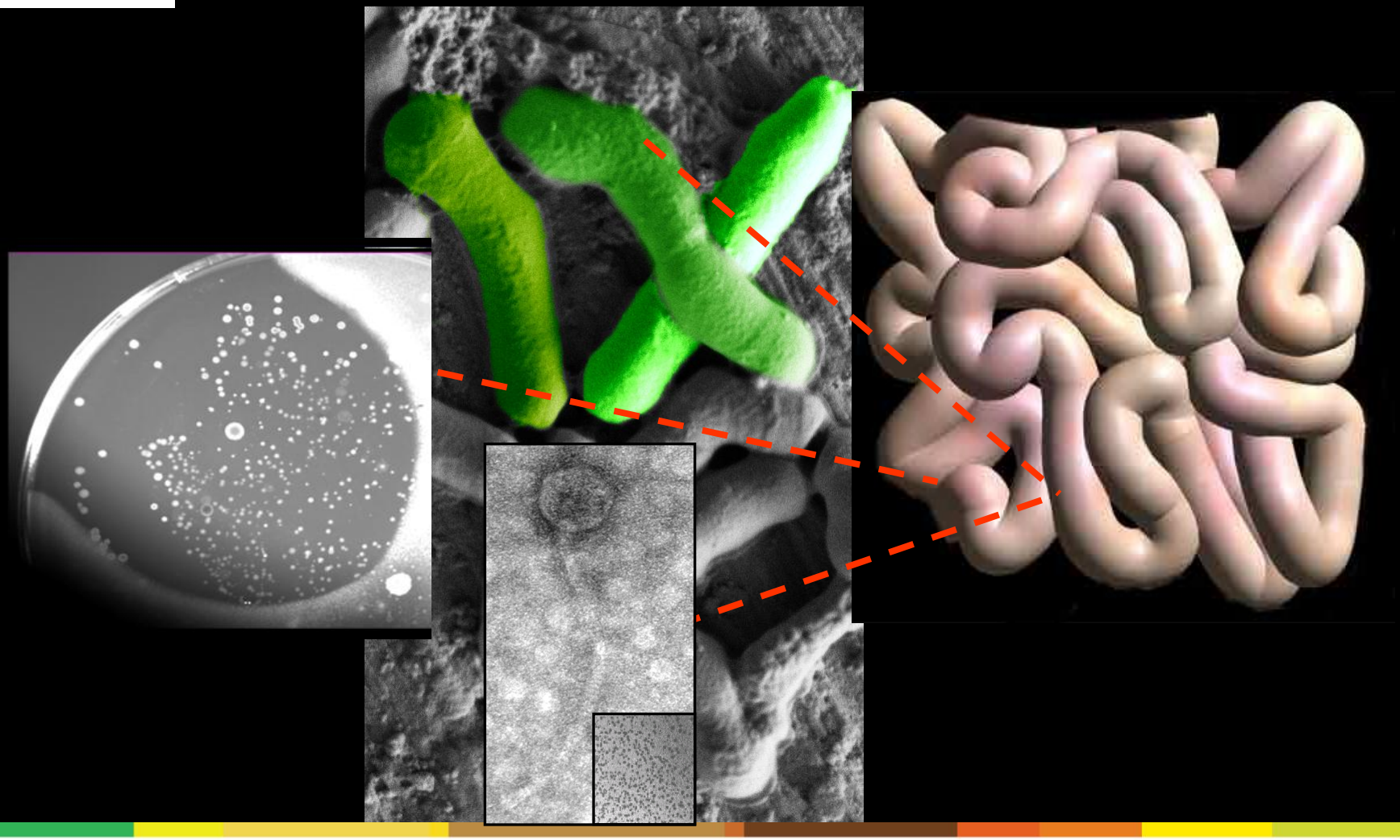
APC
Alimentary Pharmabiotic Centre
Interfacing Food and Medicine

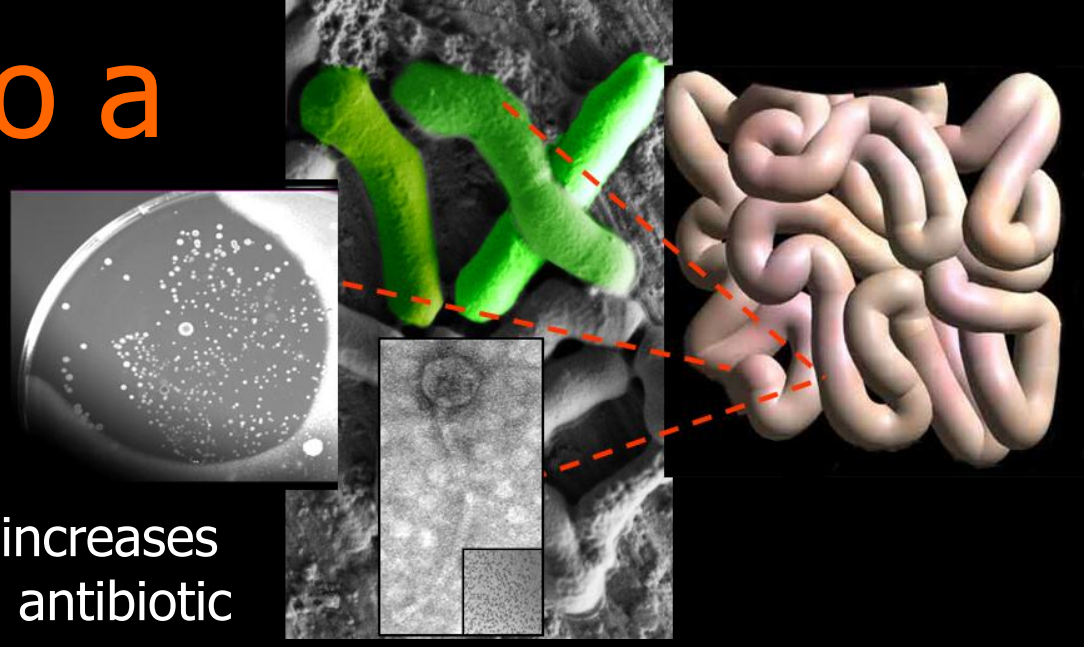
Probiotics, Bacteriocins and Bacteriopgahe for limiting *C. difficile* infection



teagasc
Agriculture and Food Development Authority

The Irish Agriculture and Food Development Authority

Gut Soutions to a Gut Problem



Gut Problem: *C. difficile* carriage increases with gut inflammation, aging and antibiotic usage

