The effect of probiotics on animal health: a focus on host's natural intestinal defenses

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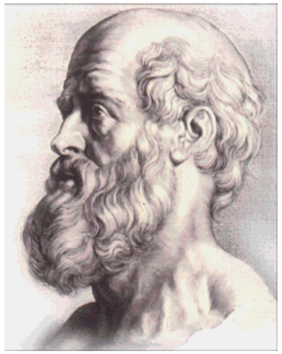
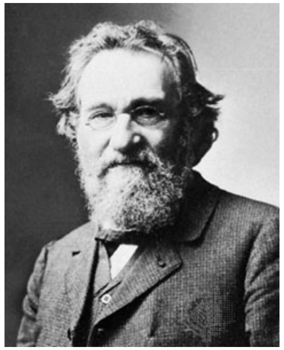
History of probiotics and definition



Hippocrates of Cos

« ***The death sits in the bowel; a bad digestion is the root of all evil*** »

He stated that it was the humble duty of the physician to facilitate that healing power firstly by means of ***dietary approaches***, and if that did was not enough, by means of ***natural medicines***



The concept of probiotics is back dated over 100 years ago to Elie Metchnikoff (Nobel Prize 1908):

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

→ Probiotics consumption alters commensal microflora and resistance to pathogenic bacteria

Roy Fuller (1992): «***live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance***»

→ Living microorganisms (either bacteria or yeast) exerting a **proven benefit** on the target species.

- Feed regulation: group of **feed additives** for stabilizing microbiota of monogastric and ruminants
- Functional view: digestive **bioregulators**
- WHO: One, or a few, **well defined strains of microorganisms**

Rationale for Probiotics usage :

- EU limitation of antibiotics usage for growth prospects (but also for therapeutic infect. Dis.)
- Intensive farming systems

→ need for alternative strategies to strengthen animals resistance to infections or pathologies associated with farming systems

Probiotics commonly used in farm animals (not exhaustive):

Table 1. Micro-organisms authorized for the use as feed additives in the EU, Simon et al 2003.

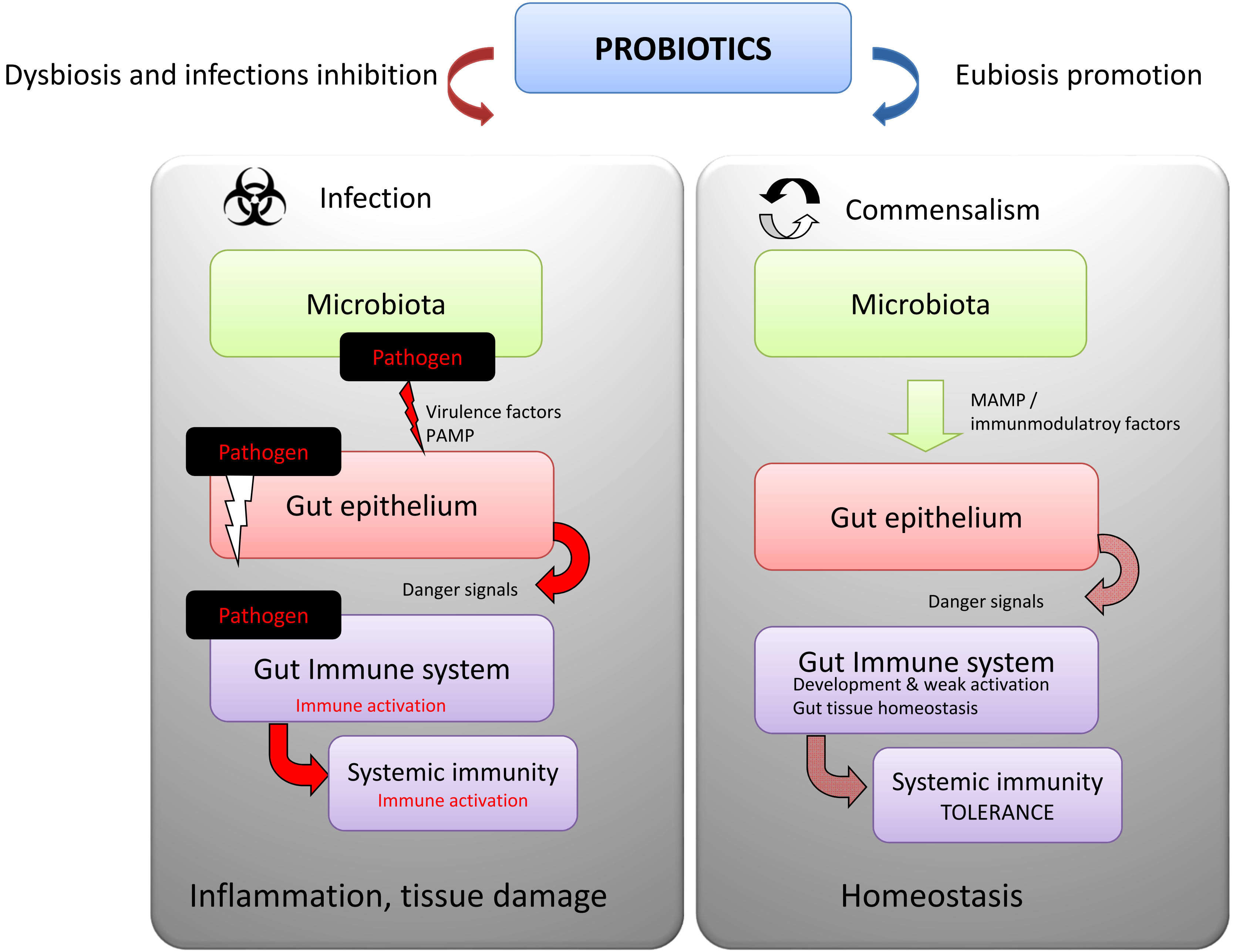
Micro-organism	Strain	Species or category of animal
Bacillus cereus var. toyoi	NCIMB 40112/ CNCM I 10121	Chickens for fattening, laying hens, calves, cattle for fattening, breeding does, rabbits for fattening, piglets, saw.
Saccharomyces cerevisiae	NCYC sc 47	Rabbits for fattening, sow, piglets, dairy cows.
Saccharomyces cerevisiae	CBS 493.94	Calves, cattle for fattening, dairy cows.
Saccharomyces cerevisiae	CNCM I- 1079	Sows, piglets.
Saccharomyces cerevisiae	CNCM I- 1077	Dairy cows, cattle for fattening
Enterococcus faecium *Enterococcus faecium*	ATCC 53519 ATCC 55593	Chickens for fattening
Pediococcus acidilactici	CNCM MA 18/5M	Chickens for fattening, pigs, piglets for fattening
Enterococcus faecium	NCIMB 10415	Chickens for fattening, pigs for fattening, sows, cattle for fattening, piglets, calves.
Enterococcus faecium	DSM 5464	Piglets, chickens for fattening, calves.
Lactobacillus farciminis	CNCM MA 67/4R	Piglets
Enterococcus faecium	DSM 10663 NCIMB 10415	Piglets, calves, chickens for fattening.
Saccharomyces cerevisiae	MUCL 39885	Piglets, cattle for fattening
Enterococcus faecium	NCIMB 11181	Calves, piglets.
Enterococcus faecium *Lactobacillus rhamnosus*	DSM 7134 DSM 7133	Calves, piglets.
Lactobacillus casei *Enterococcus faecium*	NCIMB 30096 NCIMB 30098	Calves
Enterococcus faecium	CECT 4515	Calves, piglets.
Streptococcus infantarius *Lactobacillus plantarium*	CNCM I-841 CNCM I-840	Calves
Bacillus licheniformis *Bacillus subtilis*	DSM 5749 DSM 5750	Sow, piglets, pigs for fattening, chickens for fattening, turkeys for fattening, calves.
Enterococcus faecium	DSM 3530	Calves.

