"Key Scientific Drivers Behind Probiotic and Prebiotic Applications"
International Symposium of the International Scientific Association of Probiotics and Prebiotics
June 5-6, 2018, Furama Riverfront Hotel, Singapore

If You Could Design A Probiotic, What Would It Look Like?

Sarah LEBEER
Belgium

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If You Could Design A Probiotic, What Would It Look Like?

Prof. Sarah Lebeer

ENdEMIC
Environmental Ecology and Applied Microbiology
University of Antwerp

06/06/2018

What are probiotics?

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 and Mary Ellen Sanders

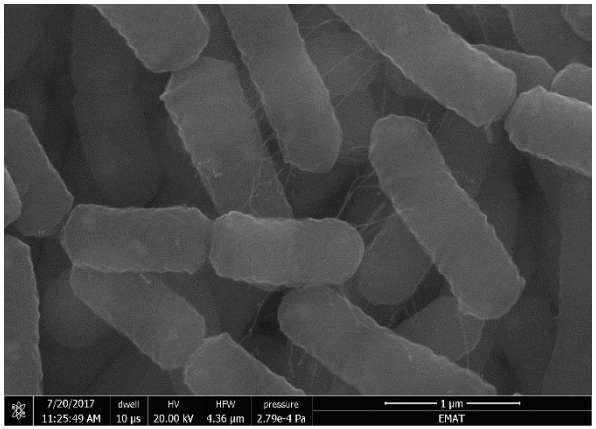
nature REVIEWS GASTROENTEROLOGY & HEPATOLOGY

Box 1 | Consensus panel recommendations for the scope of probiotics

- Retain the FAO/WHO definition[1] for probiotics, with a minor grammatical correction as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”; inconsistences between the Expert Consultation[1] and the FAO/WHO Guidelines[2] were clarified
- Include in the framework for definition of probiotics microbial species that have been shown in properly controlled studies to confer benefits to health

Objectives: predict & explain

Live micro-organism ⟷ Health benefit



Which properties?

Microbial probiotic properties?

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Shared mechanisms among probiotic taxa: implications for general probiotic claims

Mary Ellen Sanders[1], Andrew Benson[2], Sarah Lebeer[3], Daniel J Merenstein[4] and Todd R Klaenhammer[5]

Available online at www.sciencedirect.com

ScienceDirect

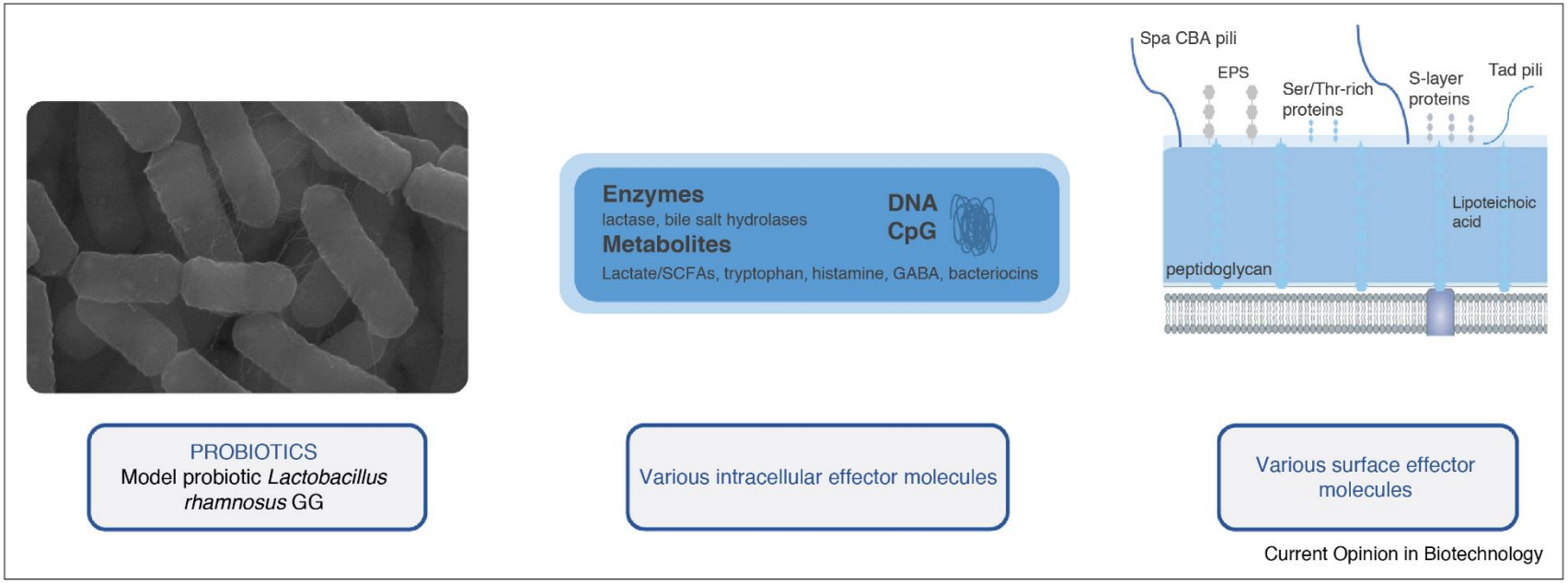
Current Opinion in Biotechnology

Identification of probiotic effector molecules: present state and future perspectives

Sarah Lebeer[1], Peter A Bron[2], Maria L Marco[3], Jan-Peter Van Pijkeren[4], Mary O'Connell Motherway[5], Colin Hill[5], Bruno Pot[6,7], Stefan Roos[8] and Todd Klaenhammer[9]

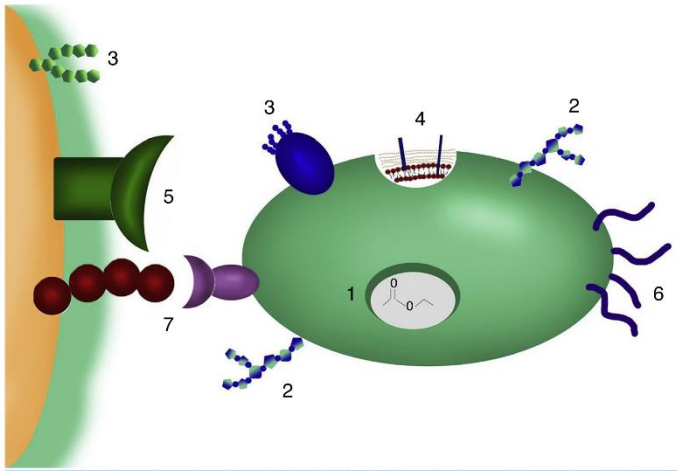
Probiotics = “bags of effector molecules”