Gut Solution: Pharmabiotics

Pagebiotics:

Probiotics:

Bacteriocins:

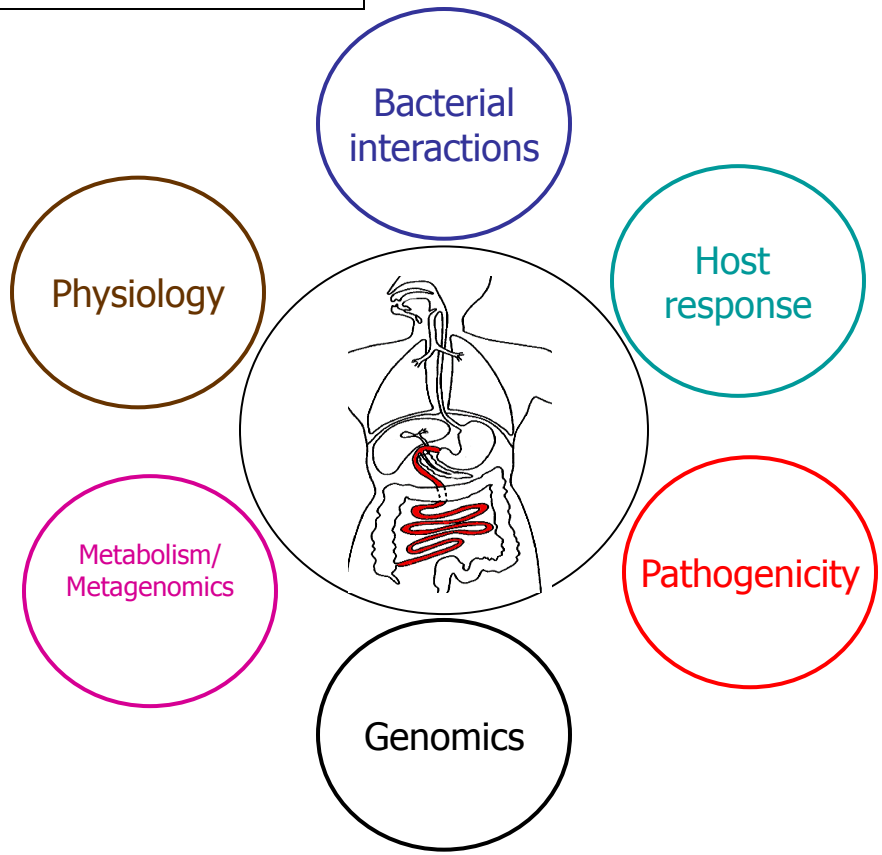
Prospects for managing infection using pharmabiotic solutions

From bugs to drugs

Alimentary Pharmabiotic Centre
Interfacing Food and Medicine

Fergus Shanahan

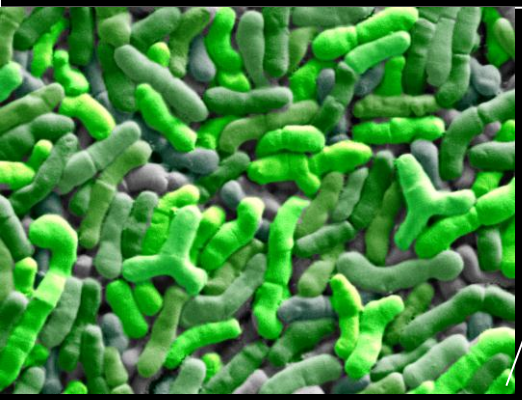
science foundation ireland

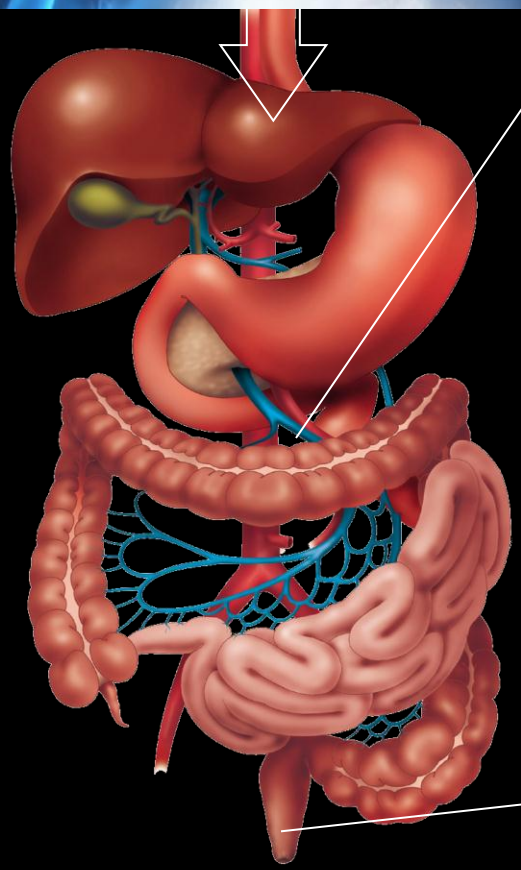


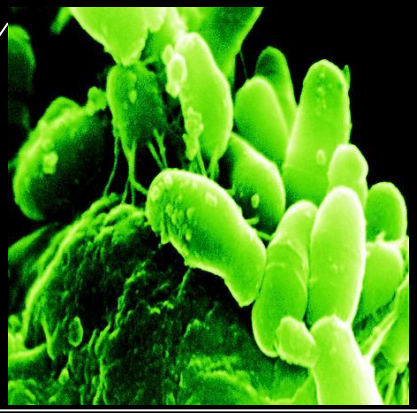
Probiotics V Resident Microbiota

Probiotics

- Probiotics have mainly transient effects – "tourists"
- Anti-infective Immuno-modulatory effects
- Out-compete pathogens







