Paul Ross

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Life in a crowded Space:

Is there room for probiotics in a Microbiome Space

SDT
Passion for Dairy

teagasc

ucc
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

Microbiome Institute

April 14th and 15th Spring conference 2016

Room for Probiotics in a Crowded Space

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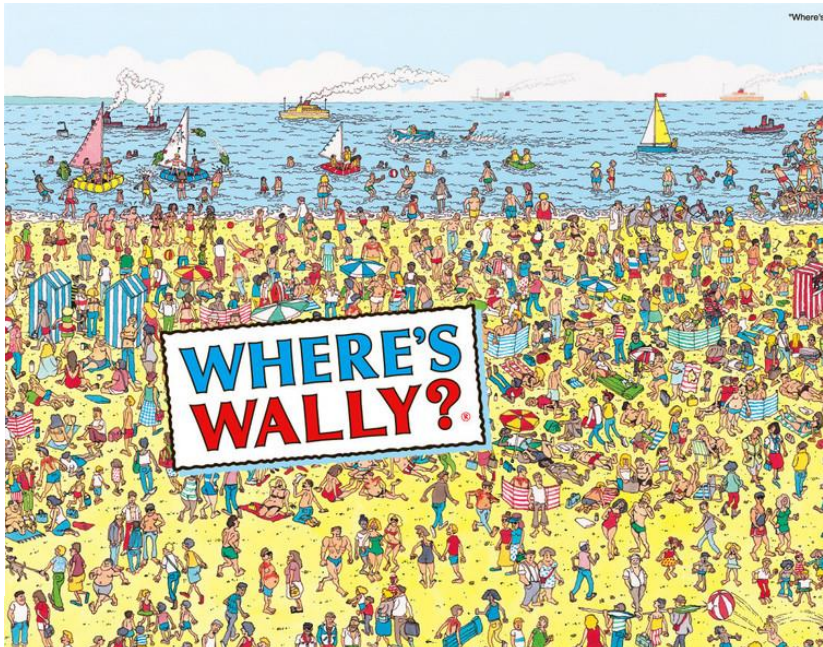
- APC Microbiome Institute
- Microbiota Complexity – Extremes of Life
- A Microbiological Conundrum
- 4 Differing Examples of Economic Significance
- Conclusions



Defining in Terms of Diet and Health

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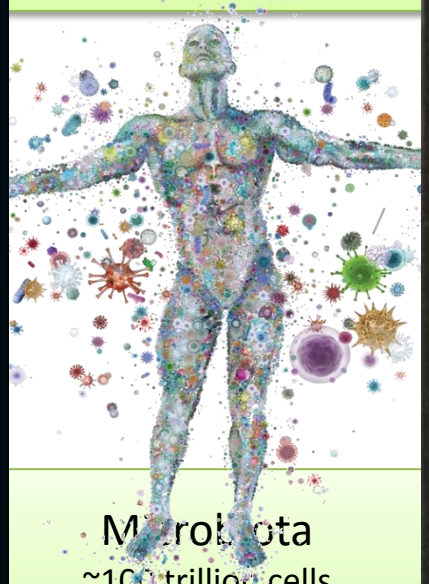
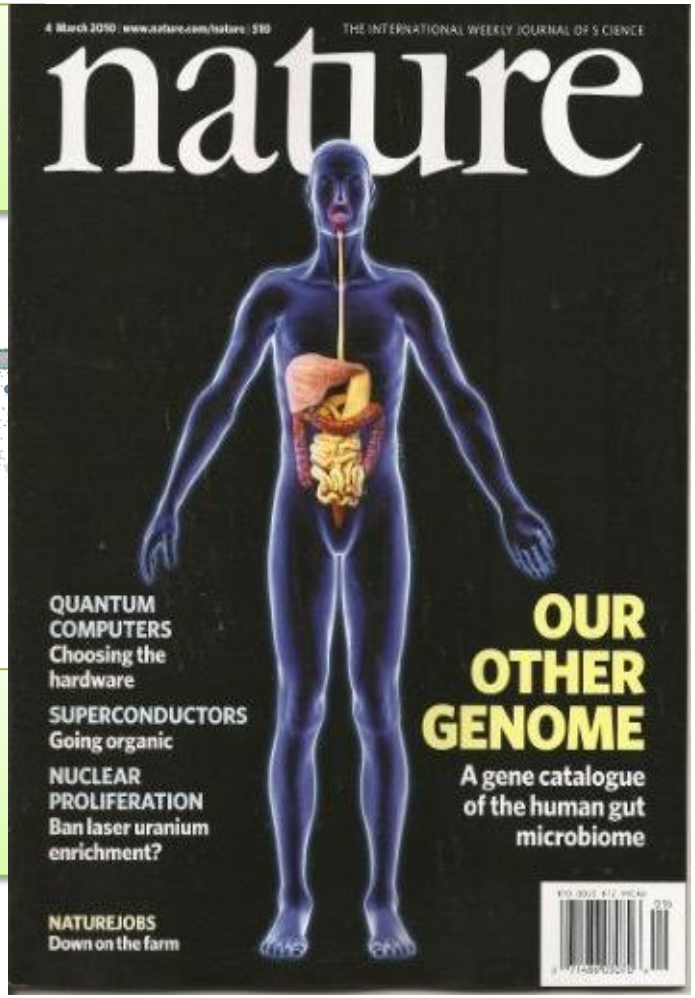
Human
~10 trillion cells
~23 thousand genes



Microbiota
~100 trillion cells
~3 million genes



APC Microbiome Institute

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Interfacing Food & Medicine

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~270 APC-Institute Staff

68 APC Faculty

Multiple basic research and clinical disciplines applied to same problem

Microbiology, immunology, pharmacology, neuroscience, food science, nutrition, biochemistry, medical microbiology, pharmacy

Gastroenterology, psychiatry, cardiovascular health, rheumatology, radiology, pathology, gerontology, neonatology, metabolic health

UCC Coláiste na hOllscoile Corcaigh, Éire University College Cork, Ireland

Teagasc

CIT Institiúid Teicneolaíochta Chorcaí Cork Institute of Technology

Mercy University Hospital

CUH Ospidéal Ollscoil Chorcaí Cork University Hospital

APC Research Themes

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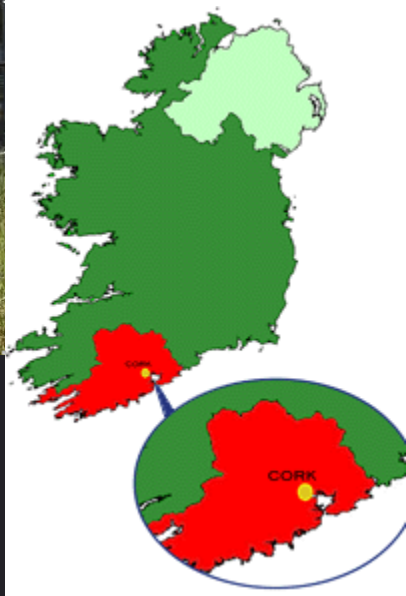

ONE	TWO
Microbes to Molecules	Diet & Microbes at the Extremes of Life
THREE	FOUR
Brain- Gut Axis	Host-Microbe Dialogue

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Extremes of Life

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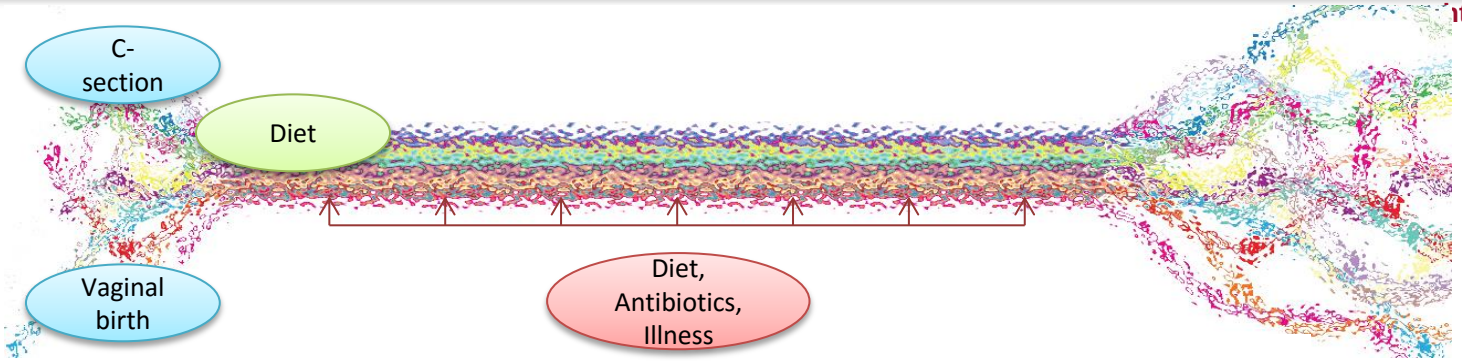


Formula

More complex
B. fragilis
E. coli
C. difficile

Breast

Bifidobacterium
Ruminococcus

Stable core genome

Bacteroides
Clostridium
Ruminococcus
Eubacterium
Parabacteroides
Coprococcus
Dorea
Alistipes
Collinsella
Lachnospira
Roseburia
Faecalibacterium

Old age

↑ *Fusobacterium*
Clostridium
Eubacterium
Facultative anaerobes

↓ *Bacteroides*
Bifidobacterium
SCFA

After Power et al, BNJ (2014) 111:387

A-V4
B-V4
C-V4
D-V4

eldermet

infantmet

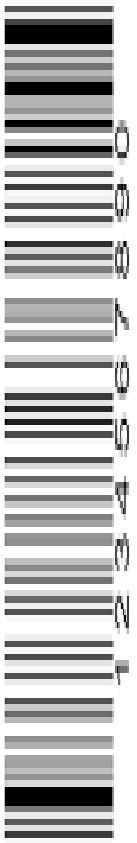
Ion DNA Seq Platforms
(PGM & Proton)
Illumina MiSeq/Hi Seq

454 SEQUENCING

239 samples

~1000 species

Barcode



INFANTMET Study Design

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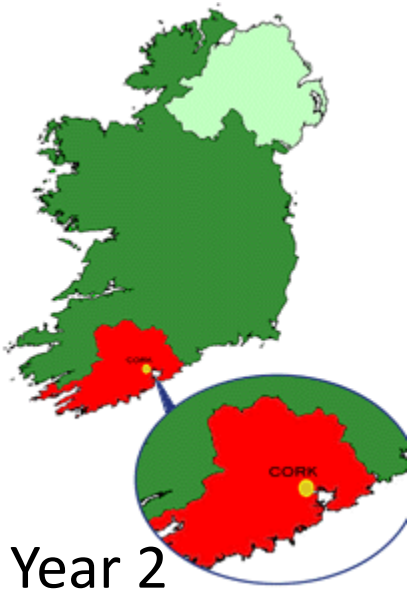
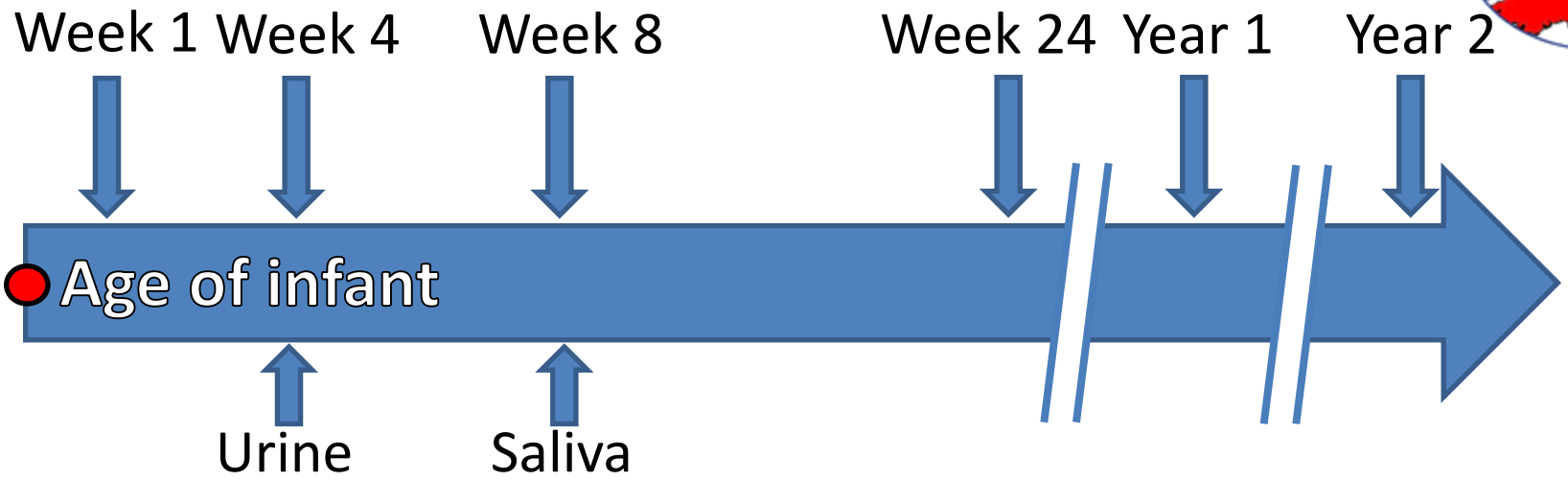
- Microbiota of breast fed infants:
  - Pre-term (<1500g or <35 weeks)
  - Caesarean section (full term)
  - Natural vaginal delivery (full term)
- n = 50 per group