Figure 1



Shared probiotic functions = CORE

Figure 2

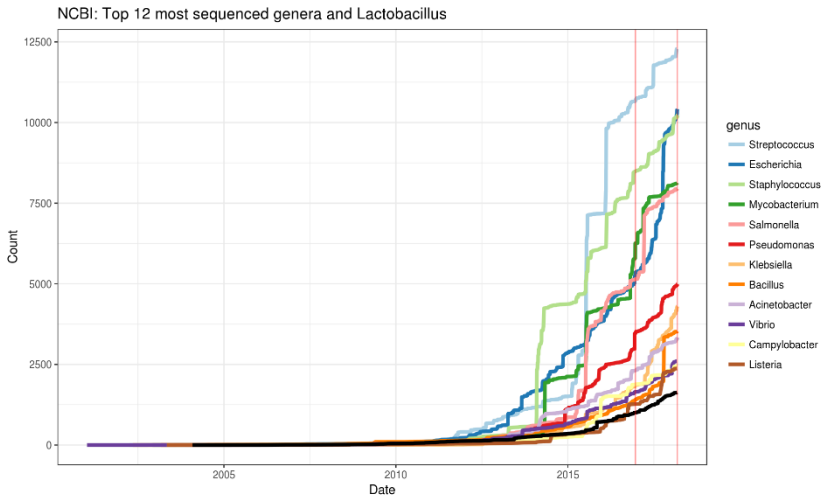


Mechanism	Gram+	*Bifidobacterium* genus wide	*Bifidobacterium* - some species	*Lactobacillus* genus wide	*Lactobacillus* – some species
1. *Bifidobacterium* shunt pathway,leading to acetate and lactate		•			
1. Lactate (not via *Bifidobacterium* shunt)				•	
2. Surface-layer-associated proteins					•
3. Mucus binding protein		•		•	
4. Lipoteichoic acid	•				
5. TLR receptors	•				
6. Sortase-dependent pilin proteins			•		•
7. Fibronectin-binding proteins					•
Lactocepin					•
CpG DNA			•		•
Vitamins			•		•

Current Opinion in Chemical Biology

Shared probiotic mechanisms and their taxonomic distribution.

Current focus = LAB

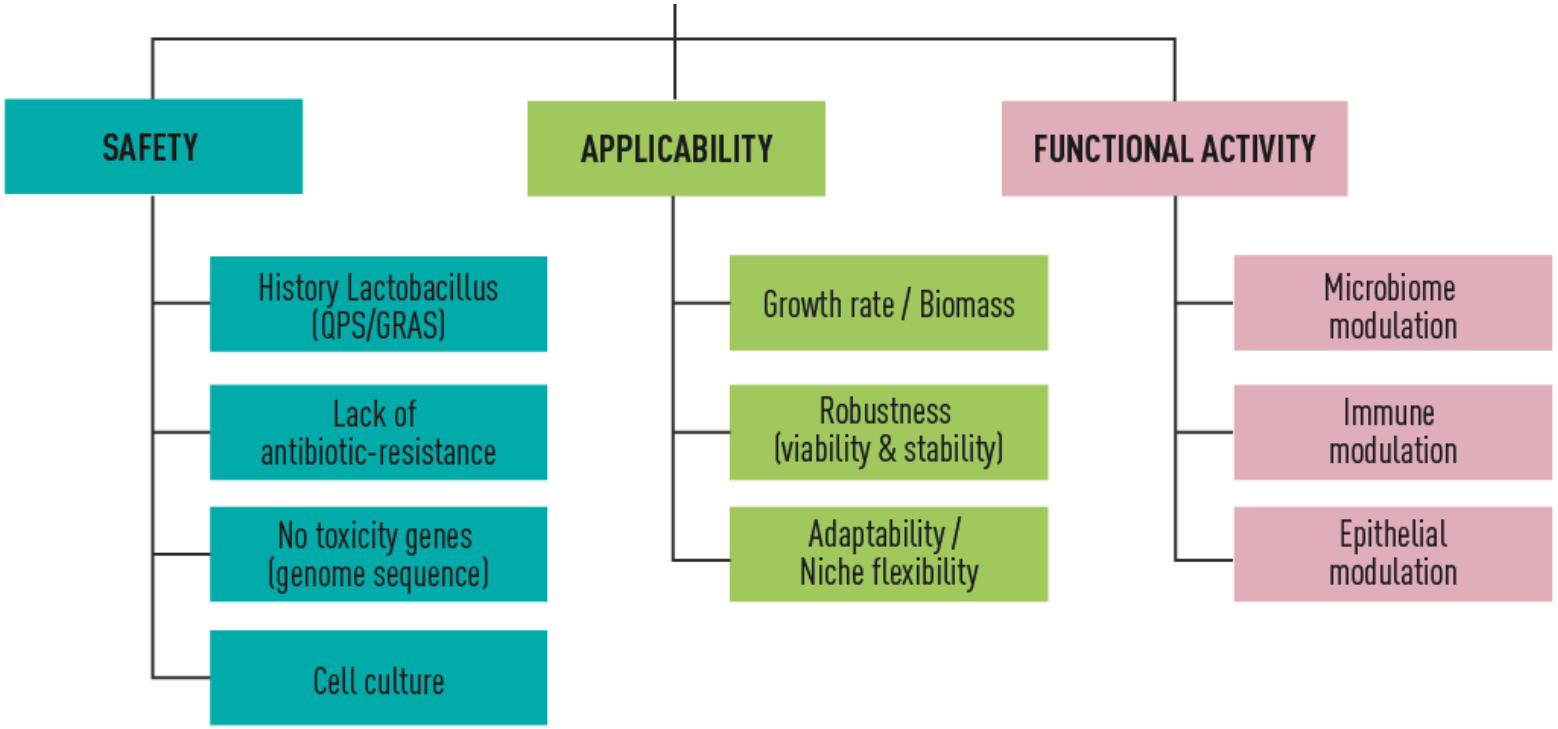


Lactobacillus: the most commonly sequenced non-pathogenic 'genus'

https://swuyts.wordpress.com/

Long history of safe use in fermented foods

Rationale for probiotic strain selection



animal models

human studies

Simplied overview health effects

Box 1 Probiotic mechanisms of action from a host perspective.

