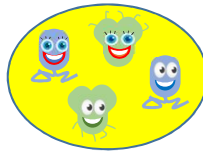




The Metabolic Products of Probiotic Bacteria with Potential for Improving Human Health



Colin Hill
APC Microbiome Ireland
@colinhillucc

“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




Life, health, disease (and everything else) is all about molecules

- Every biological phenomenon is mediated by molecules interacting with other molecules
- Probiotics can only affect a host (immune system, enteric nervous system, host physiology, microbiome, etc) by producing molecule(s) which interact with resident molecule(s)
- These molecules may have been produced prior to administration, or may be produced post administration

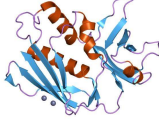
Lessons from clinical microbiology



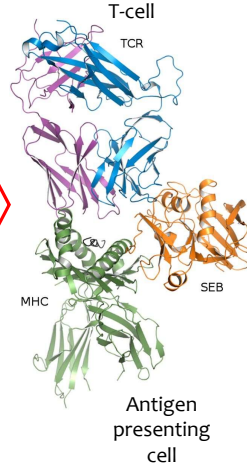
Staphylococcus aureus
>10⁵/g



Heat stable toxin

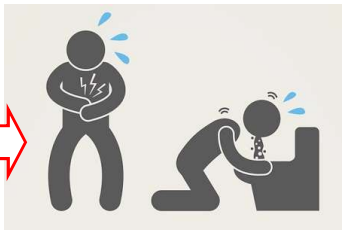


T-cell



MHC
Antigen presenting cell


Massive disruption to host physiology



No contact required between live bacterium (pathogen) and host
the health impact is mediated by a single molecule


Relatively low amounts of microbial molecules can have a significant impact on the host

Lessons from clinical microbiology




Pathogens

Infectious

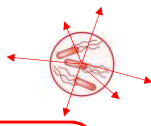


Living bacteria required for physiological effect, mediated by molecules

Probiotics




Intoxicative



Bacterial products required for physiological effect, mediated by molecules


Probiotic Products


- Paraprobiotics
- Parabiotics
- Pharmabiotics
- Postbiotics



Probiotics

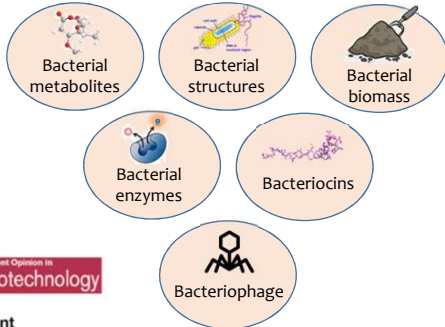
Probiotic products






Probiotics


Probiotic Products (molecules)






Available online at www.sciencedirect.com
ScienceDirect

Current Opinion in
Biotechnology



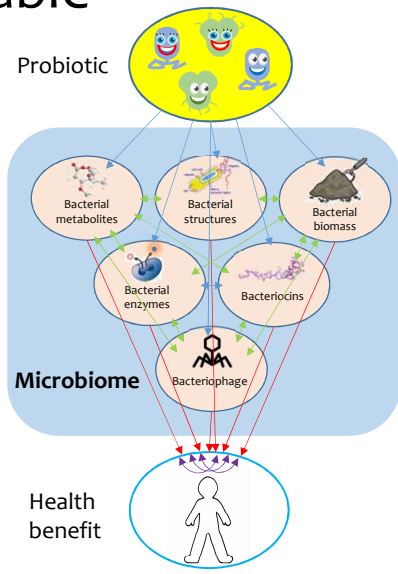
Identification of probiotic effector molecules: present state and future perspectives
 Sarah Lebeer¹, Peter A Bron², Maria L Marco³, Jan-Peter Van Pijkeren⁴, Mary O'Connell Motherway², Colin Hill⁵, Bruno Pot^{6,7}, Stefan Roos⁸ and Todd Klaenhammer³

Complexity is inevitable




Every probiotic produces multiple molecules which can interact with the microbiome and the host at multiple points, with cascading effects on host systems

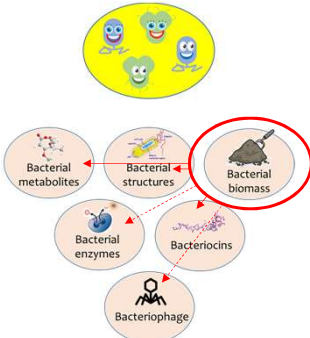
- Probiotic molecules acting on host receptors
- Probiotic molecules acting on microbiome receptors
- Crosstalk between probiotic molecules and between receptors