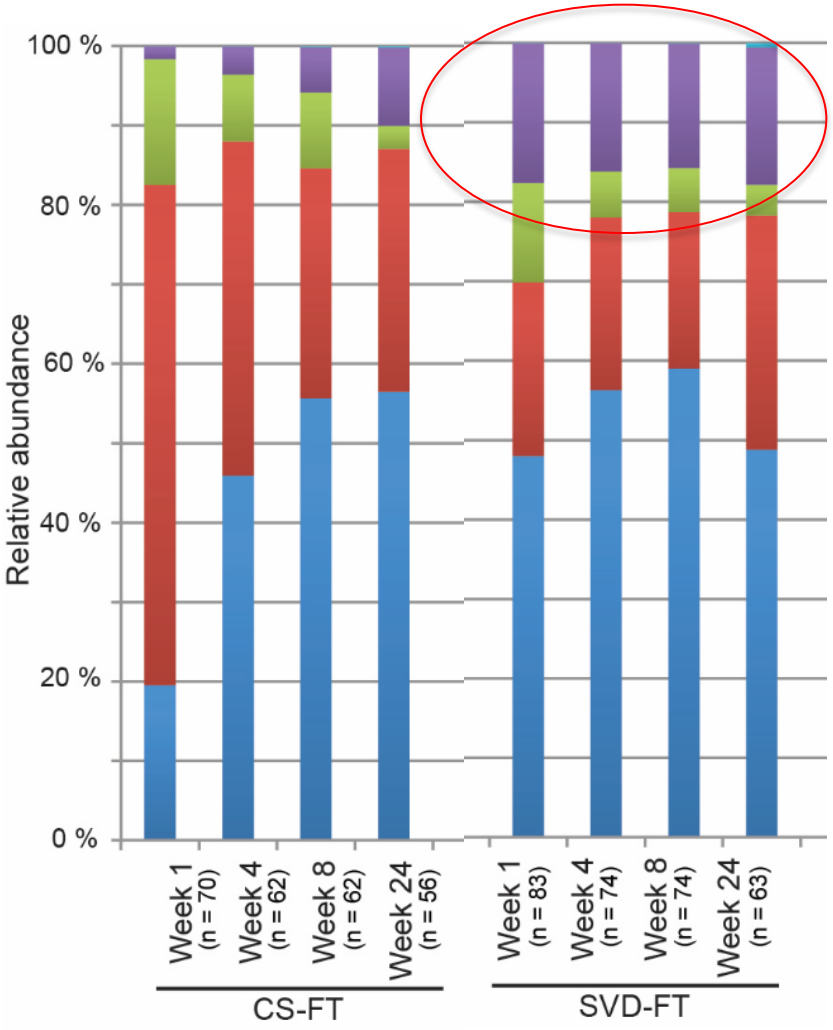




- Health questionnaire at year 1 and year 2

infantmet

At Phylum Level



C-section

Vaginal Delivery

Microbial Shifts Associated with Age

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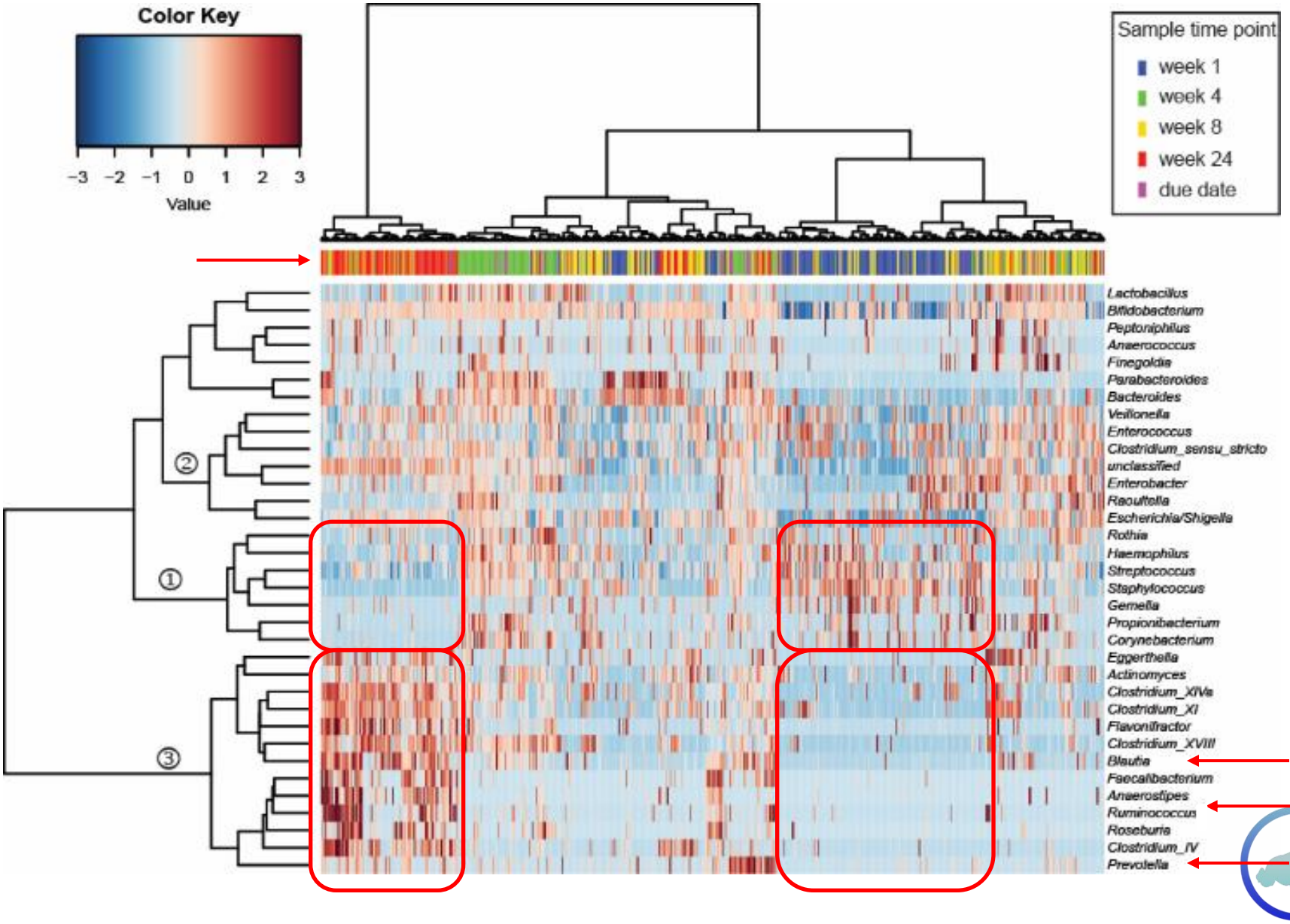


infantmet

ARTICLE

nature

Gut microbiota composition correlates with diet and health in the elderly

Marcus J. Claesson[1,2]*, Ian B. Jeffery[1,2]*, Susana Conde[3], Susan E. Power[1], Eibhlís M. O'Connor[1,2], Siobhán Cusack[1], Hugh M. B. Harris[1], Maireád Coakley[4], Bhuvaneswari Lakshminarayanan[4], Orla O'Sullivan[4], Gerald F. Fitzgerald[1,2], Jennifer Deane[1], Michael O'Connor[5,6], Norma Harnedy[5,6], Kieran O'Connor[6,7,8], Denis O'Mahony[5,6,8], Douwe van Sinderen[1,2], Martina Wallace[9], Lorraine Brennan[9], Catherine Stanton[2,4], Julian R. Marchesi[10], Anthony P. Fitzgerald[3,11], Fergus Shanahan[2,12], Colin Hill[1,2], R. Paul Ross[2,4] & Paul W. O'Toole[1,2]

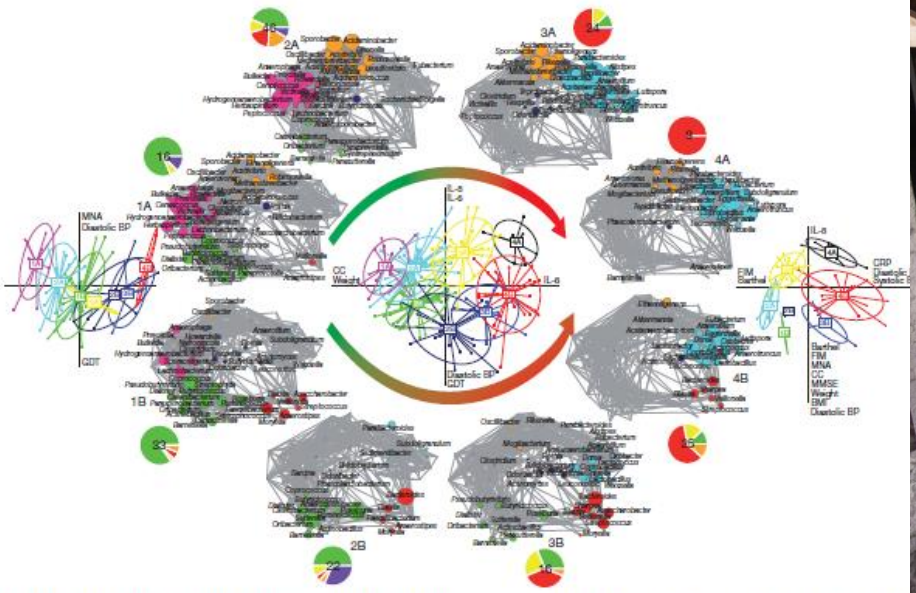
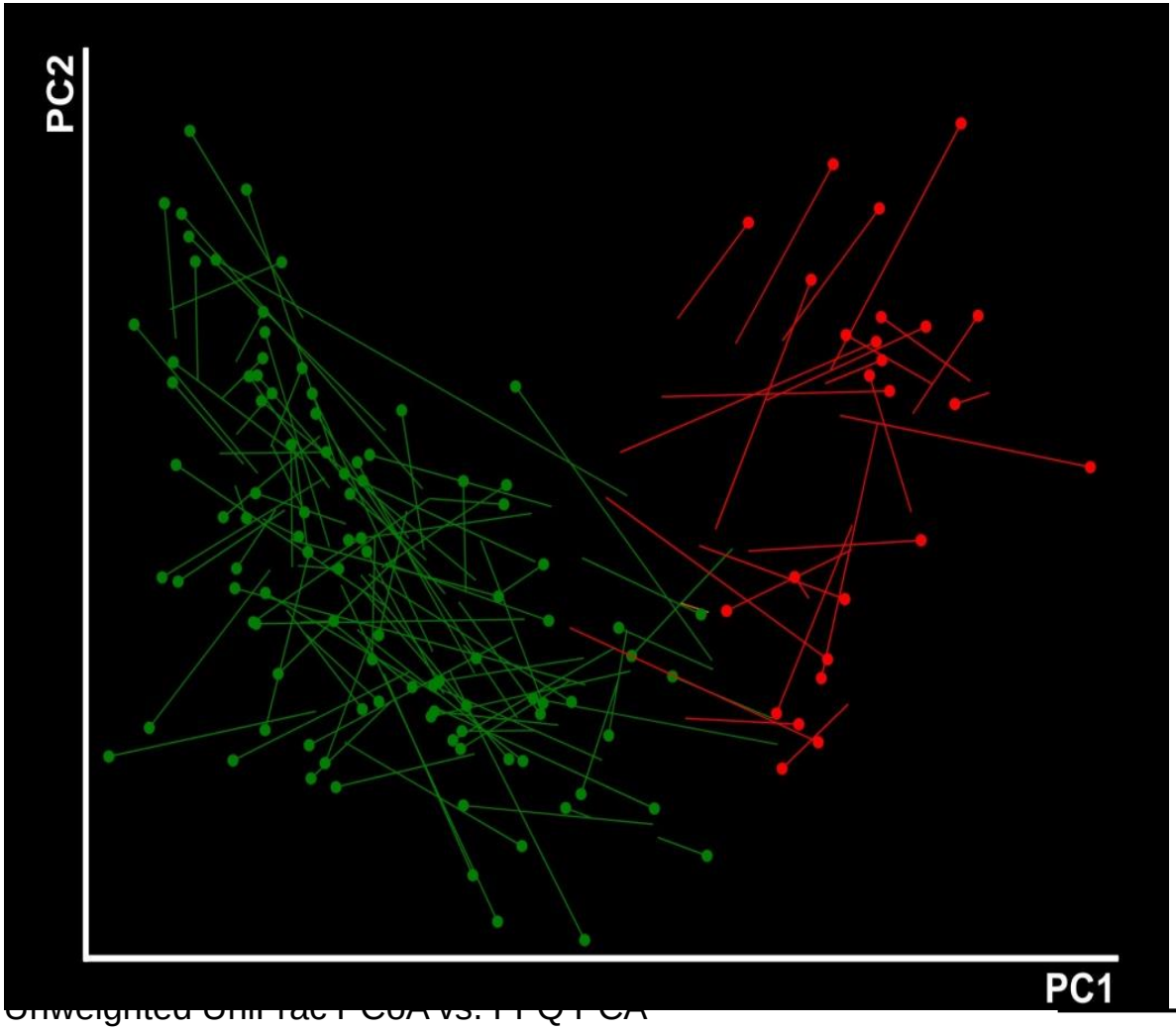


Figure 4 | Transition in microbiota composition across residence location is mirrored by changes in health indices. The PCoA plots show 8 groups of relative to background. The pie charts show residence location proportions (colour coded as in Fig. 1c) and number of subjects per subject group. Curve

Elderly: Microbiota & diet correlate and by community location

dietary groups were associated with separations inmicrobiota composition

Long-stay
Rehab
Day Hospital
Community
Young control

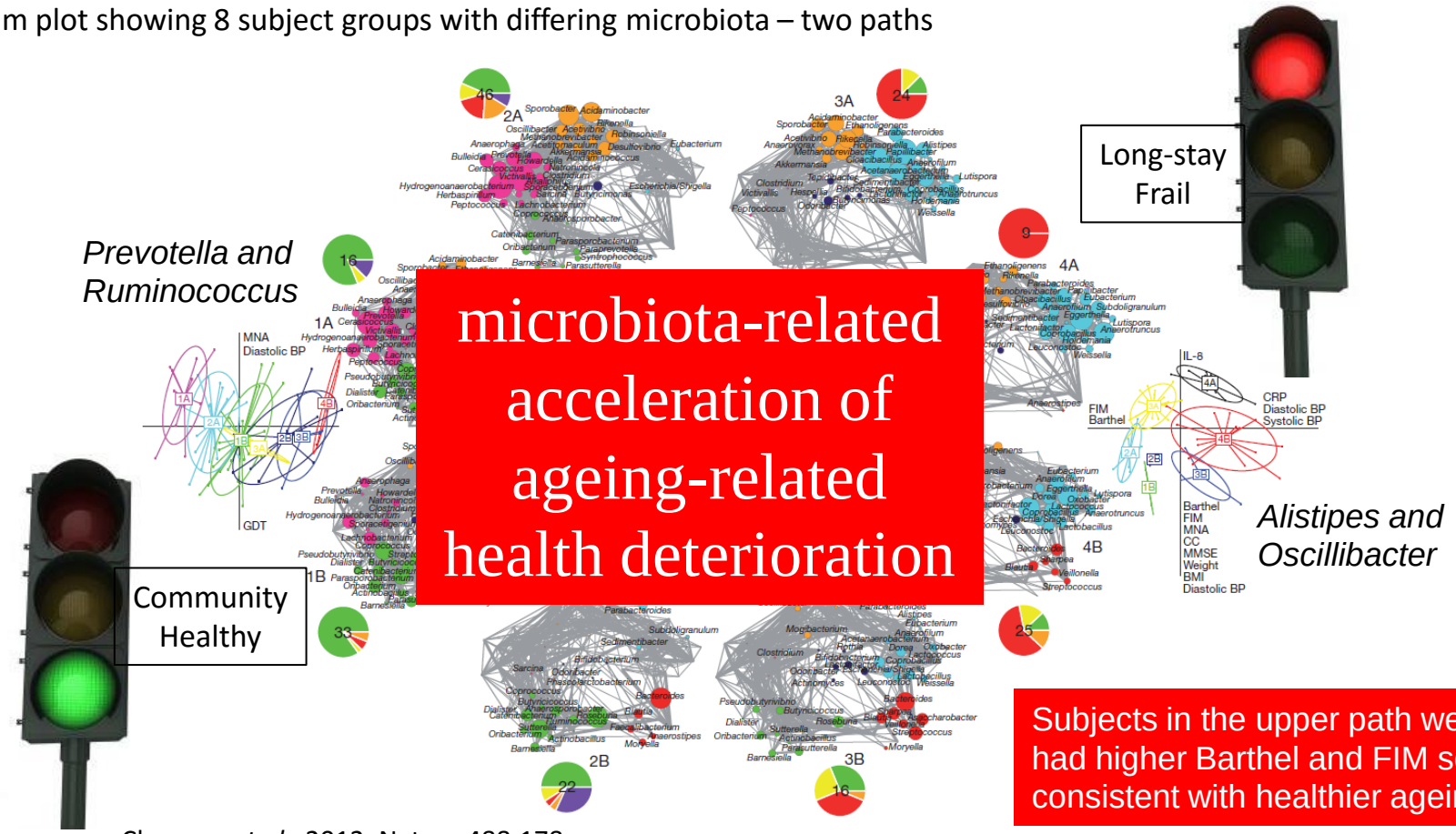


Microbiota — diet

(length of string indicative of relationship)

Claesson *et al*., 2012. Nature 488:178.