While the major part of the manuscript is focused on probiotic mechanisms of action from a microbiological perspective, possible molecular mechanisms of action of probiotics from a host perspective can be broadly divided into the following categories:

(1) Modulation of the composition and activity of the indigenous microbiota — at least temporarily

(2) Enhancement of epithelial barrier function

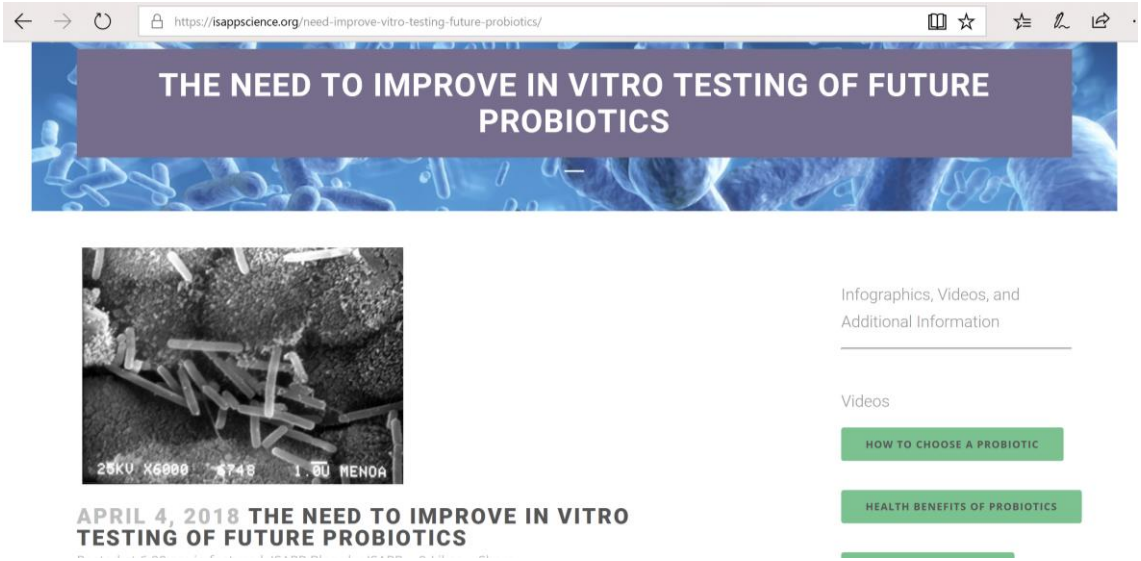
(3) Modulation of the immune system

(4) Modulation of systemic metabolic responses — e.g. bile salt hydrolases

(5) Signaling via the central nervous system — e.g. GABA

Lebeer *et al.*, 2018 Current Opinion in Biotechnology

Check blogs


https://isappscience.org/need-improve-vitro-testing-future-probiotics/
THE NEED TO IMPROVE IN VITRO TESTING OF FUTURE PROBIOTICS
Infographics, Videos, and Additional Information
Videos
HOW TO CHOOSE A PROBIOTIC
HEALTH BENEFITS OF PROBIOTICS
APRIL 4, 2018 THE NEED TO IMPROVE IN VITRO TESTING OF FUTURE PROBIOTICS


cdrf.org/2018/03/01/probiotic-screening-vitro-tests-informative/
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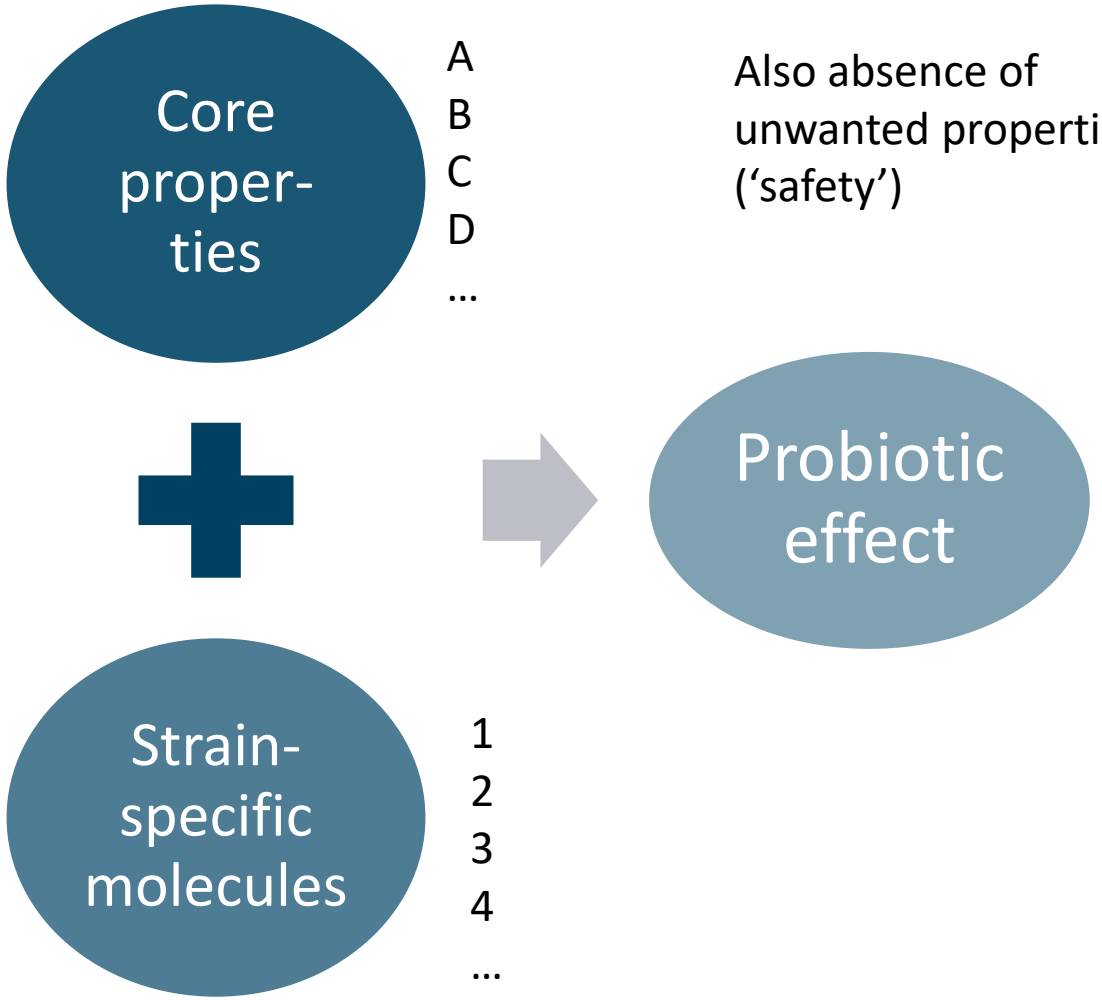
usprobiotics.org
Probiotic Screening: Are in vitro Tests Informative?
March 1, 2018
By Mary Ellen Sanders, PhD , Dairy & Food Culture Technologies
In 2002, the FAO/WHO published guidelines for probiotics. In it, a table lists laboratory tests commonly used to characterize probiotics, including: resistance to gastric acidity; bile acid resistance; immunomodulatory activity; adherence to mucus and/or to cells in cell culture; antimicrobial activity; and bile salt hydrolase activity. Some in the field insist that such tests are essential to proper probiotic strain characterization (although the FAO/WHO paper states that these tests need to be validated). Indeed, the literature is replete with papers reporting results of such tests, which aim to justify that some strain has 'probiotic properties', which make it a better choice than another strain.
NEWS
Dairy News
Photo Gallery
Dairy Research Bulletin – Jan 2018
Publications

