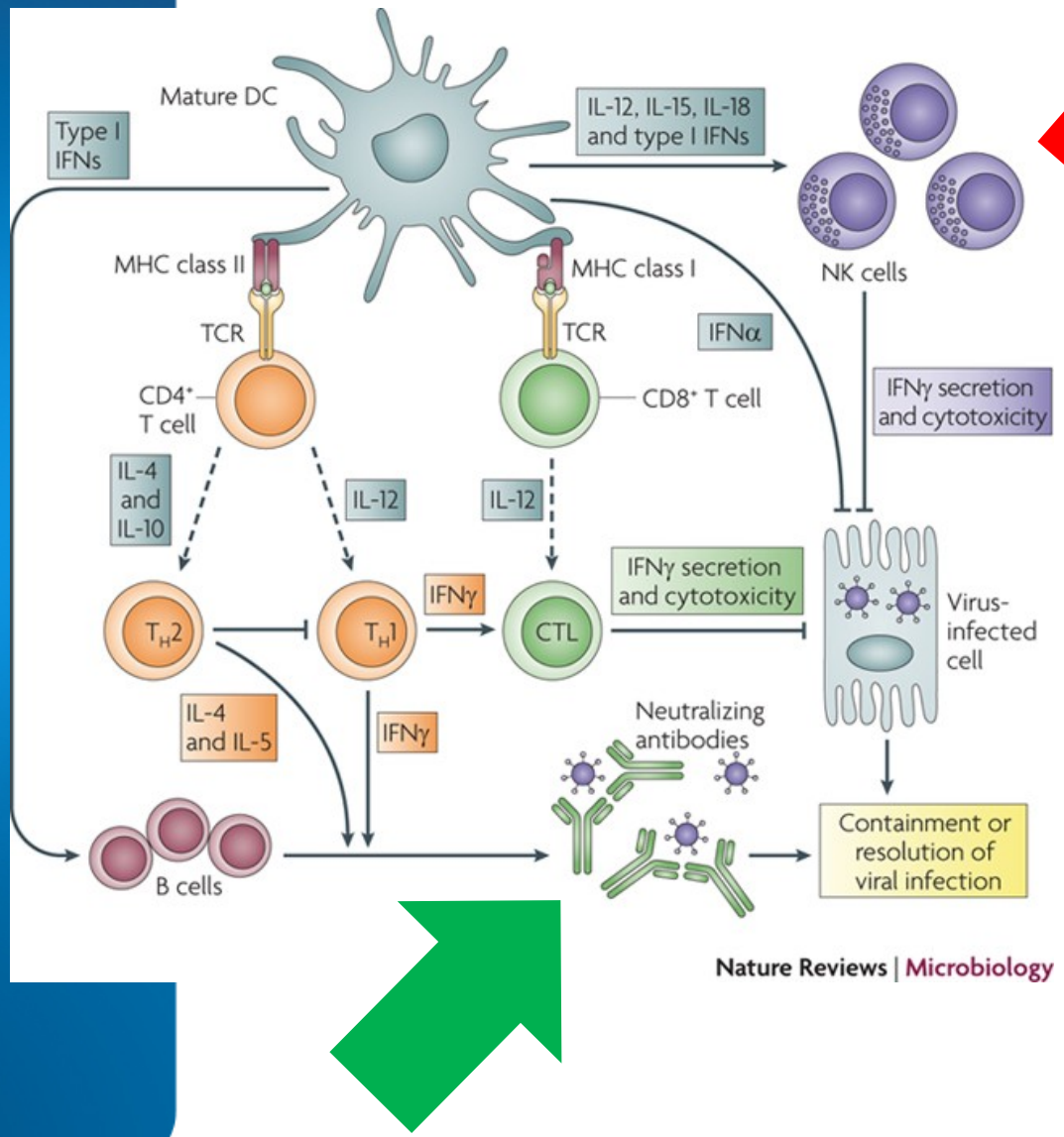
1. The immune function.

- If an immune response is to be shown in a *Healthy Population*, people can be selected in which the immune system does not function at maximum efficiency. Possible situations are:
  - Old age
  - Chronic illness
  - Psychological stress
  - Physical stress

- Probiotics can then help to boost the immune system again

- Some examples…

In COVID-19 times: Signalling pathways important for viral infections



- **Effects on Natural Killer Cells (NK-cells)**
- **Increased (secretorial) Immunoglobulin A (s) IgA**
- Increased phagocytic activity of white blood cells
- Proliferation of intra-epithelial lymphocytes

Mechanisms that may be relevant in the current corona pandemic.

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Reduced (viral) infections in athletes

International Journal of Sport Nutrition and Exercise Metabolism, 21, 2011, 55-64

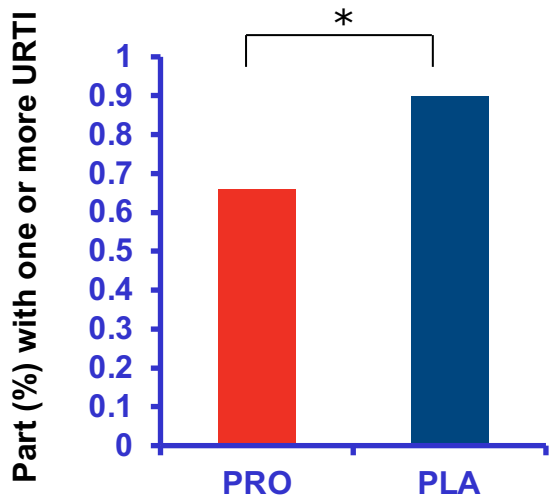
Daily Probiotic's (*Lactobacillus casei* Shirota) Reduction of Infection Incidence in Athletes

Michael Gleeson, Nicolette C. Bishop, Marta Oliveira, and Pedro Tauler

- Observation: Athletes in heavy training programs experience declined immune function (despite they are\ considered healthy)
- Incidence of upper respiratory-tract infections (URTI) and immune markers can be analysed in athletes engaged in endurance-based physical activity (with or without a probiotic)

- RDBPC-trial (n=84); 58 completed the study (LcS n=32, PB n=26)
- *L. casei* Shirota (2 x 6.5 x 10^9 CFU) or placebo, daily for 16-weeks

- **Result 1**: 27% less athletes in the probiotic experienced 1 or more weeks with URTI symptoms as compared to placebo (0.66 vs 0.90 resp., P=0.021)
- The mean number of URTI episodes was lower in the probiotic group compared to placebo (1.2 vs 2.1 respectively, P<0.01).



Gleeson M, Bishop NC, Oliveira M, Tauler P (2011) Daily probiotic's (*Lactobacillus casei* Shirota) reduction of infection incidence in athletes. International Journal of Sport Nutrition & Exercise Metabolism 21(1):55-64.

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Reduced infections in athletes

International Journal of Sport Nutrition and Exercise Metabolism, 21, 2011, 55-64
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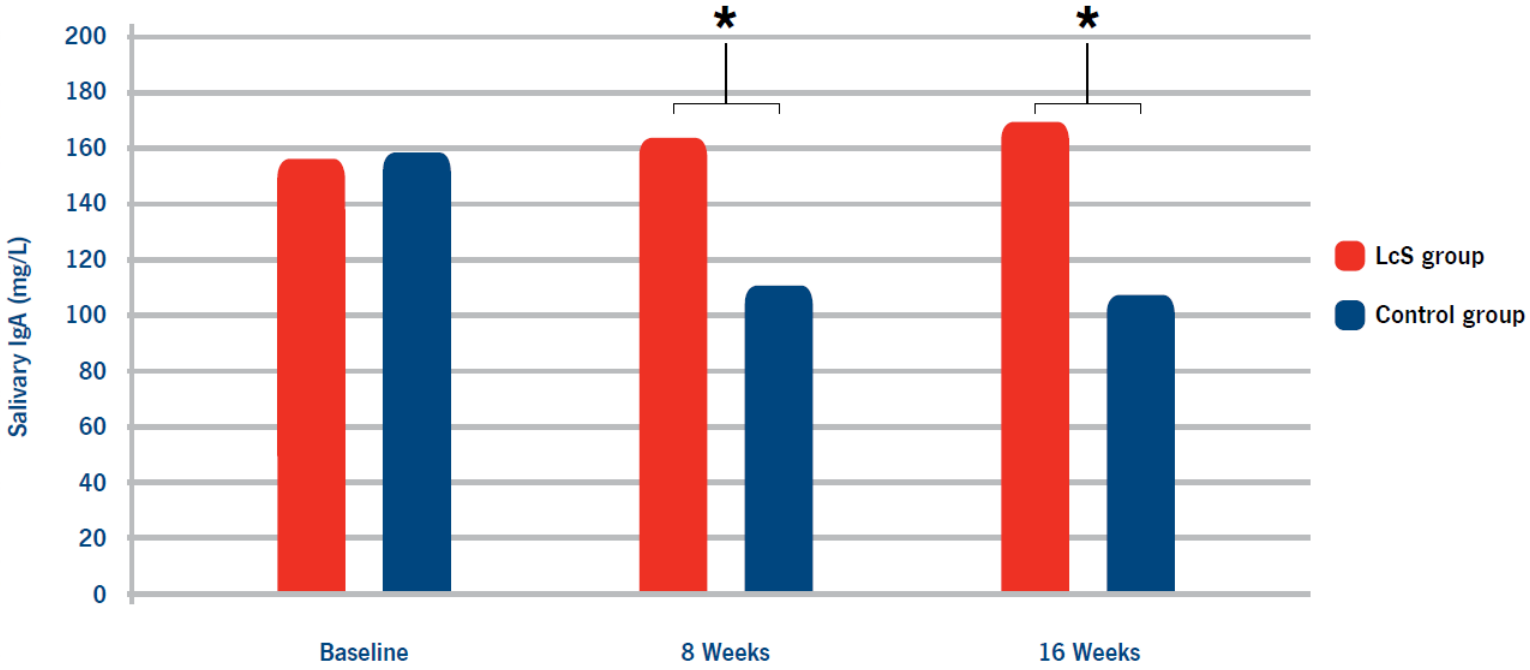
Daily Probiotic's (*Lactobacillus casei* Shirota) Reduction of Infection Incidence in Athletes

Michael Gleeson, Nicolette C. Bishop, Marta Oliveira, and Pedro Tauler

- **<u>Result 2</u>**: immune analysis:
- salivary IgA concentrations were significantly higher in the probiotic group than the placebo group at both week 8 (P=0.03) and 16 (P=0.01).



Gleeson M, Bishop NC, Oliveira M, Tauler P (2011) Daily probiotic's (*Lactobacillus casei* Shirota) reduction of infection incidence in athletes. International Journal of Sport Nutrition & Exercise Metabolism 21(1):55-64.

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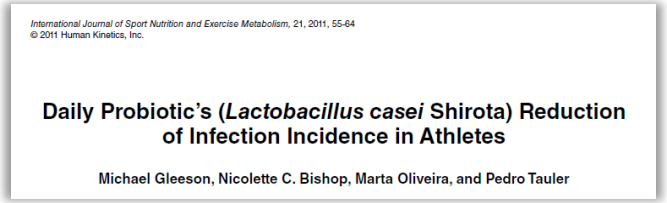
Reduced infections in athletes



Conclusion

- Daily consumption of LcS reduced the frequency of URTI in a group of athletes.
- This is likely attributable to the maintenance of salivary IgA levels which would otherwise have decreased during a winter period of intense sports training and competition, considered 'natural' causes of immune depression.

- Gleeson and co-workers also showed that probiotic intake reduced plasma Cytomegalovirus and Epstein Barr virus antibody-titres (Gleeson et al., 2016)

Gleeson M, Bishop N.C., Struszczak L. (2016) Effects of *Lactobacillus casei* Shirota ingestion on common cold infection and herpes virus antibodies in endurance athletes: a placebo-controlled, randomized. *European Journal of Applied Physiology* **116(8)**:1555-1563.
Gleeson M, Bishop NC, Oliveira M, Tauler P (2011) Daily probiotic's (*Lactobacillus casei* Shirota) reduction of infection incidence in athletes. International Journal of Sport Nutrition & Exercise Metabolism 21(1):55-64.