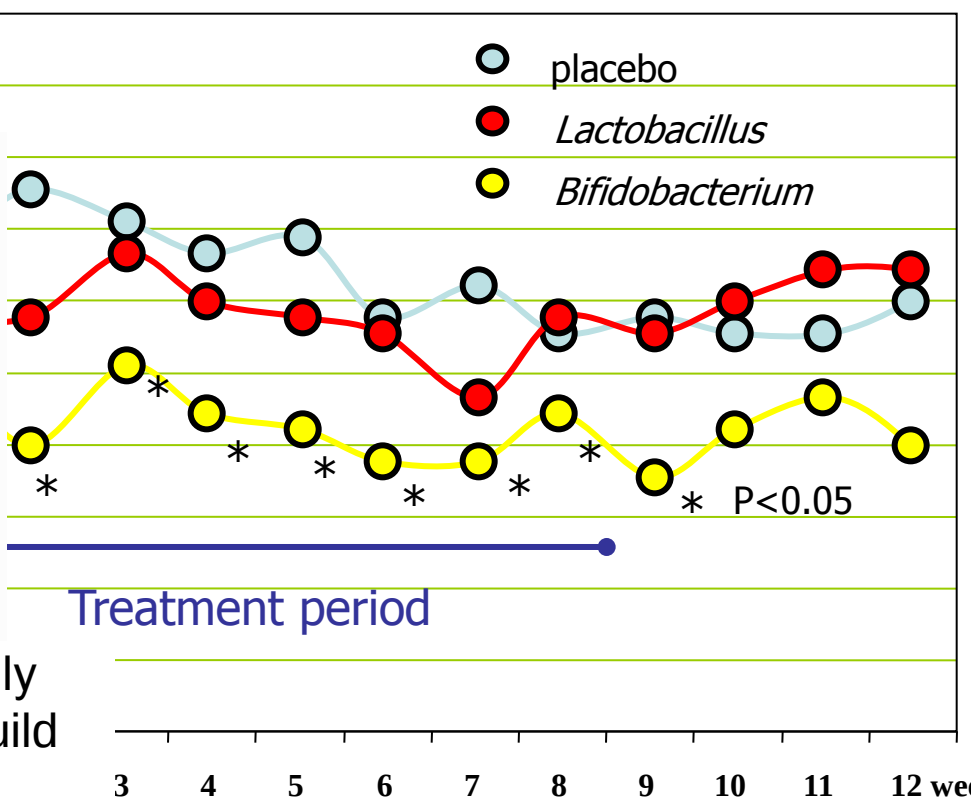
Resident microbiota

- Anti-infective/Immuno-modulatory effects
- Key long term role in digestion
- Production of essential nutrients
- Production of bioactive substances

Probiotic IBS Treatment Study

Alimentary Pharmabiotic Centre
nd Medicine

easy-to-swallow capsule that you take just once a day, every day to help even out the ups and downs of common digestive upsets such as constipation, diarrhea, abdominal discomfort, urgency, gas, and bloating.

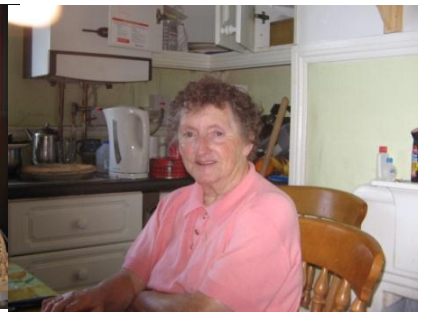


Align containing Bifantis™ is a daily probiotic supplement that helps build and maintain a healthy, balanced digestive system when taken daily. T

Mahoney et al, Gastro 2006

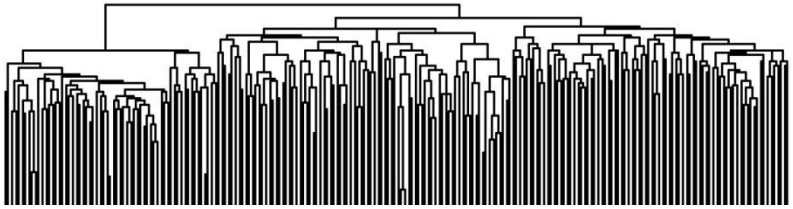
A-V4
B-V4
C-V4
D-V4

eldermet



>1,000 species

Community strata are separated by microbiota

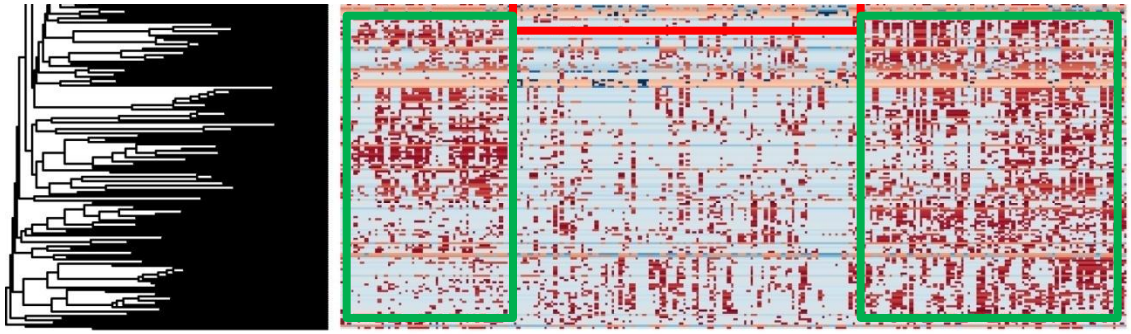


ARTICLE

doi:10.1038/nature11319

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], Mairead 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]



