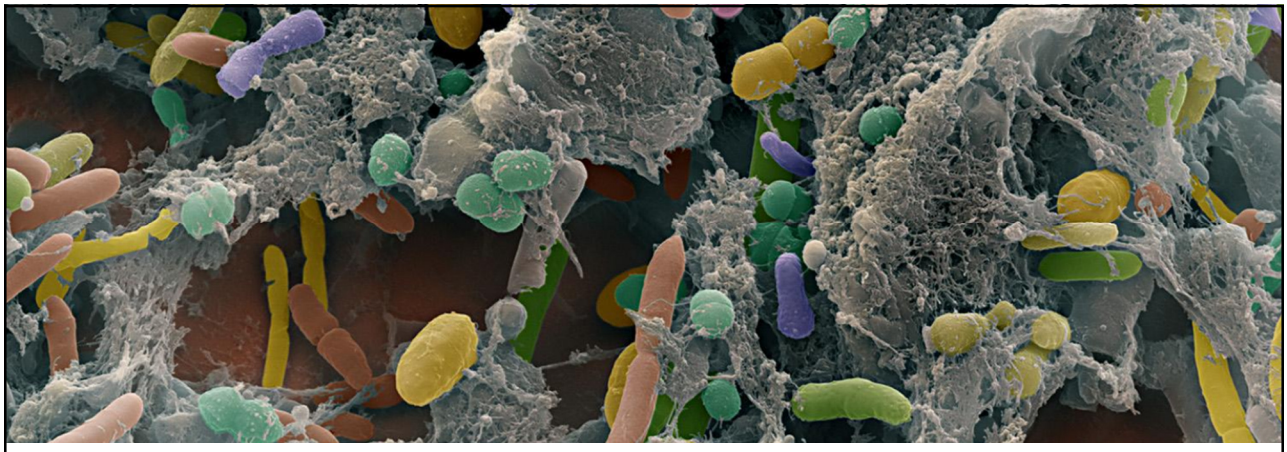
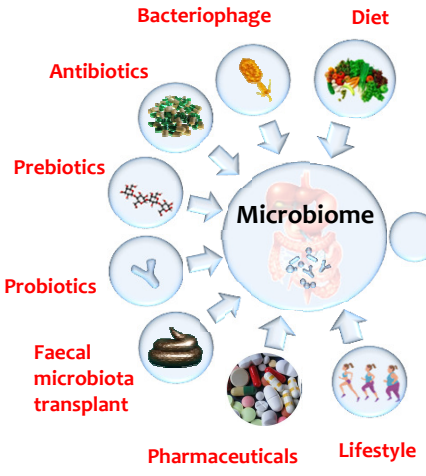


# Using the microbiome to treat resistance?

Colin Hill
APC Microbiome Ireland, University College Cork
Ireland
@colinhillucc

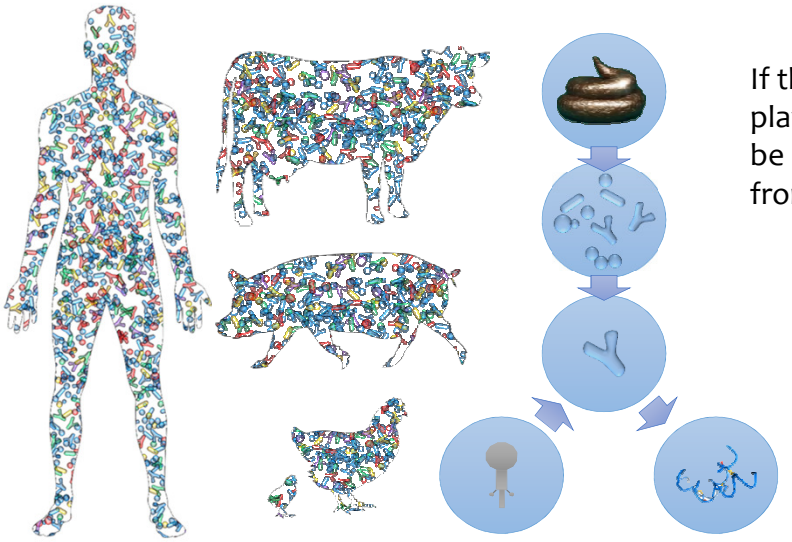
## Why the microbiome?




- A rich source of alternative **targeted** antimicrobials
- More opportunities for intervention to improve or protect health

- Treating infection (pharma/vet)
- Preventing infection (food and feed)
- Performance (animals)

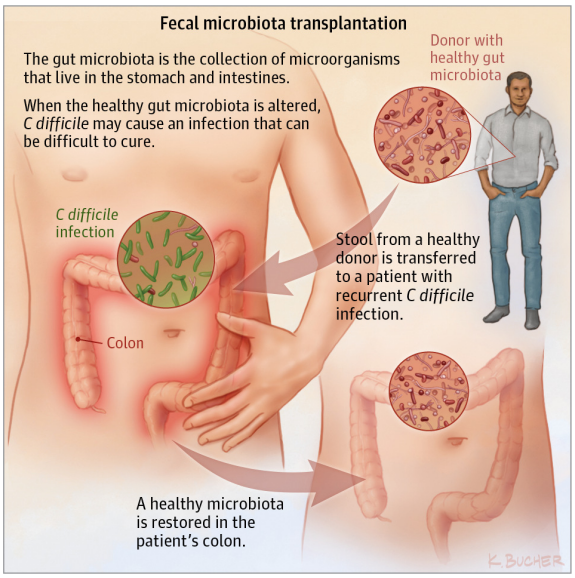
# ‘Mine’ the Microbiome for anti-infectives

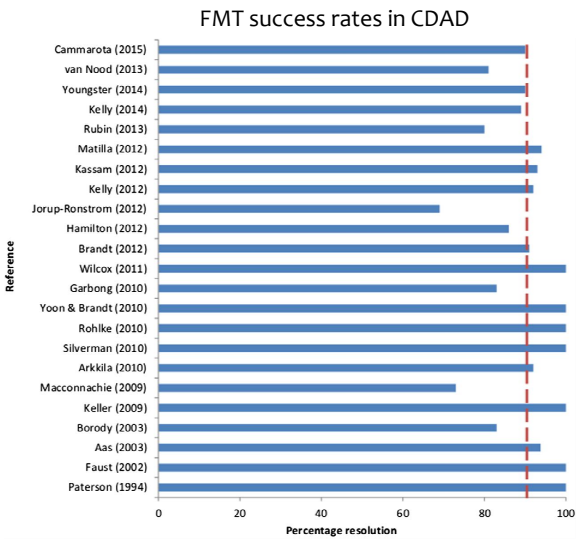


If the microbiome is a significant player in infection, then it should be possible to mine interventions from this niche

- **faecal microbiota transplants**
- probiotics
- bacteriophage
- pharmabiotics
  - (bioactives, bacteriocins, etc.)