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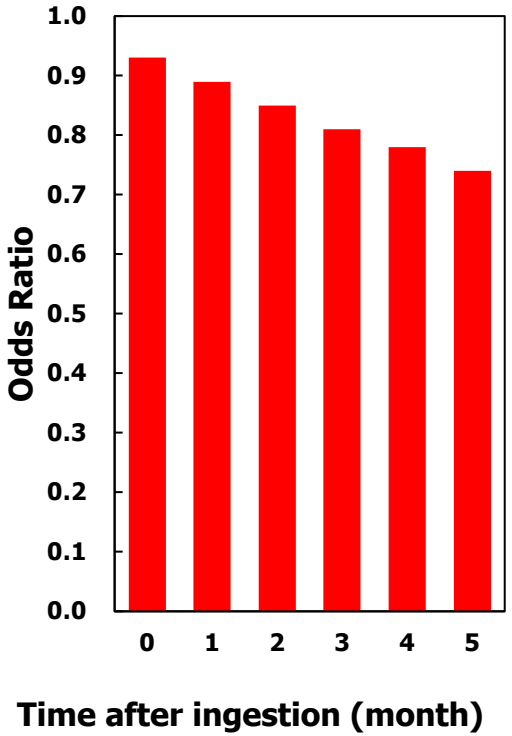
2nd target group: URT infection in elderly

Belgian study
Age: >65 years
Intervention: 6 months

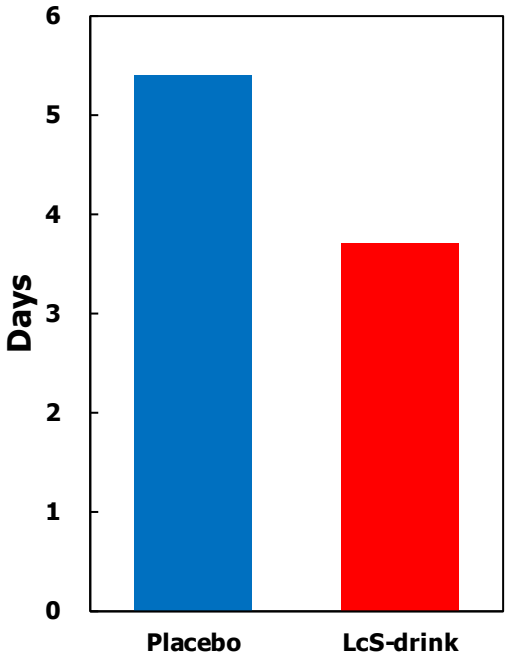
Risk of RTI



Van Puyenbroeck K et al. (2012)

Japanese study
Age: Mean 83 years
Intervention: 7 months

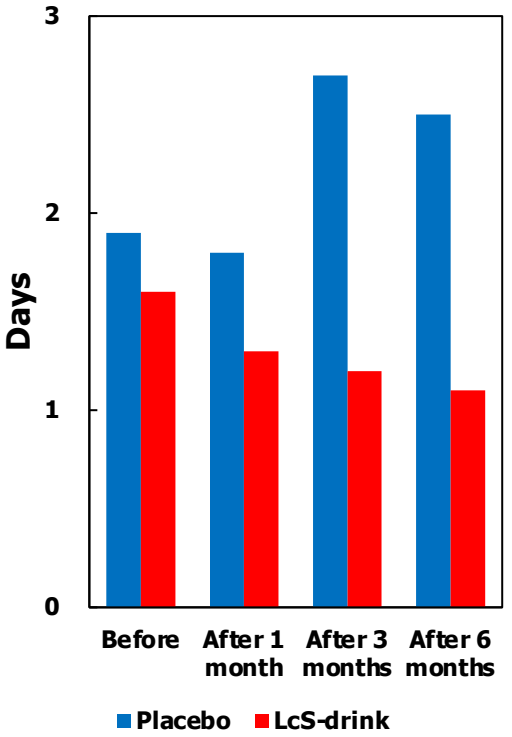
Duration of URTI



Fujita R et al. (2013)

Japanese study
Age: Mean 85 years
Intervention: 6 months

Duration with fever



Nagata S et al. (2016)

Slide with courtesy of Dr. M. Nanno, Yakult Central Institute

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3rd example: Immune effects in viral infections

Probiotics enhance the clearance of human papillomavirus-related cervical lesions: a prospective controlled pilot study

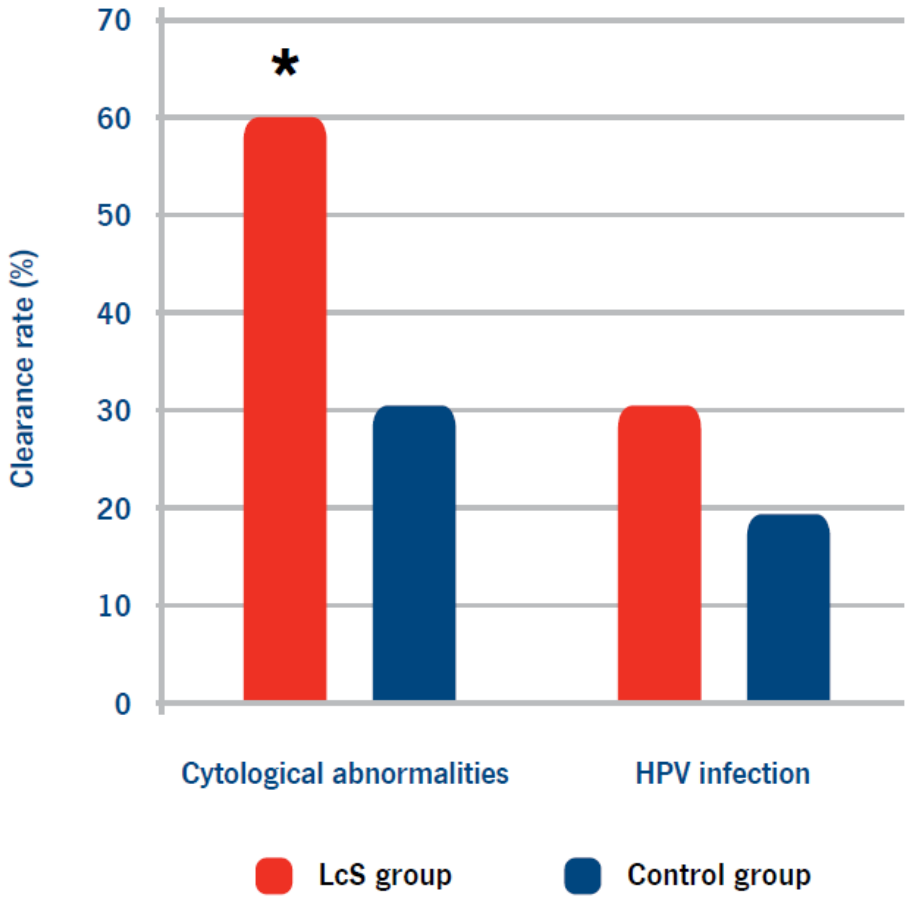
Veronique Verhoeven[a], Nathalie Renard[a], Amin Makar[d], Paul Van Royen[a], John-Paul Bogers[a,b], Filip Lardon[c], Marc Peeters[c] and Marc Baay[c]

- Clearance of HPV infection and associated pre-cancerous abnormalities was followed for 6 months (low-grade squamous intraepithelial lesions; LSIL)
- Open Label (no placebo) but controlled study (probiotic n=24; control n=27) in Belgium
- *L. casei* Shirota (6.5 x 10^9 CFU) daily for 6 months

Result:

- LcS users after 6 months had a twice as high chance of clearance of **cytological abnormalities** (60% vs. 31%) as compared to the controles (detected by PAP smear test and human papilloma virus (HPV) status).

- **HPV** was cleared in 19% of control patients versus 29% in the probotic group, suggesting that LcS promoted the immune-mediated clearance of HPV-related cytological abnormalities.



Verhoeven V, Renard N, Makar A *et al* (2012) Probiotics enhance the clearance of human papillomavirus-related lesions: a prospective controlled pilot study. *European Journal of Cancer Prevention* **22(1)**:46-51

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Effect of LcS on Natural Killer (NK) cell activity (innate immunity) in healthy adults

Subjects	Country	Intake	Results	Reference
Healthy adults (40-65 years)	The Netherlands	Placebo vs LcS 4 weeks	No difference	Eur J Clin Nutr (1998)
Healthy adults (20-40 years)	Japan	Placebo vs LcS 3 weeks	Increased by LcS	Biosci Biotechnol Biochem (2000)
Healthy adults (69-97 years)	Japan	Placebo vs LcS 3 weeks	Increased by LcS	Clin Exp Immunol (2006)
Healthy adults (18-60 years)	Germany	Placebo vs LcS 4 weeks	No difference	J Nutr (2011)
Healthy adults (40-60 years)	Italy	Placebo vs LcS 3 weeks	Increased by LcS	Br J Nutr (2012)
Healthy adults (55-74 years)	United Kingdom	Placebo vs LcS 4 weeks	Increased by LcS	Eur J Nutr (2013)
Healthy adults (30-49 years)	Japan	Milk vs LcS 12 weeks	Increased by LcS	Eur J Nutr (2017)

Slide with courtesy of Dr. M. Nanno, Yakult Central Institute

What's it all about?

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- Probiotics: What can they do ?
- Clinical studies as the ultimate proof
  - The immune effects
  - The microbiological effects
  - The metabolic effects
- Conclusions

Effects of probiotics in a healthy population:

1. The immune function.

2. The microbiological function.

- Very similarly as for immune function, as we are considering a FOOD, it is important to perform clinical studies with people that are either "sub healthy" or "at risk", but not with patients.
- A very nice example is antibiotic associated diarrhea: people with a healthy microbiota will take an antibiotic (it could be for any reason other than intestinal problems) which may pose a risk for their microbiota "balance".
- Can probiotics protect against that disturbance?
- Some examples…

Antibiotic Associated Diarrhoea

British Journal of Nutrition (2014), **111**, 672–678 doi:10.1017/S0007114513002973

A *Lactobacillus casei* Shirota probiotic drink reduces antibiotic-associated diarrhoea in patients with spinal cord injuries: a randomised controlled trial

Samford Wong[1,2,3*], Ali Jamous[1], Jean O'Driscoll[4], Ravi Sekhar[5], Mike Weldon[5], Chi Y. Yau[6], Shashivadan P. Hirani[2], George Grimble[3] and Alastair Forbes[3]

- Subjects of the National Spinal Injuries Centre at Stoke Mandeville Hospital with a Spinal Cord Injury who were on antibiotics (n=164)
- Randomized Controlled trial (probiotic or no probiotic, antibiotic regime only)
- *L. casei* Shirota (6.5 x 10^9 CFU) daily for the duration of their antibiotic course and for 7 days thereafter
- Endpoint: Bowel movements, monitored for the presence of AAD, and where necessary detection of *C. difficile* toxin.
- **Result**: significantly lower incidence of AAD (17.1% vs. 54.9%, P=0.001).

	LcS group (*n* 76)		Control group (*n* 82)		
	n	%	*n*	%	*P*
Parameters					
Age (years)	52·5		51		0·657
Male	62	81·6	69	84·1	0·948
Caucasian	67	88·1	72	84·7	0·524
Primary diagnosis on recruitment					
Onset of SCI (days)	71		60		0·474
Tetraplegia	48	63·2	52	63·4	0·631
Complete SCI	27	35·5	35	42·7	0·502
Median number of laxatives	2		2		0·735
Median number of drugs	12		12		0·537
Median number of antibiotics	1		1		0·949
Antibiotic route: oral	26	34·2	24	29·3	0·126
Risk of antibiotics causing diarrhoea*					
Low	5	6·6	19	23·1	0·004
Medium	25	32·9	24	29·3	0·622
High	48	63·2	39	47·6	0·049
Indication of antibiotics					
Urinary tract infection	32	42·1	42	50·0	0·320
Respiratory tract infection	20	26·3	26	31·7	0·456
Use of PPI†	14	18·9	17	21·8	0·532
Risk of undernutrition‡	51	68	53	62·4	0·759
Nutrient intake					
Energy (kJ)	5777·7		5442·8		0·536
Dietary fibre (g)	12·4		11·6		0·606
BMI (kg/m^2)	24·2		23·7		0·589
Use of enteral feeding tube	9	11·8	13	15·2	0·589

Wong SS, Jamous A, O'Driscoll JO *et al* (2014) A *Lactobacillus casei* Shirota probiotic drink reduces antibiotic-associated diarrhoea in patients with spinal cord injuries: a randomized controlled trial. *British Journal of Nutrition* **111(4)**:672-678.