Lactobacillus*, *Streptococcus*, *Enterococcus*, *Bacillus*, *Saccharomyces cerevisiae

Bacterial probiotics have been effective in chickens, pigs and pre-ruminant calves
Yeast and fungal probiotics have given better results in adult ruminants
R. Fuller (1999) ISBN:1898486

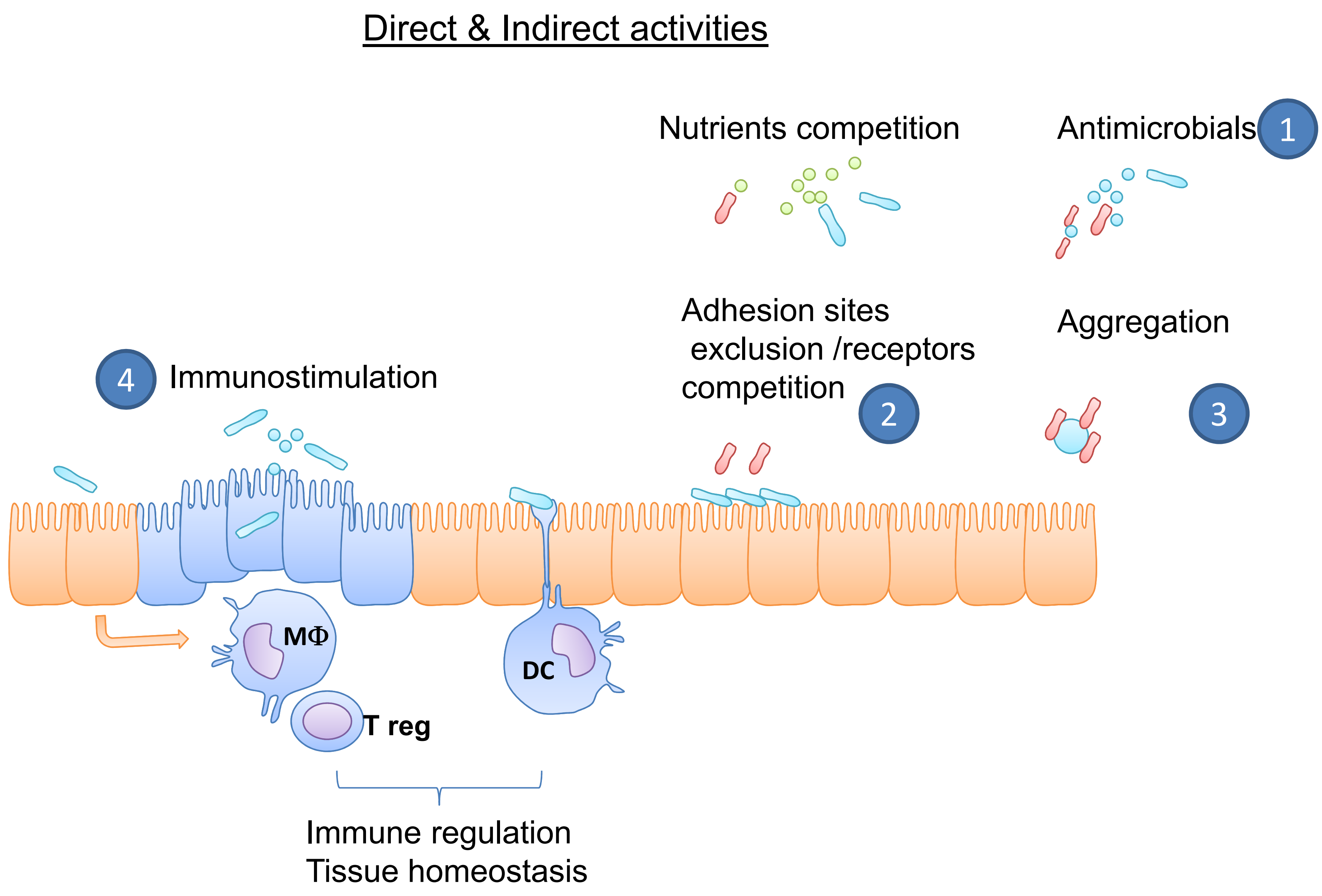
Probiotics criteria

- Normal component of the target specy microbiota
- Survive and « grow » in their respective ecological niches
- Able to utilize nutrients and substrates in a normal diet
- Capacity to adhere and colonize the epithelial cells of the gut
- Non pathogenic – non toxic
- Able to exert a beneficial effect on the host biology


PROBIOTICS
Dysbiosis and infections inhibition
Eubiosis promotion
Infection
Microbiota
Pathogen
Virulence factors
PAMP
Pathogen
Gut epithelium
Danger signals
Pathogen
Gut Immune system
Immune activation
Systemic immunity
Immune activation