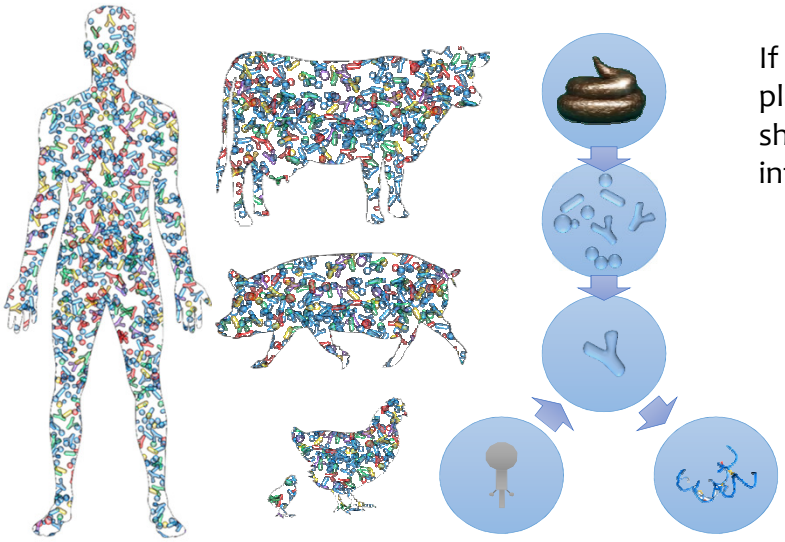
# FMT Faecal microbiota transplants




Dowle, 2016. Bioscience Horizons: The International Journal of Student Research

# 'Mine' the Microbiome for anti-infectives



If the microbiome is a significant player in health and disease, then it should be possible to mine interventions from this niche

- faecal microbiota transplants
- **probiotics**
- bacteriophage
- pharmabiotics
  - (bioactives, bacteriocins, etc.)

# Synbiotic (probiotic and prebiotic)

ARTICLE

24 AUGUST 2017 | VOL 548 | NATURE | 407 doi:10.1038/nature23480

## A randomized synbiotic trial to prevent sepsis among infants in rural India

Pinaki Panigrahi[1,2], Sailajanandan Parida[3], Nimai C. Nanda[4], Radhanath Satpathy[5], Lingaraj Pradhan[6], Dinesh S. Chandel[7], Lorena Baccaglini[1], Arjit Mohapatra[5], Subhranshu S. Mohapatra[5], Pravas R. Misra[5], Rama Chaudhry[8], Hegang H. Chen[9], Judith A. Johnson[10], J. Glenn Morris Jr[10], Nigel Paneth[11] & Ira H. Gewolb[12]

4,556 infants

**We observed a significant (40%) reduction in the primary combined outcome of death and neonatal sepsis,** from 9% in the placebo arm to 5.4% in the treatment arm

Significant reductions were also observed for culture-positive and culture-negative sepsis and lower respiratory tract infections.

*Lactobacillus plantarum* and FOS

The synbiotic preparation consisted of a capsule containing ~$10^9$ *Lactobacillus plantarum* ATCC strain 202195 and 150 mg of fructooligosaccharide with 100 mg maltodextrin as excipient

**Table 2 | Effect of synbiotic treatment on sepsis and other morbidities in the first 60 days of life**

| Outcome variables | Control n = 2,278 (%) | Synbiotic n = 2,278 (%) | RR (95% CI) |
|---|---|---|---|
| Death and sepsis (primary outcome) | 206 (9.0) | 123 (5.4) | 0.60 (0.48, 0.74) |
| Deaths | 4 (0.2) | 6 (0.3) | 1.50 (0.42, 5.31) |
| Sepsis (A + B + C) | 202 (8.9) | 117 (5.1) | 0.58 (0.46, 0.72) |
| A. Sepsis/pSBI—culture-positive septicaemia | 27 (1.2) | 6 (0.3) | 0.22 (0.09, 0.53) |
| Gram-negative sepsis | 16 (0.7) | 4 (0.2) | 0.25 (0.08, 0.75) |
| Gram-positive sepsis | 11 (0.5) | 2 (0.1) | 0.18 (0.04, 0.82) |
| B. Sepsis/pSBI—culture-negative sepsis (Culture-negative clinical sepsis warranting hospitalization and IV antibiotics) | 36 (1.6) | 19 (0.8) | 0.53 (0.30, 0.92) |
| C. Sepsis/pSBI—LRTI (LRTIs requiring antibiotic therapy) | 139 (6.1) | 92 (4.0) | 0.66 (0.51, 0.88) |
| Diarrhoea | 59 (2.6) | 12 (0.5) | 0.20 (0.11, 0.38) |
| Local infections (including >10 pustules, oral thrush, conjunctivitis) | 33 (1.5) | 16 (0.7) | 0.48 (0.27, 0.88) |
| Abscess/ otitis media | 11 (0.5) | 5 (0.2) | 0.45 (0.16, 1.33) |
| Omphalitis | 13 (0.6) | 3 (0.1) | 0.23 (0.07, 0.81) |