Wiggum plot showing 8 subject groups with differing microbiota – two paths



Subjects in the upper path were older - had higher Barthel and FIM scores consistent with healthier ageing.

Claesson *et al.*, 2012. Nature 488:178.

Microbiota Versus Probiotics

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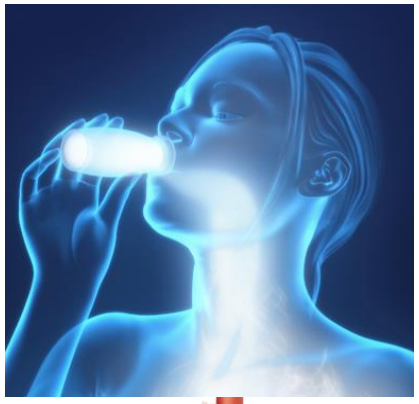
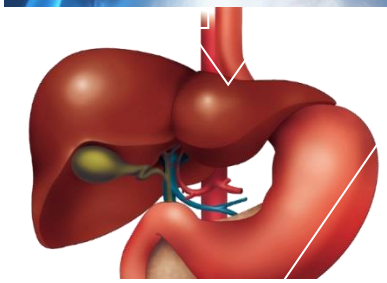
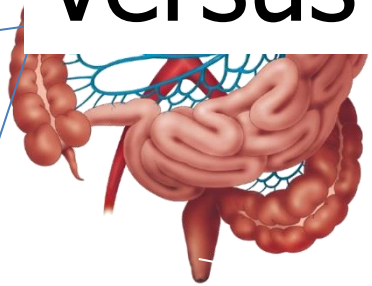
Gut Microbiota

- 100 trillion bacteria
- 10X human cells
- 100X genome
- role in digestion and Energy harvesting
- Production of essential nutrients
- Inate immunity -Anti-infective/Immuno-modulator
- Production of bioactive substances
- Composition/diversity associated with health and disease





Versus



Probiotics

- Ingested live bacteria
- 10 Billion
- Don't colonize - tourists
- Probiotics have mainly transient effects
- Range of health effects from lactose intolerance to Immuno-modulatory and antimicrobial
- Out-compete pathogens

Conundrum:

How could probiotics "work" when they represent such a tiny fraction of the gut microbiota?

But Not in Upper GIT

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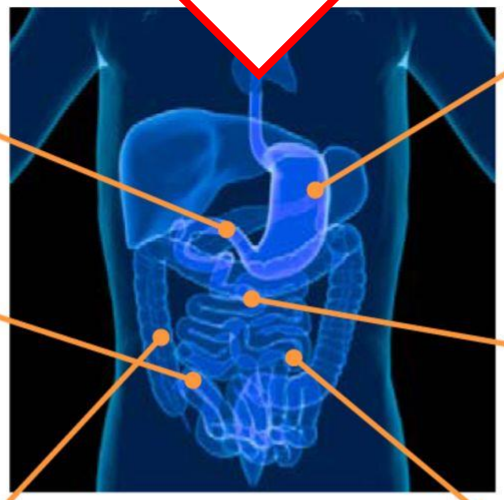
"tiny fraction"

Take lac acilli…..

10^{10}



Duodenum
10^1-10^3 cfu/ml
- Lactobacillus
- Streptococcus

Distal Ileum
10^7-10^8 cfu/ml
- Clostridium
- Bacteroides sp
- Coliforms

Colon
10^{11}-10^{12} cfu/ml
- Clostridium coccoides
- Clostridium leptum
- Fusobacterium
- Bacteroides
- Bifidobacterium

Lactobacilli 0.01-0.01% of microbiota in stool

Stomach
10^1-10^3 cfu/ml
- Lactobacillus
- Candida
- Streptococcus
- Helicobacter pylori
- Peptostreptococcus

Jejunum
10^2 cfu/ml
- Lactobacillus
- Streptococcus

Lactobacilli 16% of microbiota in jejunum/Ileal lumen

Proximal Ileum
10^3 cfu/ml
- Lactobacillus
- Streptococcus
- Actinobacteria

Conclusion: they are a dominant population where it matters

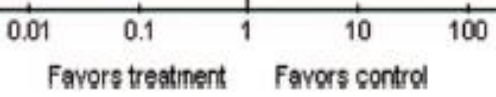
AEM 2008 Jens Walter

But Probiotcs can “work”

Review: Probiotics for prevention of necrotizing enterocolitis
Comparison: 01 NEC
Outcome: 01 Definite NEC

Study or sub-category	Probiotic n/N	no probiotic n/N	RR (fixed) 95% CI	Weight %	RR (fixed) 95% CI
Kitajima 1997	0/45	0/46			Not estimable
Dani 2002	4/295	8/290		11.15	0.49 [0.15, 1.61]
Costalos 2003	5/51	6/36		9.72	0.59 [0.19, 1.78]
Bin Nun 2005	1/72	10/73		13.73	0.10 [0.01, 0.77]
Lin 2005	2/180	10/187		13.56	0.21 [0.05, 0.94]
Manzoni 2006	1/39	3/41		4.04	0.35 [0.04, 3.23]
Mohan 2006	2/21	1/17		1.53	1.62 [0.16, 16.37]
Stratiki 2007	0/38	3/31		5.31	0.12 [0.01, 2.19]
Lin 2008	4/217	14/217		19.35	0.29 [0.10, 0.85]
Samanta 2008	5/91	15/95		20.29	0.35 [0.13, 0.92]
Rouge 2009	2/45	1/49		1.32	2.18 [0.20, 23.21]
Total (95% CI)	1094	1082		100.00	0.35 [0.23, 0.55]

Total events: 26 (Probiotic), 71 (no probiotic)
Test for heterogeneity: $Chi^2 = 7.66$, df = 9 (P = 0.57), $I^2 = 0\%$
Test for overall effect: Z = 4.64 (P < 0.00001)



Deshpande et al. 2010

Single Dose In Early Life

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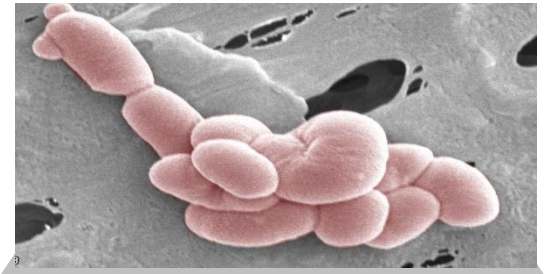
Prolonged faecal excretion following a single dose of probiotic in low birth weight infants

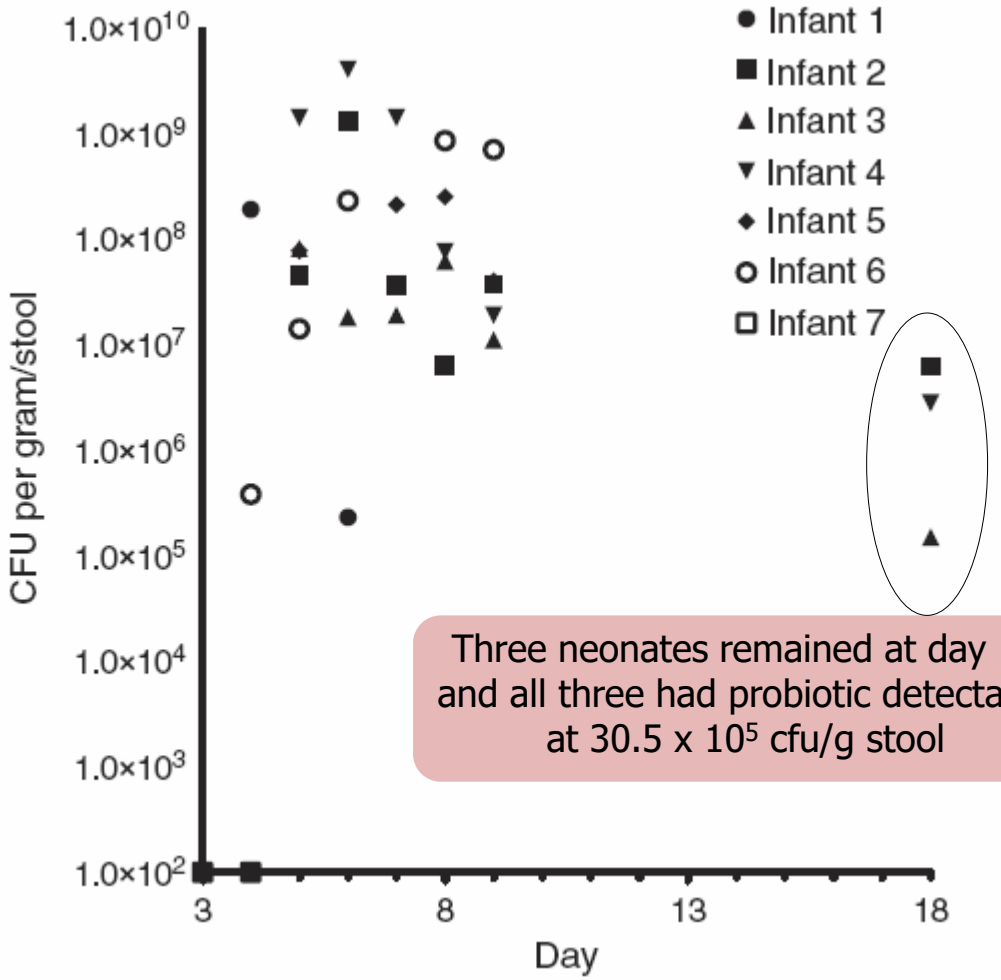
Richard Mc Gee[1,2], Paula M O'Connor[3], David Russell[3], Eugene M Dempsey[1,2], Anthony C Ryan (tony.ryan@hse.ie)[1,2], Paul R Ross[3], Catherine Stanton[3]

ACTA PÆDIATRICA
NURTURING THE CHILD



1 x 10^9 cfu on Day 4 of Life

Stools collected on Days of Life: 3 (control), 5,6,7, 8,9,18 or until discharge



Three neonates remained at day 18 and all three had probiotic detectable at 30.5 x 10^5 cfu/g stool

Screen for Strains with Properties Relevant to End Application

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Pharmabiotics: Bioactives from Mining Host–Microbe–Dietary Interactions

Fergus Shanahan, MD, Catherine Stanton, PhD, Paul Ross, PhD, Colin Hill, PhD

APC Research Themes

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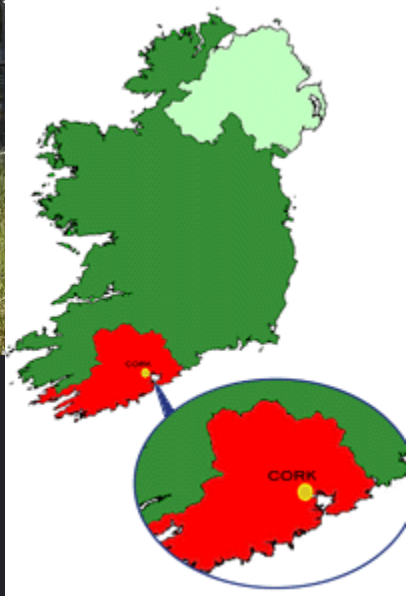

ONE	TWO
Microbes to Molecules	Diet & Microbes at the Extremes of Life
THREE	FOUR
Brain- Gut Axis	Host-Microbe Dialogue

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Probiotic 1 : Lb. mucosae for Heart Health

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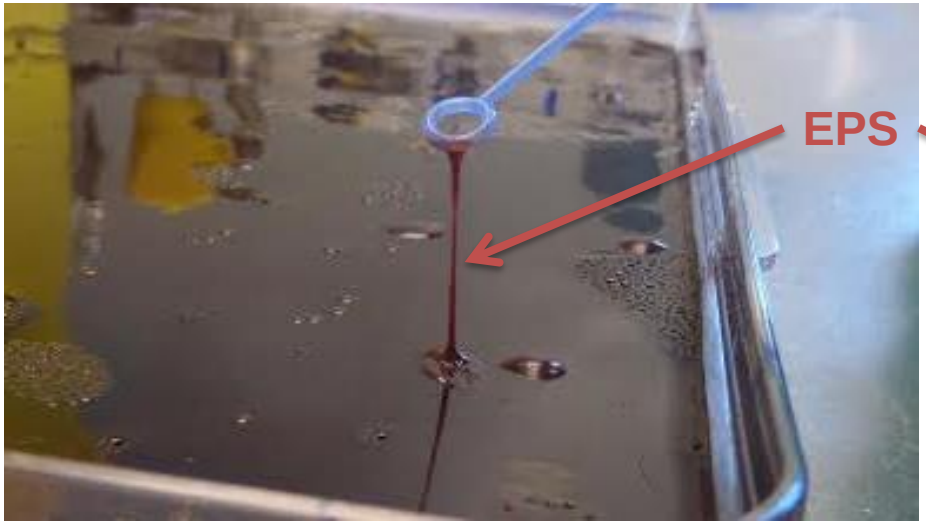