Inflammation, tissue damage
Commensalism
Microbiota
MAMP /
immunmodulatroy factors
Gut epithelium
Danger signals
Gut Immune system
Development & weak activation
Gut tissue homeostasis
Systemic immunity
TOLERANCE
Homeostasis

Probiotics modes of action

1. Enzymatic contribution to digestion
2. Production of inhibitory compounds (antimicrobial): antagonism
3. Competition for chemicals/available energy
4. Competition for adhesion sites (exclusion)
5. Enhancement of the immune response



Direct & Indirect activities
Nutrients competition
Antimicrobials
1
Adhesion sites
exclusion /receptors
competition
2
Aggregation
3
4
Immunostimulation
MΦ
DC
T reg
Immune regulation
Tissue homeostasis

1 **ANTIMICROBIALS**

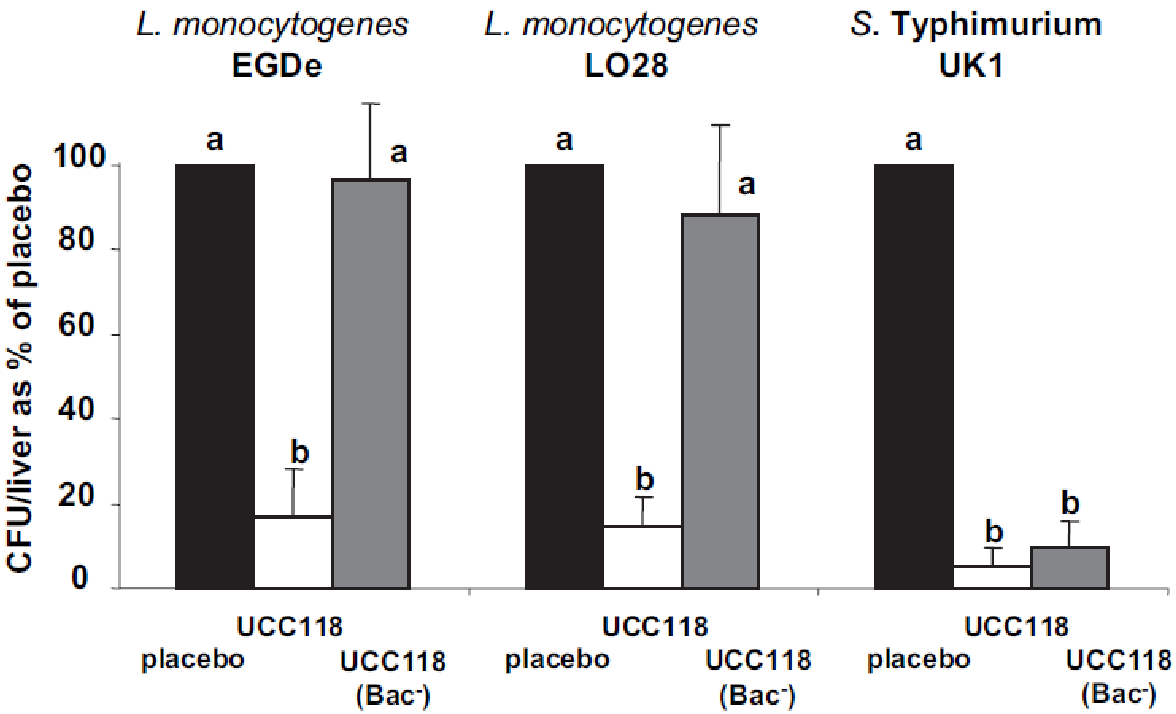
PNAS

Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118

Sinéad C. Corr*, Yin Li*†, Christian U. Riedel*, Paul W. O'Toole*, Colin Hill*†, and Cormac G. M. Gahan*§