# Probiotics and acute diarrhoea

**Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials**

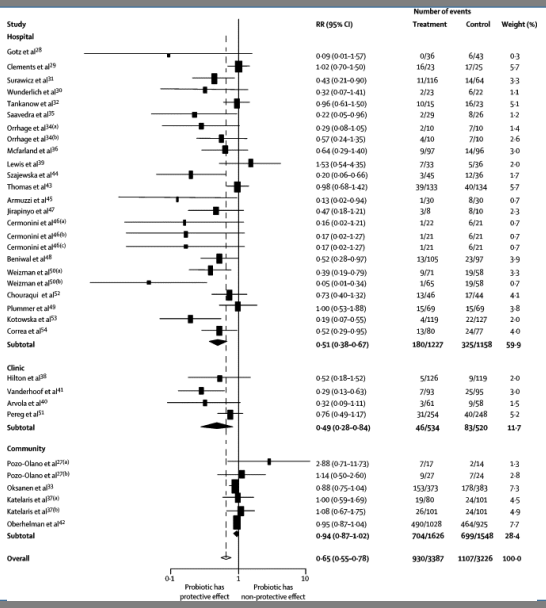
Sunil Sazawal, Girish Hiremath, Usha Dhingra, Pooja Malik, Saikat Deb, Robert E Black

*Lancet Infect Dis* 2006; 6: 374–82

Evaluating the evidence by types of acute diarrhoea suggests that probiotics significantly **reduced antibiotic-associated diarrhoea by 52%** (95% CI 35–65%), **reduced the risk of travellers' diarrhoea by 8%** (–6 to 21%), and that of **acute diarrhoea of diverse causes by 34%** (8–53%).

Probiotics reduced the associated risk of acute diarrhoea among children by 57% (35–71%), and by 26% (7–49%) among adults.

**The effect on acute diarrhoea is dependent on the age of the host and genera of strain used.**




# Probiotics and infections in older people

*Age and Ageing* 2018; **47:** 527–536
doi: 10.1093/ageing/afy006
Published electronically 3 February 2018



**Effectiveness of probiotics on the occurrence of infections in older people: systematic review and meta-analysis**

Patrick Alexander Wachholz[1], Vânia dos Santos Nunes[2], Adriana Polachini do Valle[2], Alessandro Ferrari Jacinto[2], Paulo José Fortes Villas-Boas[2]

[1]Departament of Public Health, São Paulo State University (UNESP), School of Medicine, Botucatu—São Paulo, Brazil
[2]Departament of Internal Medicine, São Paulo State University (UNESP), School of Medicine, Botucatu—São Paulo, Brazil

Address correspondence to: P. A. Wachholz, Av. Prof. Mário Rubens Guimarães Montenegro, s/n. UNESP— Campus de Botucatu, 18618687 Botucatu, SP, Brazil. Tel: (+55 014) 3880-1001; Fax (+55 014) 3880-1020. Email: pwachholz@alunos.fmb.unesp.br

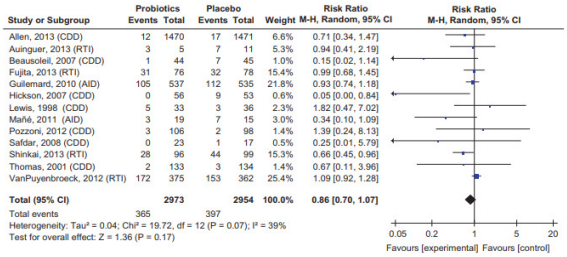
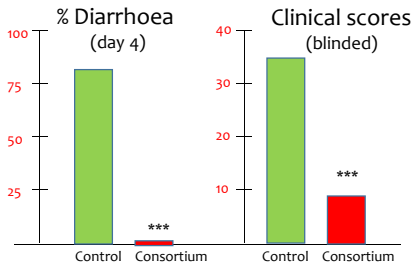


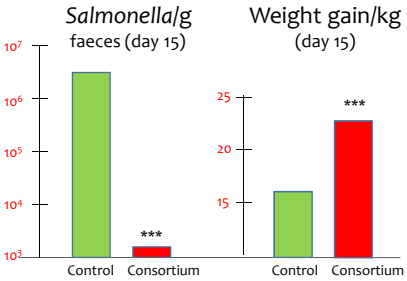

**Figure 2.** Comparison between intervention (probiotics) versus placebo for the primary outcome of *occurrence of infection* (alphabetically ordered studies). CDD; *Clostridium difficile* diarrhoea; RTI; respiratory tract infection; AID; all infectious diseases.

- The current evidence, which is of low quality, does not support the use of probiotics for the reduction of the occurrence of infection in older people.
- The current evidence, which is of very low quality, suggests that the mean duration of an infection episode is not affected by probiotics.
- The evidence, which is of low quality, suggests that probiotics have a safety profile similar to placebo.

# Probiotics and porcine salmonellosis

- Isolated 10,000 strains from uninfected pigs in a *Salmonella* infected herd
- Chose 5 strains with *in vitro* anti-*Salmonella* activity (4 x *Lactobacillus*, 1 *Pediococcus*)
- Conducted a blinded trial (N=10) where pigs were fed the microbial consortia before being deliberately infected with *Salmonella* on 3 consecutive days



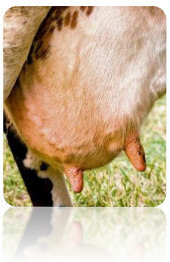

Casey et al. 2007 Appl. Environ. Microbiol. 73:1858-1863 Walsh et al. 2008 FEMS Microbiol. Ecol. 64:317-327

# Probiotics and bovine mastitis

Bovine mastitis is the most persistent disease in dairy cattle.

Treated with broad spectrum antibiotics, milk must be withheld.

Billions of euros every year.



Infused infected quarters with *Lactococcus lactis* DPC3147 (produces lacticin 3147).

A single application led to complete recovery within 3 days (11/11 animals).

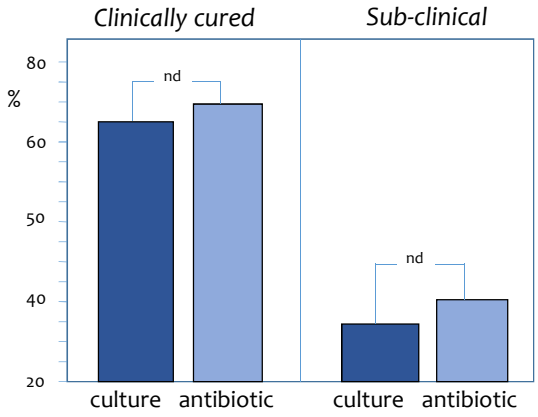
No withholding of milk.