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Antibiotic Associated Diarrhoea

- The above is only one of the many studies (next slides), with only one of the many strains that seems to be effective.
- World-wide MD's and dieticians are giving the advice to take probiotics when taking antibiotics…
- If so universal, what is the underlying mechanism?

reference	population			treatment				result
	age	n	health status	antibiotics	probiotics	Control	method	resilience
		total (Probiotics group) N		name Dose per day and duration	Name Dose Form Duration		IPA-EU Science Committee Dossier (unpublished)	
Adults								
Black 1991	adults mean age 35	20 (N=10)	healthy	1.5g ampicillin / d 7 days	L. acidophilus ($9x10^9$) +B. bifidum ($1.4x10^9$) daily in a capsule 7 days	Capsule	culture	Partial resilience: anaerobic gram-positive cocci, lactobacilli, clostridia and eubacteria increased faster in probiotic group while bifidobacteria increased faster in placebo. No significant differences between groups on the number of *Veillonella*-cocci and *Bacteroides. Bacteroides* were recovered in higher numbers in probiotic
Engelbrekston 2006 (same study also published in 2009)	adults mean age 37	40	healthy	1.75 g amoxicillin-clavulanic acid /d 7 days	B. lactis Bl-04 ($5x10^9$) +B.lactis Bi-07 ($5x10^9$) + L. acidophilus NCFM ($5x10^9$) + L. paracasei - 37 ($5x10^9$) + B.bifidum 02 ($5x10^8$) Total: $4x10^{10}$ CFU / d Capsule 10 days	Maltodextrin, Capsule	TRFLP + PCR for L.a NCFM + culture at genus level	Resilience: probiotics limit the disruption of microbiota due to AB (p=0,046 with culture data and p=0,066 with molecular data)
Forssten 2014	adults mean age 32	80 (N=36)	healthy	amoxicillin-0,875g/d + clavulanic acid (0,125g/d 7 days)	12.5 × 10^9 CFU/d L. acidophilus NCFM and 12.5 × 10^9 CFU/d B. lactis Bi-07 Capsule 14 days	No control for intake of probiotics	RT PCR	No conclusion, but trend towards enhanced resilience: AB-induced changes are very minimal in both groups, but *Clostridium* cluster XIV recovered more quickly in probiotic group
Imase 2008	adults 32 to 71	19 (N=5-7)	H pylori positive	amoxicillin (1500 mg), and clarithromycin (800 mg) 7 days	CBM588 (Clostridium butyricum) Tablets 7 days	No control for intake of probiotics	culture + detection of C Diff toxin	Resilience occurs in all groups, incl the control group. But the decrease in obligate anaerobes is less in the probiotic group

reference	population			treatment				result
	age	n	health status	antibiotics	probiotics	Control	method	resilience
		total (Probiotics group) N		name Dose per day and duration	Name Dose Form Duration			
Adults								
Jernberg 2005	adults	8 (N=4)	healthy	clindamycin 600 mg /d 7 days	Yoghurt: Lactobacillus acidophilus NCFB 1748, Lactobacillus paracasei F19, and Bifidobacterium lactis Bb12 $5x10^{10}$ CFU each / d Dairy product 14 days	unflavored yoghurt	TRFLP+ culture	Resilience in probiotic group, for 3/4 subjects microbiota at day 21 was closed to the one at day 0. In the placebo group, no resilience was observed.
Kabbani 2016	Adults mean age 30	48 (N=12)	healthy	amoxicillin-clavulanate (875/125 mg, twice daily)	S. boulardii CNCM I-745 1g/d (10^{11} cfu/day)	No control product	16s rRNA gene pyrosequencing (bTEFAP).	Resilience: S. boulardii can mitigate some antibiotic-induced microbiota changes; among others less over growth of Escherichia
Lidbeck 1988	adults mean 20 to 55	20 (N=5 / antibiotic)	healthy	800 mg /denoxacin (N=10) 600 mg clindamycin 150 mg /d (N=10) 7 days	L. acidophilus $1.25x10^{11}$ cfu/d Fermented milk) 7 days	No control for intake of probiotics	culture	Resilience in the enoxacin-treated group; no resilience in the clindamycin treated group.
Madden 2005	adults 33 to 70	22 (N=6-9)	H pylori positive	2 g amoxycillin / d, 1.2 g metronidazole /dt. 7 days	two strains of Lactobacillus acidophilus (CLT60 and CUL21) and two strains of Bifidobacterium bifidum (CUL17 and B. bifidum Rhodia) Total of $2.5x10^{10}$ Capsule 7 to 14 days	maltodextrin, Capsule	culture	Resilience: no change in AB+probio group whereas in the placebo group total anaerobes and total facultative anaerobes remain high
					Lactobacillus	No control for		

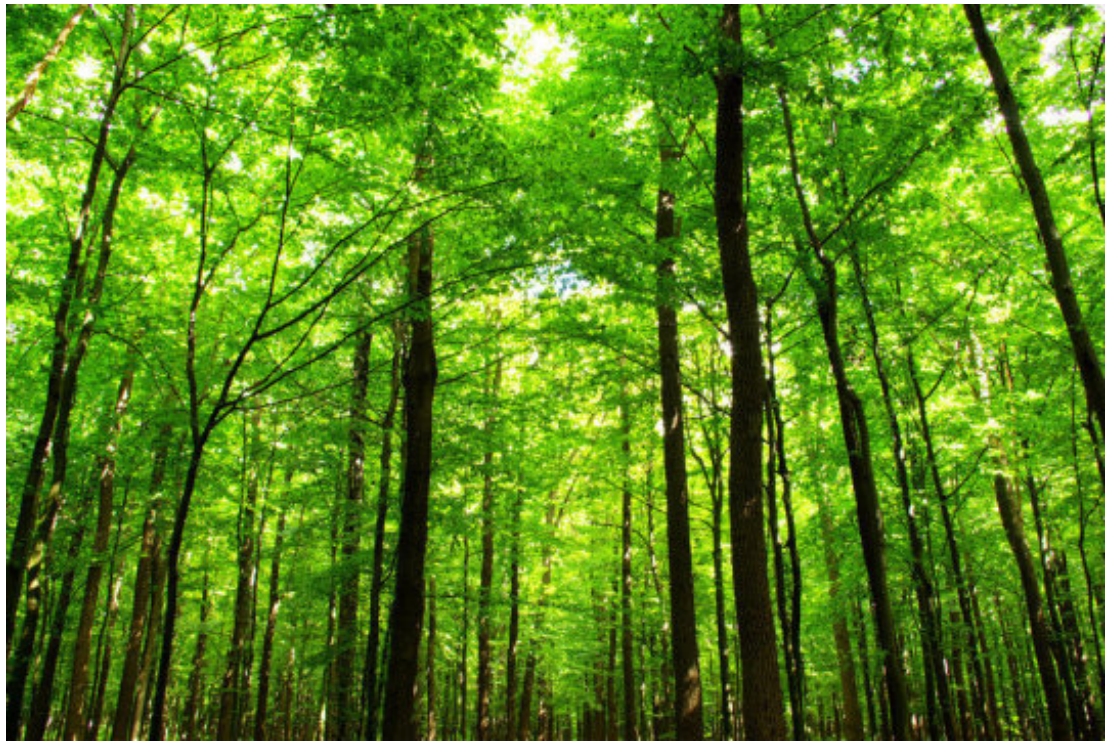
reference	population			treatment				result
	age	n	health status	antibiotics	probiotics	Control	method	resilience
		total (Probiotics group) N		name Dose per day and duration	Name Dose Form Duration			
Adults								
Myllyluoma 2007	adults mean age 57	47 (N=19)	H pylori positive	H pylori positive only 2 g amoxycillin /d + 1 g clarithromycin (/d 7 days	Lactobacillus rhamnosus GG (ATCC 53103) and L. rhamnosus LC705 (DSM 7061), Propionibacterium freudenreichii ssp. shermanii JS (DSM 7067) and Bifidobacterium breve Bb99 Total of 1×10^9 cfu Milk-based fruit drink 28 days	No control for intake of probiotics	FISH and culture	<u>Resilience: better restoration of total aerobes in probiotic treated grpup vs control group.</u>
Oh 2016	adults mean 44 to 55	6 (N=3)	H. pylori positive	1 g Clarithromyci /d2 g Amoxicillin / d 14 days	Medilac-S ; Enterococcus faecium 9 x 10^8 and Bacillus subtilis 1 x 10^8 Powder 14 days	No control for intake of probiotics	metagenomic D sequencing ; functio metagenome profiling	<u>Resilience</u> in the probiotic-treated group vs placebo group (in terms of gene expression, OTUs and diversity)
Oh 2016b	adults mean 44 to 55	23 (N=12)	H. pylori positive	1 g Clarithromyci /d2 g Amoxicillin / d 14 days	Medilac-S ; Enterococcus faecium 9 x 10^8 and Bacillus subtilis 1 x 10^8 Powder 14 days	No control for intake of probiotics	16S rRNA	
Orrhage 1994	adults mean age 37	30 (N=10/group)	healthy	600 mg clindamycin/d 7 days	gp I: B. longum BB 536 (2.5 x 10^8) and L. acidophilus NCFB 1748 (10^9) gp II: B. longum BB 536 (2.5 x 10^8) Fermented milk	Fermented milk product with yogurt starters	culture	<u>Resilience:</u> no effect of probiotic on aerobic flora, but Bifidobacteria are decreased in control group and not in probio ones. Total bacteroides were higher probiotic groups.

reference	population			treatment				result
	age	n	health status	antibiotics	probiotics	Control	method	resilience
		total (Probiotics group) N		name Dose per day and duration	Name Dose Form Duration			
Adults								
Orrhage 2000	adults mean age 28	30 (N=10/group)	healthy	cefpodoxim proxetil 100 mg	gp A: B. longum BB 536 (2.5×10^8) and L. acidophilus NCFB 1748 (10^9) plus 15g fructo-oligosaccharides gp B: 15g fructo-oligosaccharides control group: no probiotic, no fructo-oligosaccharides; in 500 ml Fermented milk21 days	Fermented milk product with yogurt starters	culture plus PFGE for strain identification	Resilience: more lactobacilli at the end of treatments in the probiotic group vs the placebo group.
Pirker 2012	adults mean age 70	678 (N=340; 56 microbiota analysed)	infectious diseases	Various (penicillins; cephalosporins; quinolone, vancomycine, clindamycine) X days	L. casei Shirota (6.5 x 10^9); fermented milk 5 days or more	No control for intake of probiotics	PCR; DNA (and d… butyryl coA transferase ge…es	Resilience. Higher diversity in probiotic -treated group.
Plummer 2005	no info	155 (N=76)	H. pylori positive	2 g amoxicillin / d 1 g clarithromycin/d 7 days	two strains of Lactobacillus acidophilus (CUL60 and CUL21) and two strains of Bifidobacterium 2.5×10^{10}cfuCapsule 21 days	Maltodextrin, Capsule	culture	Resilience: there was recovery of the majority of the components of the microbiota in both groups. A noticeable difference occurred with the enterobacterial component which was subject to disturbance in the placebo group, but not in the probiotic-treated group
Sullivan 2003	adults mean age 28	24 (N=12)	healthy	600 mg clindamycin / d 7 days	L. acidophilus NCFB 1748, B. lactis Bb12 and Lactobacillus paracasei F19 (Arla Foods $5x10^{10}$ cfu each strain In milk matrix (yogurt)	Milk matrix (yogurt)	culture	Resilience: Bifidobacteria and veillonella decreased in both groups, but lactobacilli and bacteroides remained stable in the probiotic groups whereas they decreased in the placebo group.