Bacterial biomass



- Per definition, inactivated cells cannot be probiotics (**live** microorganisms that, ...)
- But, just because they cannot be probiotics does not mean they can't have beneficial effects
- The terms 'paraprobiotics' and 'postbiotics' have been mooted for inactivated cells with a health benefit
- Inactivated cells could have significant advantages over probiotics in terms of production, formulation, shelf life, safety and dosing regimes





Review
Paraprobiotics: Evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods
Caroline N. de Almada, Carine N. Almada, Rafael C.R. Martinez, Anderson S. Sant'Ana*

>50 studies

Bacillus

- amyloliquefaciens*
- subtilis*
- pumilis*

Bifidobacterium

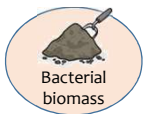
- longum*

Lactobacillus

- fermentum*
- bulgaricus*
- paracasei*
- salivarius*
- acidophilus*
- brevis*
- casei*
- gasseri*
- pentosus*
- plantarum*
- rhamnosus*
- sakei*

Heat

Sonication
γ-Irradiation
UV



Bacterial biomass

Enterococcus

- faecalis*

Leuconostoc

- mesenteroides*

Lactococcus

- lactis*


Saccharomyces

- boulardii*

Streptococcus

- thermophilus*

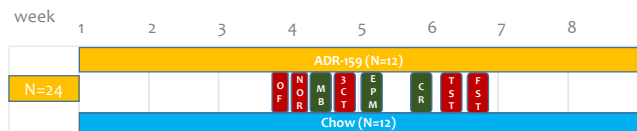
- Immunostimulatory
- Anti-inflammatory
- Cholesterol reduction
- Diarrhoea in IBS
- Rhinitis
- Reduction in cavities
- Constipation
- Anti-enteric pathogens
- NAFLD
- Colitis
- Allergy
- Respiratory infections
- Anti-proliferation
- Visceral pain
- NEC
- Anti-aging
- Barrier function



Inactivated cells (biomass)



male C57BL/6 mice



- Open field
- Novel object recognition
- Marble burying
- 3 chamber test
- Elevated plus maze
- Carmine red transit
- Tail suspension test
- Forced swim test

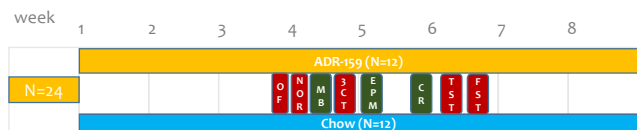
- Put mice on a standard diet supplemented with ADR-159 (5%)
- ADR-159 contains heat killed lactobacilli (*Lb. fermentum* and *Lb. delbreuckii*) and spent growth medium
- Animals were subjected to a battery of 8 behavioural tests
- Corticosterone (stress hormone) levels were tested at week 6
- Faecal samples were collected weekly for microbiome analysis