Challenge probiotic properties



My experience: LGG as model

TOOLBOX

- Genome editing tools: mostly loss-of function mutants
- Biochemical tools: extraction & characterization of cell surface & secreted molecules (EPS, pili, secreted (glyco) proteins
- Comparative genomics of lactobacilli
- Microbiome analysis mainly by 16S amplicon sequencing
- Array of phenotypic tests: niche adaptation, metabolism, adhesion, immunomodulation, formulation, etc.
- *In vivo* tests: mice models & human intervention studies (urogenital, skin & upper respiratory tract >< gut)

LGG : documented health benefits

- Promotion of gastro-intestinal health in children and adults
- Reduce respiratory infections in children
- Lower eczema risk in family with history
- Protect hospitalized patients against ventilator-associated pneumonia
- Promote oral health
- Vaccine adjuvans (e.g. polio)
- ….

Antimicrobial activity LGG

Lactate

QS? AI-2?

Bacteriocins?

- R-IVET: only in vivo?

Lectins

Secreted enzymes & EPS

Kinetic analysis of the antibacterial activity of probiotic lactobacilli towards *Salmonella enterica* serovar Typhimurium reveals a role for lactic acid and other inhibitory compounds

Lefteris Makras [a], Vagelis Triantafyllou [a,b], Domitille Fayol-Messaoudi [c], Tom Adriany [a], Georgia Zoumpopoulou [b], Effie Tsakalidou [b], Alain Servin [c], Luc De Vuyst [a,*]

Beneficial Microbes, 2018 online ARTICLE IN PRESS Wageningen Academic Publishers

Multifactorial inhibition of lactobacilli against the respiratory tract pathogen *Moraxella catarrhalis*

M.F.L. van den Broek[1], I. De Boeck[1], I.J.J. Claes[1], V. Nizet[2,3] and S. Lebeer[1*]

JOURNAL OF BACTERIOLOGY, Feb. 2007, p. 860–871 Vol. 189, No. 3
0021-9193/07/$08.00+0 doi:10.1128/JB.01394-06
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Functional Analysis of *luxS* in the Probiotic Strain *Lactobacillus rhamnosus* GG Reveals a Central Metabolic Role Important for Growth and Biofilm Formation▽

Sarah Lebeer, Sigrid C. J. De Keersmaecker, Tine L. A. Verhoeven, Abeer A. Fadda, Kathleen Marchal, and Jos Vanderleyden*

Centre of Microbial and Plant Genetics, K. U. Leuven, Kasteelpark Arenberg 20, 3001 Leuven, Belgium

PLOS ONE

RESEARCH ARTICLE

Lectin-Like Molecules of *Lactobacillus rhamnosus* GG Inhibit Pathogenic *Escherichia coli* and *Salmonella* Biofilm Formation

Mariya I. Petrova[1,2*], Nicole C. E. Imholz[1], Tine L. A. Verhoeven[1], Jan Balzarini[3], Els J. M. Van Damme[4], Dominique Schols[3], Jos Vanderleyden[1], Sarah Lebeer[1,2*]

1 KU Leuven, Centre of Microbial and Plant Genetics, Kasteelpark Arenberg 20, box 2460, B-3001, Leuven, Belgium, 2 University of Antwerp, Department of Bioscience Engineering, Groenenborgerlaan 171, B-2020, Antwerp, Belgium, 3 KU Leuven, Rega Institute for Medical Research, Minderbroedersstraat 10, B-3000, Leuven, Belgium, 4 Ghent University, Laboratory of Biochemistry and Glycobiology, Department of Molecular Biotechnology, Coupure Links 653, B-9000, Ghent, Belgium

* sarah.lebeer@uantwerpen.be (SL); mariyapetrova3@gmail.com (MIP)

microbial biotechnology Open Access

Interplay between *Lactobacillus rhamnosus* GG and *Candida* and the involvement of exopolysaccharides

Camille N. Allonsius,[1] Marianne F. L. van den Broek,[1] Ilke De Boeck,[1] Shari Kiekens,[1,2] Eline F. M. Oerlemans,[1] Filip Kiekens,[2] Kenn Foubert,[3] Dieter Vandenheuvel,[1] Paul Cos,[4] Peter Delputte[4] and Sarah Lebeer[1,*]

Gut microbiome modulation capacity

ARTICLE

DOI: 10.1038/s41467-018-03157-4 OPEN

Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by *Lactobacillus* supplementation

Juliana Durack[1], Nikole E. Kimes[1,4], Din L. Lin[1], Marcus Rauch[1,5], Michelle McKean[2], Kathryn McCauley[1], Ariane R. Panzer[1], Jordan S. Mar[1,6], Michael D. Cabana[2,3] & Susan V. Lynch[1]

European Journal of Nutrition
October 2017, Volume 56, Issue 7, pp 2245-2253 | Cite as

Probiotics modulate gut microbiota and health status in Japanese cedar pollinosis patients during the pollen season

Authors Authors and affiliations

Gaku Harata, Himanshu Kumar, Fang He, Kenji Miyazawa, Kazutoyo Yoda, Manabu Kawase, Akira Kubota, Masaru Hiramatsu, Samuli Rautava, Seppo Salminen

RESEARCH ARTICLE

Lactobacillus rhamnosus GG Intake Modifies Preschool Children's Intestinal Microbiota, Alleviates Penicillin-Associated Changes, and Reduces Antibiotic Use