reference	population			treatment				result
	age	n	health status	antibiotics	probiotics	Control	method	resilience
		total (Probiotics group) N		name Dose per day and duration	Name Dose Form Duration			
Adults								
Sullivan 2004	adults mean age 60	87 (N=8 to 10)	infectious disease	41 with penicillin (3g/d) and 23 with quinolones [cipro- (1 to 1.5 g/d)or norflaxin(0.8g/d)] 7 days	L paracasei F19 (arla) 10^{10} cfu In powdered milk 14 days	Powdered milk	culture	Resilience: In the placebo group, the numbers of B. fragilis, the total number of anaerobic microorganisms and of resistant strains increased. No statistically significant changes were seen in the intestinal microflora in the active group.
Swidinski 2016	Adult women	56 (N=37)	Bacterial vaginosis	metronidazole 3 x 500 mg/d) and ciproflaxin (2x 500 mg/d)2 weeks	S. boulardii CNCM I-745 (10^{11} cfu/day) (2 capsule/day)	No control product	FISH	Resilience: concentrations of essential bacteria (E. rectale, C. coccoides, Bacteroidaceae, F. prausnitzii) reached pre-antibiotic values at the end of the observation
Taibi 2016 (abstract only)	adults	24	healthy	875 mg of amoxicillin and 125 mg of clavulanic acid twice a day	Lacidofil STRONG® (Lactobacillus rhamnosus R0011 and Lactobacillus helveticus R0052)		microRNA	partial answer, consistent with resilience. The AB-associated alteration of the fecal miRNA signature is alleviated by probiotic.
Children								
Korpela 2016	children mean age 5	88 with fecal sample (N=8-13 / antibiotic)	healthy	Various (penicillins; cephalosporins; macrolides , sulfonamide-trimethoprim)	L. rhamnosus GG Approx. 4.10^8 Milk	Milk	micro-array (human intestinal track chip)	Resilience: LGG appears to counteract some of the changes associated with penicillin use, but does not protect against macrolide-associated changes
Zakordonets 2016	children 3 to 14	40	tonsitllitis	ceftrifaxone	multiprobiotic Symbiter: lactobacilli and lactococci: $1.0x10^9$; Bifidobacterium: $1.0x10^8$; propionate-oxidising bacteria: $3.0x10^7$; acetic acid bacteria: $1.0x10^5$: Saccharomyces		culture (not detailed)	Resilience: just after AB cessation, there is more E.coli in probiotic treated group vs control.

reference	population			treatment				result
	age	n	health status	antibiotics	probiotics	Control	method	resilience
		total (Probiotics group) N		name Dose per day and duration	Name Dose Form Duration			
Children								
Korpela 2016	children mean age 5	88 with fecal sample (N=8-13 / antibiotic)	healthy	Various (penicillins; cephalosporins; macrolides , sulfonamide-trimethoprim)	L. rhamnosus GG Approx. 4.10^8 Milk	Milk	micro-array (human intestinal track chip)	<u>Resilience:</u> LGG appears to counteract some of the changes associated with penicillin use, but does not protect against macrolide-associated changes
Zakordonets 2016	children 3 to 14	40	tonsitllitis	ceftrifaxone	multiprobiotic Symbiter: lactobacilli and lactococci: $1.0x10^9$; Bifidobacterium: $1.0x10^8$; propionate-oxidising bacteria: $3.0x10^7$; acetic acid bacteria: $1.0x10^5$:		culture (not detailed)	<u>Resilience</u>: just after AB cessation, there is more E.coli in probiotic treated group vs control.
Zoppi 2001	children mean age 5	51	respiratory infections	ceftriaxone parenterally (50 mg/kg)	Saccharomyces boulardii (therapy 2); Enterococcus species (therapy 3); lactulose (therapy 4); Lactobacillus rhamnosus GG (therapy 5); Lactobacillus rhamnosus, Lactobacillus bifidus, and Lactobacillus acidophilus (therapy 6); Bifidobacterium bifidum and L. acidophilus (therapy 7); or a mixture of various lactobacilli and bifidobacteria at high concentrations (therapy 8).		culture	<u>Resilience</u> is observed with some probiotics (L. rhamnosus; L. rhamnosus+L. bifidus; L. acidophilus; S. boulardii; L. acidophilus+B. bifidum)

Resilience and diversity are key.



Larger diversity: will compensate for lost functionality!

This is called resilience!

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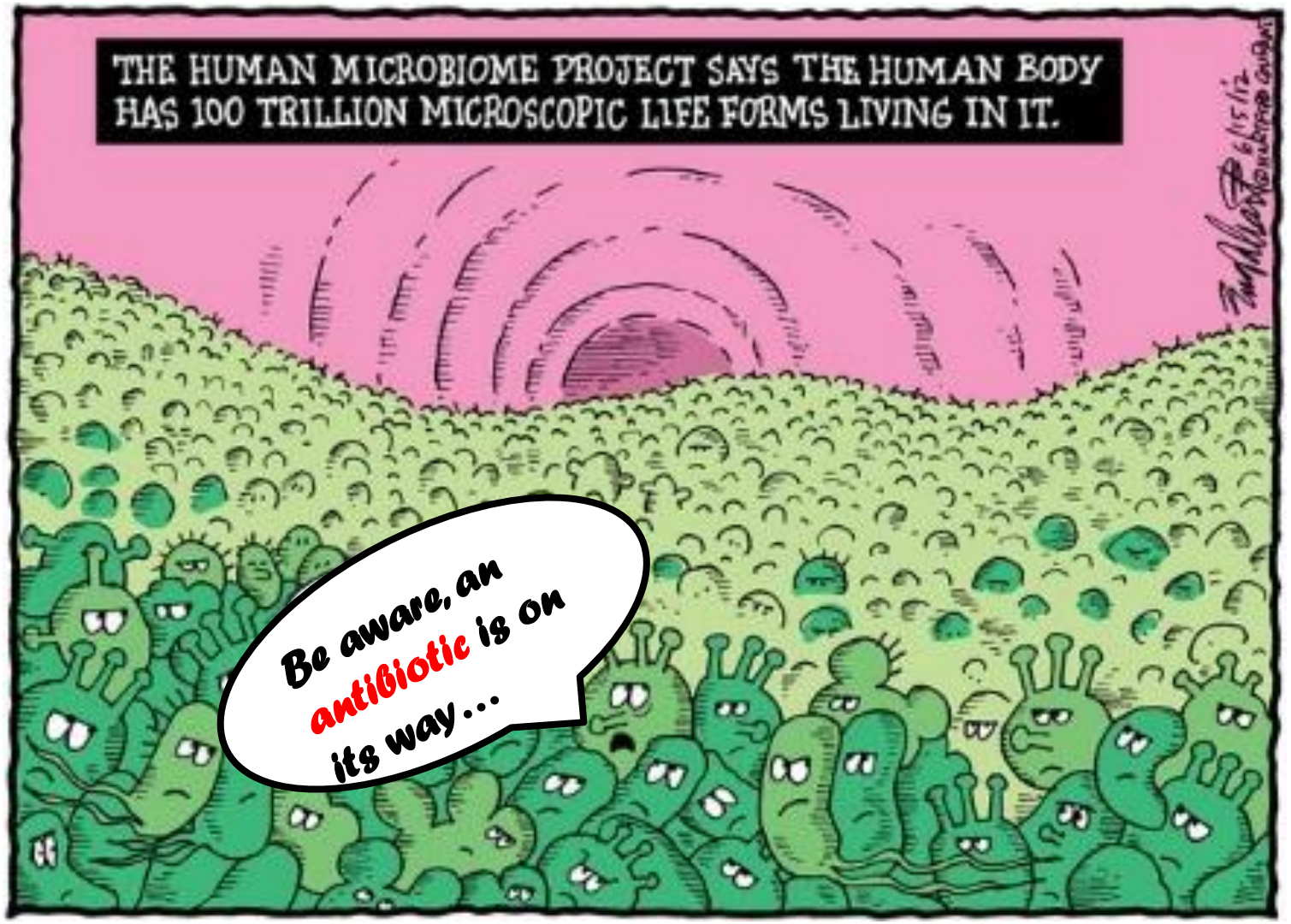
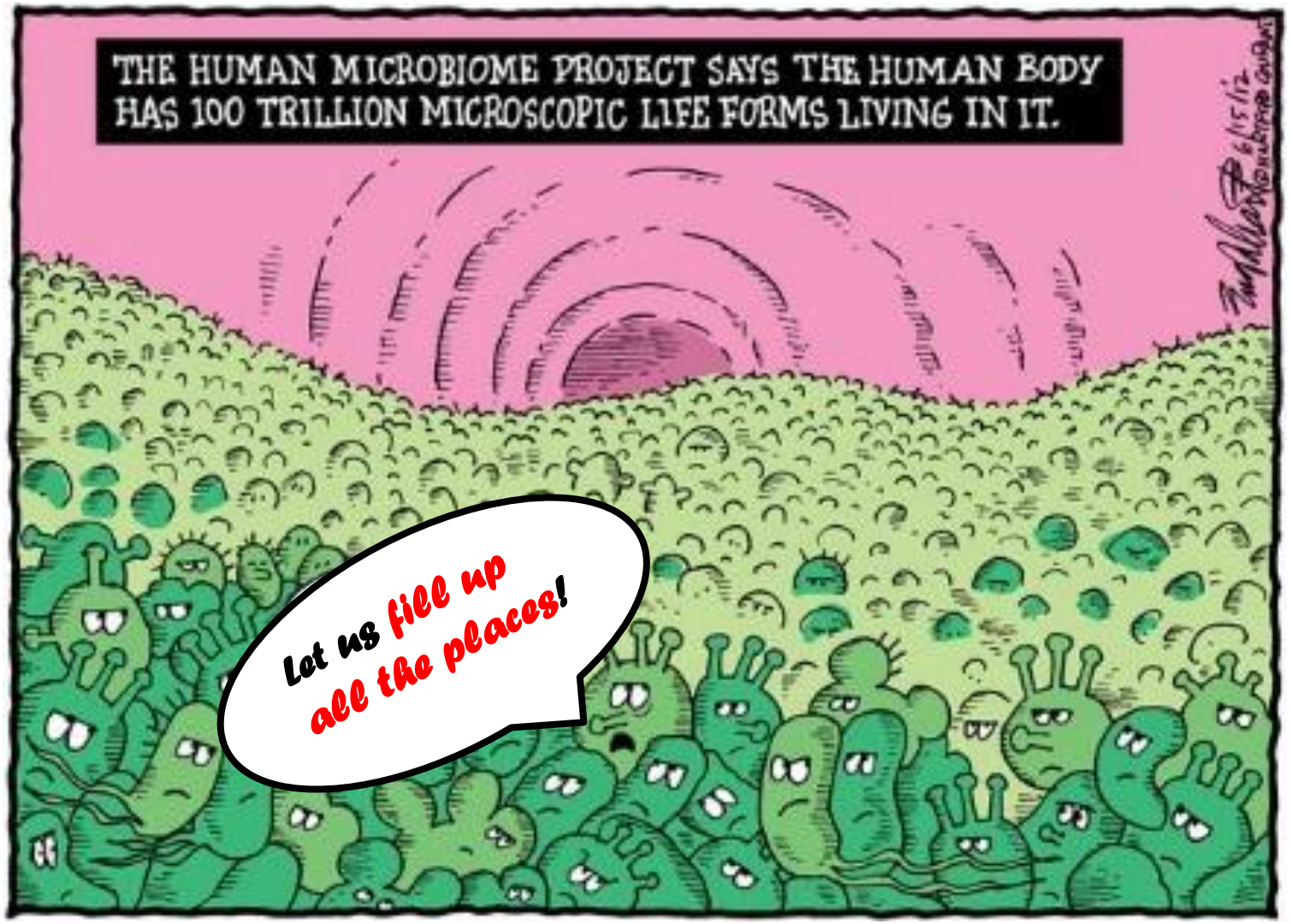
What is the underlying phenomenon?



What is the underlying phenomenon?