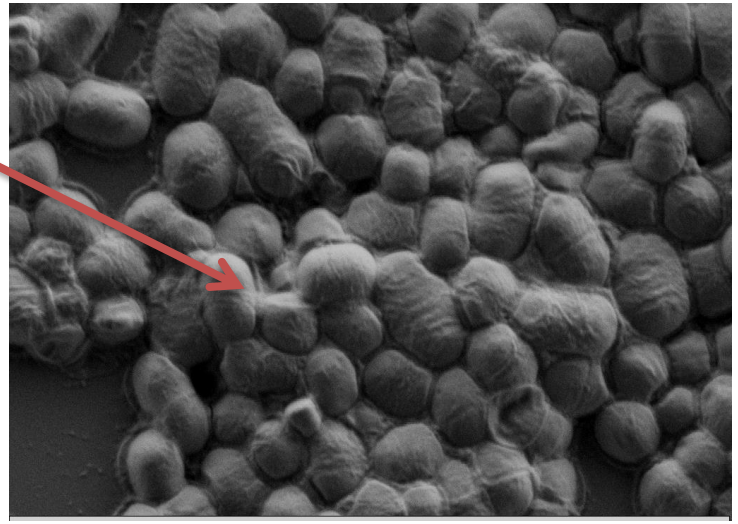

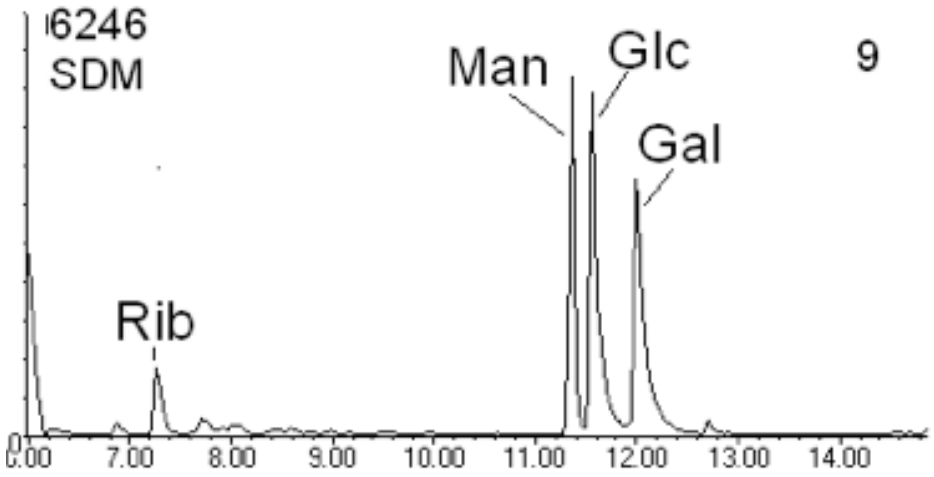

Gluc/Man/Gal
α
1
↓
2

Man
α
1
↓
6

Gluc/Man/Gal (α1→2)**Man** (α1→2)**Man**(α1→2)

Lb. mucosae Draft Genome Sequence

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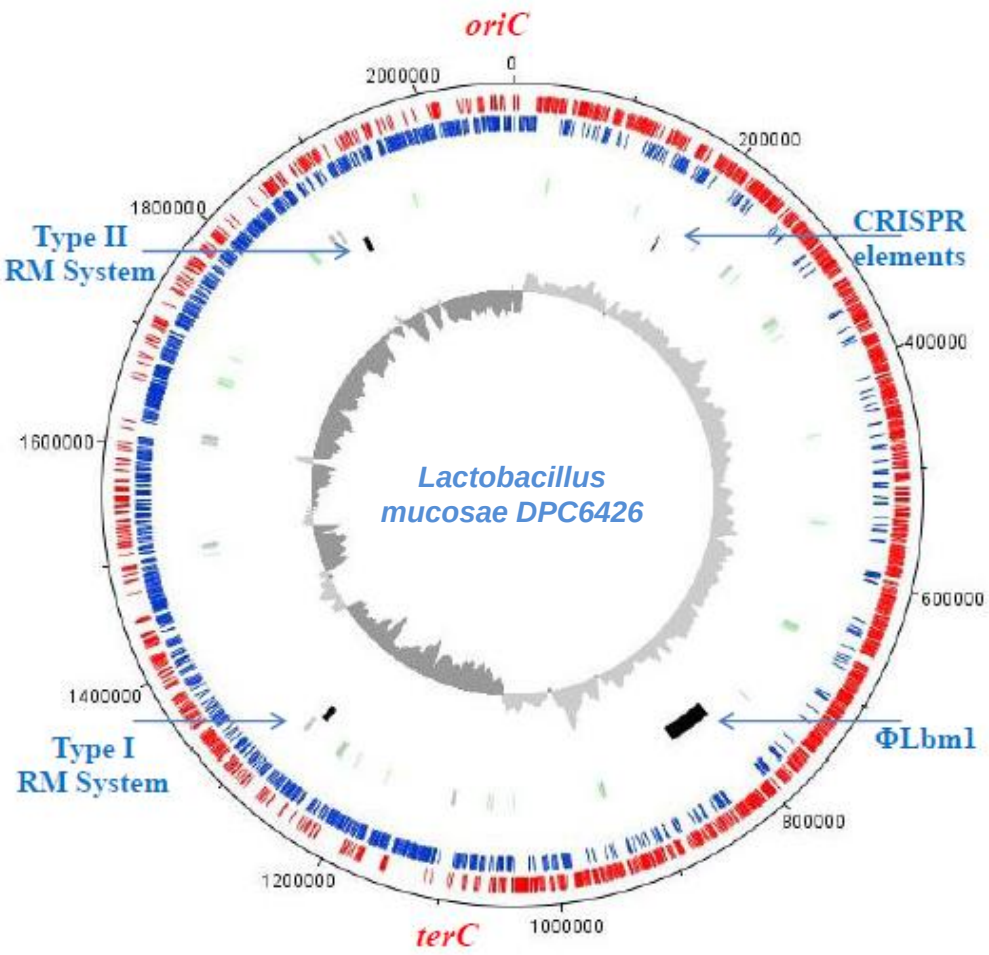
genomeA
Journals.ASM.org

Genome Sequence of the Heteropolysaccharide-Producing Strain *Lactobacillus mucosae* DPC 6426

Paul M. Ryan,[a,b] Caitriona M. Guinane,[a] Lis E. E. London,[c,d] Philip R. Kelleher,[b] Gerald F. Fitzgerald,[b,c] Noel M. Caplice,[c] R. Paul Ross,[d] Catherine Stanton[a,c]



The genome revealed a plethora of homologues linked to carbohydrate metabolism (EPS operon) and mucin binding. In addition, several putative bile salt hydrolase genes (BSH) were identified, along with a potential novel phage and CRISPR elements.

DPC6426 Reduces Atherosclerosis – *In Vivo*

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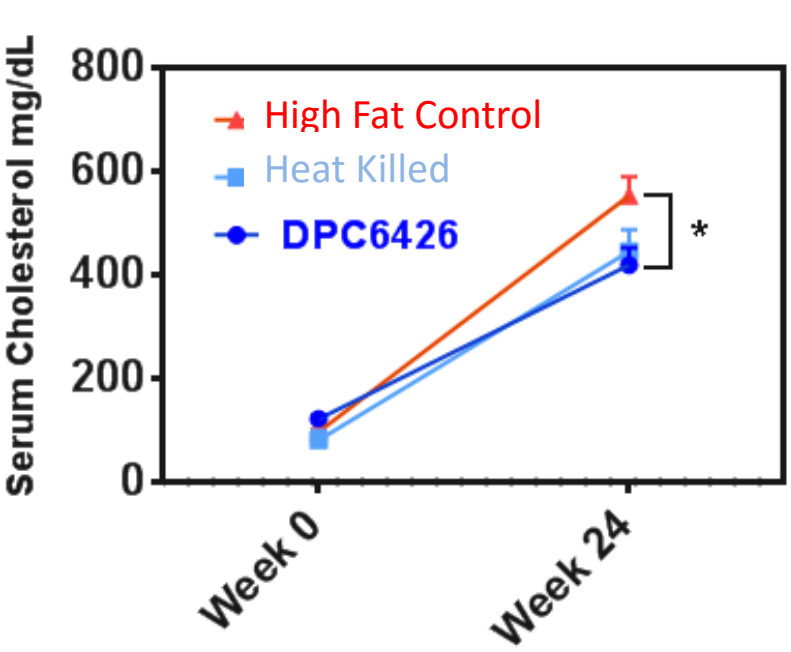

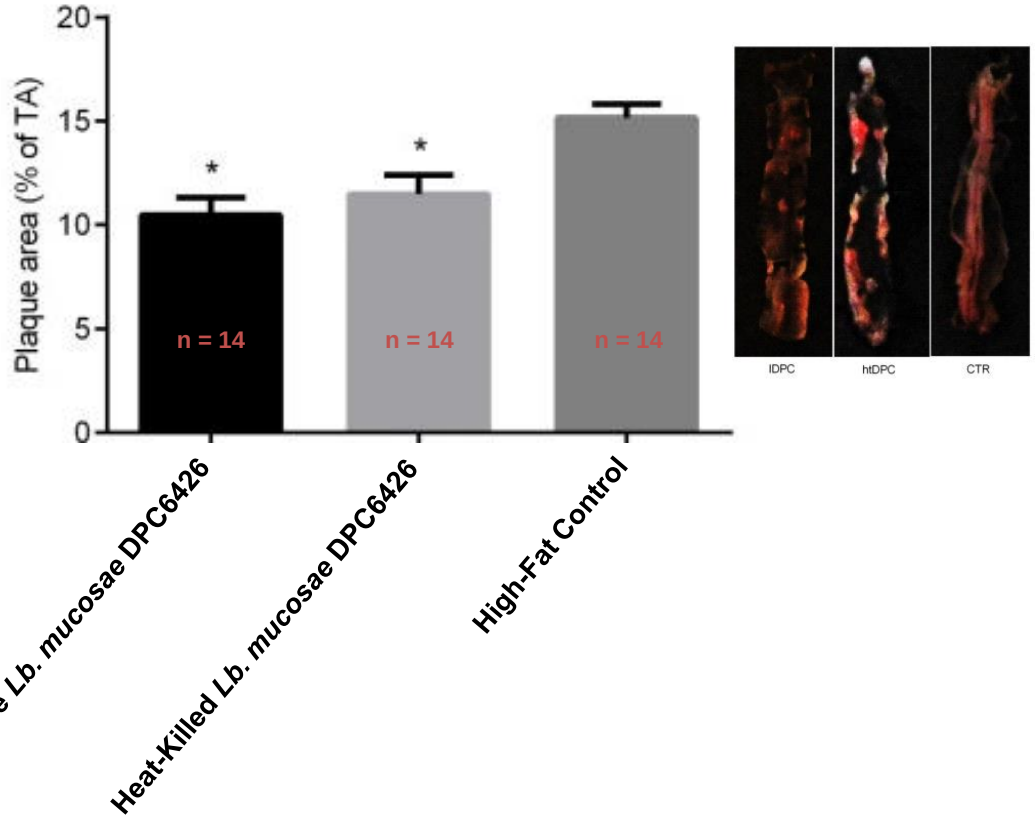

A 24-week intervention with either Live (**IDPC**) or Heat-Killed (**hkDPC**) *Lb. mucosae* DPC6426 fed in conjunction with a high-fat diet significantly attenuated cholesterol (and in particular LDL-C) accumulation when compared to the high-fat control (**HFC**). In turn, the strain reduced the development of aortic plaque in the atherosclerosis-prone apolipoprotein-E-deficient (apo-$E^{-/-}$) mouse model.

Antiatherogenic Pathways of DPC6426

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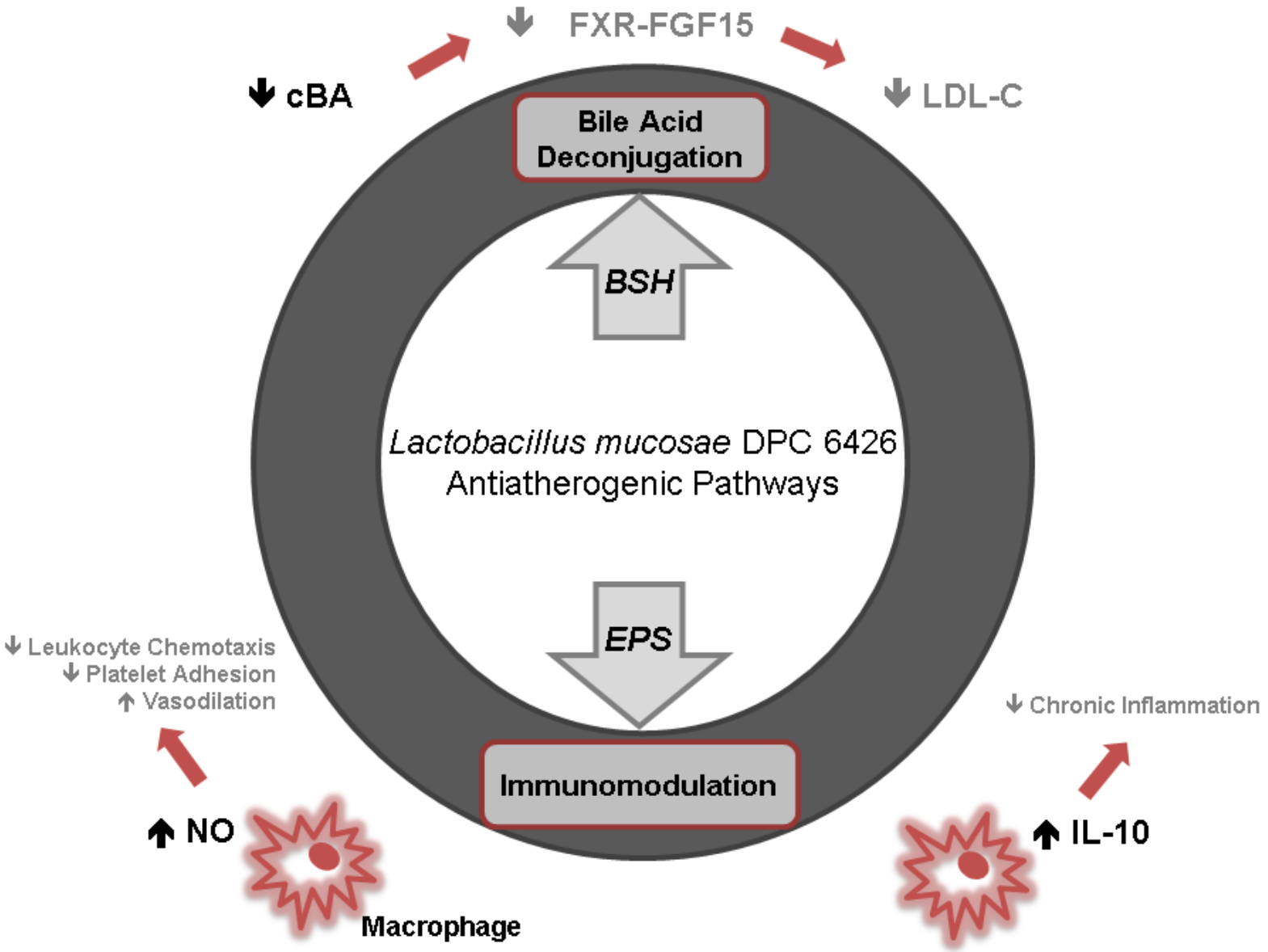