Katri Korpela[1]*, Anne Salonen[1], Lauri J. Virta[2], Minna Kumpu[3], Riina A. Kekkonen[3], Willem M. de Vos[1,4]

1 Department of Bacteriology and Immunology, Immunobiology Research Programme, Faculty of Medicine, University of Helsinki, Helsinki, Finland, **2** Research Department, Social Insurance Institution, Turku, Finland, **3** R&D, Valio Limited, Helsinki, Finland, **4** Laboratory of Microbiology, Wageningen University, Wageningen, the Netherlands

* katri.korpela@helsinki.fi

LGG effector molecules → host cells

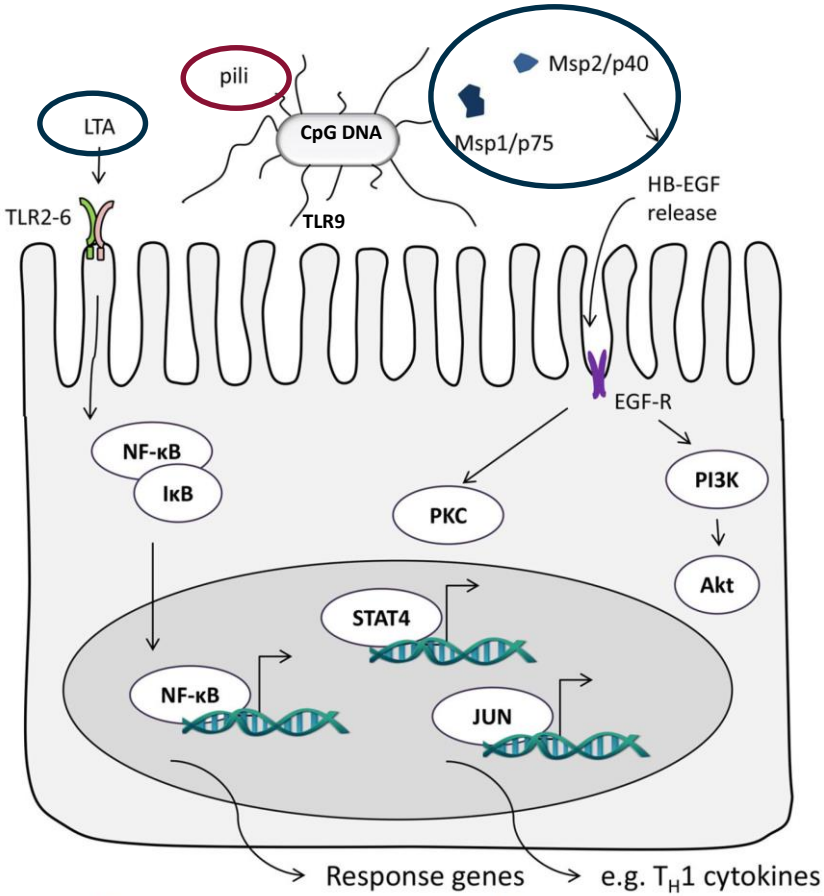

Figure 2 Molecular interactions of LGG with intestinal epithelial cells. LTA as a MAMP interacts with TLR2-6, activating NF-κ signaling [43]. Secreted protein Msp2/p40 induces release of HB-EGF that causes phosphorylation of EGF-R, activating downstream protein kinase C (PKC) and phosphoinositide 3-kinase (PI3K) -Akt signaling [51,53,54]. A recent human duodenal transcriptome study indicates that JUN and STAT4 transcription factors play a central role in downstream signaling after consumption of LGG, leading to mainly T_H1 cytokine production and activating pathways involved in cellular growth and proliferation, wound healing, angiogenesis, interferon-mediated responses, calcium signaling and ion homeostasis [92]. Adapted from [96]

Epithelial interaction

+ EPS

Segers and Lebeer Microbial Cell Factories 2014, 13(Suppl 1):S7
http://www.microbialcellfactories.com/content/13/S1/S7

MICROBIAL CELL FACTORIES

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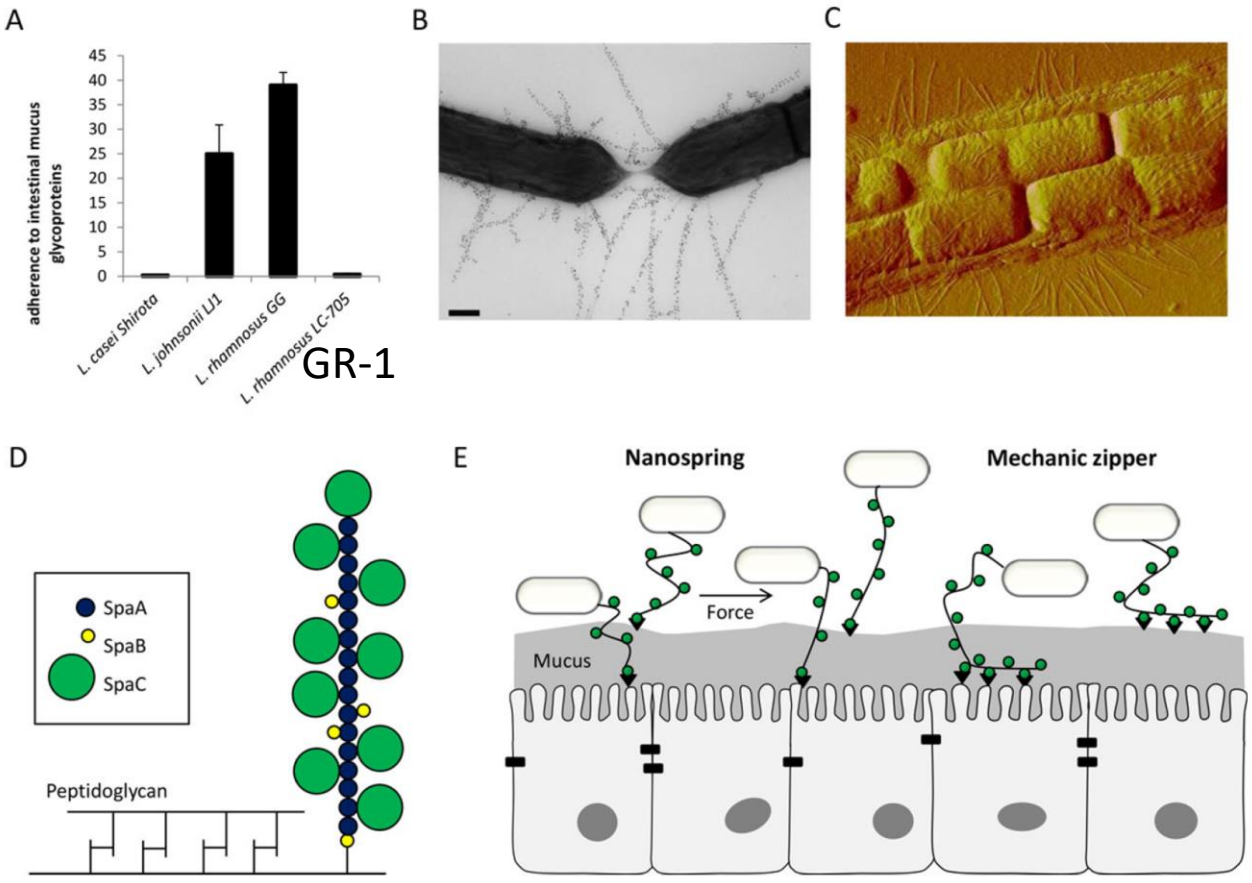
Towards a better understanding of *Lactobacillus rhamnosus* GG - host interactions