232 subjects

Sample Index

Cummunity → Longstay

Paul O'Toole

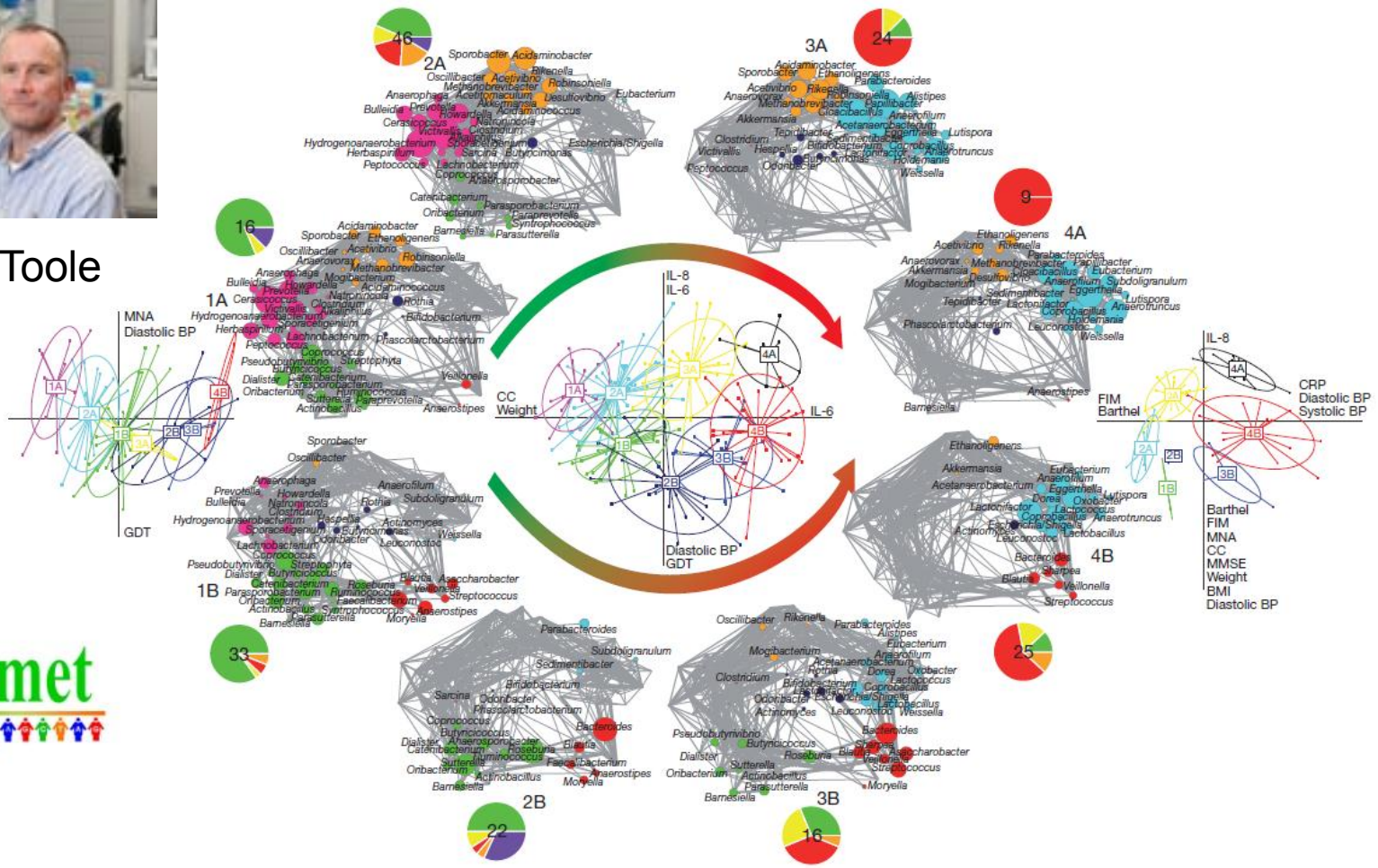


Figure 4 | Transition in microbiota composition across residence location is mirrored by changes in health indices. The PCoA plots show 8 groups of subjects defined by unweighted UniFrac microbiota analysis of community subjects (left), the whole cohort (centre), and long-stay subjects (right). The main circle shows the Wiggum plots corresponding to the 8 groups from whole-cohort analysis, in which disc sizes indicate genus over-abundance relative to background. The pie charts show residence location proportions (colour coded as in Fig. 1c) and number of subjects per subject group. Curved arrows indicate transition from health (green) to frailty (red). FIM, functional independence measure; MNA, mini nutritional assessment; GDT, geriatric depression test; CC, calf circumference; CRP, C-reactive protein; IL, interleukin; BP, blood pressure; MMSE, mini-mental state examination.