*Alimentary Pharmabiotic Centre and Department of Microbiology and §School of Pharmacy, University College Cork, Cork, Ireland

Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved March 1, 2007 (received for review January 17, 2007)



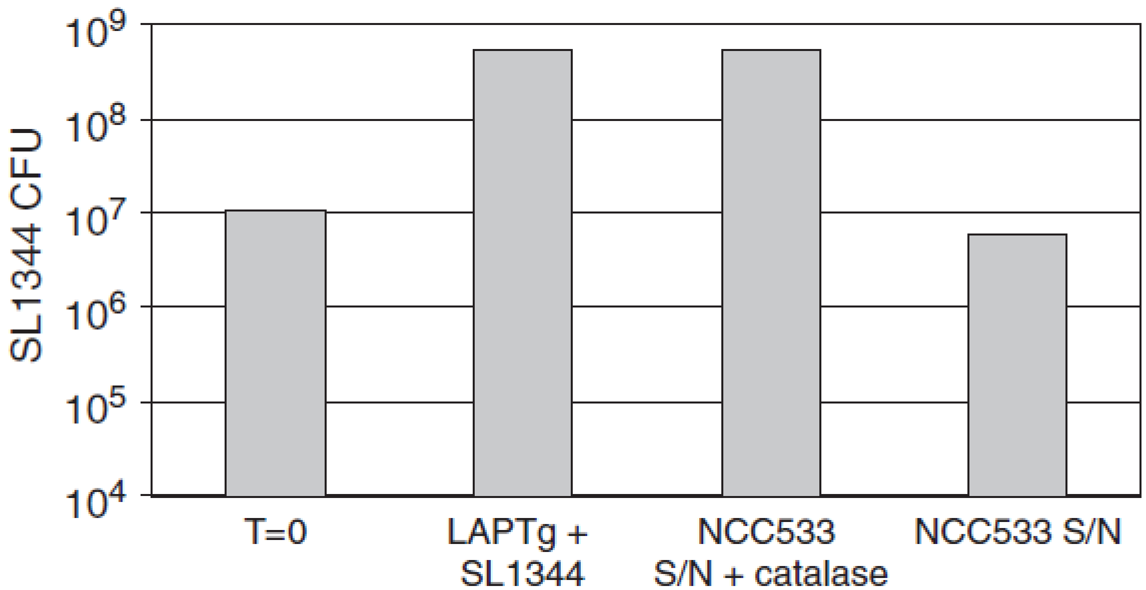
1

RESEARCH LETTER

Hydrogen peroxide production by *Lactobacillus johnsonii* NCC 533 and its role in anti-*Salmonella* activity

Raymond David Pridmore, Anne-Cécile Pittet, Fabienne Praplan & Christoph Cavadini

Department of Nutrition and Health, Nestlé Research Center, Vers-chez-les-Blancs, Lausanne, Switzerland

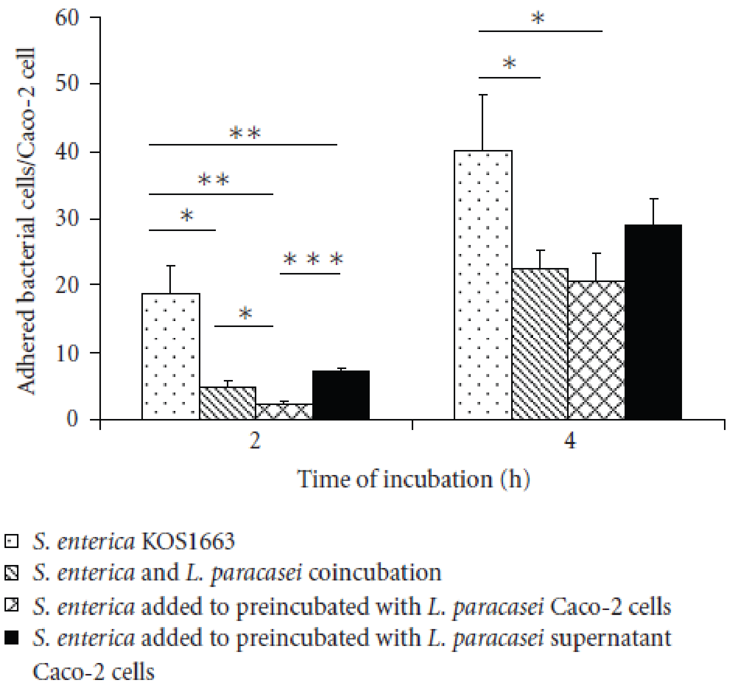


2 ADHESION SITES COMPETITION

Research Article

Competition of *Lactobacillus paracasei* with *Salmonella enterica* for Adhesion to Caco-2 Cells

Alicja Jankowska,[1] Daniel Laubitz,[1] Hanna Antushevich,[1] Romuald Zabielski,[2] and Elżbieta Grzesiuk[3]



2 PROBIOTIC STRAINS COMBINATIONS TO SATURATE ADHESION SITES

Curr Microbiol (2007) 55:260–265
DOI 10.1007/s00284-007-0144-8

Probiotic Strains and Their Combination Inhibit *In Vitro* Adhesion of Pathogens to Pig Intestinal Mucosa

M. C. Collado · Łukasz Grześkowiak ·
Seppo Salminen

J. Dairy Sci. 90:2710–2716
doi:10.3168/jds.2006-456

Development of New Probiotics by Strain Combinations: Is It Possible to Improve the Adhesion to Intestinal Mucus?