Warda et al, unpublished

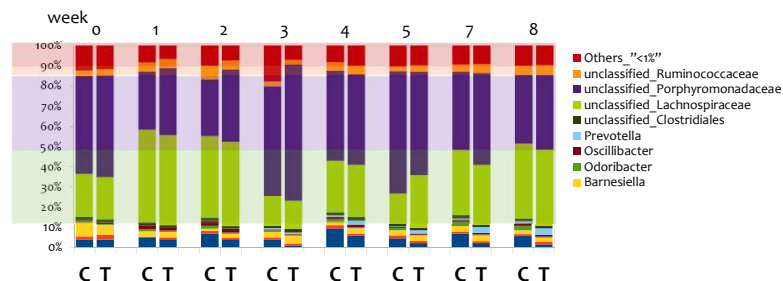
Inactivated cells & microbiome



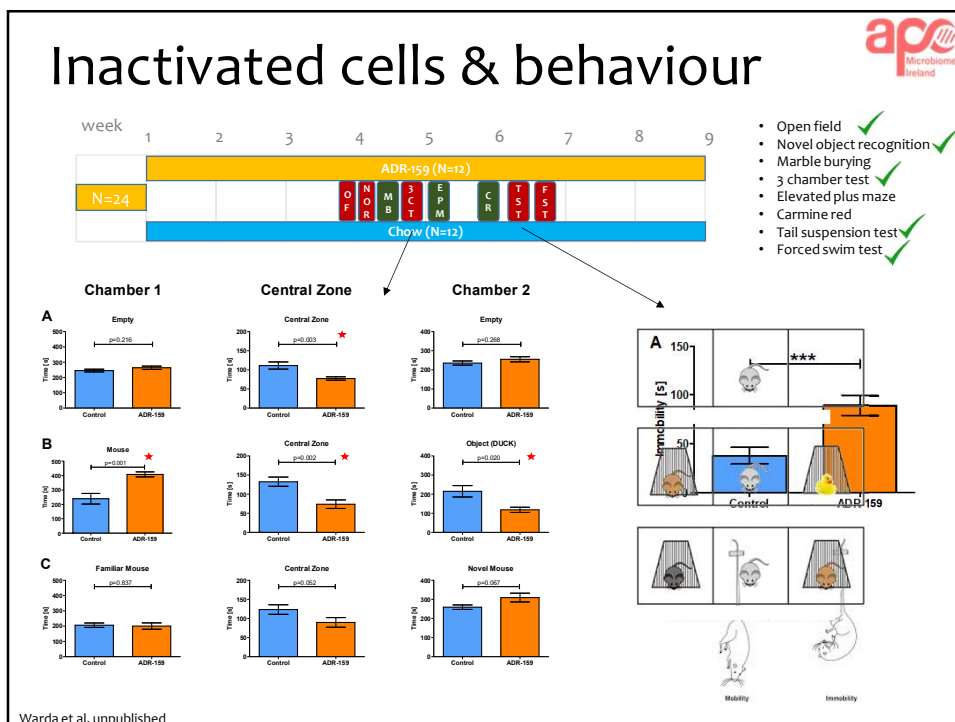
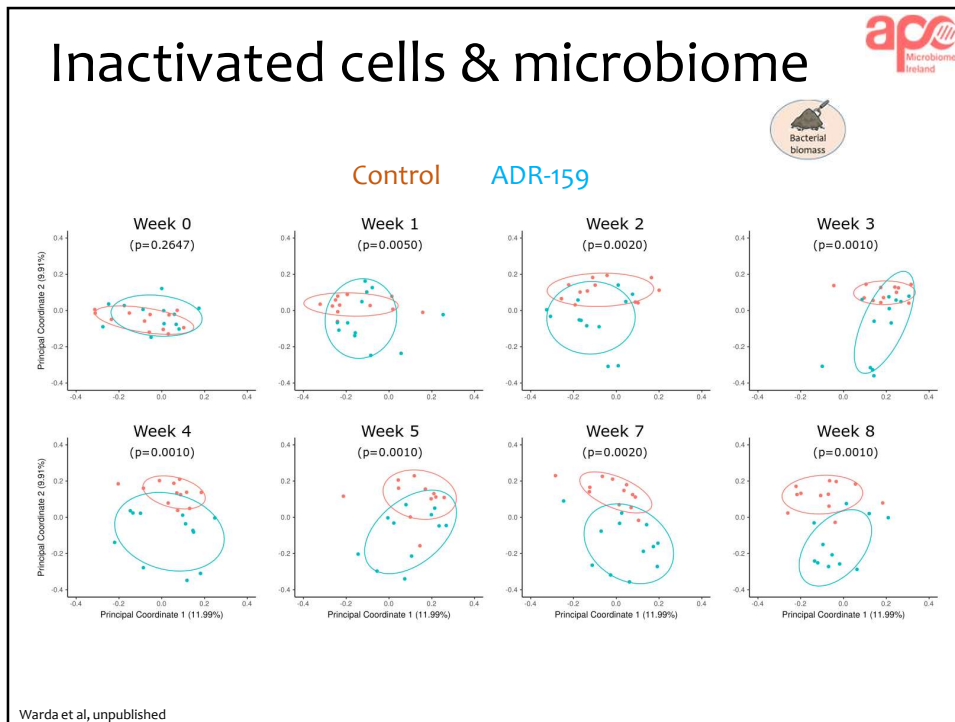
male C57BL/6 mice