JCM Journals.ASM.org 0095-1137/12/$12.00 Journal of Clinical Microbiology p. 867–875

Clostridium difficile Carriage in Elderly Subjects and Associated Changes in the Intestinal Microbiota

Mary C. Rea,[a,b] Orla O'Sullivan,[a,b] Fergus Shanahan,[b,c] Paul W. O'Toole,[b,d] Catherine Stanton,[a,b] R. Paul Ross,[a,b] and Colin Hill[b,d]

Teagasc Food Research Centre, Moorepark, Fermoy, County Cork, Ireland[a]; Alimentary Pharmabiotic Centre, University College, Cork, Ireland[b]; Department of Medicine, University College, Cork, Ireland[c]; and Department of Microbiology, University College, Cork, Ireland[d]

Ir J Med Sci
DOI 10.1007/s11845-009-0361-1

ORIGINAL ARTICLE

Asymptomatic carriage of *Clostridium difficile* in an Irish continuing care institution for the elderly: prevalence and characteristics

J. Ryan, C. Murphy , C. Twomey, RP Ross, MC Rea, J MacSharry B. Sheil, F. Shanahan

The American Journal of GASTROENTEROLOGY VOLUME 104 | MAY 2009 www.amjgastro.com

The Vexed Relationship Between *Clostridium Difficile* and Inflammatory Bowel Disease: An Assessment of Carriage in an Outpatient Setting Among Patients in Remission

Evelyn M. Clayton, BSc[1,2], Mary C. Rea, BSc, MSc[1,2], Fergus Shanahan, MD, BSc, FRCP (UK), FRCPI, FACP, FRCP(C)[2], Eamonn M.M. Quigley, MD, FRCP, FACP, FACG[2], Barry Kiely, BSc, PhD[3], Colin Hill, BSc, PhD[2] and R. Paul Ross, BSc, PhD[1,2]

Journal of Medical Microbiology (2012), 61, 1290–1294 DOI 10.1099/jmm.0.040568-0

Carriage of *Clostridium difficile* in outpatients with irritable bowel syndrome

Evelyn M. Clayton,[1,2] Mary C. Rea,[1,2] Fergus Shanahan,[2,3] Eamonn M. M. Quigley,[2,3] Barry Kiely,[4] R. Paul Ross[1,2] and Colin Hill[2,5]

Eldermet study (65years +)

Healthy in community

n = 123 **1.6%**

Out-patients

n = 43 **9.5%**

Short or long stay hospital

n = 151 **21%**

Ribotypes isolated:

027, 078, 014, 018, 072, 220, 026, 216, 010 308, 050, 002

IBD Study

Ulcerative colitis/Crohns

n = 122 **8.2%**

Controls

n = 99 **1.0%**

Ribotypes isolated

015, 005, 020, 062, 050, 003

IBS Study

IBS outpatients

n = 87 **5.7%**

Controls

n = 88 **1.1%**

Ribotypes isolated

050, 005, 060, 062

Cystic Fibrosis

CF patients

n = 27 **55%**

Ribotypes isolated

001, 014, 002, 039,126,140,078, 010, 046

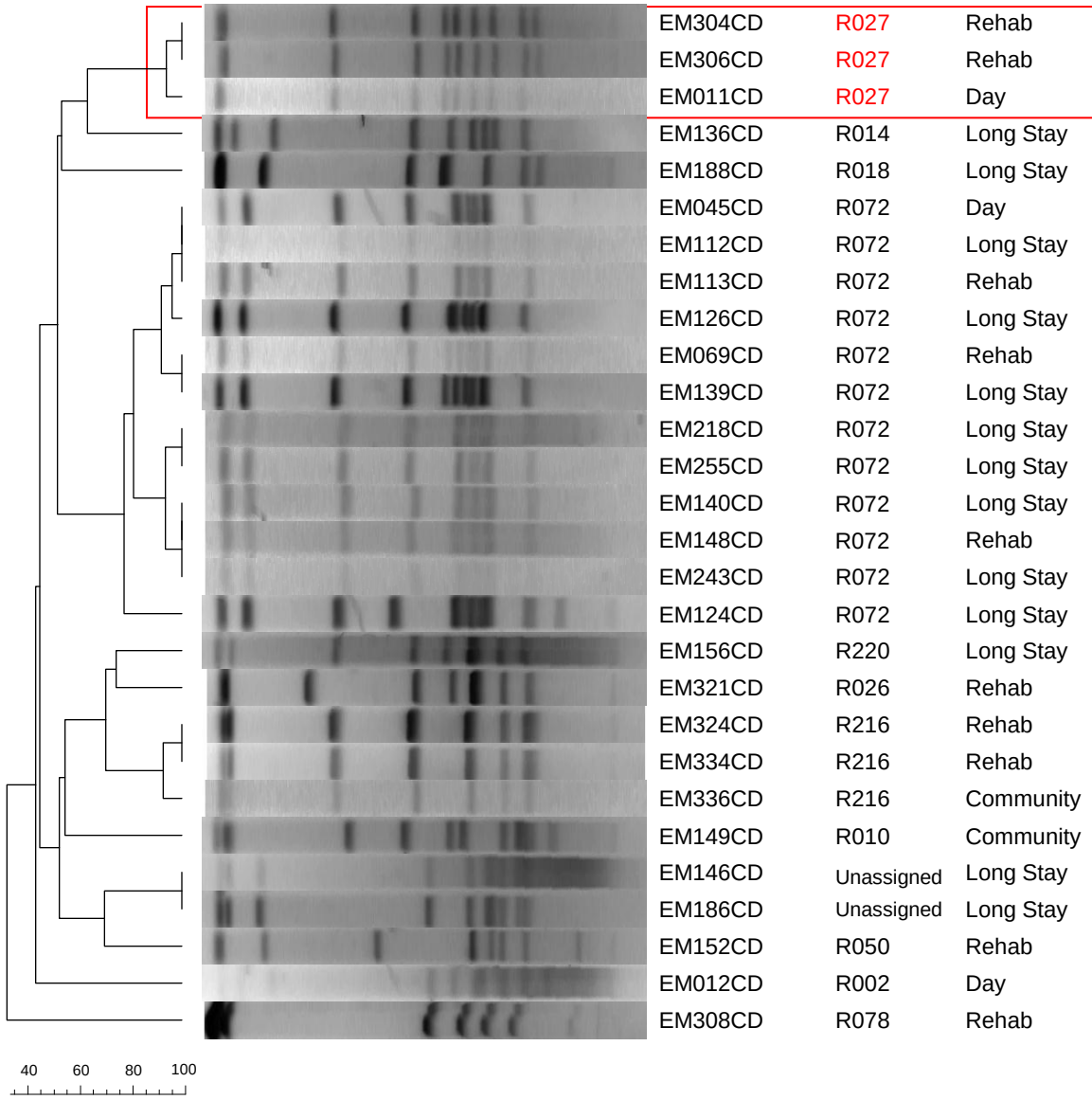
C. difficile strains from the elderly


grow old in Ireland than the
nd Age Action Ireland
of the elderly. Indeed, with
place in September, what
uality of life of our elderly

apidly thanks to advances in
atic increases in life
early one-third of the
enomenon that can also be
d, it is predicted that the 20-
9%) (Table 1). Such a pattern
ealthcare resources and create
therefore vital that efforts are
such as osteoporosis,
and cancer, to ensure that
nd, thus, healthy living.

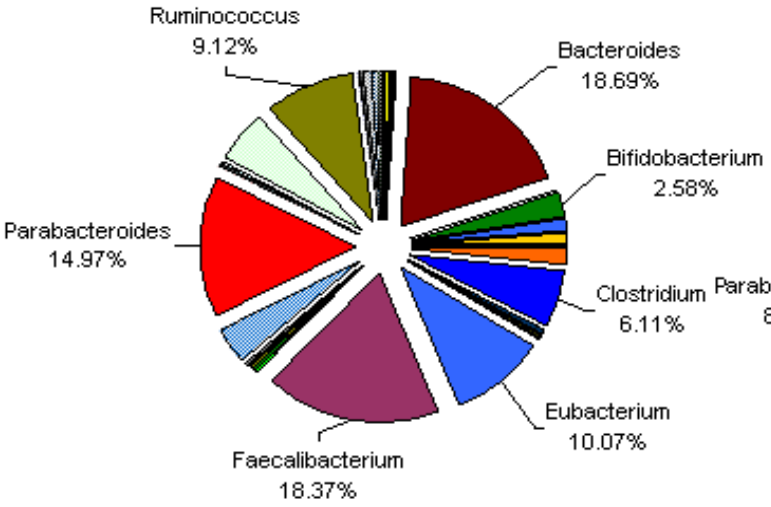
experience the benefits of a
ensuring a healthy ageing
intestinal microbiota is
elationship between gut
e Science
g and
tic
Cork

Mark Wilcox

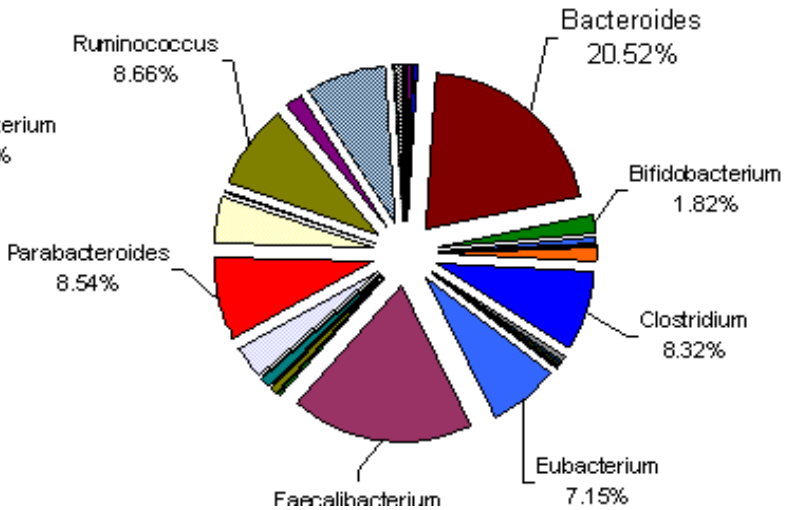


EM304CD	R027	Rehab
EM306CD	R027	Rehab
EM011CD	R027	Day
EM136CD	R014	Long Stay
EM188CD	R018	Long Stay
EM045CD	R072	Day
EM112CD	R072	Long Stay
EM113CD	R072	Rehab
EM126CD	R072	Long Stay
EM069CD	R072	Rehab
EM139CD	R072	Long Stay
EM218CD	R072	Long Stay
EM255CD	R072	Long Stay
EM140CD	R072	Long Stay
EM148CD	R072	Rehab
EM243CD	R072	Long Stay
EM124CD	R072	Long Stay
EM156CD	R220	Long Stay
EM321CD	R026	Rehab
EM324CD	R216	Rehab
EM334CD	R216	Rehab
EM336CD	R216	Community
EM149CD	R010	Community
EM146CD	Unassigned	Long Stay
EM186CD	Unassigned	Long Stay
EM152CD	R050	Rehab
EM012CD	R002	Day
EM308CD	R078	Rehab

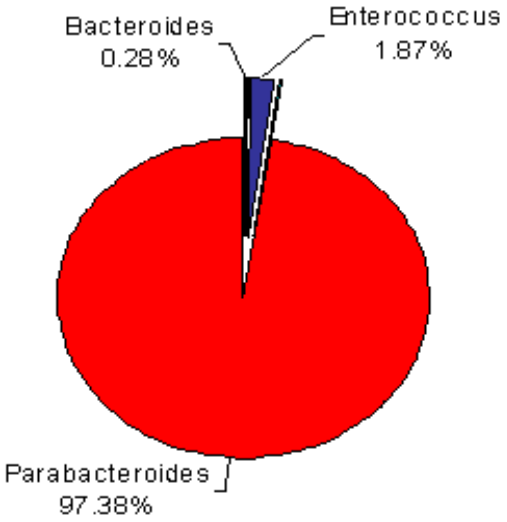
Gut microbiota different with active C. difficile infection