1. Competitive exclusion!

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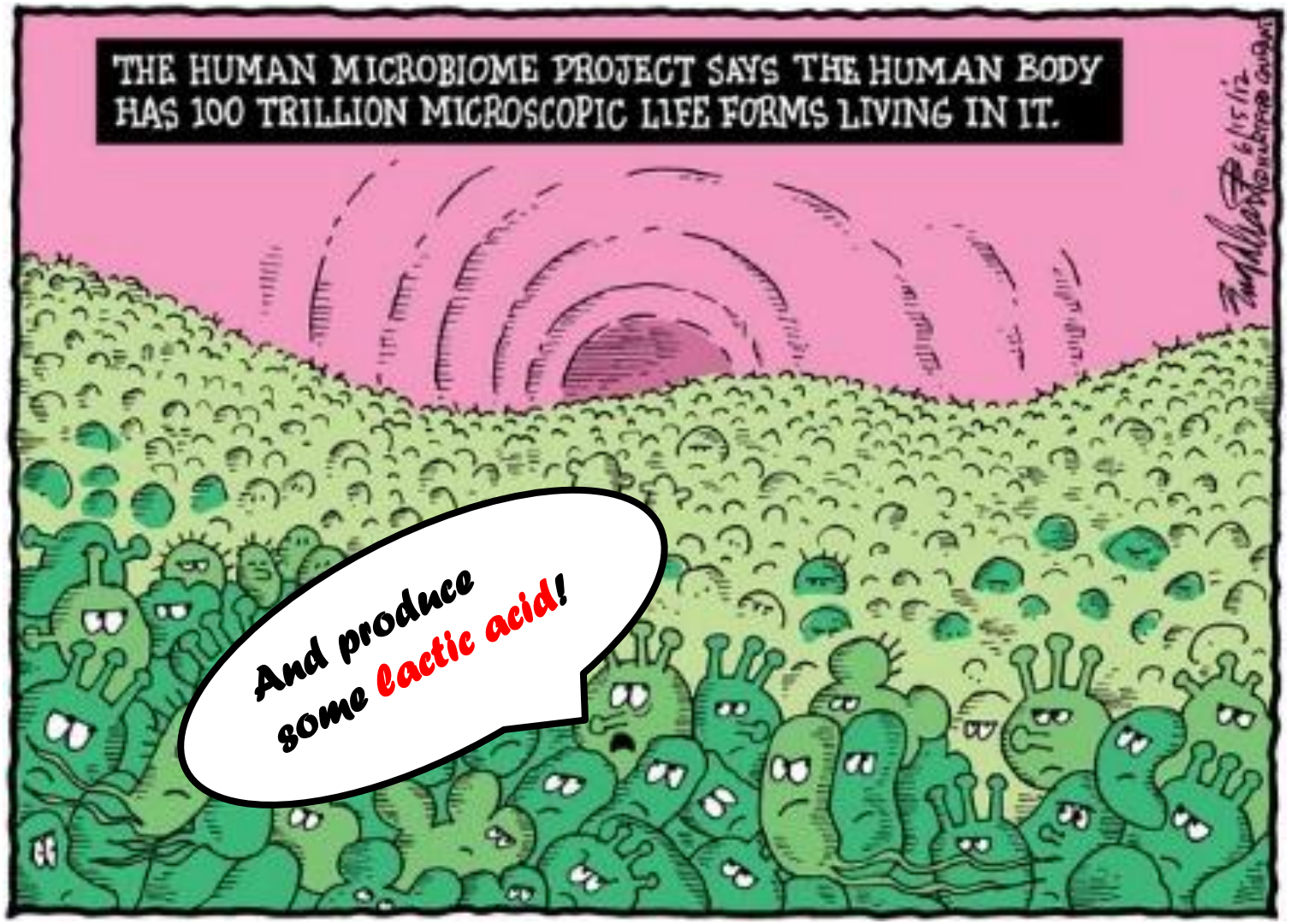
What is the underlying phenomenon?



2. Metabolic exclusion!

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What's it all about?

- Probiotics: what are they ?
- The importance of the microbiota.
- Probiotics: What can they do ?
- Clinical studies as the ultimate proof
  - The immune effects
  - The microbiological effects
  - The metabolic effects
- Conclusions

Effects of probiotics in a healthy population:

1. The immune function.

2. The microbiological function.

3. The metabolic function.

- This brings me to their third role: probiotics can assist the metabolism and physiology of the host.

- Again there is a strong link with the diet and the health status of the host (diabetics, overweight, …)

- An increasing number of studies is looking to metabolic parameters

metabolites

MDPI

Review

The Application of Metabolomics to Probiotic and Prebiotic Interventions in Human Clinical Studies

Thomas M. O'Connell

Department of Otolaryngology—Head & Neck Surgery, Indiana University School of Medicine, Indianapolis, IN 46202, USA; thoconne@iu.edu; Tel.: +1-919-621-1074

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Metabolism besides composition

Subjects	Intervention	Study Design	N	Duration	Samples	Metabolomics Platform	Clinical Outcomes
Effects on healthy adults							
Healthy adults	Prebiotic: chicory derived inulin	R, DB, PC, CR	44	2 week run in 2 week intervention 2 week washout crossover	feces	GC/MS focus on volatiles	bowel function, stool consistency, constipation
Healthy adults	Prebiotic: wheat bran	R, DB, PC, CR	20	1 week run in 3 week intervention 3 week washout crossover	feces	untargeted GC/MS	colonic fermentation patterns, fecal water cytotoxicity
Healthy elderly age 65-80	Prebiotic: galactooligosaccharides	R, DB, PC, CR	40	10 week intervention 4 week washout crossover	feces	^{1}H NMR	age assocated alterations in microbiota, improvements in immune markers e.g. IL-8, IL-10 and IL-1β
Overweight/Obesity							
Obese women BMI > 30 kg/m^2	Prebiotic: inulin-type fructans	R, DB, PC	15 placebo 15 inulin	3 months	plasma urine	^{1}H NMR	alterations in gut microbiota and metaboli profiles in urine and serum
erweight/obese adults BMI 28.0 - 34.9	1. Prebiotic polydextrose 2. Probiotic with B. animalis 3. Combined pro & prebiotic	R, DB, PC	36 placebo 36 prebiotic 25 probiotic 37 combined	6 months	feces plasma	^{1}H NMR targeted UHPLC/MS bile acids	alterations in host energy metabolism and correlations to baseline e.g. waist/hip ratio, DXA measurements
Infant/Pediatric Health							
Healthy infants	Baby formula supplemented with: B. bifidum, B. breve, B. longum	R, DB, PC	49 standard formula 48 probiotic formula 9 breast fed	12 months	feces	untargeted UPLC/MS targeted SCFAs	composition and function of microbiota of infants in first year of life
infants with colic	Probiotic mixture	R, DB, PC	8 placebo 11 probiotic	21 days	feces	^{1}H NMR	reduction of colic symptoms e.g. crying time, QoL score

Chlidren with recurrent respiratory infections age 3-6 years	1. Pidotimod (immunostimulant) 2. Pidotimod + Bifidobacteria mixture 3. Bifidobacteria mixture	R, DB, PC	16 controls 13 Pidotimod 13 Bifidobacteria 13 Pidotimod + Bifidobacteria	first 10 days of each month for 4 months	urine	untargeted UPLC/MS	respiratory symptom free days, number of days with common cold
Pre-term infants < 32 weeks gestation	Probiotic containing: L. acidophilus-NCIMB701748 B. bifidum-ATCC15696	R, DB, PC	3 controls 7 probiotic	9-68 days	feces	untargeted UPLC/MS	probiotic colonization and metabolic function of the pre-term gut
Irritable Bowel Syndrome							
IBS patients	Probiotic: fermented milk	R, DB, PC	37 placebo 37 probiotic	8 weeks	feces serum	^{1}H NMR	Improvements in IBS symptoms e.g. abdominal pain, bloating
Diarrhea predominant IBS patients	Synbiotic yogurt containing Probiotic: L. plantarum & L. fermentum Prebiotic: xylooligosaccharides	CT	16 healthy controls 8 IBS patients	4 weeks	serum urine	^{1}H NMR	Metabolic alterations in serum and urine
Womens Health							
Post-menopausal women	Vaginal probiotic containing: L. rhamnosis GR-1 & L. reuteri RC-14	R, DB, PC, CR	7 placebo 7 probiotic	3 days treatment 17 days washout crossover	vaginal swabs	untargeted GC/MS	Nugent score for bacterial vaginosis
Pregnant women	Probiotics containing: L. rhamnosis GR-1 & L. reuteri RC-14	R, DB, PC	13 placebo 8 probiotic	1 month	vaginal swabs	untargeted GC/MS	probiotic treatment compliance pre-term birth
Other							
Relapsing-remitting multiple sclerosis patients	Probiotic containing: Lactobacillus, Bifidobacterium and Streptococcus	CT	9 healthy 9 MS	2 months	feces	^{1}H NMR	immune modulatory markers microbiotia alterations
Atopic dermatitis in adults	Probiotic formulation	R, DB, PC	22 placebo 22 probiotic	8 weeks	feces	CE-ToFMS	itching & QoL scores

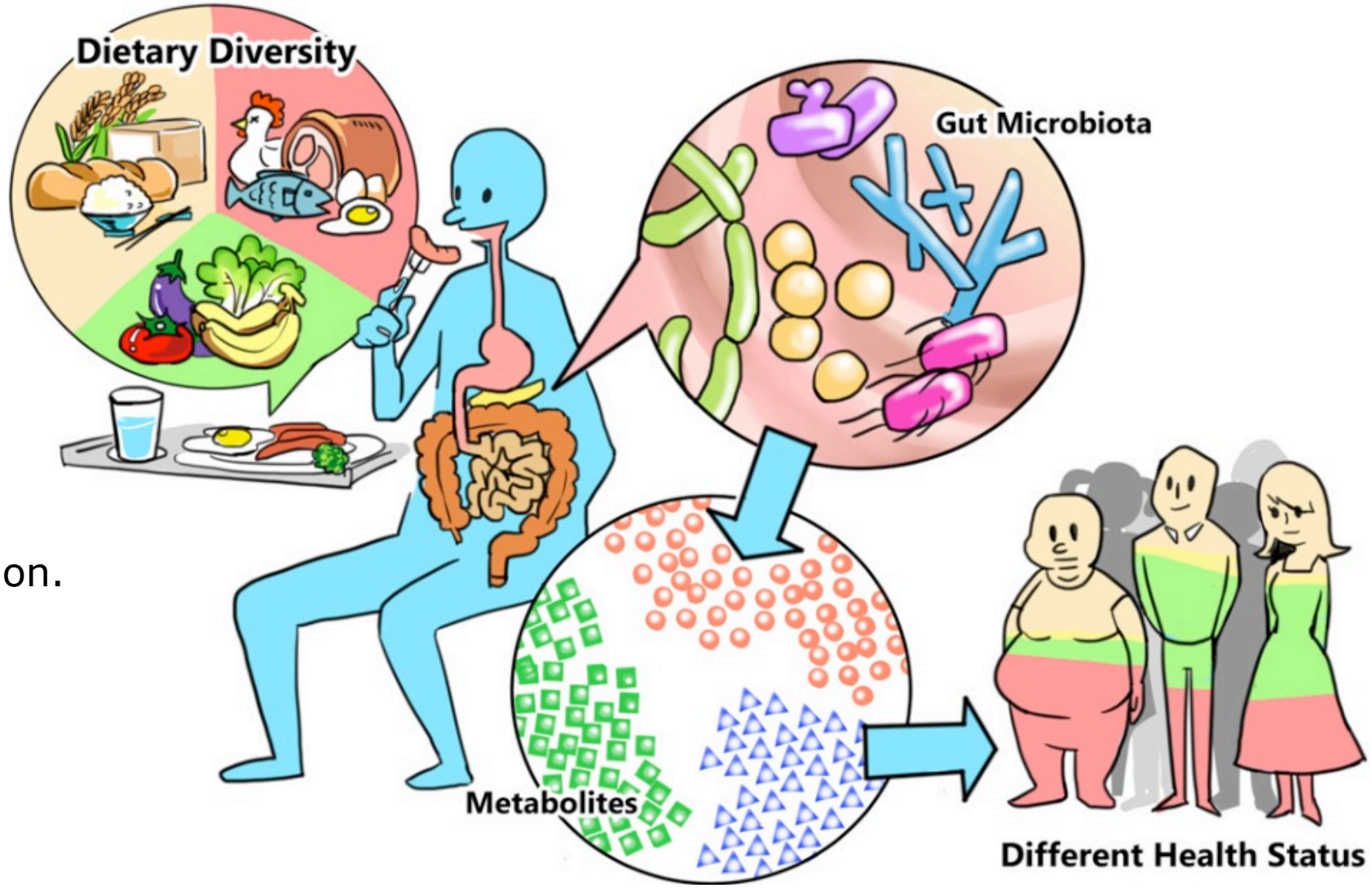
Description of human clinical studies of probiotic and prebiotic interventions that included the use of a discovery-based metabolomics analysis. Abbreviations: R, randomized; DB, double blind; PC, placebo controlled; CT, clinical trial.