Marijke E Segers[1,2], Sarah Lebeer[1,2*]

Segers & Lebeer, 2014, MCF

SpaCBA pili as important modulators



Segers & Lebeer, 2014

Segers and Lebeer Microbial Cell Factories 2014, 13(Suppl 1):S7
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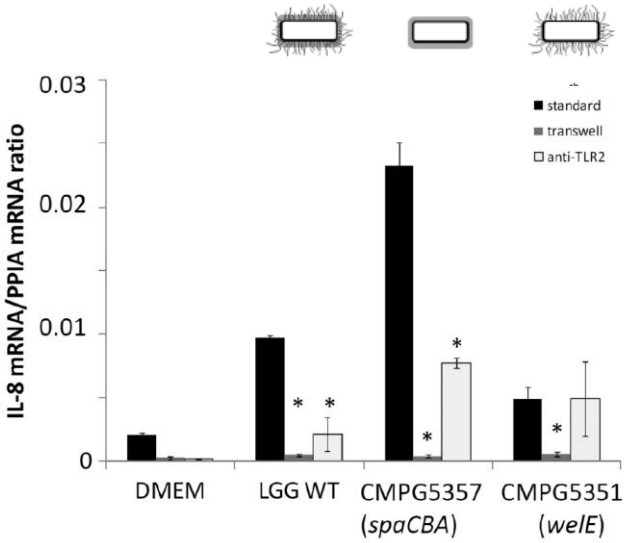
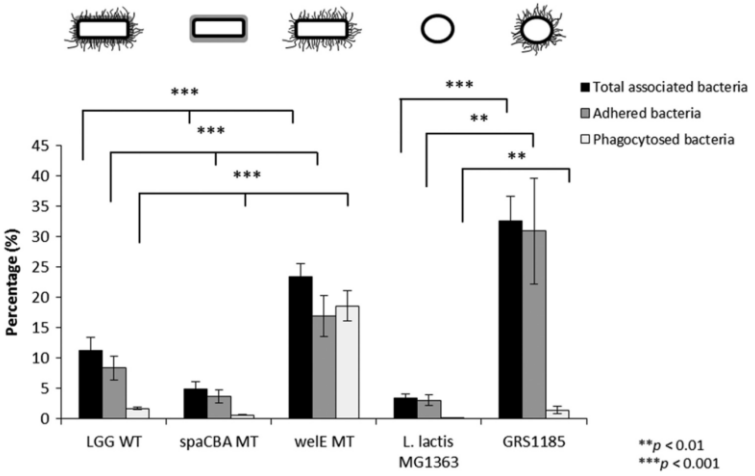
Marijke E Segers[1,2], Sarah Lebeer[1,2*]

Presence of pili determines interaction





IECs

macrophages

AEM
Journals.ASM.org

Functional Analysis of *Lactobacillus rhamnosus* GG Pili in Relation to Adhesion and Immunomodulatory Interactions with Intestinal Epithelial Cells

Sarah Lebeer,[a,b] Ingmar Claes,[a] Hanne L. P. Tytgat,[a] Tine L. A. Verhoeven,[a] Eyra Marien,[a] Ingemar von Ossowski,[c] Justus Reunanen,[c] Airi Palva,[c] Willem M. de Vos,[c,d] Sigrid C. J. De Keersmaecker,[a] and Jos Vanderleyden[a]

Centre of Microbial and Plant Genetics, K.U. Leuven, Leuven, Belgium[a]; Department of Bioscience Engineering, University of Antwerp, Antwerp, Belgium[b]; Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland[c]; and Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands[d]

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Piliation of *Lactobacillus rhamnosus* GG Promotes Adhesion, Phagocytosis, and Cytokine Modulation in Macrophages

Cynthia E. Vargas García,[a,b] Mariya Petrova,[a,b] Ingmar J. J. Claes,[a,b] Ilke De Boeck,[a,b] Tine L. A. Verhoeven,[a] Ellen Dilissen,[c] Ingemar von Ossowski,[d] Airi Palva,[d] Dominique M. Bullens,[c] Jos Vanderleyden,[a] Sarah Lebeer[a,b]

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Comparative genomics: how unique?