M. C. Collado,*[1] J. Meriluoto,† and S. Salminen*
*Functional Foods Forum, University of Turku, Itäinen Pitkäkatu 4A, 20520 Turku, Finland
†Department of Biochemistry and Pharmacy, Åbo Akademi University, Tykistökatu 6A, 20520 Turku, Finland

➡ **Probiotics binding to mucosa or mucus**

3

Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by *Saccharomyces* probiotics

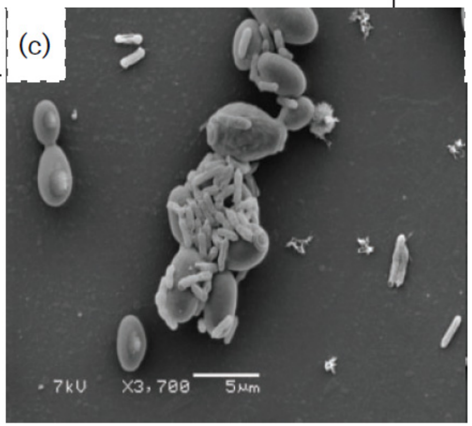
F. C. P. Tiago,[1] F. S. Martins,[1] E. L. S. Souza,[1] P. F. P. Pimenta,[2] H. R. C. Araujo,[2] I. M. Castro,[3] R. L. Brandão[3] and Jacques R. Nicoli[1]

Journal of Medical Microbiology, 2012

+, Adhesion observed in 15 min; + +, adhesion observed after 1 h; −, absence of adhesion after 3 h. All bacterial counts in the supernatants of yeast–bacteria associations were significantly different from those of the counterpart control without yeast (Student's *t*-test, $P<0.05$).

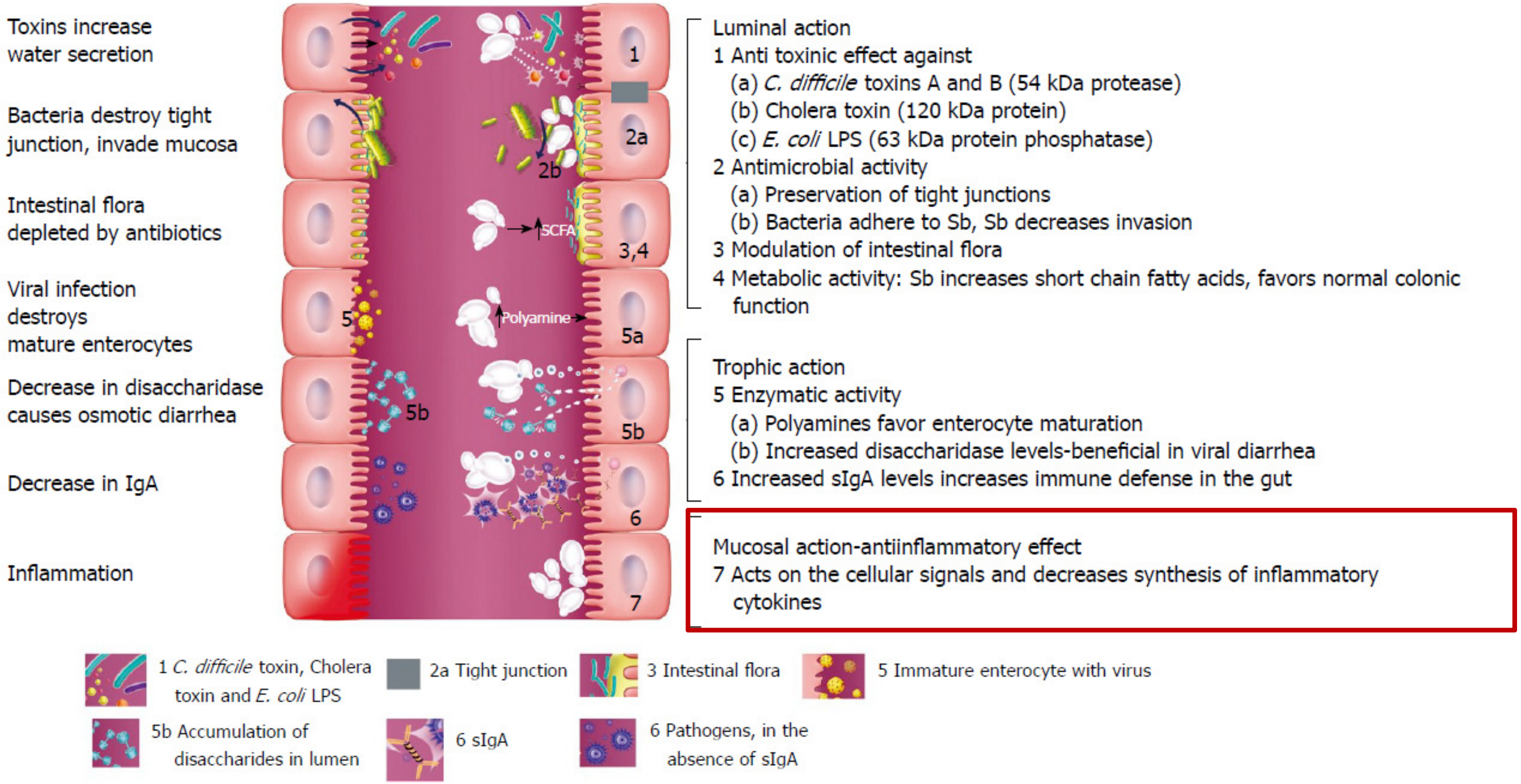
Indicator strain	Adhesion/bacterial count [log (c.f.u. ml^{-1})]					
	Control	*Saccharomyces boulardii* (live)	*Saccharomyces cerevisiae* UFMG 905 (live)	*Saccharomyces cerevisiae* W303 (live)	*Saccharomyces boulardii* (dead)	*Saccharomyces cerevisiae* UFMG 905 (dead)
Salmonella Typhimurium ATCC 14028	6.20	+ +/5.62	+ +/5.78	−	+ +/5.03	+ +/5.68
Salmonella Typhimurium (human origin)	6.43	+ +/5.77	+ +/5.87	−	+ +/5.63	+ +/5.71
Escherichia coli ATCC 25723	6.46	+ +/5.45	+ +/5.83	−	+ +/5.61	+ +/5.54
Salmonella Typhi ATCC 19430	7.36	+/6.72	−/6.51	−	+/5.61	+/5.43
Shigella sonnei ATCC 11060		−	−	−	−	−
Enterococcus faecalis ATCC 19433		−	−	−	−	−
Listeria monocytogenes ATCC 15313		−	−	−	−	−
Bacillus cereus ATCC 11778		−	−	−	−	−
Vibrio cholerae (human origin)		−	−	−	−	−
Clostridium difficile ATCC 9689		−	−	−		
Clostridium perfringens ATCC 13124		−	−	−		