Talking about Metabolism: Diet and Microbes are very closely linked!

A diverse diet, rich in fermented foods and probiotics, will increase the metabolic diversity and therefore also the metabolic resilience.

This combination is determining to a certain extent your health situation.

Metabolic Syndrome as an example.



Metabolic Syndrome: systematic review

Report of the National Heart, Lung, and Blood Institute/American Heart Association, identified 6 components of the metabolic syndrome that relate to CVD:

- Abdominal obesity
- Atherogenic dyslipidemia
- Raised blood pressure
- Insulin resistance ± glucose intolerance
- Proinflammatory state
- Prothrombotic state

Table 1. Definitions of metabolic syndrome

	NCEP ATP III (2005 revision)	WHO (1998)	EGIR (1999)	IDF (2005)
Absolutely required	None	Insulin resistance* (IGT, IFG, T2D or other evidence of IR)	Hyperinsulinemia‡ (plasma insulin >75th percentile)	Central obesity (waist circumference§): ≥94 cm (M), ≥80 cm (F)
Criteria	Any three of the five criteria below	Insulin resistance or diabetes, plus two of the five criteria below	Hyperinsulinemia, plus two of the four criteria below	Obesity, plus two of the four criteria below
Obesity	Waist circumference: >40 inches (M), >35 inches (F)	Waist/hip ratio: >0.90 (M), >0.85 (F); or BMI >30 kg/m^2	Waist circumference: ≥94 cm (M), ≥80cm (F)	Central obesity already required
Hyperglycemia	Fasting glucose ≥100 mg/dl or Rx	Insulin resistance already required	Insulin resistance already required	Fasting glucose ≥100 mg/dl
Dyslipidemia	TG ≥150 mg/dl or Rx	TG ≥150 mg/dl or HDL-C: <35 mg/dl (M), <39 mg/dl (F)	TG ≥177 mg/dl or HDL-C <39 mg/dl	TG ≥150 mg/dl or Rx
Dyslipidemia (second, separate criteria)	HDL cholesterol: <40 mg/dl (M), <50 mg/dl (F); or Rx			HDL cholesterol: <40 mg/dl (M), <50 mg/dl (F); or Rx
Hypertension	>130 mmHg systolic or >85 mmHg diastolic or Rx	≥140/90 mmHg	≥140/90 mmHg or Rx	>130 mmHg systolic or >85 mmHg diastolic or Rx
Other criteria		Microalbuminuria†		

*IGT, impaired glucose tolerance; IFG, impaired fasting glucose; T2D, type 2 diabetes; IR, insulin resistance; other evidence includes euglycemic clamp studies.
†Urinary albumin excretion of ≥20 μg/min or albumin-to-creatinine ratio of ≥30 mg/g.
‡Reliable only in patients without T2D.
§Criteria for central obesity (waist circumference) are specific for each population; values given are for European men and women.
Rx, pharmacologic treatment.

nutrients

MDPI

Review

Effects of Probiotics on Metabolic Syndrome: A Systematic Review of Randomized Clinical Trials

Carmen Tenorio-Jiménez [1,*], María José Martínez-Ramírez [2,3], Ángel Gil [4,5,6,7] and Carolina Gómez-Llorente [4,5,6,7,*]

P.L. Huang. A comprehensive definition for metabolic syndrome. Dis Model Mech. 2009 May-Jun; 2(5-6): 231–237.

science for health

Table 2. Main characteristics of the nine included articles evaluating the effect of probiotics on metabolic syndrome parameters.

Author	*n* (Sample Size)	Age Range	Probiotic Strain	Period of Intervention (Weeks)	Probiotic Dose	Primary Outcomes	Secondary Outcomes
Leber et al. [16]	28	Control group: 54.5 ± 8.9 Probiotic group: 51.5 ± 11.4	*Lactobacillus casei* Shirota	12	milk (65 mL bottles × 3/day) 10^8 cells/mL	No changes were found in BMI, BP, waist circumference, triacylglycerols, TC, and fasting glucose levels.	High-sensitive CRP (1.86 mg/L in the probiotic group vs. −1.60 mg/L in the placebo group, $p = 0.016$) and LBP levels (5827 ng/mL in the probiotic group vs. −1510 ng/mL in the placebo group, $p = 0.023$) increased within the probiotic group
Sharafedtinov et al. [21]	40	Control group: 51.7 ± 12.1 Probiotic group: 52 ± 10.9	*Lactobacillus plantarum* TENSIA	3	cheese (50 g/day) 1.5×10^{11} CFU/g	BMI was significantly reduced in the probiotic group. (BMI variation in probiotic group −2 vs. −1.6 kg/m^2 in the placebo group, $p = 0.031$).	A positive association was detected between TENSIA colonization and the extent of change of morning diastolic BP ($r = 0.617$, $p = 0.0248$)
Tripolt et al. [17]	28	Control group: 55 ± 9 Probiotic group: 51 ± 11	*Lactobacillus casei* Shirota	12	milk (65 mL bottles × 3/day) 10^8 cells/mL	No changes were found in BMI, fasting plasma glucose levels, and HOMA-IR index.	Probiotic supplementation resulted in a significant reduction in sVCAM-1 level (−195 ng/mL in the probiotic group vs. 30 ng/mL in the placebo group, $p = 0.008$) and a significant increase in high-sensitive CRP level (1.86 mg/L in the probiotic group vs. −1.60 mg/L in the placebo group, $p = 0.002$)
Barreto et al. [22]	24	Control group: 63 ± 7.6 Probiotic group: 62 ± 4.35	*Lactobacillus plantarum*	12	milk (80 mL bottles × 1/day) 10^7 CFU/g	Glucose levels showed a significant reduction in the FM group compared with the NFM group (Glucose variation in FM −10.5 vs. −3 mg/dL in NFM group, $p = 0.037$).	Homocysteine levels showed a significant reduction in the FM group compared with the NFM group $p = 0.019$).
Stadlbauer et al. [18]	28	Control group: 55 ± 9 Probiotic group: 51 ± 11	*Lactobacillus casei* Shirota	12	milk (65 mL bottles × 3/day) 10^8 cells/mL	No changes were found in BMI, BP, waist circumference, triacylglycerols, and TC blood levels.	LcS administration was associated with subtle microbiota changes at a genus level (enrichment of Parabacteroidetes)
Bernini et al. [23]	51	No data	*Bifidobacterium lactis* HN019	6	milk(80 mL bottle × 1/day) 3.4×10^8 CFU/mL	Significant differences in BMI variation (Probiotic group −1.3 vs. −0.3 kg/m^2 in control group; $p = 0.017$); TC variation (probiotic group −15 vs. 6 mg/dL in control group, $p = 0.09$) and LDLc variation (probiotic group −17.5 vs. −2 mg/dL in control group, $p = 0.08$)	Significant decrease in TNFα and IL−6 ($p < 0.05$) in the probiotic group.
Szulinska et al. [19]	81	Control group: 58.72 ± 7.25 Low dose group: 56.38 ± 6.55 High dose group: 55.16 ± 6.87	*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, and *Lactococcus lactis* W58	12	lyophilisate powder Low dose (2.5×10^9 CFU/day) or High dose (1×10^{10} CFU/day)	Significant differences were found in glucose variation (HD vs. placebo −0.61 mg/dL, $p = 0.0272$; HD vs. LD −0.72 mg/dL, $p = 0.0043$), Insulin (HD vs. placebo −0.83 UI/L, $p = 0.0002$; HD vs. LD −0.40 UI/L, $p = 0.0155$), and HOMA-IR (HD vs. placebo −0.90, $p = 0.0005$; HD vs. LD −0.54 mg/dL, $p = 0.0127$).	Significant differences were found in uric acid (HD vs. placebo −0.73 mmol/L, $p = 0.0109$; HD vs. LD −0.92 mmol/L, $p = 0.0016$) and LPS levels (HD vs. placebo −0.99 ng/mL, $p = 0.001$).

Author	*n* (Sample Size)	Age Range	Probiotic Strain	Period of Intervention (Weeks)	Probiotic Dose	Primary Outcomes	Secondary Outcomes
Szulinska et al. [20]	81	Control group: 58.72 ± 7.25 Low dose group: 56.38 ± 6.55 High dose group: 55.16 ± 6.87	*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, and *Lactococcus lactis* W58	12	lyophilisate powder Low dose (2.5×10^9 CFU/day) or High dose (1×10^{10} CFU/day)	No changes were found in BMI and BP.	Significant differences were found in the pulse wave analysis systolic pressure (HD vs. placebo −1 mmHg, $p = 0.0054$; HD vs. LD −0.91 mmHg, $p = 0.0057$), the pulse wave analysis augmentation index (HD vs. placebo −0.55, $p = 0.0079$), the pulse wave velocity (HD vs. placebo −0.82 m/s, $p = 0.0045$; HD vs. LD −0.55 m/s, $p = 0.0189$), VEGF (HD vs. placebo −1.09 pg/mL, $p = 0.0001$; HD vs. LD −1.10 pg/mL, $p = 0.0007$), TNFα (HD vs. placebo −1.03 pg/mL, $p = 0.0009$; HD vs. LD −0.68 pg/mL, $p = 0.0471$), and thrombomodulin levels (HD vs. placebo −0.78 ng/mL, $p = 0.0194$).
Rezazadeh et al. [24]	44	Control group: 44.55 ± 5.70 Probiotic group: 44.05 ± 6.60	*Lactobacillus acidophilus* La5, *Bifidobacterium lactis* Bb12	8	yogurt containing 6.45×10^6 CFU/g of *L. acidophilus* and 4.94×10^6 CFU/g of *B. lactis* Bb12	Consumption of probiotic yogurt resulted in a significant reduction in the level of blood glucose (Mean difference: −3.80, $p = 0.01$)	Consumption of probiotic yogurt resulted in a significant reduction in the level of VCAM-1 (Mean difference −463.39, $p = 0.001$)

Abbreviations: SD: Standard deviation; BMI: Body mass index; BP: Blood pressure; TC: Total cholesterol; CRP: C reactive protein; LBP: Lipopolysaccharide binding protein; CFU: Colony forming units; VCAM-1: Vascular cell adhesion molecule 1; LDLc: Low-density lipoprotein cholesterol; FM: Fermented milk; NFM: Non-fermented milk; LcS: Lactobacillus casei Shirota; TNF-α: Tumor necrosis factor α; IL-6: Interleukine-6; HOMA-IR: Homeostasis model assessment-insulin resistance; LPS: Lipopolysaccharide; HD: High dose; LD: Low dose; VEGF: Vascular endothelial growth factor.

Further in the paper: **a real meta-analysis could not be performed**, due to the design and **methodology diversity** and the **small number of RCTs**.

In contrast to meta-analyses conducted in **drugs**, those conducted in **nutritional science** are not always the best method for extracting relevant information, due to the heterogeneity of interventions and protocols.

Indeed, one important issue that this review highlights is the **heterogeneity of the studies**, in terms of population, probiotic strain and genus, administered doses, and the period of the interventions.

What's it all about?

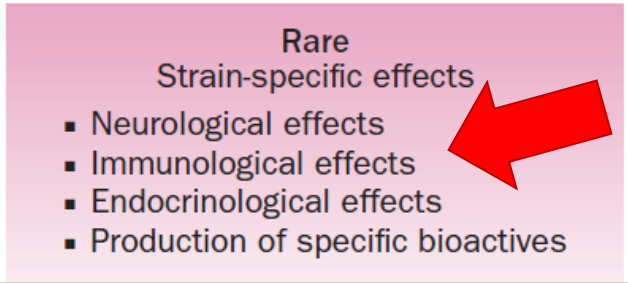
- Probiotics: what are they ?
- The importance of the microbiota.
- Probiotics: What can they do ?
- Clinical studies as the ultimate proof
  - The immune effects
  - The microbiological effects
  - The metabolic effects
- Conclusions

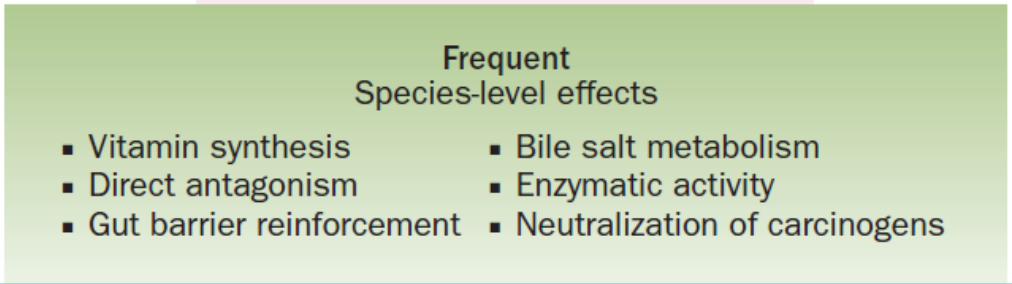
Conclusion 2.