# Probiotics and bovine mastitis

Compared live culture against leading antibiotic treatment (N=25)

## Clinical cure rates

| | |
|---|---|
| *L. Lactis* | 64% (16/25) |
| Antibiotic | 72% (18/25) |




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

# Probiotic mechanism of action

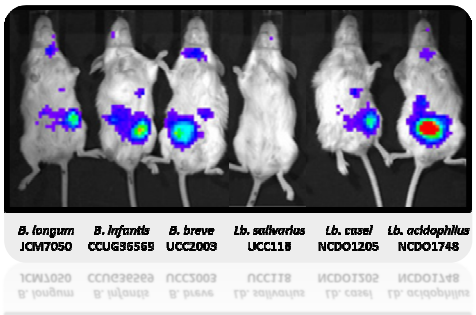
PNAS

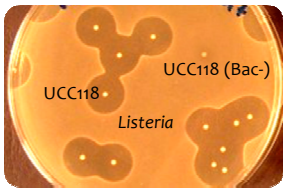
**Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118**

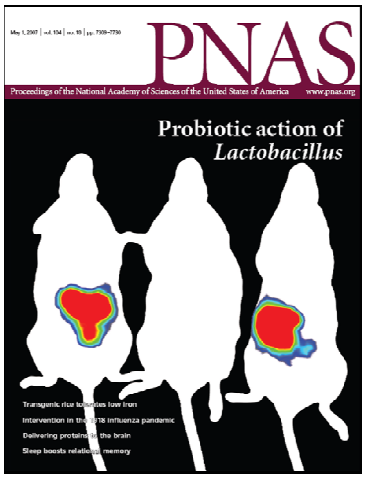
Sinéad C. Corr*, Yin Li*†, Christian U. Riedel*, Paul W. O'Toole*, Colin Hill*‡, and Cormac G. M. Gahan*§

*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)




Rare instance in which a single molecule has been identified as the probiotic mechanism

# Next Generation Probiotics

nature microbiology

PERSPECTIVE

PUBLISHED: 25 APRIL 2017 | VOLUME: 2 | ARTICLE NUMBER: 17057

## Next-generation probiotics: the spectrum from probiotics to live biotherapeutics

Paul W. O'Toole[1]*, Julian R. Marchesi[2,3] and Colin Hill[1]

**The leading probiotics currently available to consumers are generally drawn from a narrow range of organisms. Knowledge of the gut microbiota and its constituent actors is changing this paradigm, particularly given the phylogenetic range and relatively unknown characteristics of the organisms under investigation as novel therapeutics. For this reason, and because their development is likely to be more amenable to a pharmaceutical than a food delivery route, these organisms are often operationally referred to as next-generation probiotics, a concept that overlaps with the emerging concept of live biotherapeutic products. The latter is a class of organisms developed exclusively for pharmaceutical application. In this Perspective, we discuss what lessons have been learned from working with traditional probiotics, explore the kinds of organisms that are likely to be used as novel microbial therapeutics, discuss the regulatory framework required, and propose how scientists may meet this challenge.**

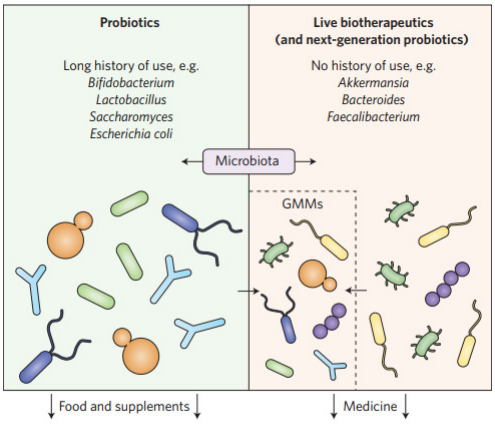
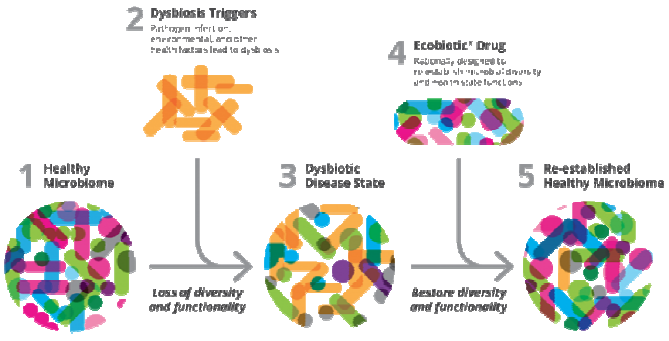



**Figure 2 |** Schematic diagram summarizing some differences in the history and route to market of probiotics, next-generation probiotics and live biotherapeutic products.

# Microbial consortia

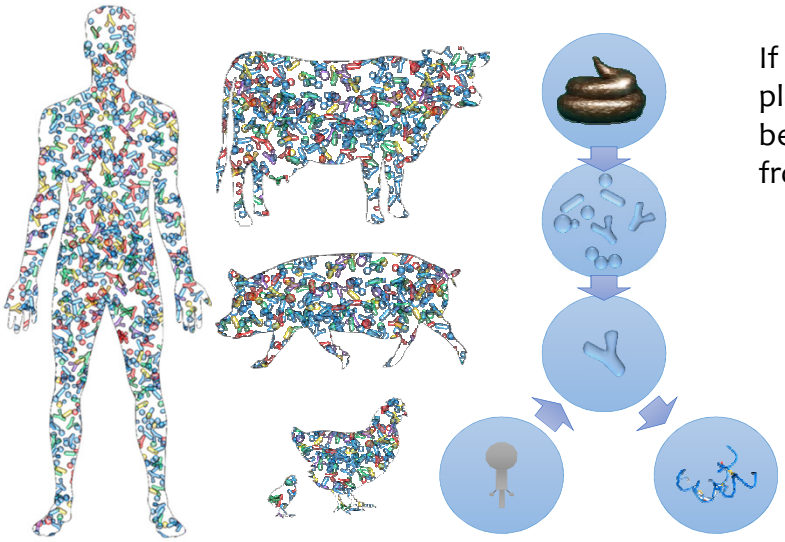
- Phase 3 development candidate, SER-109, oral microbiome therapeutic for the prevention of recurrent *Clostridium difficile* infection.
- The FDA has granted SER-109 both Breakthrough Therapy and Orphan Drug designations.
- SER-109 is an ecology of bacterial spores enriched and purified from healthy, screened human donors.