Lb. mucosae Viability in Cheese

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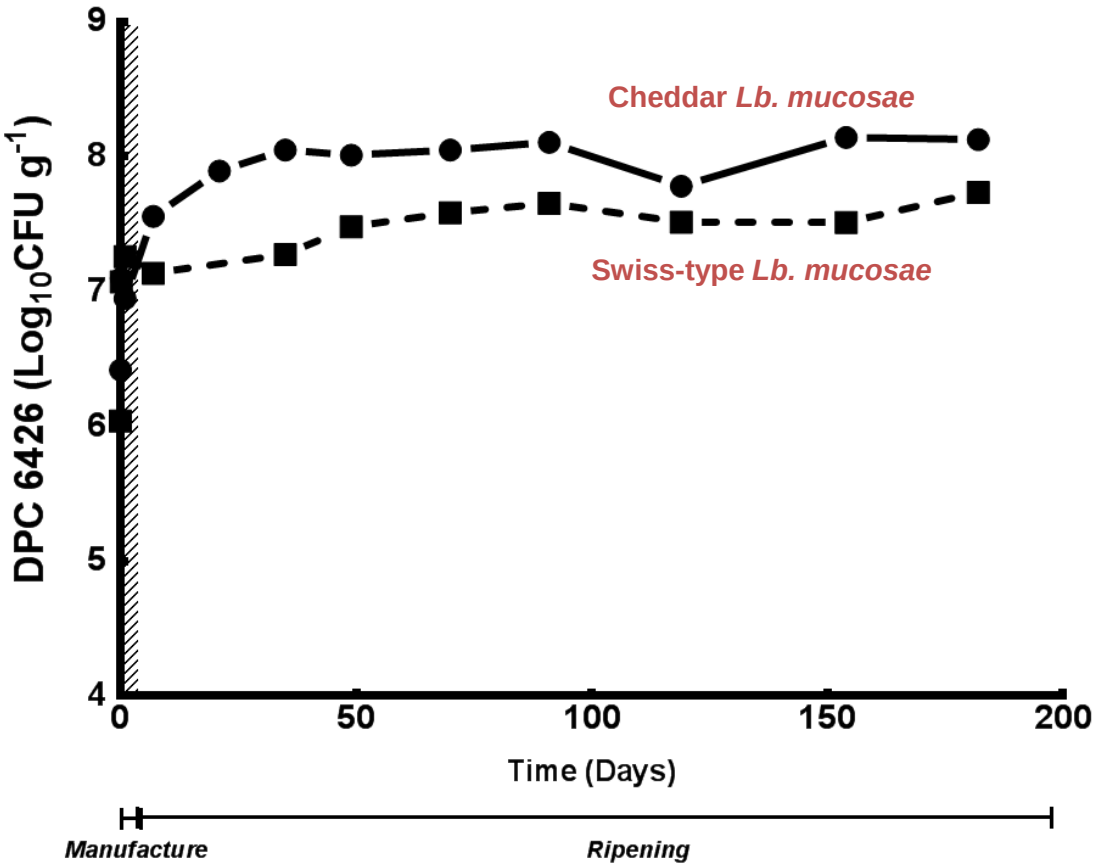
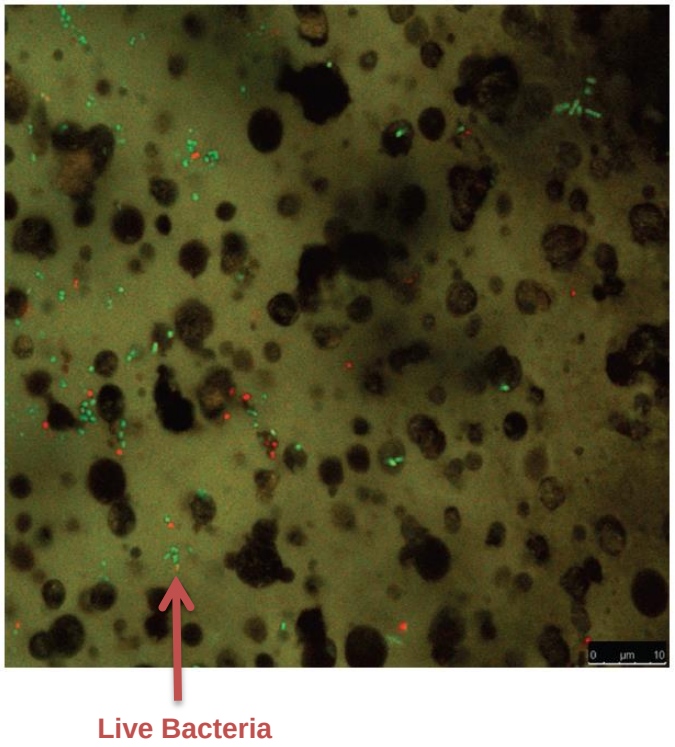
***Lb. mucosae* DPC6426 survived at desirable levels in the both cheese types – *i.e.* delivering ~10^9 CFU/30g serving.**





Textural & Sensory Attributes

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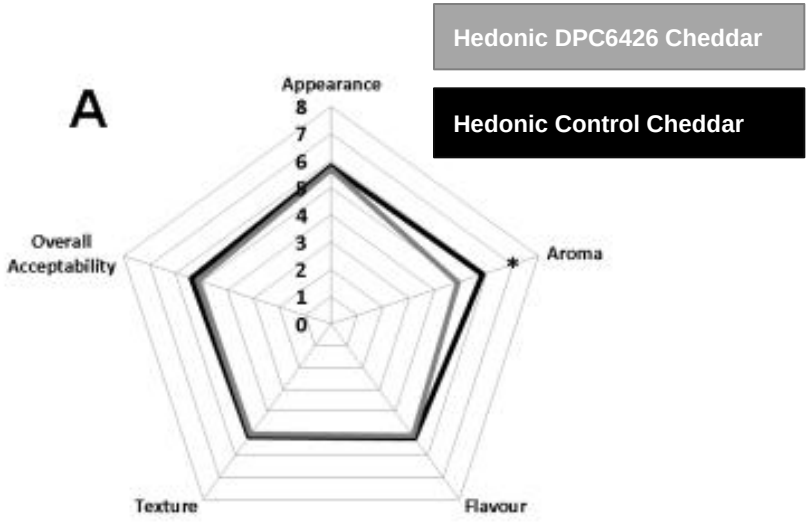
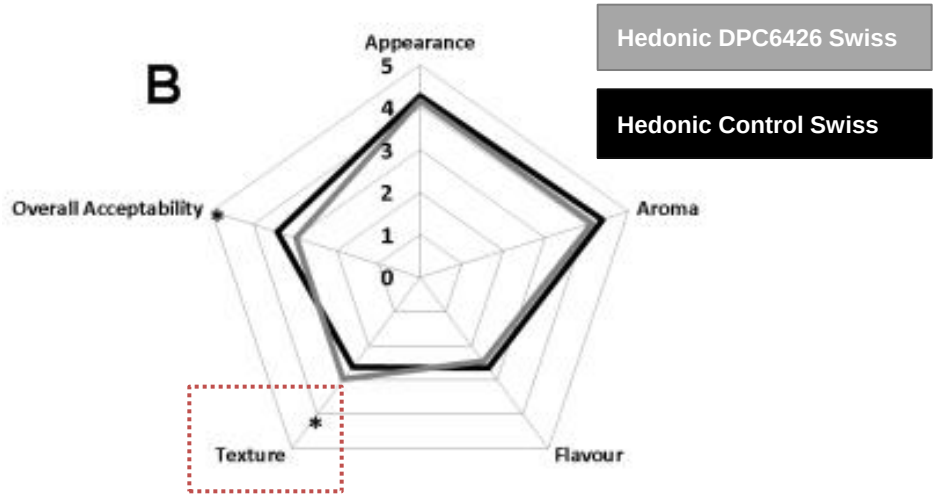
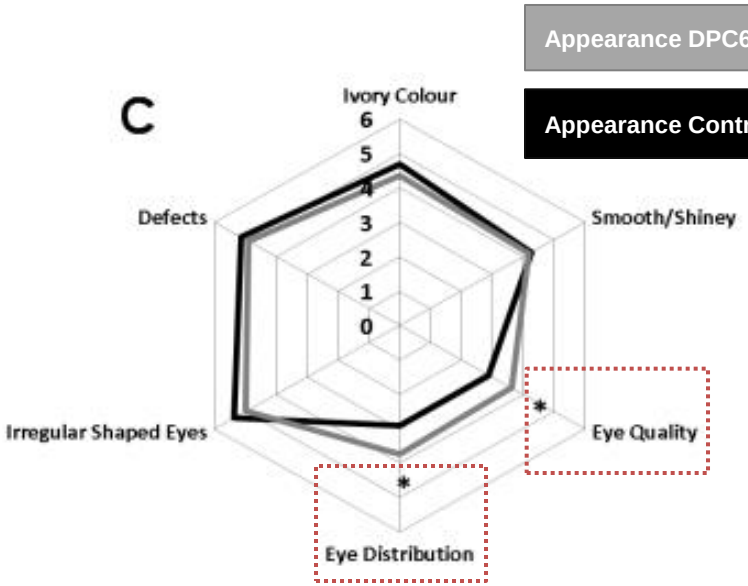


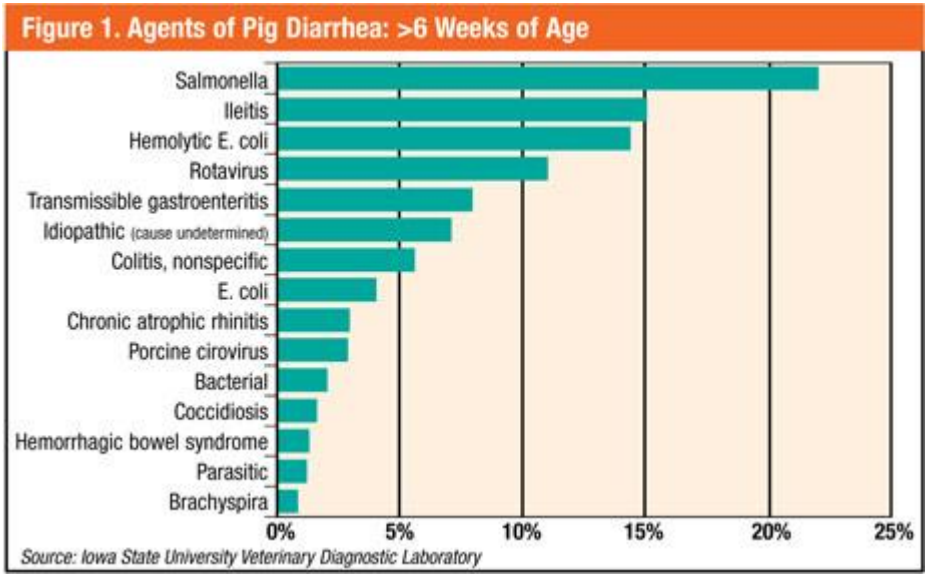




Inclusion of *Lb. mucosae* DPC6426 in cheese manufacture improve trained assessors liking of Swiss-type texture (**B**), eye-distribution and eye quality (**C**).

Salmonella in Pigs

- Animal welfare
- Zoonotic potential
- Economic costs



Probiotic 2: 5-Live

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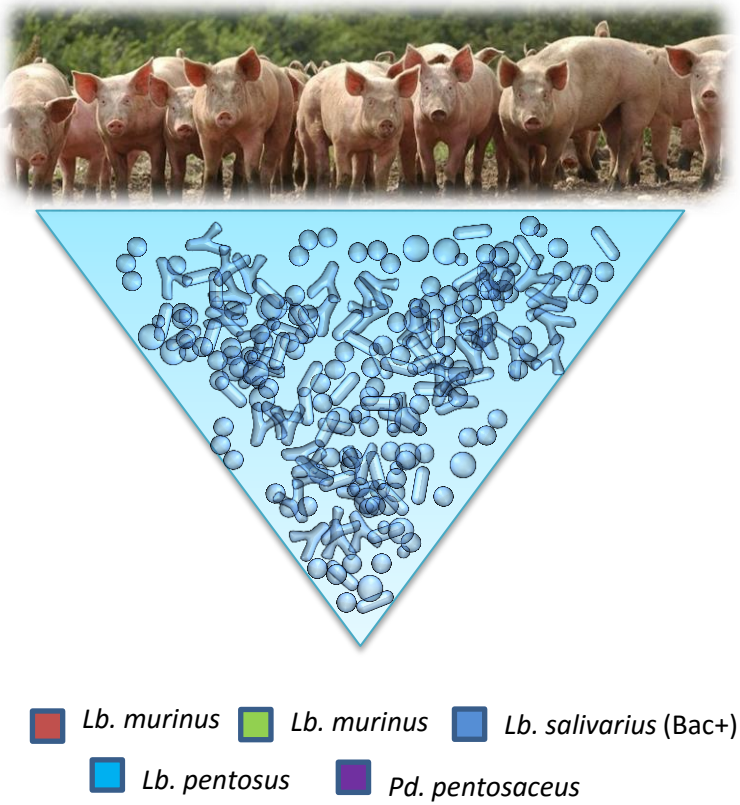
- Isolated 10,000 strains from sero-converted *Salmonella*–free pigs in infected herd
- Characterised strains for *in vitro* anti-*Salmonella* activity
- Identified 5 strains with *in vitro* efficacy (Five Live consortium)
- Performed an animal trial with deliberate infection (N=10)