→ Elimination with feces



(c)

4 Immunostimulation by probiotics: Yeast – *Saccharomyces* spp.



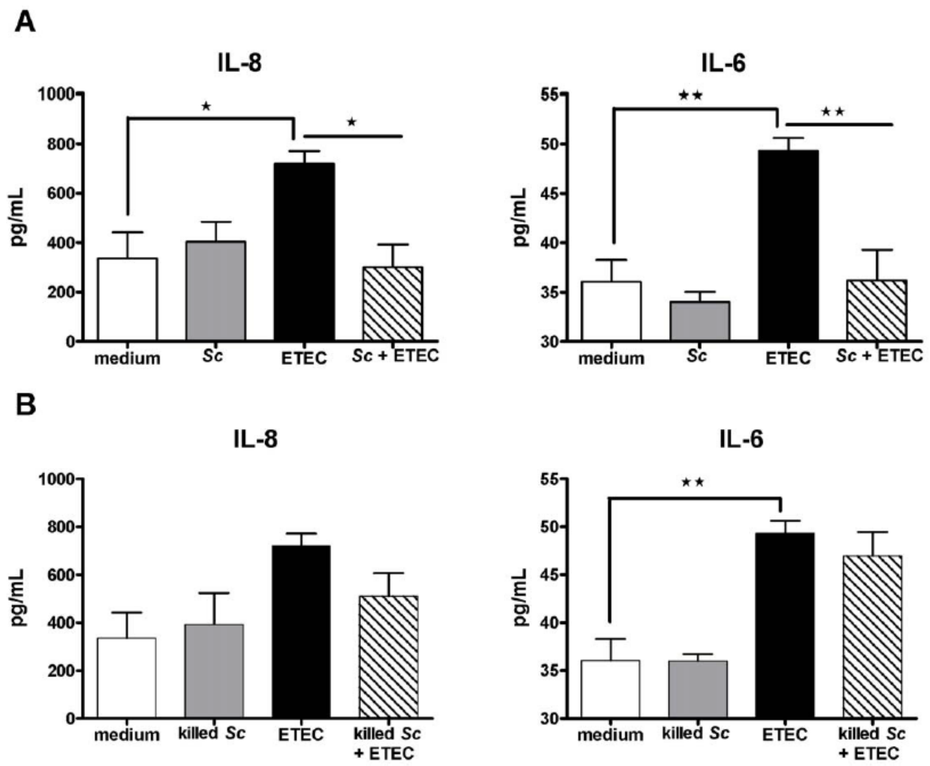
From McFarland, L. V. (2010). Systematic review and meta-analysis of Saccharomyces boulardii in adult patients. World Journal of Gastroenterology : WJG, 16(18), 2202–2222.