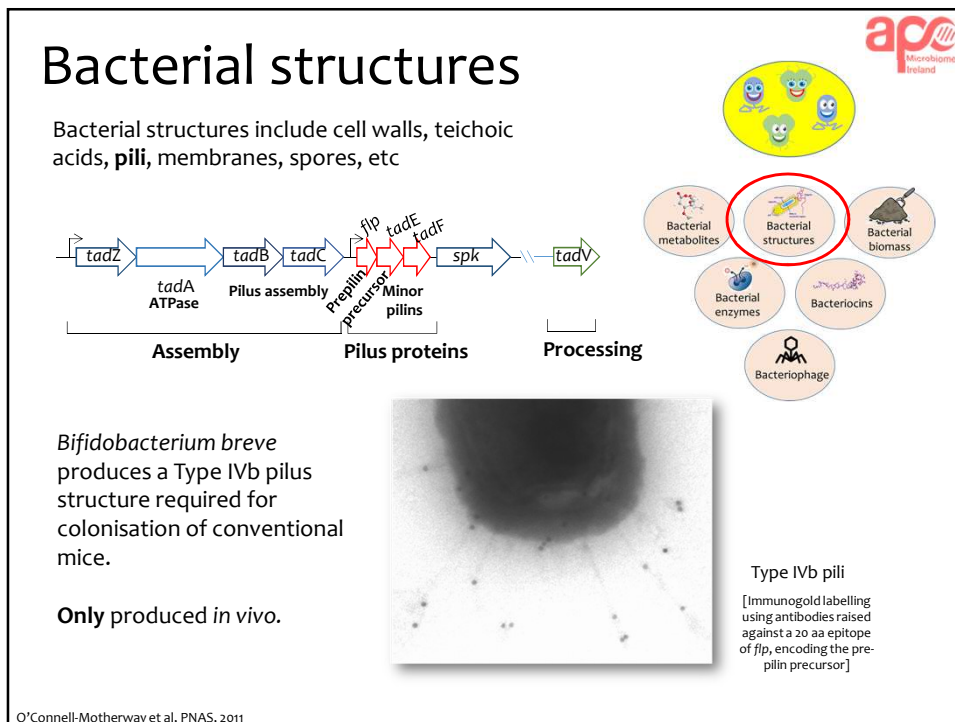
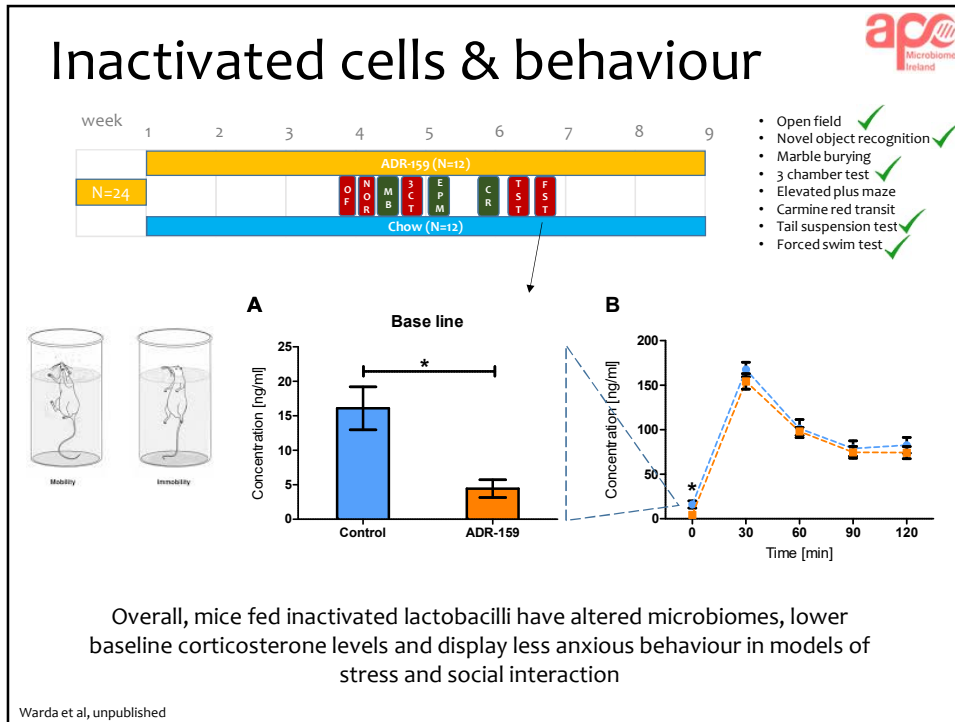


- Open field
- Novel object recognition
- Marble burying
- 3 chamber test
- Elevated plus maze
- Carmine red transit
- Tail suspension test
- Forced swim test



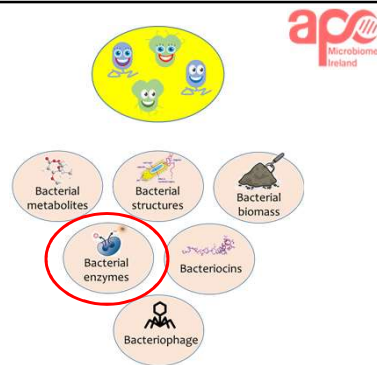
Warda et al, unpublished





Bacterial enzymes

- Probiotics contain enzymes which can influence host metabolism by consuming undesirable compounds (detoxification), or by producing desirable compounds (SCFA's).
- For example, lactase produced by yoghurt cultures.