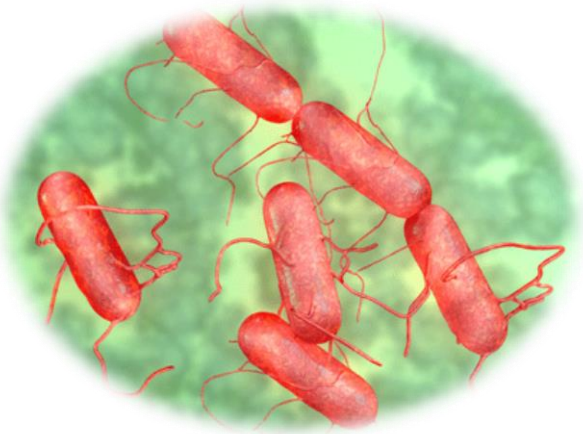


'5-live'

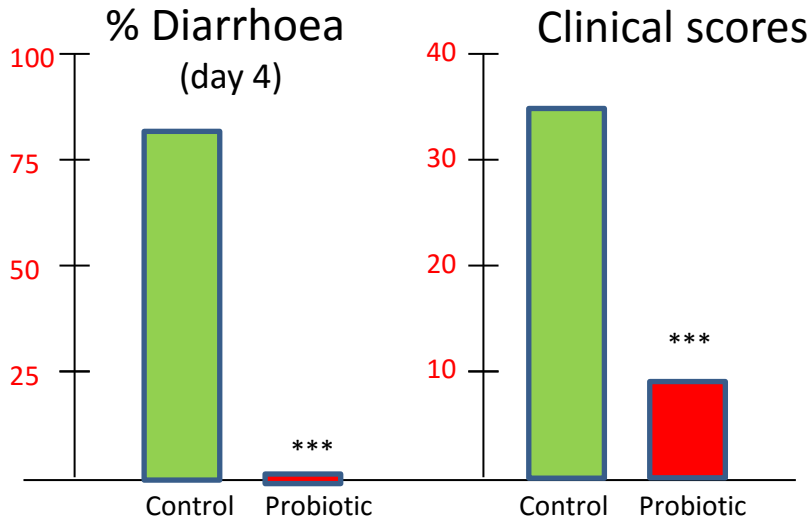
Casey et al. 2007 Appl. Environ. Microbiol. 73:1858-1863 Walsh et al. 2008 FEMS Microbiol. Ecol. 64:317-327

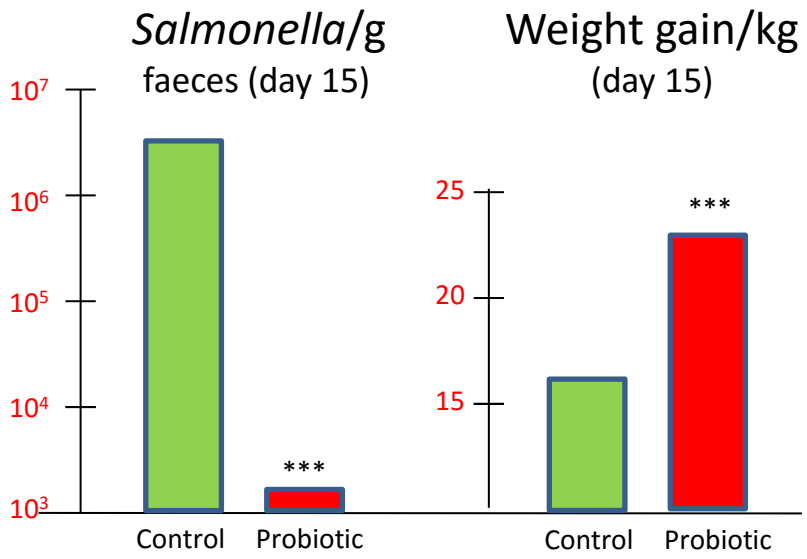
Trial

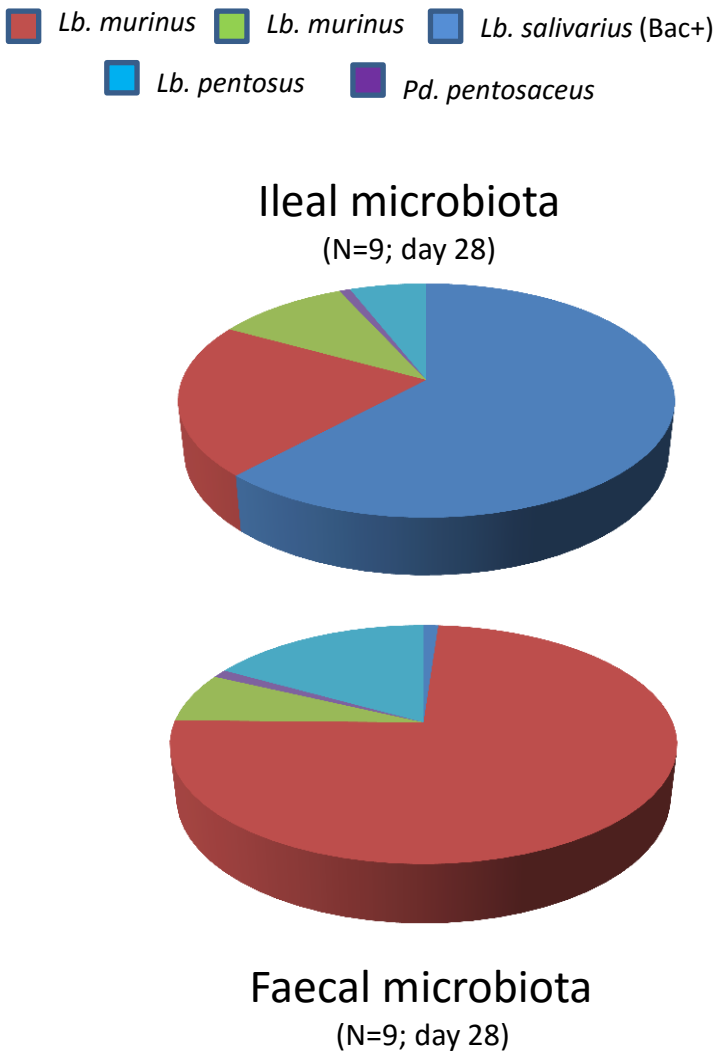
- 21 crossbred (Large White x Landrace) weaned pigs
- Trained to drink milk
- *Salmonella*-free status confirmed
- 100ml probiotic or control milk daily for 30 days. Pigs receiving probiotic culture @ 4 x 10^{10}
- Challenged orally on day 6, 7, 8 with 1 x 10^{8} *Salmonella* Typhimurium PT12



Result



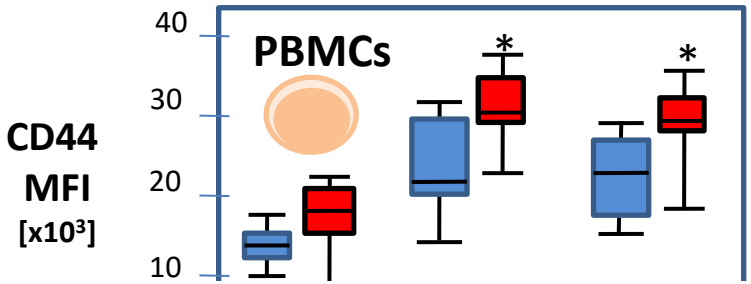




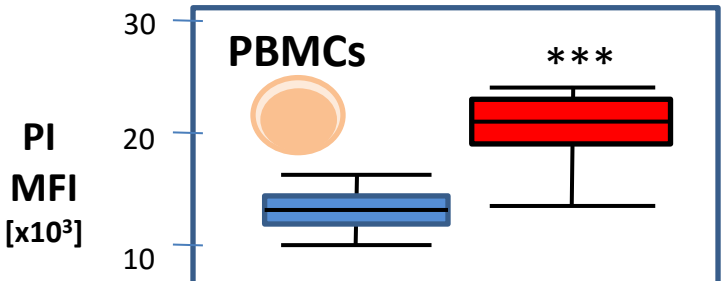
Casey et al. 2007 Appl. Environ. Microbiol. 73:1858-1863 Walsh et al. 2008 FEMS Microbiol. Ecol. 64:317-327

Probiotic feeding trial (without infection)

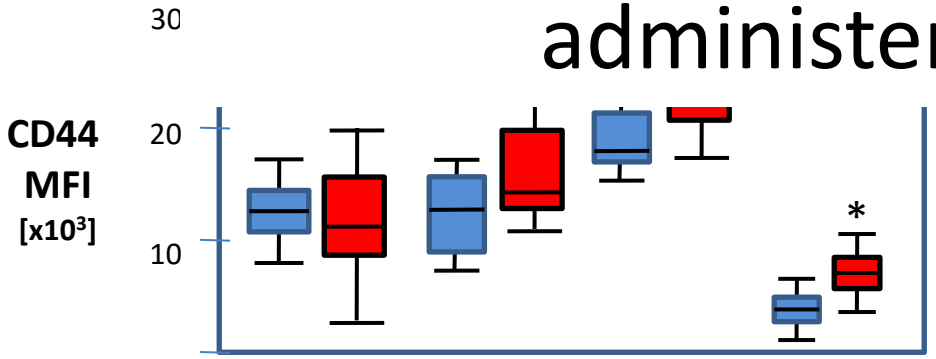
CD44 EXPRESSION

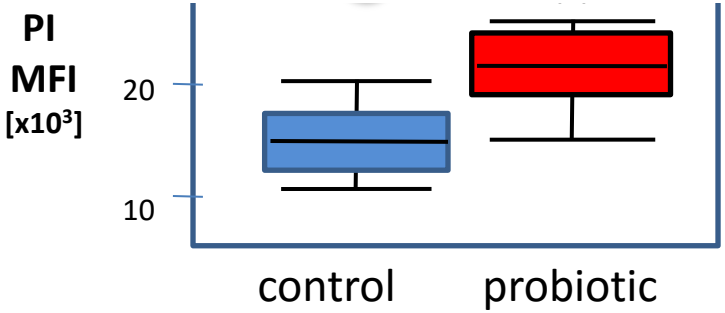


CYTOTOXICITY (v SV40 tumor cells)

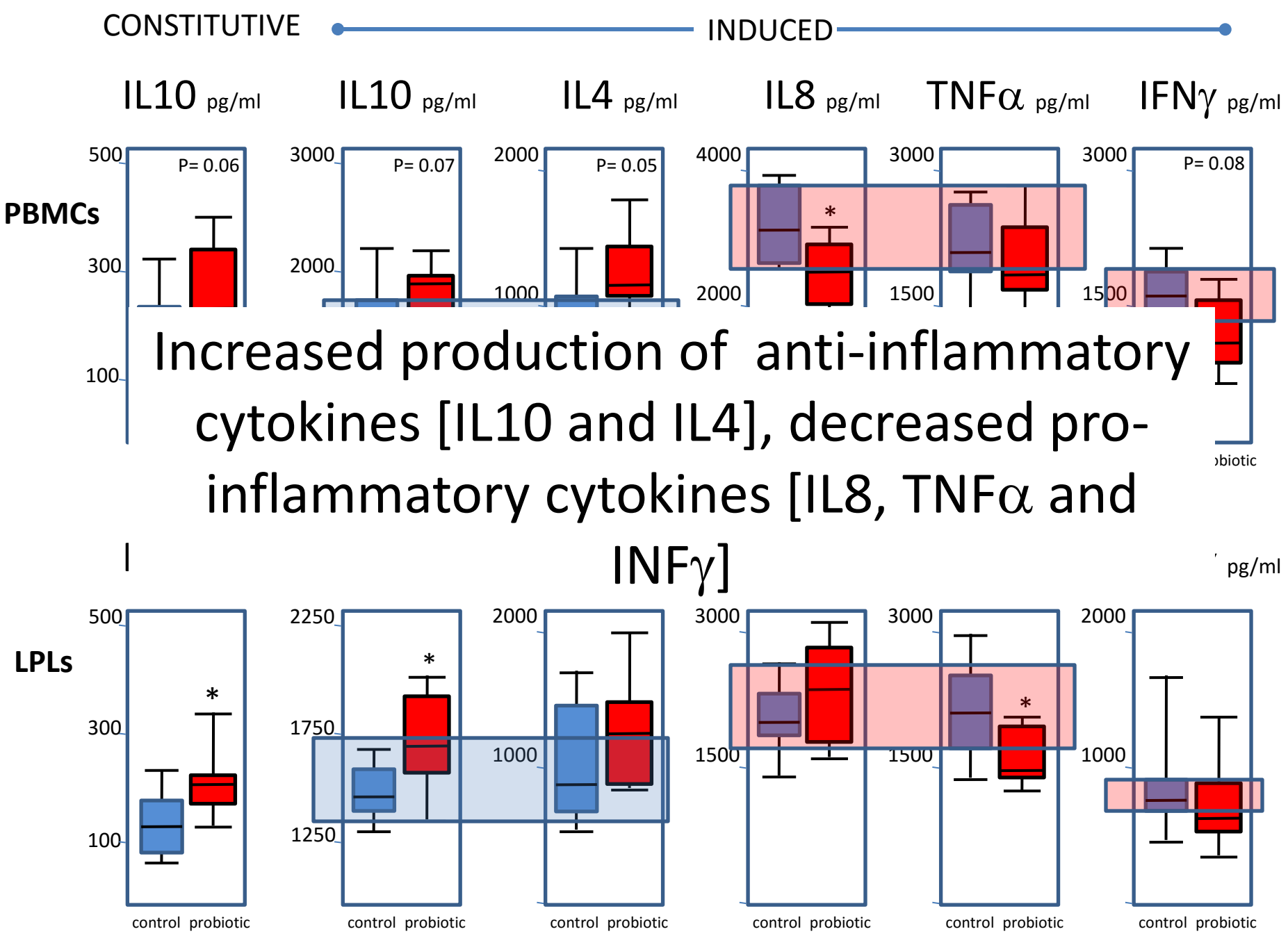


Slight but significant activation and increased cytotoxicity of T-cells in pigs administered probiotic



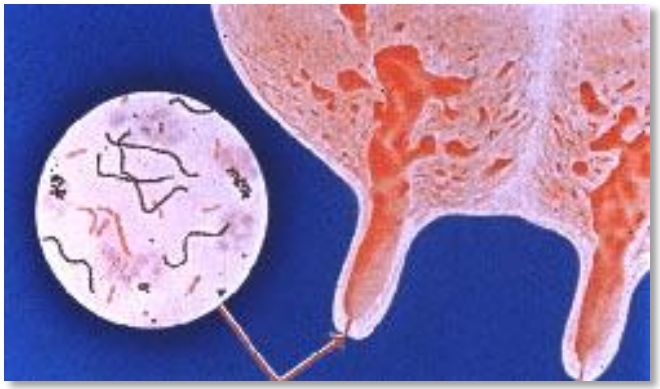


CD4+:Helper T cells; CD4+CD8+: naïve/memory T cells; CD8+: Cytotoxic T cells; CD3+CD4-CD8-: γδ-T cells


CONSTITUTIVE
INDUCED
IL10 pg/ml
IL10 pg/ml
IL4 pg/ml
IL8 pg/ml
TNFα pg/ml
IFNγ pg/ml
PBMCs
P= 0.06
P= 0.07
P= 0.05
P= 0.08
Increased production of anti-inflammatory cytokines [IL10 and IL4], decreased pro-inflammatory cytokines [IL8, TNFα and INFγ]
pg/ml
LPLs
control probiotic

Probiotic 3: Mastitis In Cows

- Inflammation of the udder
- Most persistent disease in dairy cows
  - *St. aureus*
  - *S. dysgalactiae*
  - *E. coli*
- Treatment via administration of antibiotics – leads to withdrawal of milk

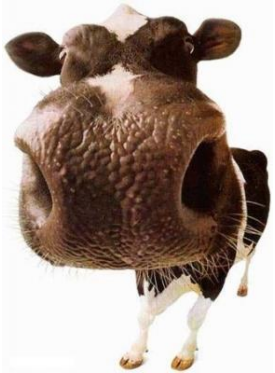


Trials

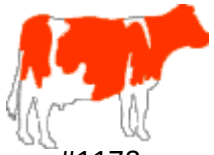
TRIAL 1

11 animals were enrolled which were suffering from severe mastitis but had not responded to antibiotic treatment.