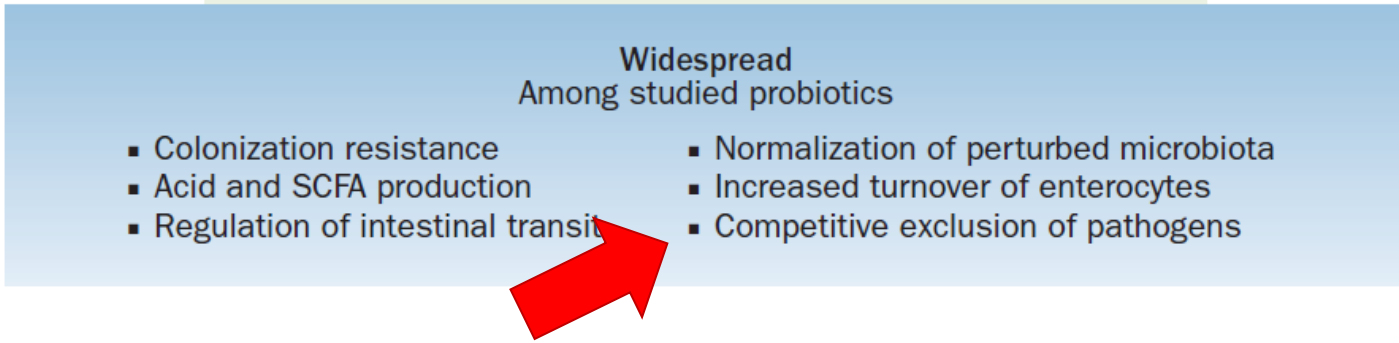
1. Strain differences are important (and are real)

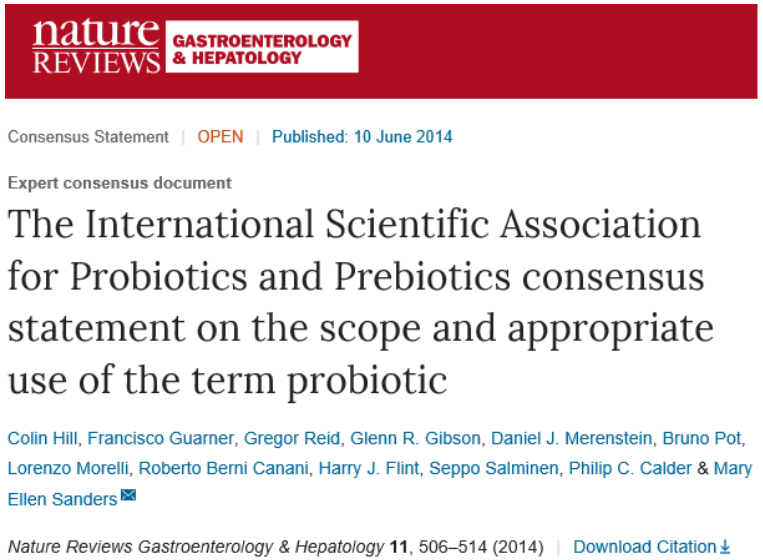
2. There are no miracle strains that can do it all…

3. Probiotics have limits: **do not expect probiotic foods to behave as (probiotic) drugs.**





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Consensus Statement | OPEN | Published: 10 June 2014

Expert consensus document

The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic

Colin Hill, Francisco Guarner, Gregor Reid, Glenn R. Gibson, Daniel J. Merenstein, Bruno Pot, Lorenzo Morelli, Roberto Berni Canani, Harry J. Flint, Seppo Salminen, Philip C. Calder & Mary Ellen Sanders

Nature Reviews Gastroenterology & Hepatology **11**, 506–514 (2014) | Download Citation

Some mechanisms might be *widespread* among commonly studied probiotic genera; others might be *frequently observed* among most strains of a probiotic species; *others may be rare*, e.g. not present among all strains of the same species.

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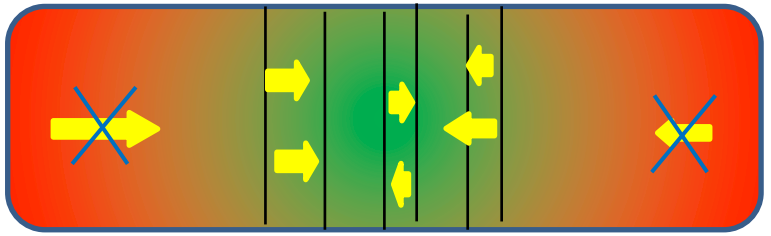
Probiotics do have limits

*They will not **cure** serious disease*

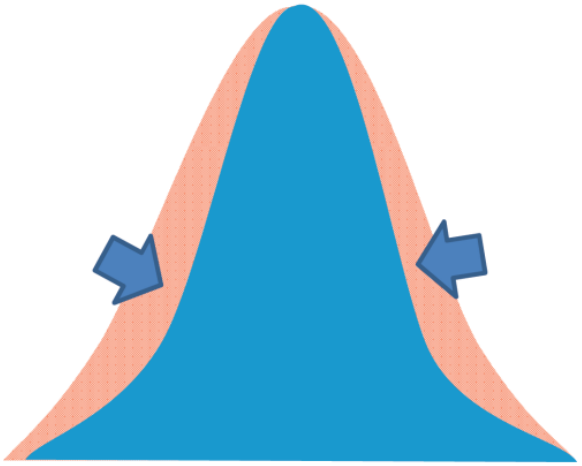
- IBD, allergy, …
- Cancer
- Liver cirrhosis



Some (red) diseases might be out of reach for probiotics

They might not be efficient for all types of patients

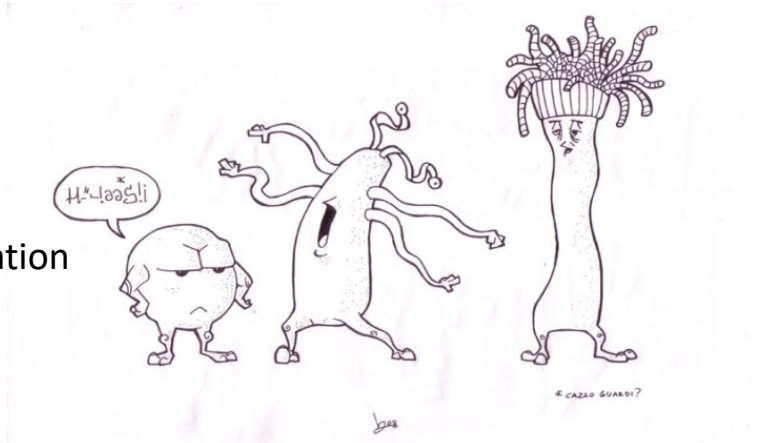
- Differences in patient types
- Differences in individual reactions, linked to
  - Differences in microbiota type (enterotypes)
  - Differences in diet
  - Differences in stress management



Some probiotic effects may only be mesurable in a sub-population

Selecting the bacteria

- Differences in probiotic strains or mixtures
- Differences in dose and mode of administration



Importance of the endpoints

- Differences in clinical end points
- Differences in treatment periods and doses administered

Study the mechanisms involved

- Differences in mechanisms...
- Identification of active compound(s)

➡ Towards drug-like applications ?

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This is also reflected in the regulation

Functional food	**PROBIOTICS**	Drug (medication)
Healthy Population	**INTENDED USE**	Diseased population
Health / Nutrition claim (Funtionality claim)	**CLAIM TYPE**	Medical claim (Curing or Risk reduction claim)
Nutrition claim: - high in … - source of… - improves … - maintains …		Medical claim: - preventing … - curing … - treating …
NO !	**SIDE EFFECTS**	YES, maybe

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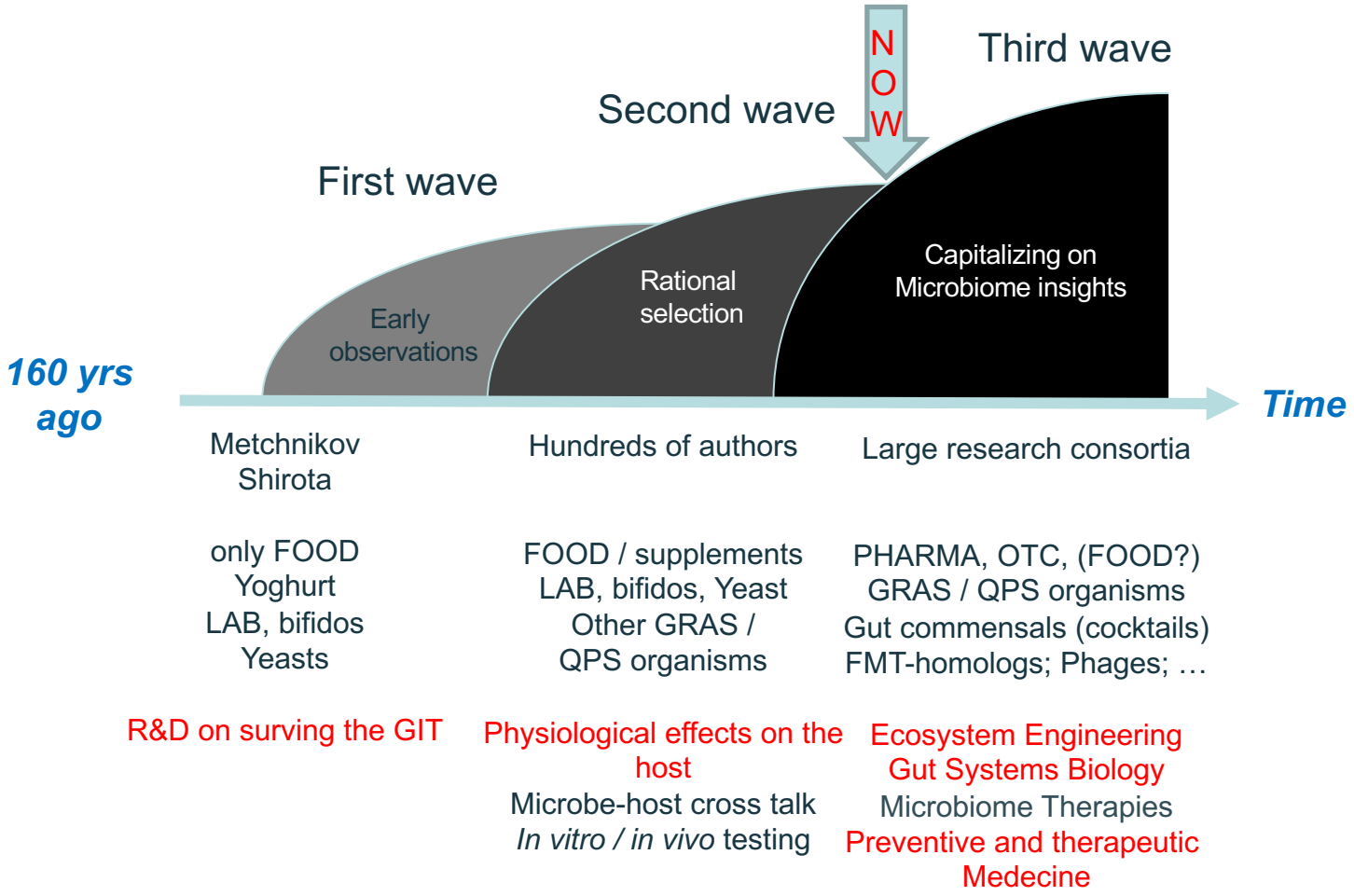
The future of probiotics