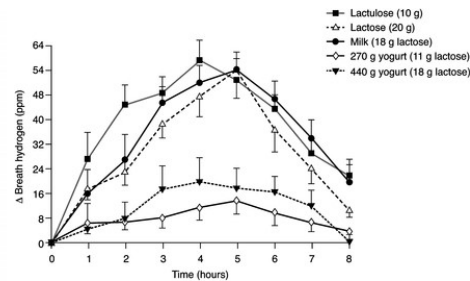


The New England Journal of Medicine

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 Volume 510 JANUARY 5, 1984 Number 1

YOGURT — AN AUTODIGESTING SOURCE OF LACTOSE

JOSEPH C. KOLANS, M.D., MICHAEL D. LEVITT, M.D., MOSTAFA AOUJ, D.A.G., AND DENNIS A. SAYALANO, PH.D.
Abstract Large quantities of yogurt are consumed by some lactase-deficient population groups. We used breath hydrogen measurements to determine whether lactase-deficient subjects absorbed lactose in yogurt better than lactose in milk. Ingestion of 18 g of lactose in yogurt resulted in only about one third as much hydrogen excretion as a similar load of lactose in milk or water, indicating a much better absorption of lactose in yogurt. Ingestion of yogurt also resulted in fewer reports of diarrhea or flatulence than did a similar quantity of lactose ingested in milk or a water solution. The enhanced absorption of lactose in yogurt appeared to result from the intraintestinal digestion of lactose by lactase released from the yogurt organisms. This autodigesting feature makes yogurt a well-tolerated source of milk for lactase-deficient persons and may explain the widespread consumption of yogurt by lactase-deficient population groups. (N Engl J Med 1984; 310:1-3)



Bacterial enzymes

- Bacterial enzymes are responsible for the only probiotic claim approved to date by EFSA
- Effects can be recapitulated by enzyme alone



European Food Safety Authority

EFSA Journal 2010;8(10):1763

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to live yoghurt cultures and improved lactose digestion (ID 1143, 2976) pursuant to Article 13(1) of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)^{2,3}

The claimed effect is "lactose digestion". The target population is individuals with lactose maldigestion. The Panel considers that improved lactose digestion is a beneficial physiological effect for individuals with lactose maldigestion.



European Food Safety Authority

EFSA Journal 2009; 7(9):1236

SCIENTIFIC OPINION

Scientific Opinion on the substantiation of health claims related to lactase enzyme and breaking down lactose (ID 1697, 1818) pursuant to Article 13(1) of Regulation (EC) No 1924/2006¹

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)²

The Panel concludes that a cause and effect relationship has been established between the consumption of lactase enzyme and breaking down lactose in individuals with symptomatic lactose maldigestion. The following wording reflects the scientific evidence: "Lactase enzyme contributes to breaking down lactose".



Bacterial enzymes



Individuals with lactase intolerance (GG genotype) and yet have a high milk intake 'select' for high levels of *Bifidobacterium* in their microbiome (lactase positive)

The effect of host genetics on the gut microbiome

Mart-Jan Bouder^{1,19}, Alexander Kurishnikov^{1,19}, Ertjo F Tigchelaar^{1,4}, Zlatan Mujagic^{4,5}, Floris Imhann⁶, Arnau Vich Vila⁶, Patrick Deelen^{1,2}, Tommi Vatanen^{6,9}, Melanie Schirmer^{6,9}, Sanne P Smeekens^{1,12}, Daria V Zernakova¹, Soesma A Jankipersadting^{1,13}, Martin Jaeger^{1,12}, Marije Oosting^{1,12}, Maria Carmen Cceni^{1,18}, Ad A M Masclee¹, Morris A Swertz^{1,7}, Yang Li¹, Vinod Kumar¹, Leo Joosten^{1,12}, Hermie Harmsen¹⁴, Rinse K Weersma⁶, Lude Franke¹, Marten H Hofker¹³, Ramnik J Xavier^{6,15-17}, Daisy Jonkers⁸, Mihai G Netea^{1,12}, Cisca Wijmenga¹, Jingyuan Fu^{1,15,20} & Alexandra Zernakova^{1,4,20}

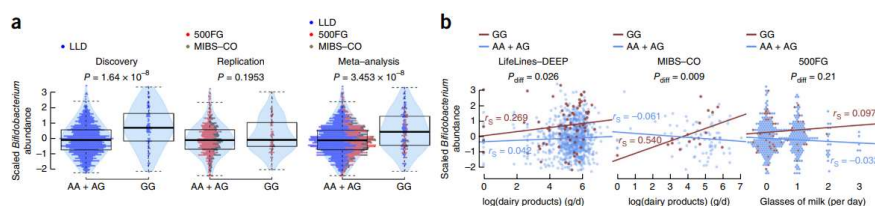


Figure 3 Complex interaction between a functional *LCT* variant, dairy intake and *Bifidobacterium* abundance. (a) Microbial QTL plots showing association of a functional *LCT* SNP (rs4988235) with the abundance of *Bifidobacterium*. The three plots show the effect of genotype at the functional SNP on *Bifidobacterium* abundance. The plots are a combination of violin plots and box plots; box plots show the median and the 25% and 75% quantiles. LLD, LifeLines-DEEP. (b) Interaction of *LCT* genotype, intake of dairy products and *Bifidobacterium* abundance in the three cohorts. Data on total dairy consumption were unavailable for the 500FG cohort, but the same trend was observed with respect to data on the number of glasses of milk drunk per day in this cohort. Pdiff shows the significance of the difference in the correlation of dairy product consumption and *Bifidobacterium* abundance between the two genotype groups, GG versus AA and AG (Online Methods).