Each infected quarter was infused with *Lactococcus lactis* DPC 3147, a harmless organism normally used in cheese manufacture.



Single application of a *Lactococcus lactis* strain in teat canal relieved clinical symptoms within 2 -3 days



#1178

#1154

#1850

Results compare to Antibiotic

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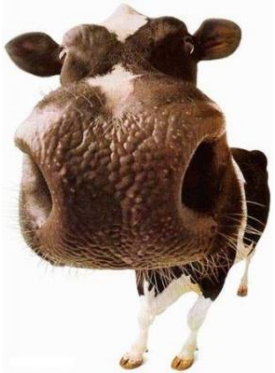
Interfacing Food & Medicine
http://apc.ucc.ie

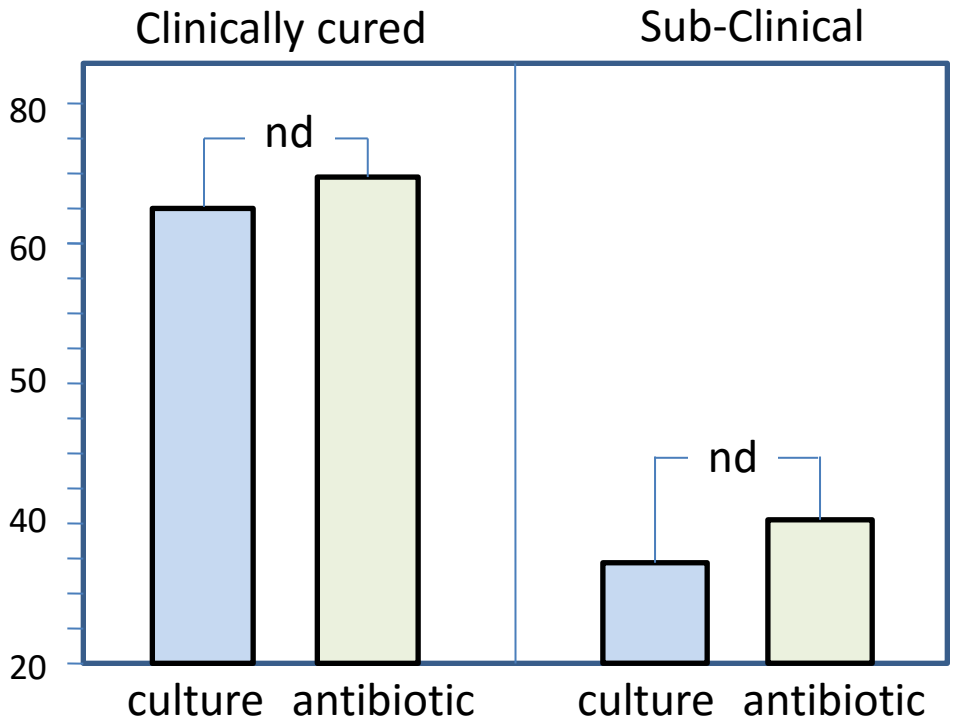
TRIAL 2: Compared live culture against leading antibiotic treatment (N=25)

Clinical cure rates (overall):

L. lactis	64%	(16/25)
Antibiotic	72%	(18/25)

ENTERPRISE IRELAND
where innovation means business





Crispie et al. J. Dairy Res. 75:374

Klosterman et al. J. Dairy Res. 75:365

Hypothesis: Infusion with *Lactococcus lactis* provokes a localised innate inflammatory response

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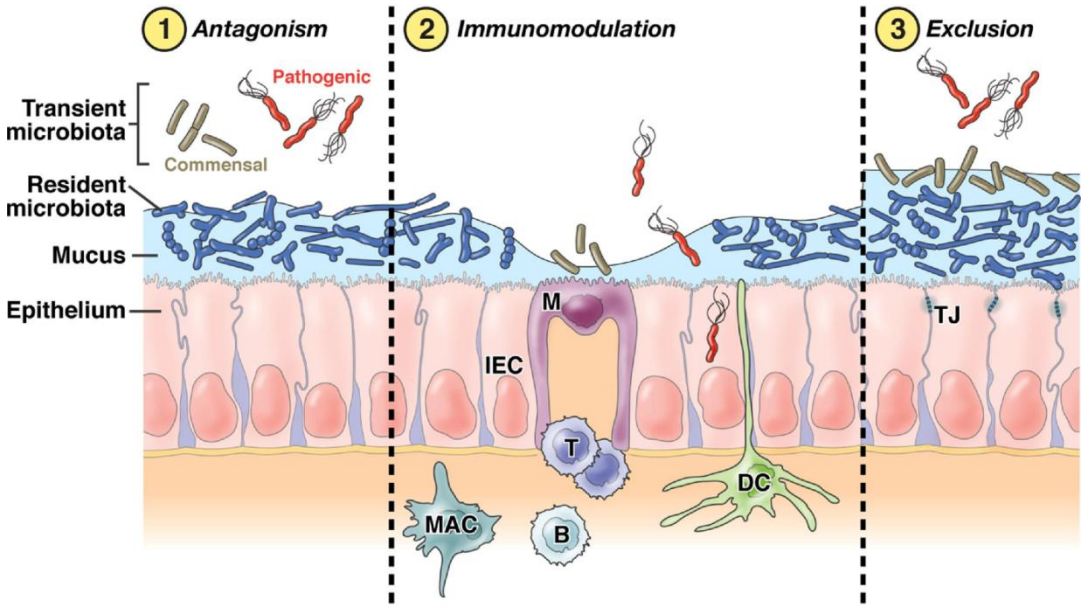


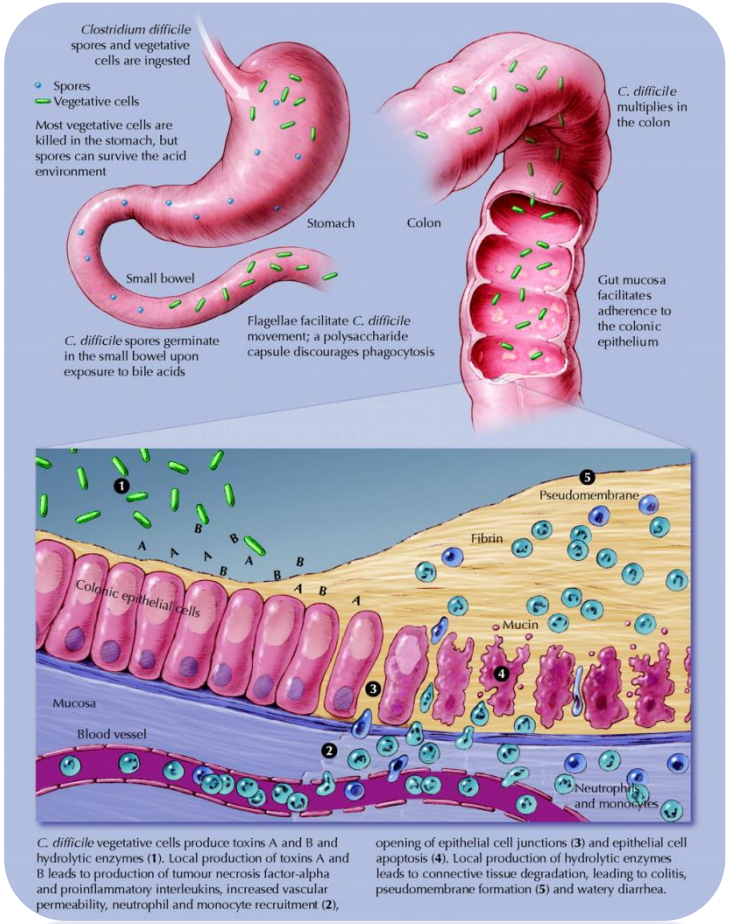
Characterised by:

- Recruitment of neutrophils and lymphocytes
- Increased acute phase proteins, and…
- … elimination of mastitic pathogens and resolution of inflammation

Clostridium difficile associated diarrhoea

Gram positive, anaerobic, sporeforming bacterium

- Antibiotic resistant 'superbug'
- Occurs when the microbiota is disrupted by antibiotics
- Often has a lethal outcome
- Relapses are common
- Evidence for interventions aimed at the microbiota



Probiotic 4: anti *C. diff* live strain

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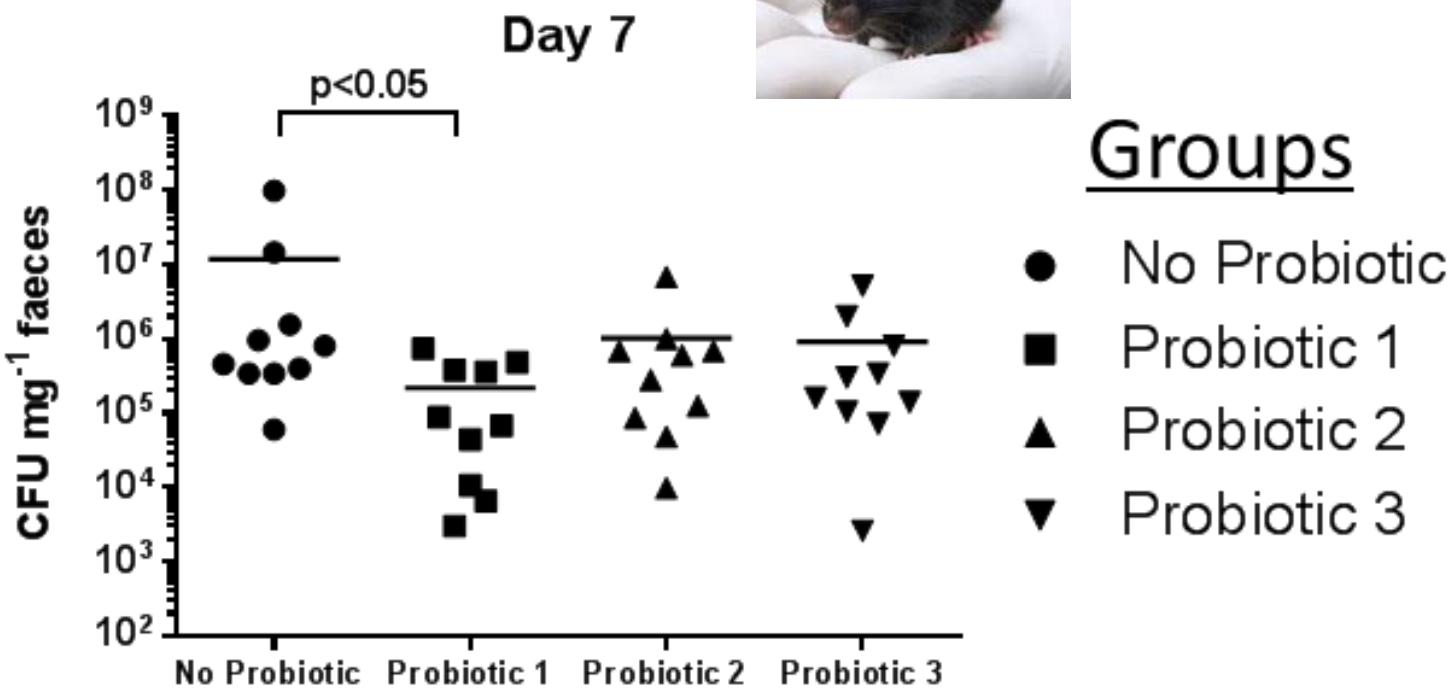
Initial screen of >1500 bacteria from
Human and animal origin
60 then in co-culture screen

3 strains

In vivo mouse model:

- *C. difficile* (5 x 10^5 CFU/mouse)
- Probiotic daily (1 x 10^8 CFU/mouse)



C. difficile detected in mouse faeces (CFU mg^{-1} faeces) during probiotic administration at 7 days

Thuricin CD (anti *C. difficile*)

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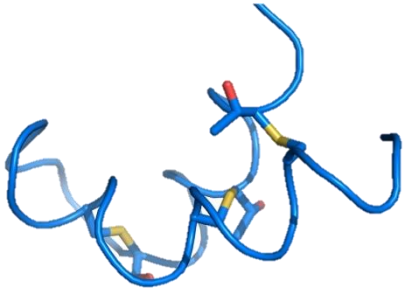
http://apc.ucc.ie

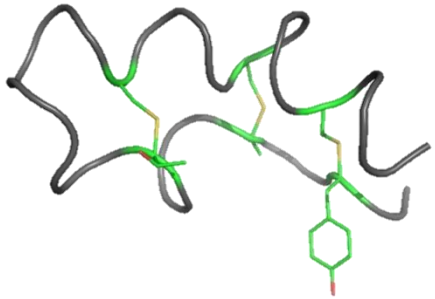
Novel, narrow spectrum, *anti-Clostridium difficile* bacteriocin.