SERES THERAPEUTICS™




www.serestherapeutics.com/pipeline/ser-109

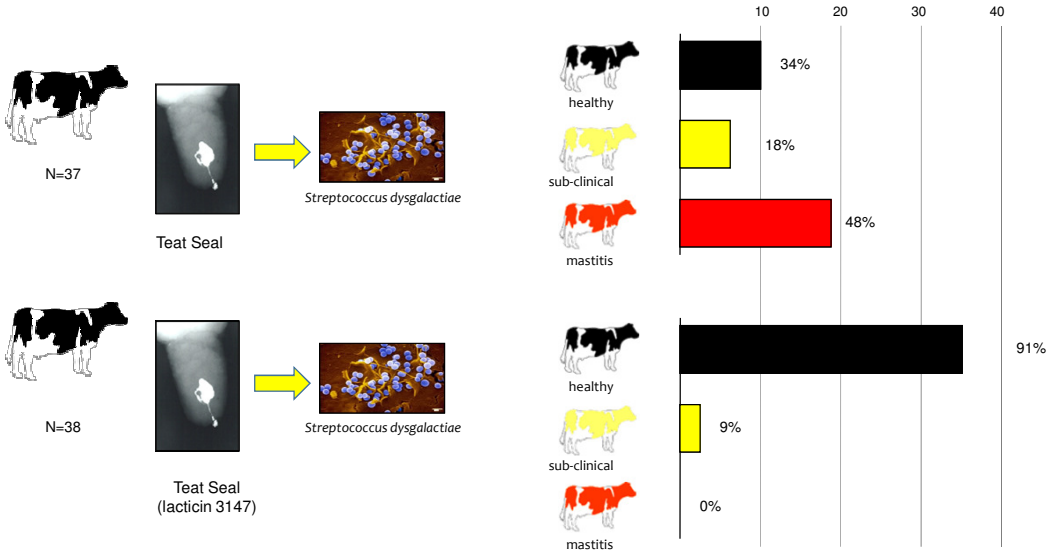
# ‘Mine’ the Microbiome for anti-infectives



If the microbiome is a significant player in infection, then it should be possible to mine interventions from this niche

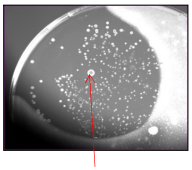
- faecal microbiota transplants
- probiotics
- bacteriophage
- **pharmabiotics**
  - (bioactives, bacteriocins, etc.)

# Bacteriocins and mastitis

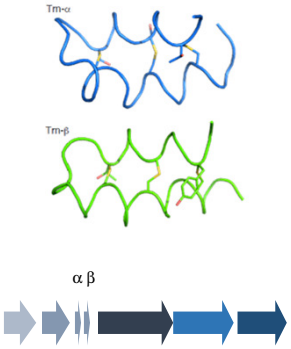


# Thuricin CD – narrow spectrum

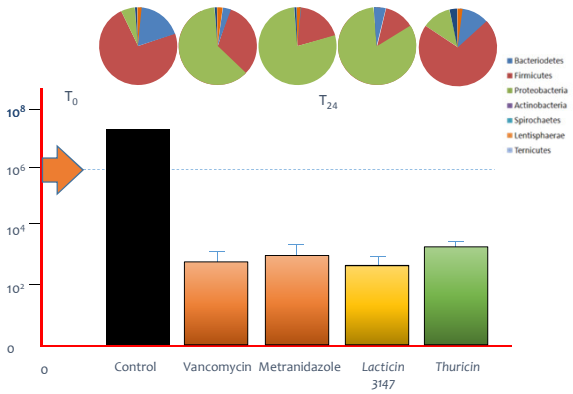
Screened >30,000 sporeformers from faecal specimens



*Bacillus thuringiensis*




Thuricin CD; a two component sactibiotic



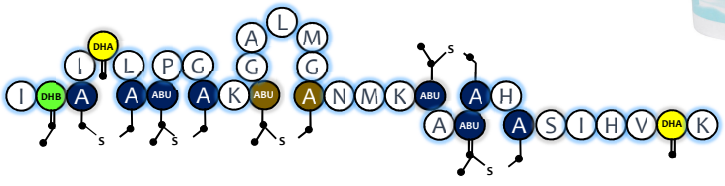

Faecal fermentation

Rea *et al.*, 2010. Proc. Natl. Acad. Sci. 107:9352-9358. Rea *et al.*, 2011. Proc. Natl. Acad. Sci. 108:4639-4644.