Bacterial enzymes/metabolites



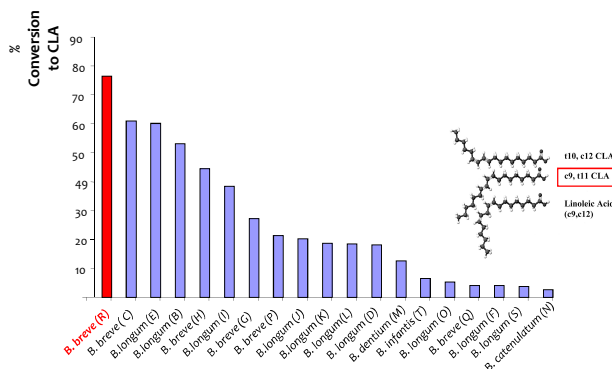
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 2007, p. 2333-2337
0099-2240/07/\$08.00 + 0 doi:10.1128/AEM.01855-06
Copyright © 2007, American Society for Microbiology. All Rights Reserved.

Vol. 73, No. 7

Rapid Screening Method for Analyzing the Conjugated Linoleic Acid Production Capabilities of Bacterial Cultures¹⁷

E. Barrett,^{1,2} R. P. Ross,^{1,2} G. F. Fitzgerald,^{2,3} and C. Stanton^{1,2*}

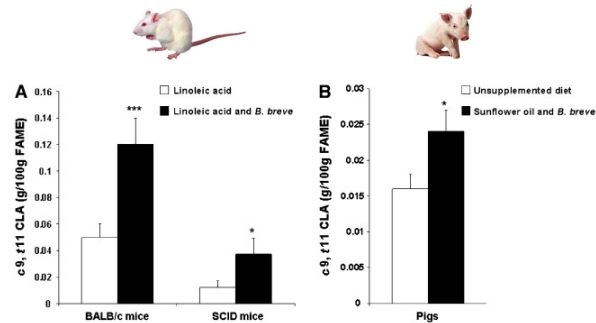
- Screened bifidobacteria for the ability to convert linoleic acid into c9, t11 conjugated linoleic acid
- Best performer was a strain of *Bifidobacterium breve*



Bacterial enzymes/metabolites



- Incorporated *Bifidobacterium breve* and linoleic acid in the diet
- Lead to a significant increase in c9,t11 CLA in the livers (and adipose tissue) of mice and pigs



Metabolic activity of the enteric microbiota influences the fatty acid composition of murine and porcine liver and adipose tissues¹⁻³

Rebecca Wall, R Paul Ross, Fergus Shanahan, Liam O'Mahony, Caitlin O'Mahony, Mairead Coakley, Orla Hart, Peadar Lawlor, Eamonn M Quigley, Barry Kiely, Gerald F Fitzgerald and Catherine Stanton

Am J Clin Nutr 2009;89:1393-401.

Bacterial enzymes/metabolites



- Effect can be recapitulated by a single gene product
- Cloned linoleic isomerase (converts LA to t10, c12 CLA) from *Propionibacterium acnes* into *Lactobacillus paracasei*.
- Increased t10, c12 CLA content of adipose tissue in mice following dietary intervention.

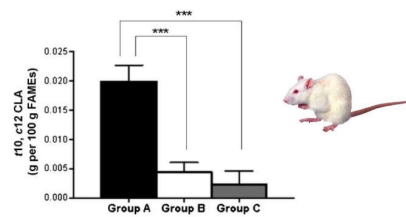


Fig. 3. t10, c12 CLA concentration of adipose tissue following 8 weeks supplementation. Group A: t10, c12 CLA-producing recombinant Lb338 in combination with linoleic acid (2%, w/w) (n=8). Group B: isogenic control strain in combination with linoleic acid (2%, w/w) (n=8). Group C: linoleic acid alone (2%, w/w) (n=5). Statistical significance was determined by ANOVA followed by Tukey's post hoc test; ***P<0.001.