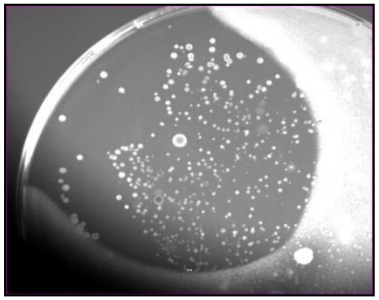
Identified in screening programme in APC2. Patented 2007, licenced (2009). IP recovered in 2014.

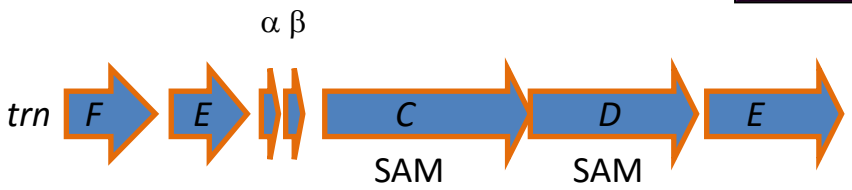
Produced by *Bacillus thuringiensis* – food grade, sporeforming Gram positive bacterium

Thuricin CD: Two peptides, unusual sulphur to α-carbon (SAC) linkages (sactibiotic)









Faecal fermentations (ex vivo colon)

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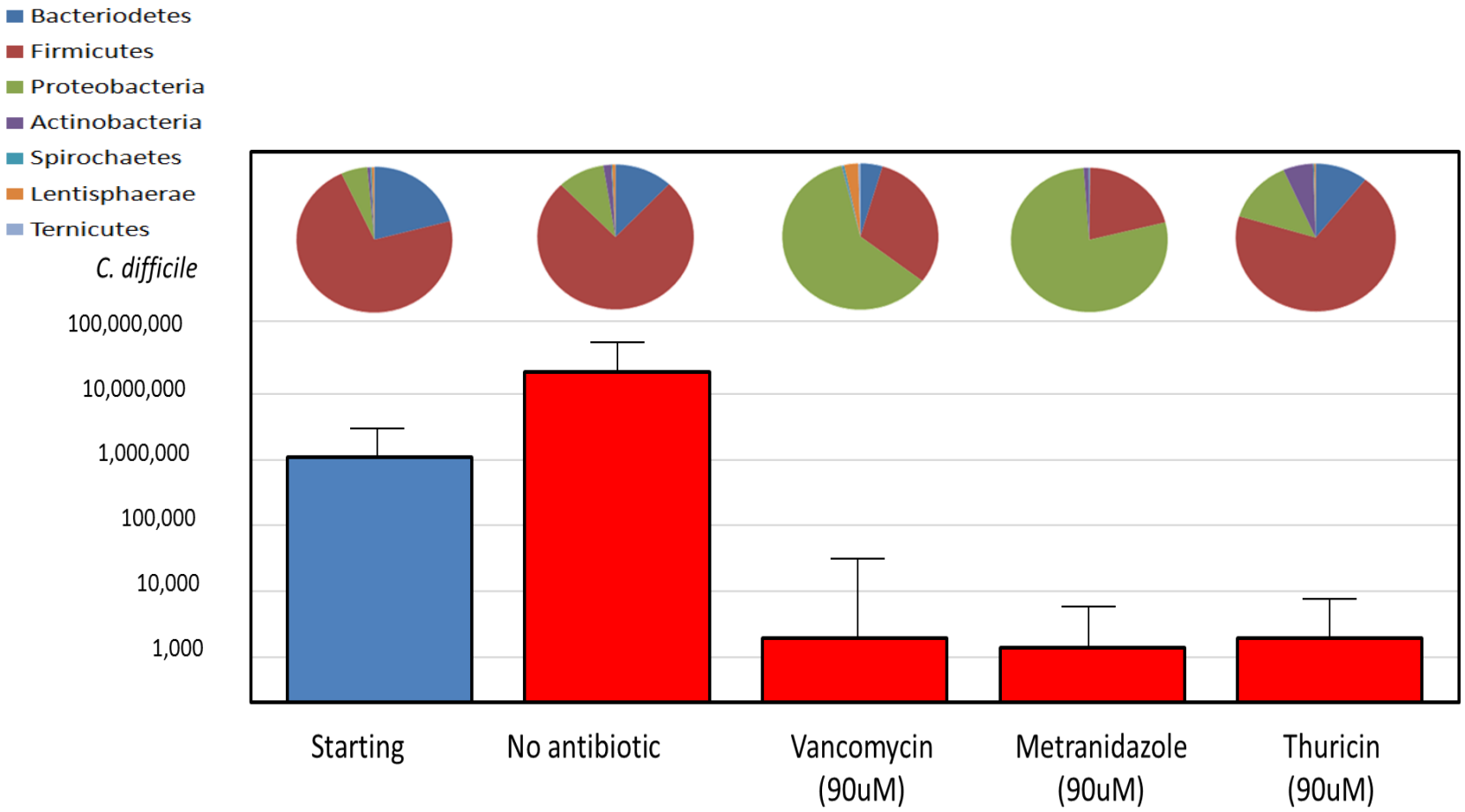
Equally effective as Vancomycin and metranidazole are effective at reducing *C. difficile*, but without collateral damage



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

Thuricin CD *in vivo*

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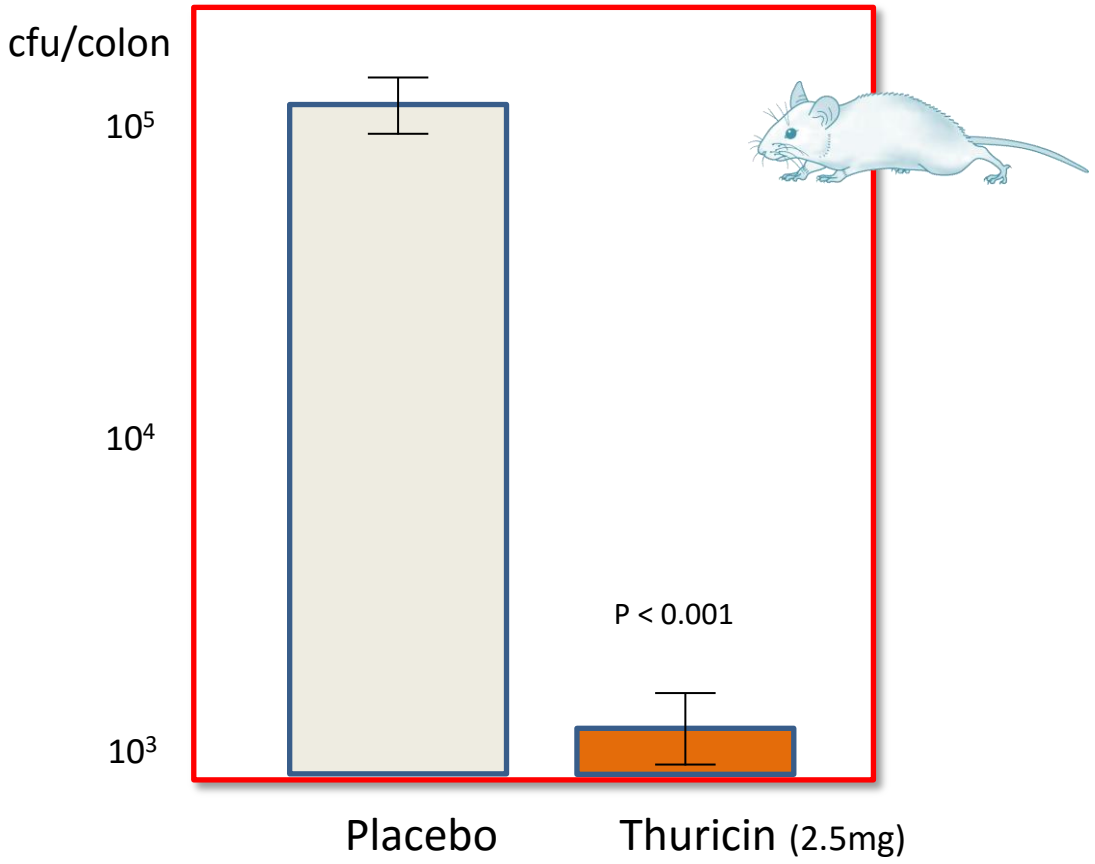
Mice rectally inoculated with *Clostridium difficile*

Thuricin added 60 minutes post-inoculation

Mice sampled 60 minutes post-treatment

Colonic contents plated for *C. difficile*

Effect of Thuricin CD added rectally post inoculation with *C. difficile* ribotype 027



Rea, Casey et al., 2015

New Spin Out Company

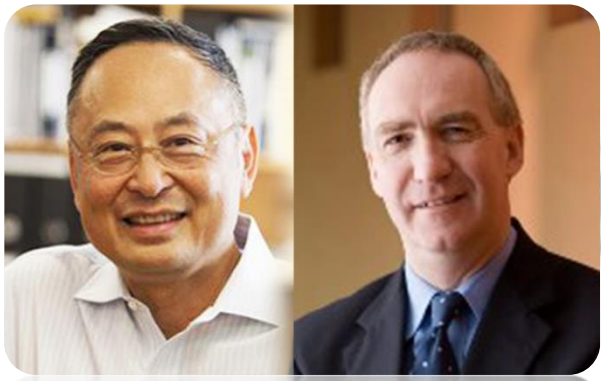
Microbiome Institute
Interfacing Food & Medicine
http://apc.ucc.ie

New Spin-out Company

€3 Million Investment

Licencing Thuricin CD

MORNINGSIDE



Gerald Chan

Ronnie Farquhar (former VP Cubist Pharma)

Artugen Therapeutics

Mission: To discover, develop and commercialise novel antimicrobials for human therapeutic use

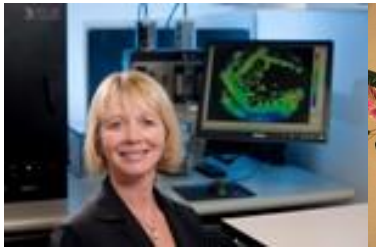
Room for Probiotics in a Crowded Space

- Microbiome Research Rapidly Expanding
- Microbiome associated with Health and Aging
- Probiotics - large biomass portion of upper GIT
- Understanding mechanisms critical

teagasc

ucc
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

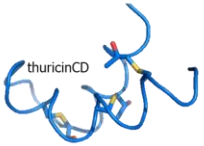
apc
Alimentary Pharmabiotic Centre
Interfacing Food & Medicine



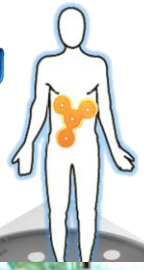


Catherine Stanton Noel Caplice Paul O'Toole Colin Hill and Paul Cotter

Lis London
Paul Ryan
Elaine O'Brien
Eoin Barrett



Cholesterol




Fergus Shanahan and Tony Ryan

Mary Rea
Paula O Connor
Fergus Collins
Evelyn Clayton
Des Field
Lorraine Draper
Pat Casey

Cork Bacteriocin Group

NDP NATIONAL DEVELOPMENT PLAN

sfi science foundation ireland

THE DEPARTMENT OF AGRICULTURE & FOOD
AN ROINN TALMHAÍOCHTA AGUS BIA

DPC6426 Alters the Caecal Microbiome

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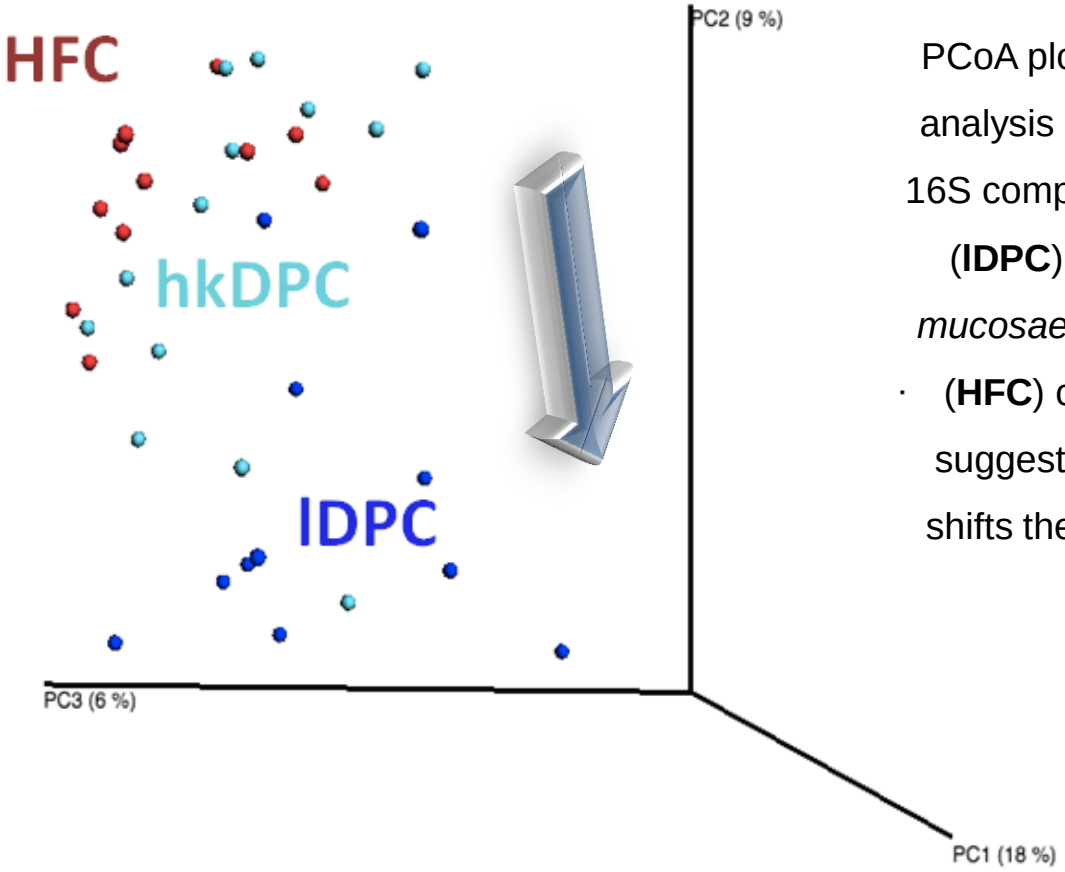

PCoA plot depicting unweighted Unifrac analysis depicting the beta-diversities of 16S compositional sequencing of the Live (**lDPC**) and Heat-Killed (**hkDPC**) *Lb. mucosae* DPC6426, and high-fat control (**HFC**) caecal microbiome. The figure suggests that solely lDPC significantly shifts the microbiome composition from that of the HFC.