# Nisin (E234)

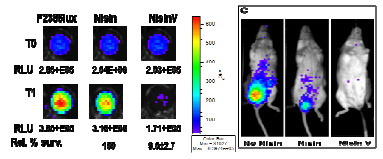
- Nisin is a 34 aa lantibiotic used in food as a preservative
- And in veterinary medicine for mastitis
- Gene encoded, can be bioengineered for additional properties



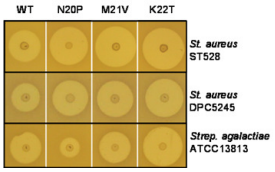


# Nisin bioengineering

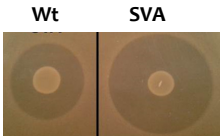
M21V Enhanced anti-*Listeria*



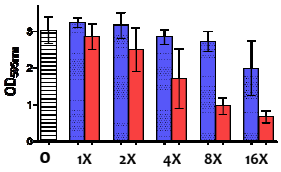
K22T Enhanced anti-*Staphylococcus*



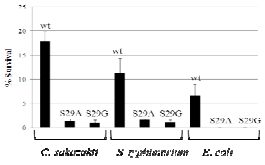

Enhanced Diffusion

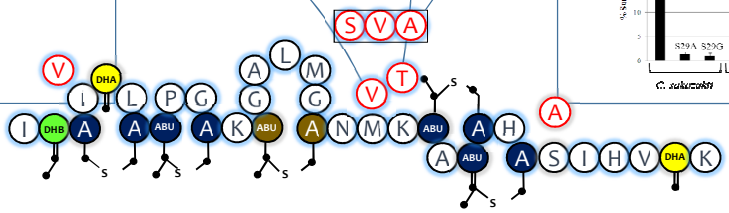



Enhanced Anti-Biofilm




Enhanced Anti-Gram Negative

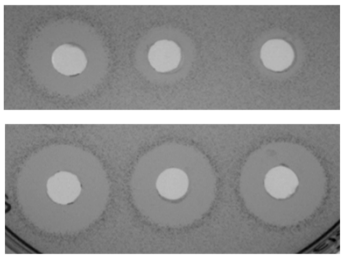


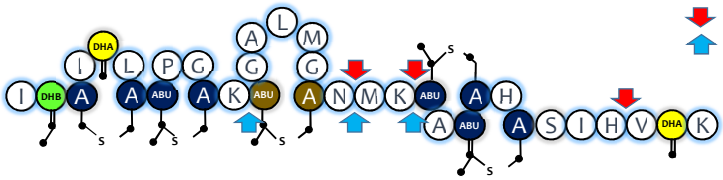



Field et al., 2008
Field et al., 2010
Field et al., 2012
Field et al., 2015
Rouse et al., 2012
Molloy et al., 2013
Healy et al., 2013
Carroll et al., 2010
Campion et al., 2013

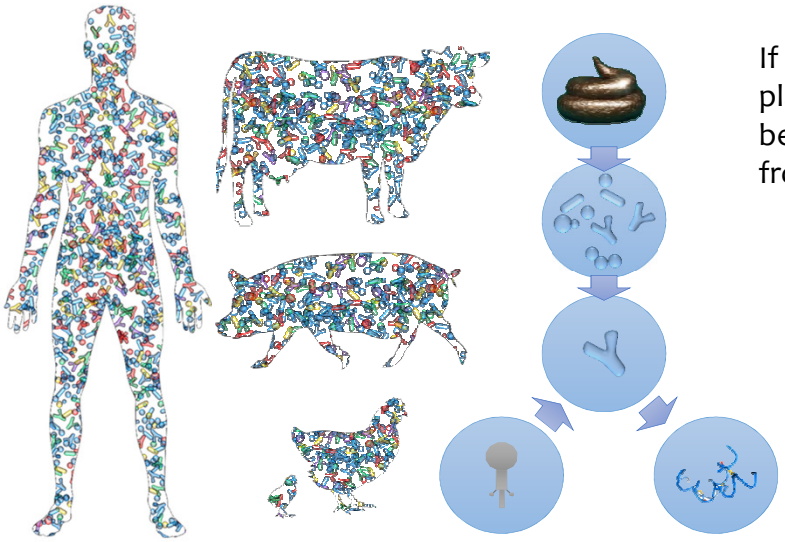
# Nisin in the gut

- Nisin cannot be used for gut applications because it is sensitive to trypsin and α-chymotrypsin (6 cleavage sites)
- Nisin S is an engineered version of nisin with all protease sites removed but with full activity





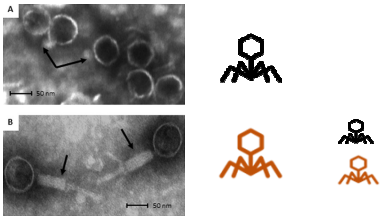
# ‘Mine’ the Microbiome for anti-infectives



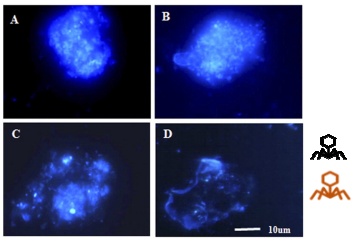
If the microbiome is a significant player in infection, then it should be possible to mine interventions from this niche

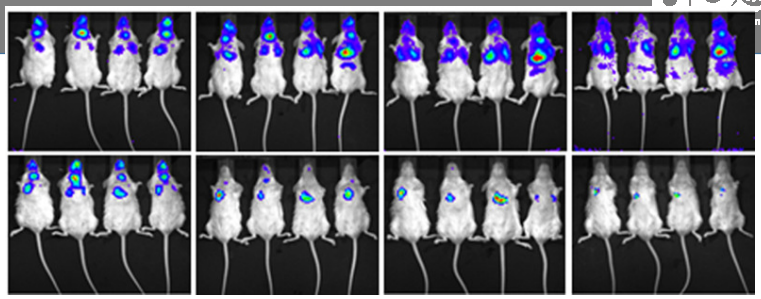
- faecal microbiota transplants
- probiotics
- **bacteriophage**
- pharmabiotics
  - (bioactives, bacteriocins, etc.)

# Phage therapy



Isolated 2 phage active against *Pseudomonas aeruginosa*



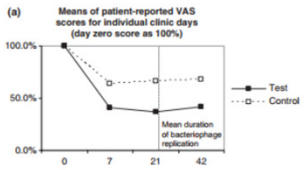


Tom Patterson USA

*Acinetobacter baumannii*

16y old male (France)

*Staphylococcus aureus*




RCT (N=24) for treatment of chronic otitis due to antibiotic-resistant *Pseudomonas aeruginosa* (United Kingdom)

Alemayehu, et al. 2012. mBio 3(2): e00029-12.