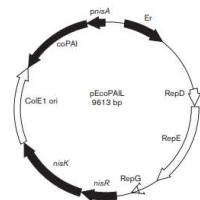


Fig. 1. Plasmid construct pEcoPAL used to transform *Lb. paracasei* NFB3 338 (Lb338). *yoPAI* denotes the codon-optimized *P. acnes* isomerase gene (t10, c12 CLA isomerase).


Microbiology (2011), 157, 609-615

DOI: 10.1099/mic.0043406-0


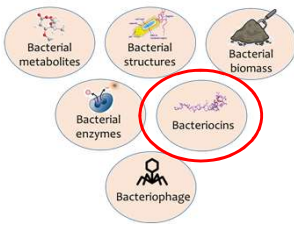
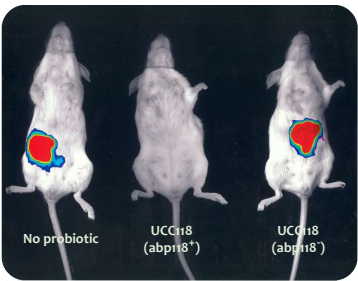
Recombinant lactobacilli expressing linoleic acid isomerase can modulate the fatty acid composition of host adipose tissue in mice

Eva Rosberg-Cody,^{1,2,3} Catherine Stanton,^{1,3} Liam O'Mahony,¹ Rebecca Wall,¹ Fergus Shanahan,¹ Eamonn M. Quigley,¹ Gerald F. Fitzgerald^{1,2} and R. Paul Ross^{1,3}

Bacteriocins




- *Lb. salivarius* UCC118 produces a bacteriocin - abp118 – which kills *Listeria*
- Made a knockout of UCC118 which no longer produces abp118
- The knockout no longer protects mice from *Listeria* infection

Corr et al., PNAS 2007

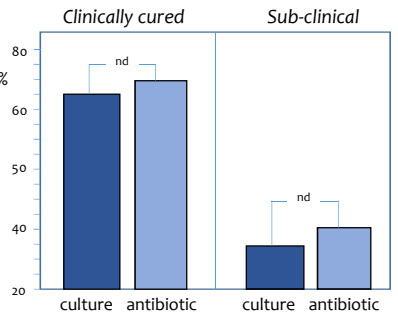

Probiotics and bovine mastitis



Introduced a viable bacteriocin-producing strain into the teat canal of mastitic animals – compared to gold standard antibiotic


Clinical cure rates (N=25)

<i>L. Lactis</i>	64% (16/25)
Antibiotic	72% (18/25)





Klostermann et al., 2008 J Dairy Res 75:365-373 Crispie et al., 2008 J Dairy Res 75:374-384


Bacteriocins and mastitis




- Can be recapitulated using bacteriocin alone




N=37



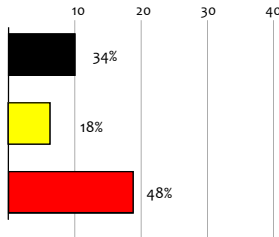
Teat Seal




Streptococcus dysgalactiae




healthy
sub-clinical
mastitis




healthy	34%
sub-clinical	18%
mastitis	48%




N=38



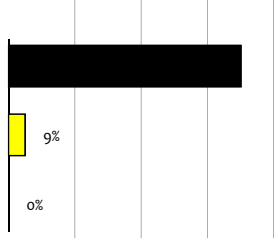
Teat Seal
(lacticin 3147)



Streptococcus dysgalactiae




healthy
sub-clinical
mastitis



healthy	91%
sub-clinical	9%
mastitis	0%

Ryan et al. (1998) Appl Environ Microbiol 64:2287-2290.

Phage & probiotic mechanisms



Beneficial Microbes, 2016, 7(2): 289-297

Wageningen Academic Publishers

Prophage-mediated modulation of interaction of *Streptococcus thermophilus* J34 with human intestinal epithelial cells and its competition against human pathogens

C. Guigas¹, K. Faulhaber², D. Duerbeck³, H. Neve¹ and K.J. Heller¹

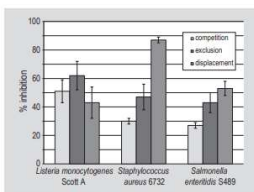


Figure 3. Inhibition of *in vitro* pathogen adhesion by *Streptococcus thermophilus* J34. For competition assay, *S. thermophilus* cells were added to enterocytes simultaneously with the three pathogens. For exclusion assay, *S. thermophilus* cells were added 30 min before the addition of pathogens. For displacement assay, *S. thermophilus* cells LAB were added 30 min after addition of pathogens. Values presented are mean \pm standard deviation from three independent experiments.