OPEN ACCESS Freely available online

PLoS one

Saccharomyces cerevisiae Modulates Immune Gene Expressions and Inhibits ETEC-Mediated ERK1/2 and p38 Signaling Pathways in Intestinal Epithelial Cells

Galliano Zanello[1,2], Mustapha Berri[2], Joëlle Dupont[3], Pierre-Yves Sizaret[4], Romain D'Inca[1], Henri Salmon[2], François Meurens[2*]

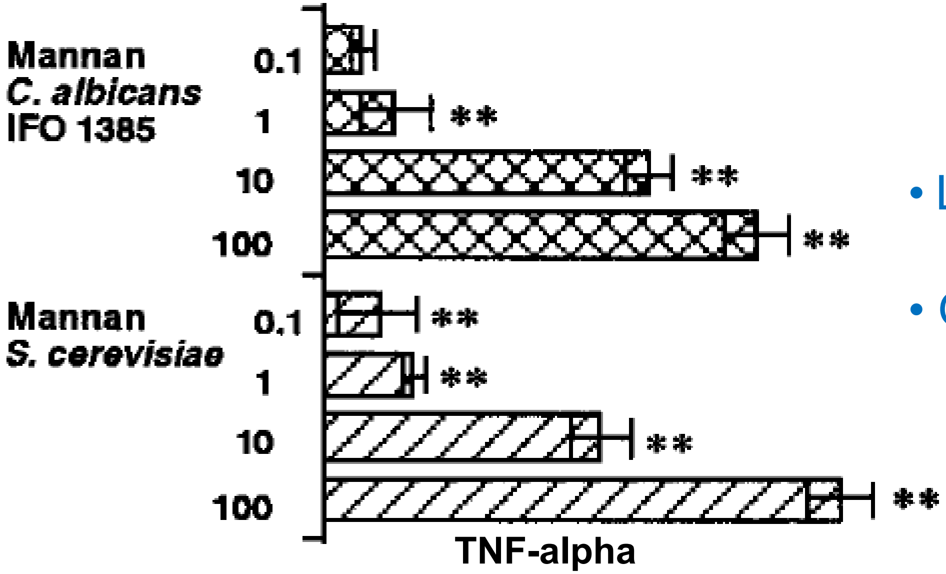


Live S. cerevisiae exerts anti-inflammatory activity

Saccharomyces cerevisiae- and *Candida albicans*-Derived Mannan Induced Production of Tumor Necrosis Factor Alpha by Human Monocytes in a CD14- and Toll-Like Receptor 4-Dependent Manner

Hiroyuki Tada[1,2], Eiji Nemoto[2], Hidetoshi Shimauchi[2], Toshihiko Watanabe[3], Takeshi Mikami [3], Tatsuji Matsumoto[3], Naohito Ohno[4], Hiroshi Tamura[5], Ken-ichiro Shibata[6], Sachiko Akashi [7], Kensuke Miyake[7], Shunji Sugawara[1], and Haruhiko Takada[*,1]

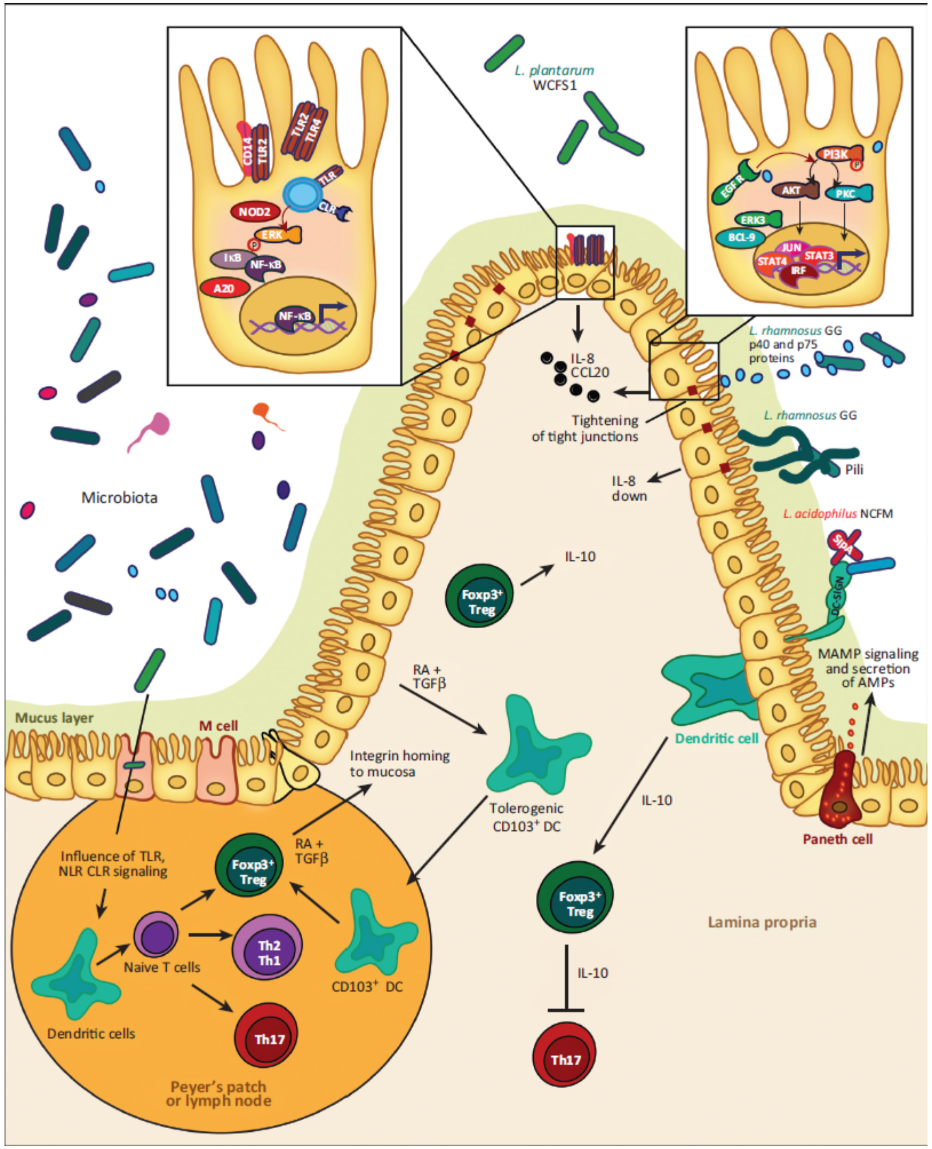
Microbiol. Immunol. (2002)