# Bacteriophage in feed and food

Research in Veterinary Science 93 (2012) 1173–1178

Use of bacteriophage for biological control of *Salmonella* Enteritidis infection in chicken

Tae-Hyun Lim [a], Myung-Seob Kim [a], Dong-Hun Lee [a], Yu-Na Lee [a], Jae-Keun Park [a], Ha-Na Youn [a], Hyun-Jeong Lee [b], Si-Yong Yang [b], Young-Wook Cho [b], Joong-Bok Lee [a], Seung-Yong Park [a], In-Soo Choi [a], Chang-Seon Song [a,*]

[a] Avian Disease Laboratory, College of Veterinary Medicine, Konkuk University, 1 Hwayangdong, Gwangjin-gu, Seoul 143-701, Republic of Korea
[b] Research Institute of Biotechnology, CJ Cheiljedang Co., Gayangdong, Gangseo-gu, Seoul 157-724, Republic of Korea

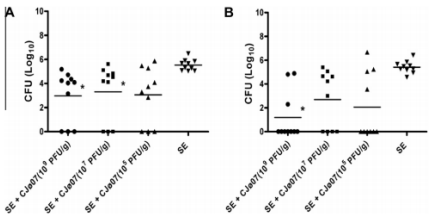
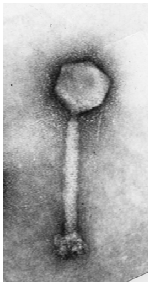
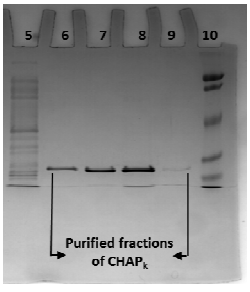
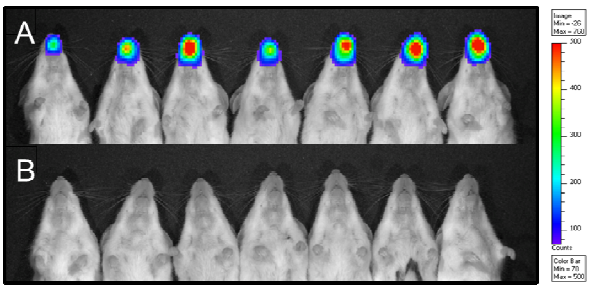


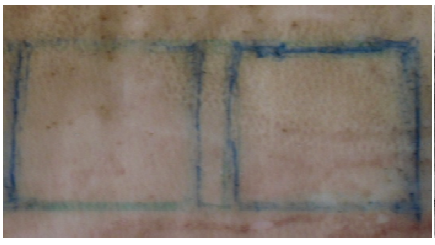

Fig. 3. Results of *Salmonella* Enteritidis counts (CFU $\log_{10}$) in intestines of challenged (A) and contact (B) chicks at 3 weeks after challenge. Asterisk (*) indicates significant difference ($P < 0.05$) between bacteriophage-treated and positive control group. Black dots represent individual chickens and horizontal bars represent averages within group.

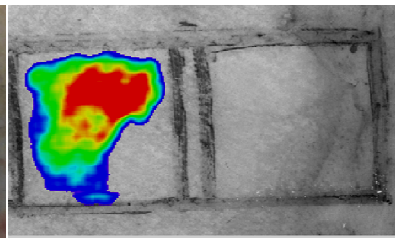

# Phage enzyme therapy

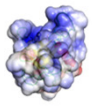




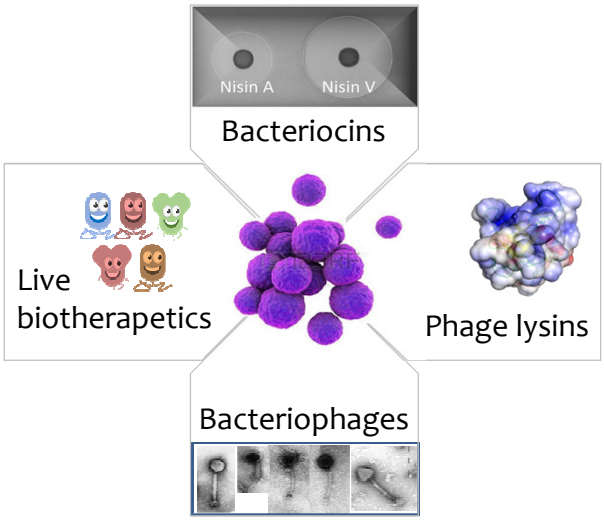




CHAP$_K$

w/ Aidan Coffey, Cork Institute of Technology

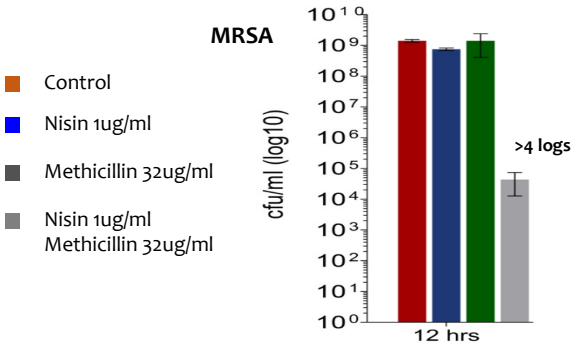
# Microbiome solutions for MRSA



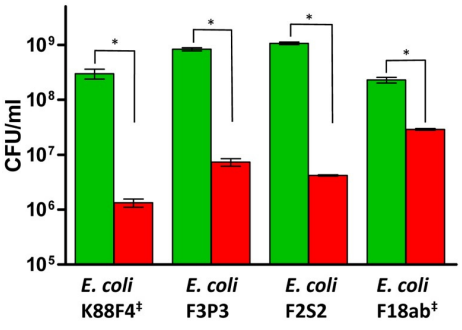

Traditional antibiotics

# Combination therapies

- Not a binary choice, could use microbiome-based solutions with standard small molecule antimicrobials as combination therapies




Nisin and methicillin




Nisin and cinnamaldehyde

Ellis et al, unpublished

Field et al, Antibiotics 2017. 6:35

# Combinations and biofilms

npj | Biofilms and Microbiomes

www.nature.com/npjbiofilms

REVIEW ARTICLE OPEN

Fighting biofilms with lantibiotics and other groups of bacteriocins

Harsh Mathur[1,2], Des Field[2,3], Mary C. Rea[1,2], Paul D. Cotter[1,2], Colin Hill[2,3] and R. Paul Ross[2,4]