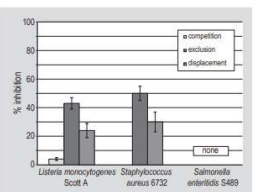
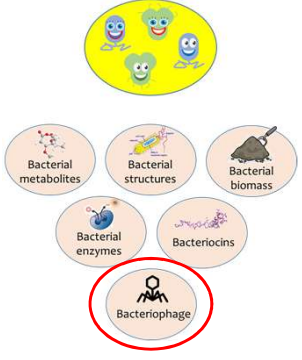


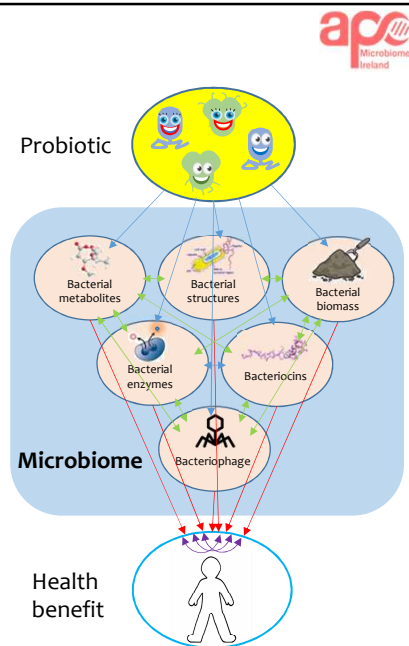
Figure 4. Inhibition of *in vitro* pathogen adhesion by *Streptococcus thermophilus* J34-6. For competition assay, *S. thermophilus* cells were added to enterocytes simultaneously with the three pathogens, for exclusion assay, *S. thermophilus* cells were added 30 min before the addition of pathogens, for displacement assay, *S. thermophilus* cells were added 30 min after addition of pathogens. Values presented are mean \pm standard deviation from three independent experiments (n=9).



- When incubated together, *S. thermophilus* J34 but not J34-6 (prophage-cured) was able to compete with three gastrointestinal pathogens for adhesion sites on the surface of HT29 cells.

Conclusions

- Probiotic mechanisms all involve molecules interacting with molecules
- Probiotic mechanisms are hard to decipher because there are so many molecular interactions (probiotic, microbiota and host)
- There are instances where single molecules seem to be largely responsible for probiotic effects
- It is reasonable to expect dead (or inactive) cells to have a beneficial effect – what is the best term for this phenomenon (ISAPP consensus?)
 - Paraprobiotic? Postbiotic? Parabiotic? Pharmabiotic?

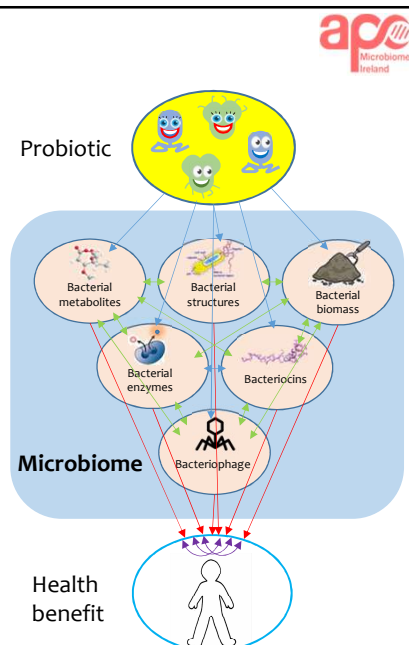


Acknowledgements

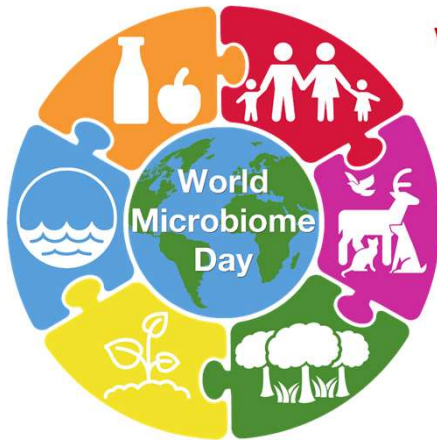
Alicja Warda
 Cara Heuston
 Ted Dinan
 Paul Ross

Catherine Stanton
 Douwe van Sinderen
 Mary O’Connell-Motherway

School of Microbiology, UCC
 APC Microbiome Ireland
 Science Foundation Ireland



World Microbiome Day



Wednesday June 27th 2018

www.worldmicrobiomeday.com

- to showcase the diverse worlds of microbiomes
- to encourage public dialogue on their critical importance to human, animal & environmental health

Please get involved: organise an event near you and register it at <http://worldmicrobiomeday.com/planned-events/>