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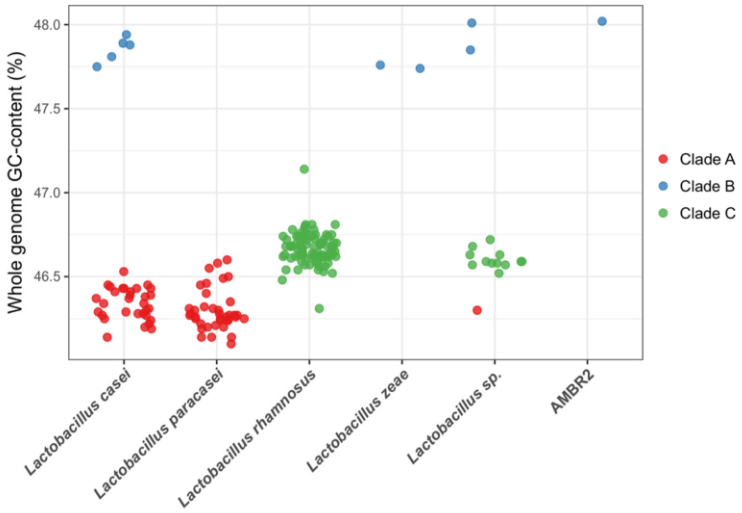
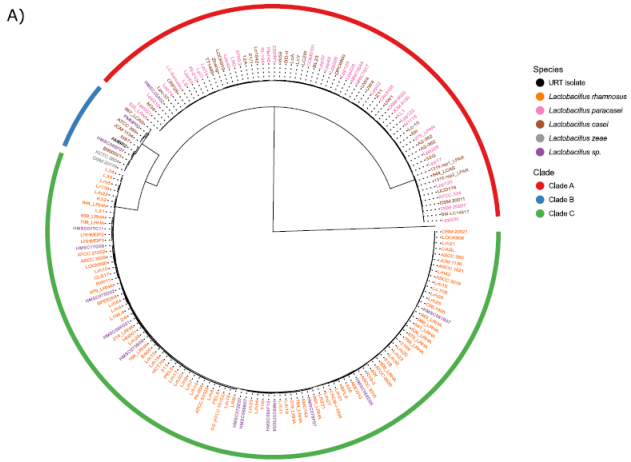
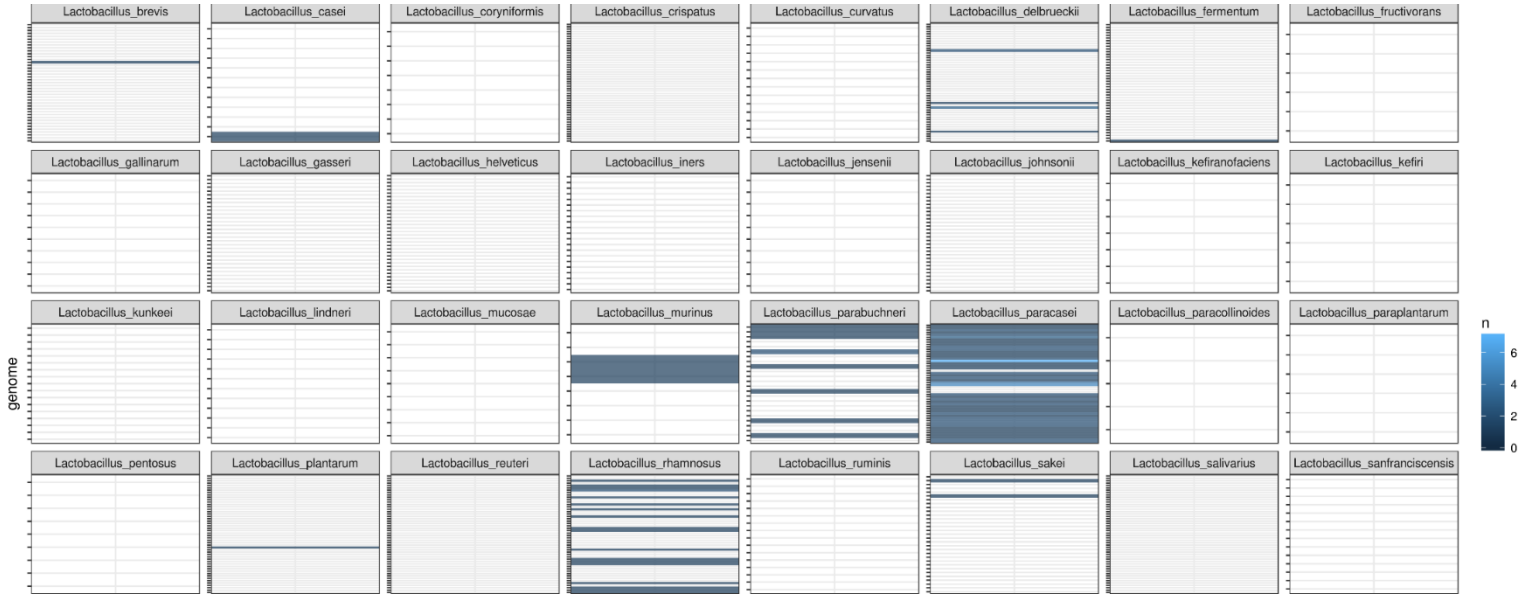
Large-Scale Phylogenomics of the *Lactobacillus casei* Group Highlights Taxonomic Inconsistencies and Reveals Novel Clade-Associated Features

Sander Wuyts,[a] Stijn Wittouck,[a] Ilke De Boeck,[a] Camille N. Allonsius,[a] Edoardo Pasolli,[b] Nicola Segata,[b] Sarah Lebeer[a]





SpaC presence in *Lactobacillus* 'genus'



What can we learn from LGG?

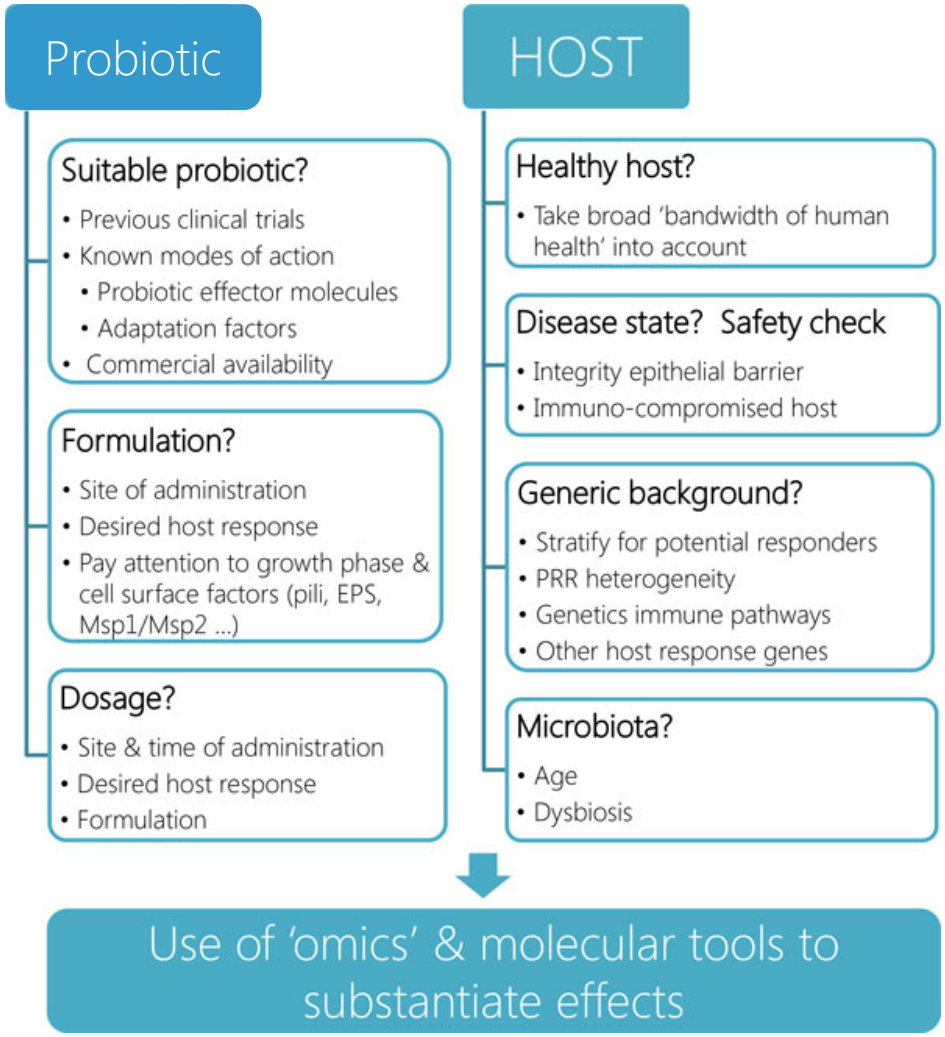
- Key effector molecules
  - Pili
  - Lactic acid
  - Msp1/2, LTA, EPS, CpG DNA