Biofilms are sessile communities of bacteria typically embedded in an extracellular polymeric matrix. Bacterial cells embedded in biofilms are inherently recalcitrant to antimicrobials, compared to cells existing in a planktonic state, and are notoriously difficult to eradicate once formed. Avenues to tackle biofilms thus far have largely focussed on attempting to disrupt the initial stages of biofilm formation, including adhesion and maturation of the biofilm. Such an approach is advantageous as the concentrations required to inhibit formation of biofilms are generally much lower than removing a fully established biofilm. The crisis of antibiotic resistance in clinical settings worldwide has been further exacerbated by the ability of certain pathogenic bacteria to form biofilms. Perhaps the most notorious biofilm formers described from a clinical viewpoint have been methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Gardnerella vaginalis* and *Streptococcus mutans*, the latter of which is found in oral biofilms. Due to the dearth of novel antibiotics in recent decades, compounded by the increasing rate of emergence of resistance amongst pathogens with a propensity for biofilm formation, solutions are urgently required to mitigate these crises. Bacteriocins are a class of antimicrobial peptides, which are ribosomally synthesised and often are more potent than their antibiotic counterparts. Here, we review a selection of studies conducted with bacteriocins with the ultimate objective of inhibiting biofilms. Overall, a deeper understanding of the precise means by which a biofilm forms on a substrate as well as insights into the mechanisms by which bacteriocins inhibit biofilms is warranted.

*npj Biofilms and Microbiomes* (2018)4:9 ; doi:10.1038/s41522-018-0053-6

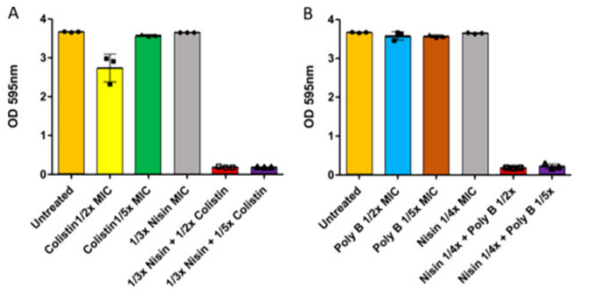
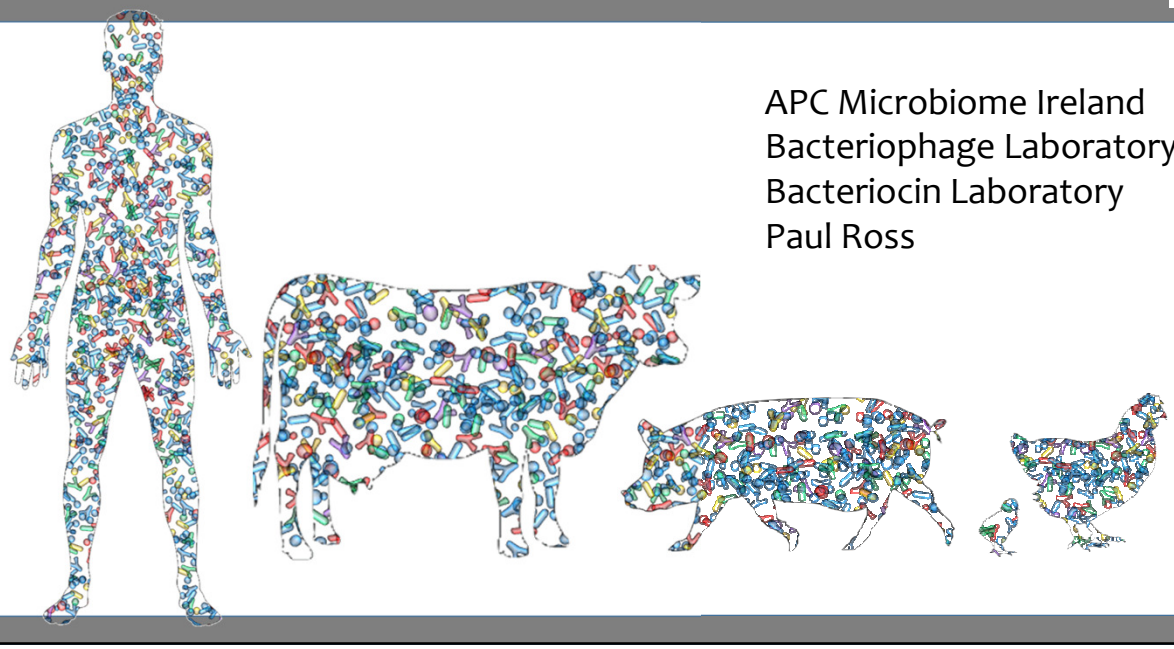



**Fig. 2** Anti-biofilm activity of nisin and polymyxins against *P. aeruginosa*: Inhibition of biofilm formation of *P. aeruginosa* PA-01 **a**) in the presence of nisin (1/3× MIC), colistin (1/2×, 1/5× MIC) and combinations thereof and **b**) in the presence of nisin (1/4× MIC) and polymyxin B (1/2×, 1/5× MIC) and combinations thereof, when assessed in microtiter plates and subjected to crystal violet (CV) staining for the detection of biofilm formation. (Adapted from Field et al. 2016b [73] under the terms of the Creative Commons Attribution License)

# Opportunities and challenges

- Microbiomes can act as a rich source of novel antimicrobials. Fewer issues with resistance?
- Microbiome-based solutions for infection could be deployed alone or in combination with antibiotics/preservatives.
- Paradigm shift needed, current regulatory framework is not appropriate for microbiome-based interventions aimed at impacting health; these will involve complex biological compositions and food and feed based solutions (FDA: CBER and CDER).
- Microbiome may deliver 'traditional' blockbusters, but is more likely to involve personalised or targeted solutions.

# Thanks



APC Microbiome Ireland
Bacteriophage Laboratory
Bacteriocin Laboratory
Paul Ross