Examples of this third wave of **potential probiotics:**

Acidaminococcus intestinalis
Akkermansia muciniphila
Bacteroides ovatus
Bacteroides fragilis
Bacteroides uniformis
Blautia producta
Clostridia clusters IV, XIVa and XVIII cocktails
Clostridium cocleatum
Collinsella aerofaciens
Dorea longicatena
Eubacterium desmolans
Eubacterium eligens
Eubacterium hallii
Eubacterium limosum
Eubacterium rectale
Eubacterium ventriosum
Faecalibacterium prausnitzii
Lachnospira pectinoshiza

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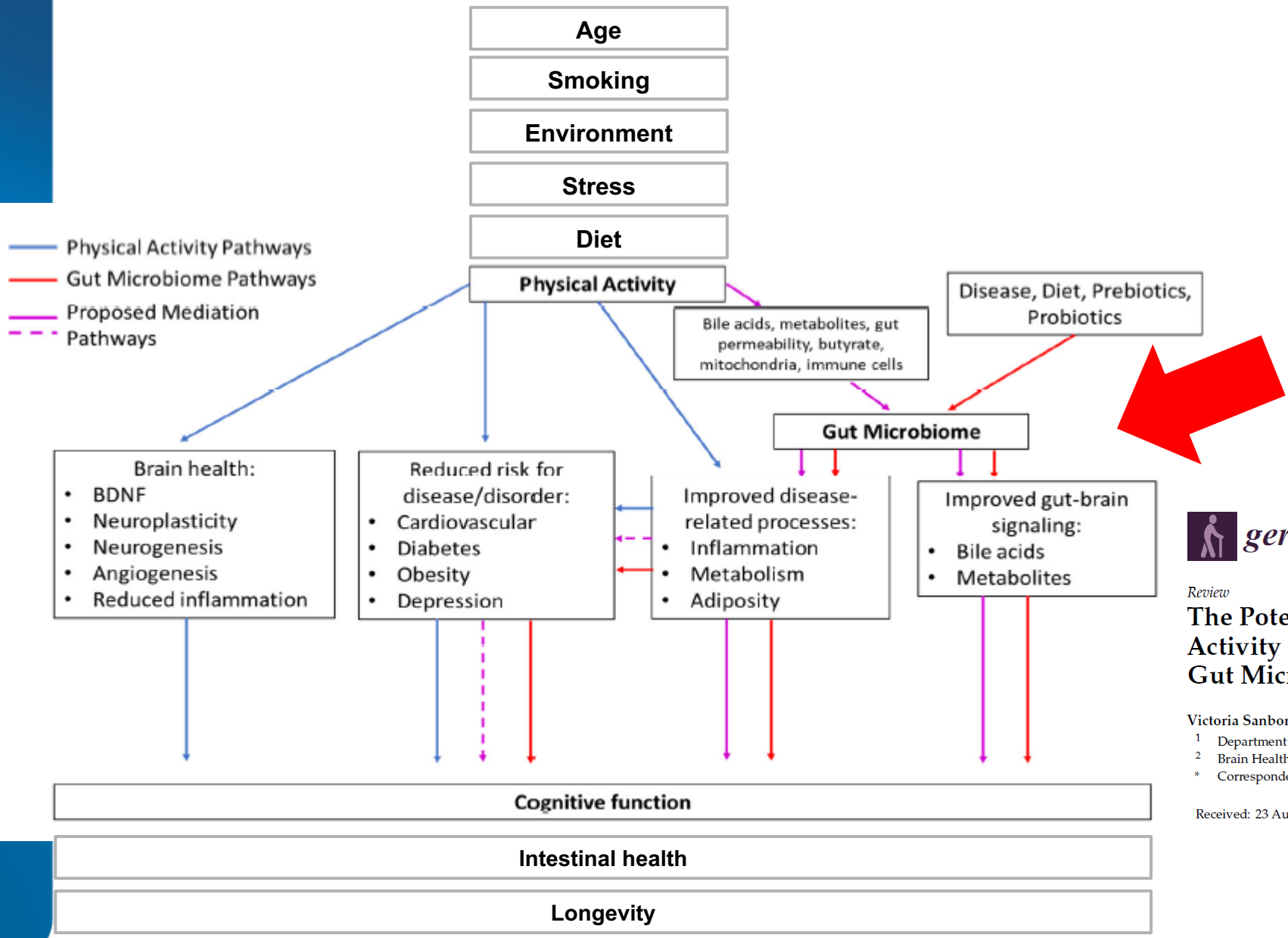
General conclusion: the complexity is enormous



The microbiome is key, … is central!

geriatrics

MDPI

Review

The Potential Mediation of the Effects of Physical Activity on Cognitive Function by the Gut Microbiome

Victoria Sanborn [1,*] and John Gunstad [1,2]

[1] Department of Psychological Sciences, Kent State University, Kent, OH 44240, USA; jgunstad@kent.edu
[2] Brain Health Research Institute, Kent State University, Kent, OH 44240, USA
* Correspondence: vsanborn@kent.edu

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check for updates

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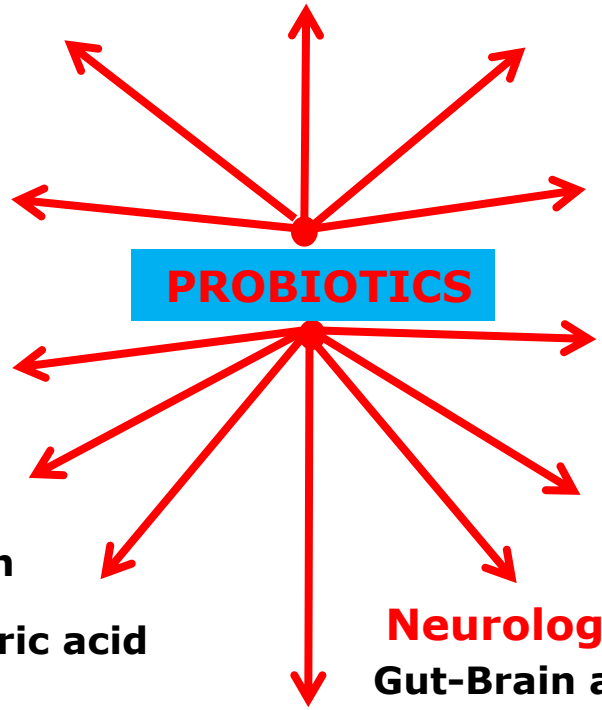
Coming back to the initial slide …



Microbiological effects

Competitive exclusion of enteric pathogens

Inhibit growth or potential pathogens by producing lactic acid, bacteriocins, etc.

Assist in restoring the normal intestinal flora (e.g. AB therapy, acute infection)

Immunological effects

Stimulate / reduce Immuno-globuline production (allergy)

Triggers cytokine synthesis from enterocytes and dendritic cells (reduce inflammation); receptor activations (TLR, leptin, …)

Endocrinological effects

Influences satiety

Barrier effects

Fortifies tight junctions

Physiological effects

Alleviation of symptoms of lactose intolerance

Intestinal transit regulation

Production of butyric acid

Neurological effects

Gut-Brain axis: anxiety, mood, depression behaviour,…

Metabolical effects

Reduce risk for diabetes and obesity (metabolic syndrome) CV disorders, …

Increased turnover of enterocytes

Neutralization of dietary carcinogens

Produce useful metabolites e.g. hydrogen peroxide, vitamins, short chain fatty acids, …

Probiotics: can they really deliver what they promise?

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… and the final answer…

- Despite the complexity of the human ecosystem (the "holobiont"), proper clinical trials can be set up in a sufficient large part of the population to show the difference of a probiotic versus a placebo

- Further lab experiments can help to find systematic effects that will help to build up knowledge on the mechanisms of action (microbiological, immunological, metabolic, … in nature.)

- While these mechanisms may be widely available, or only present in a selected number of strains, there is not a single probiotic that can do it all

- In selecting the right strain for the right application, it is important to consider the difference between probiotics as foods or probiotics as drugs, in order not to disappoint the consumer…

Then probiotics will be able to deliver what they promise!

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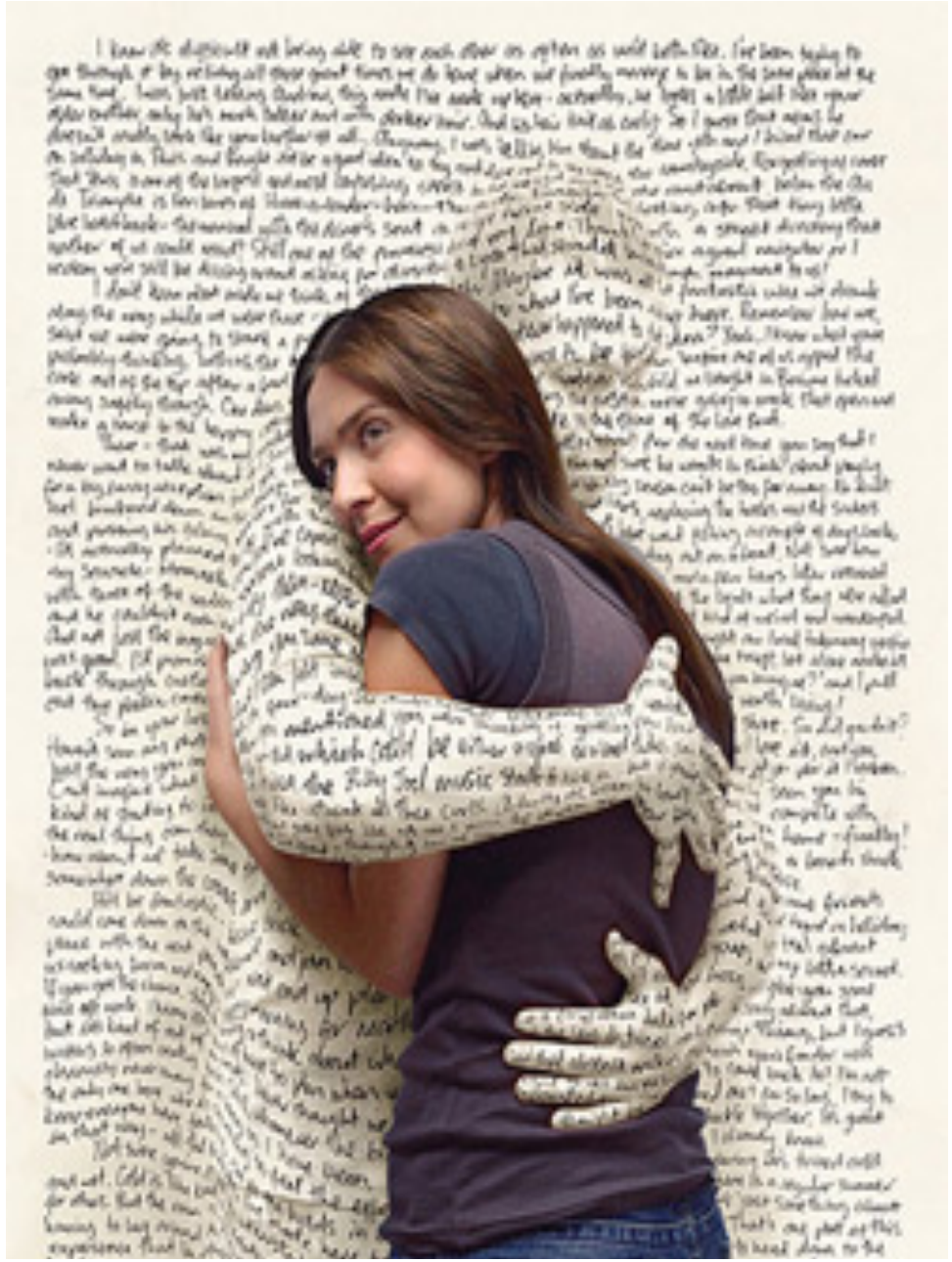

Probiotics are our invisible friends!

Thank **you!** for your attention.
Stay safe!

Questions?

Always welcome…

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Louis Pasteur

"The role of the infinitely small in nature is infinitely large."

As quoted in Biology of Microorganisms, 1994, Brock, Madigan, Martinko and Parker, Prentice-Hall, New Jersey.