- Live Sc. exerts anti-inflammatory activity
- One of the main component of Sc. wall is as pro-inflammatory as a pathogenic yeast

Secreted immunomodulatory proteins

4 Immunostimulation by probiotics: Lactobacilli



From Peter Van Baarlen *et al*. – Trends in Immunology 2013

- MAMPs recognition: TLR/NLR/CLR
- Pathogen = non self
- Probiotic lactobacilli = non self

For a similar result ?

Strain and species specific variations in the chemical structure of major MAMPS such as LTA or PGN

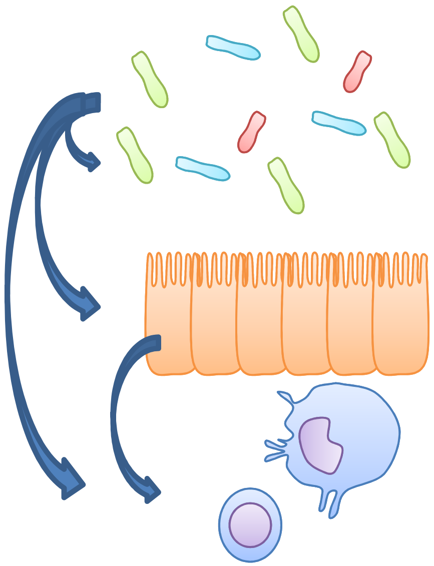
Immunomodulatory proteins (glycosylation)

→ Different responses in IEC or immune cells

Engineering of various Lactobacilli strains to promote the « desired » effect

Concluding remarks

Microbiota
+ single probiotic well defined strain
+/- pathogen



First level of interactions

Second level of interactions

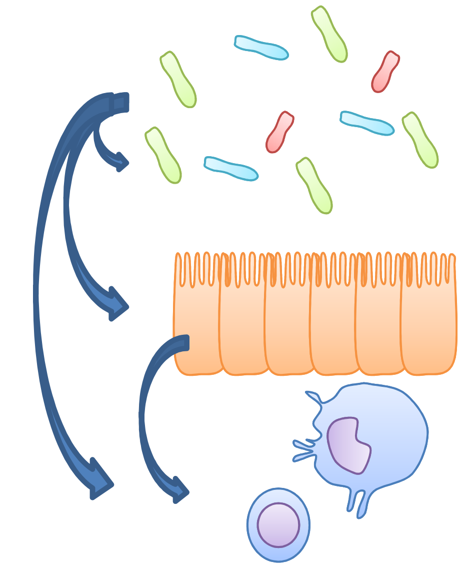
Third level of interactions

Rather complex…but what if…

Concluding remarks

Microbiota
+ single probiotic well defined strain
+/- pathogen

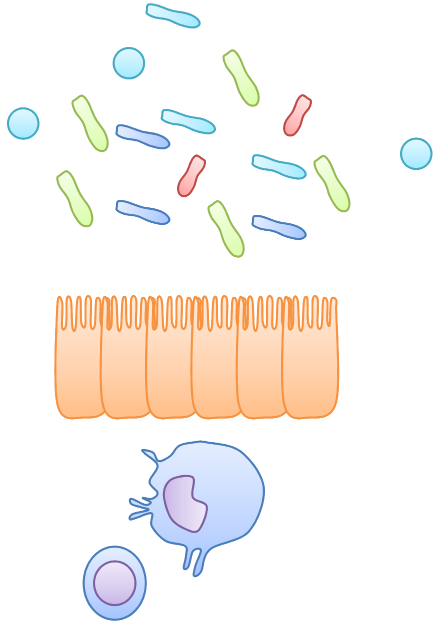
Microbiota
+ combined / engeneered probiotics
+/- pathogen



First level of interactions

Second level of interactions

Third level of interactions



+Host inter-species variations
+Host inter-individual variations

Rather complex…but what if…

A tremendous complexity

Concluding remarks and take-home message

- Existing and convincing proofs of concept of clinical efficacy of probiotics applications for various conditions
    - prevention of antibiotic associated diarrhea
    - prevention severe necrotizing enterocolitis
    - Protection against a variety of pathogens in chicken, pigs…
    - Reduction of shedding of *E. Coli* O157:H7 in cattle and calves
    - improvement of health and production criteria of various livestock animal
    - …. The list in now quite long…

- Several modes of action by which probiotics contribute to human and animal health have been proposed or established

- No single probiotic supplement drives all the mentioned effects.
- There is no common responses to different probiotics even of the same genera (i.e. lactobacili)

Consequently, there is a need for:

- for a rational selection of a specfic probiotic for defined targets (individual, specie…) and clinical indication

- for having a better konwledge in the effects (and associated molecular mechanisms) a specific probiotic will have on healthy and unhealthy individuals