  + highly robust strain= survives various stresses (e.g. formulation) >< vagina (GR-1)

- Causal molecules? Test mutants or molecules in humans
  - E.g. spontaneous pili mutant (Prof. Willem de Vos)
  - E.g. Msp2/p40 (Prof. Polk, Prof. Yan, USA)
  - Crispr-Cas mutagenesis & other recombineering tools being implemented

- Validation in larger scale human studies
  - Multi-omics approaches – holistic view – translation back to mechanisms
  - Patient stratification, dosing, formulation, timing of intervention, ….

Probiotic design



Segers and Lebeer Microbial Cell Factories 2014, 13(Suppl 1):S7
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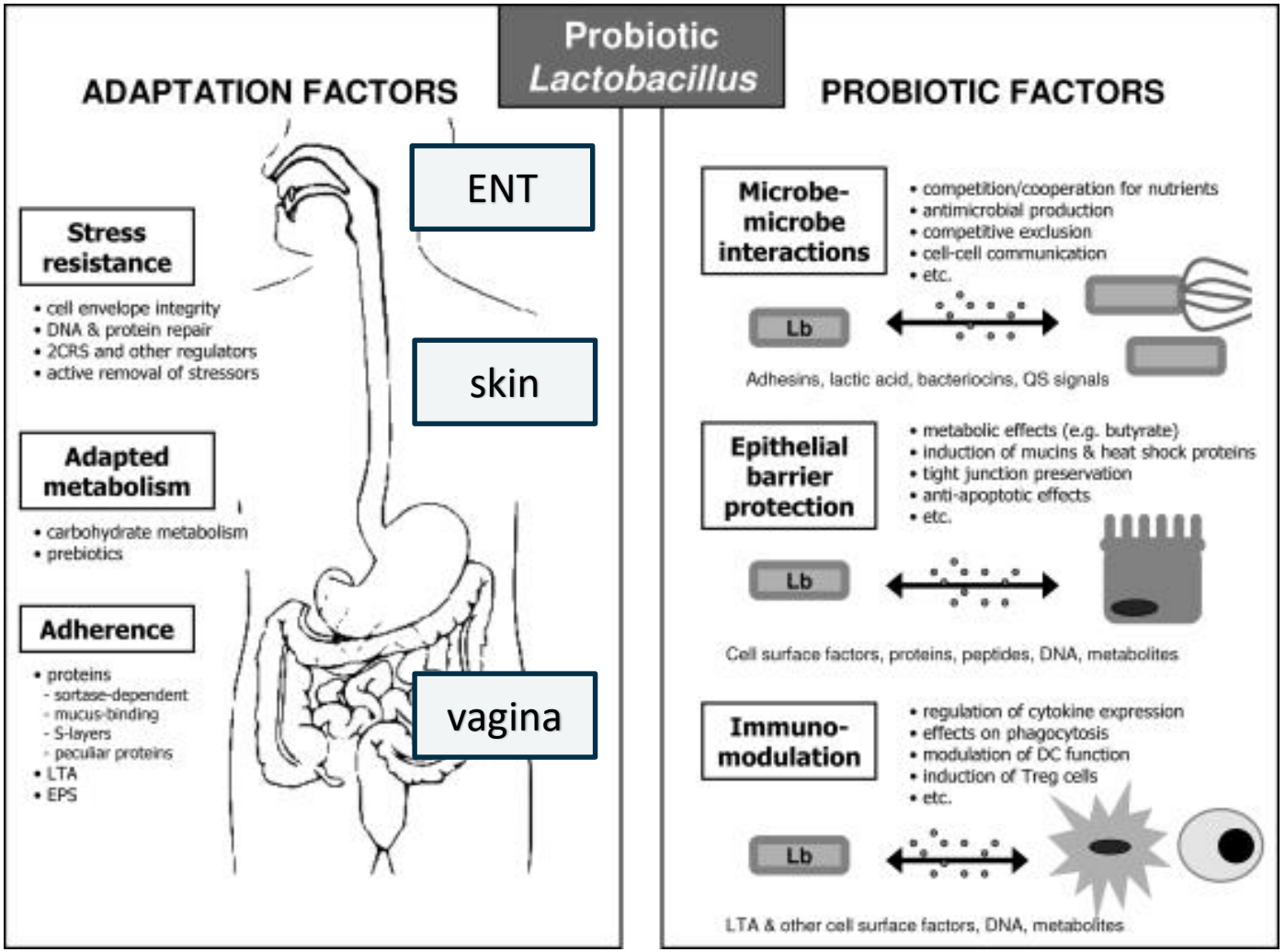
Towards a better understanding of *Lactobacillus rhamnosus* GG - host interactions

Marijke E Segers[1,2], Sarah Lebeer[1,2*]

Translation outside gut



Lebeer *et al.*, 2008, MMBR

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Prof. T. Van de Wiele
Prof. Ceuppens
Prof. Bullens
Prof. Hellings
Prof. Donders
Prof. Lambert
Prof. Vanderveken
Prof. Kiekens
….

ISAPP International Scientific Association for Probiotics and Prebiotics

ILSI

ENdEMIC Environmental Ecology and Applied Microbiology University of Antwerp

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