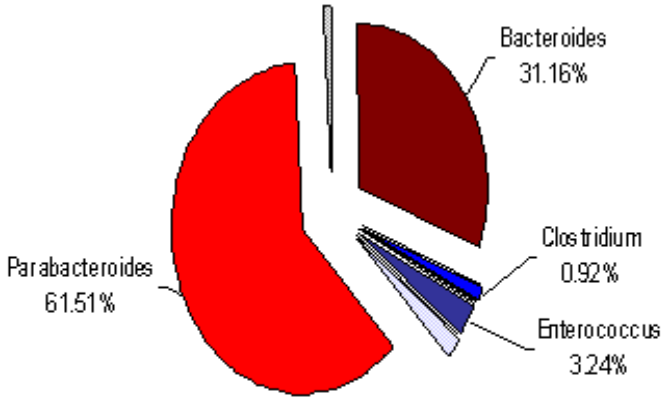


252
Controls



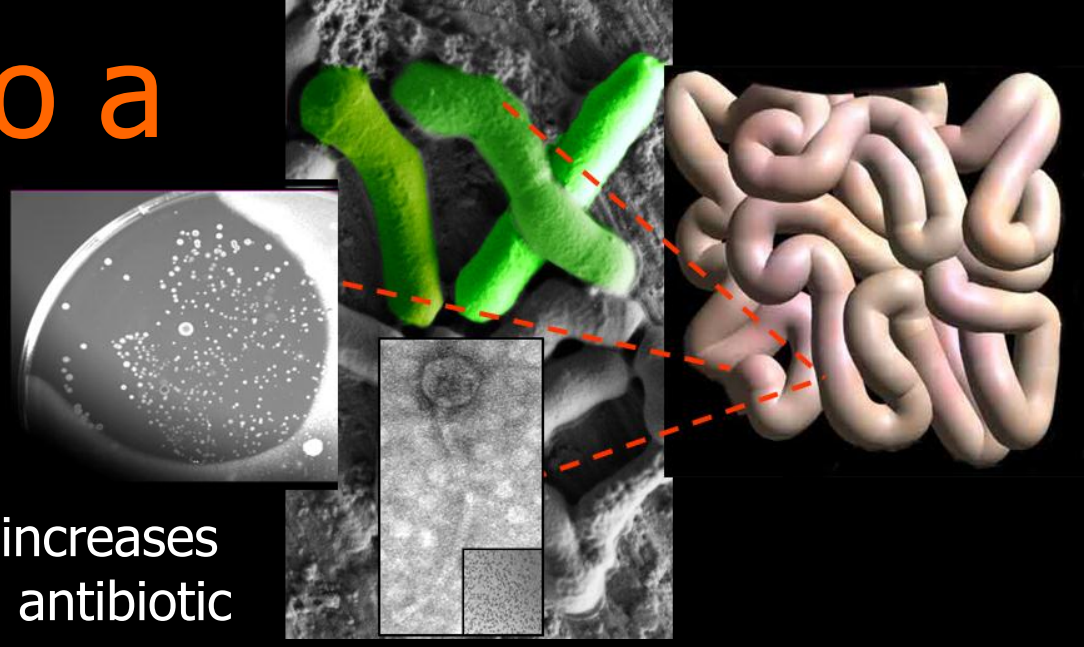
18 asymptomatic





individual subjects with active *C. difficile* infection at the time of sampling

Gut Soutions to a Gut Problem



Gut Problem: *C. difficile* carriage increases with gut inflammation, aging and antibiotic usage

Gut Solution: Pharmabiotics

Pagebiotics:

Probiotics:

Bacteriocins:

Prospects for managing infection using pharmabiotic solutions

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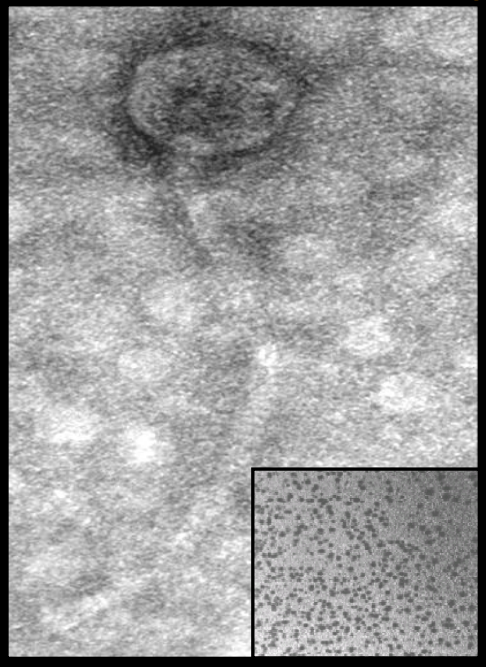
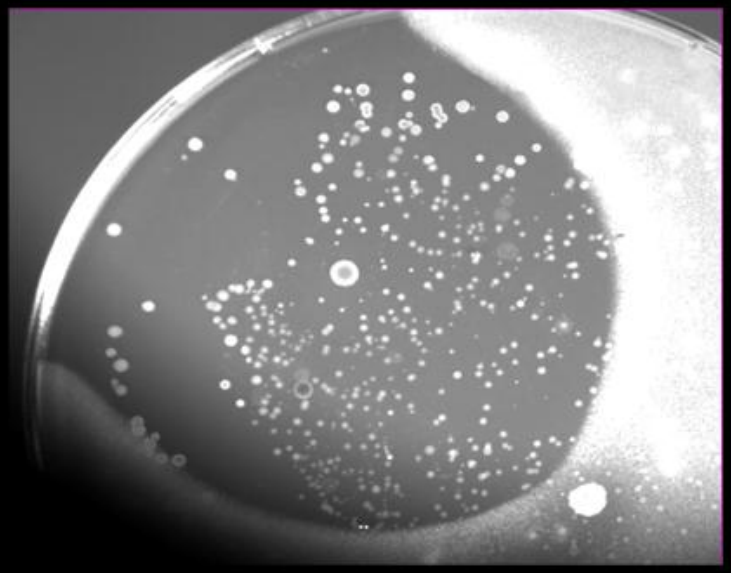
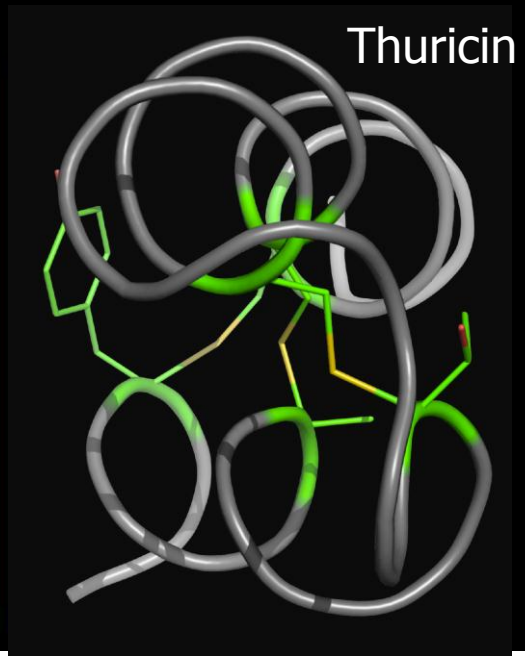
Interfacing Food and Medicine

Gut bacteria produce an almost limitless set of biopactives

Understanding these and the bacteria which produce them is key to understanding probiotic effects



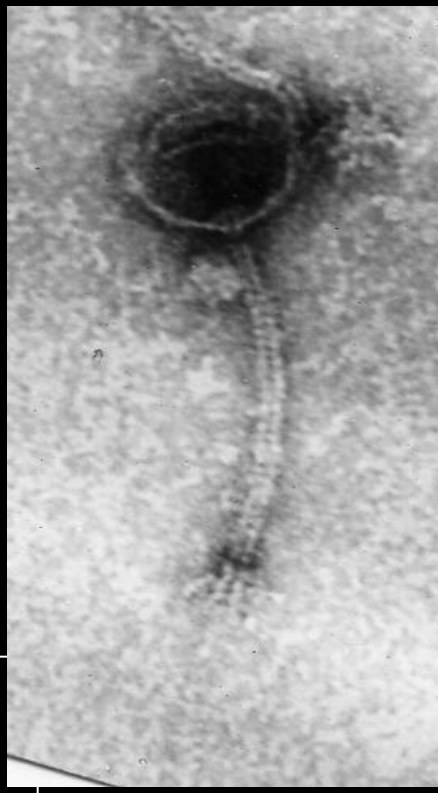




Pharmabiotics: Bioactives from Mining Host–Microbe–Dietary Interactions

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

Bacteriophages (bacterial viruses)



- Obligate intracellular parasites
- Minute particles (0.05 x 0.15μm)
- Protein and DNA

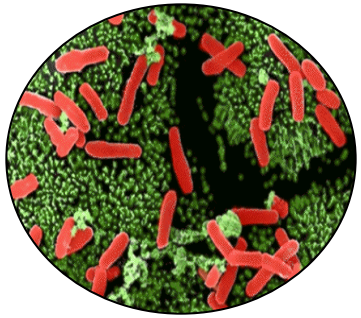
ADVANTAGES

Activity against drug-resistant microorganisms.

high specificity.

self-replicating and self-limiting

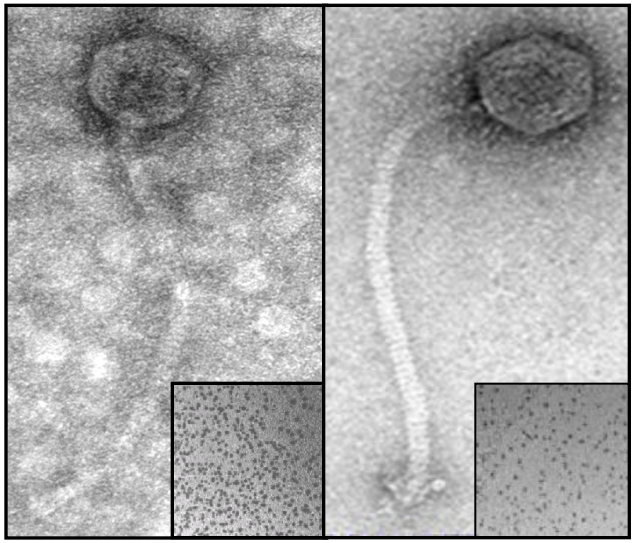
low cost



C. difficile phages induced.....

Siphoviridae family

phiC6356 **phiC6365**

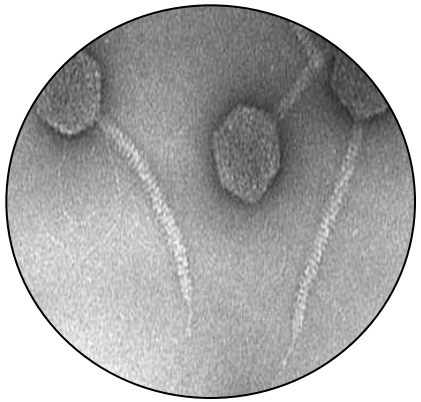


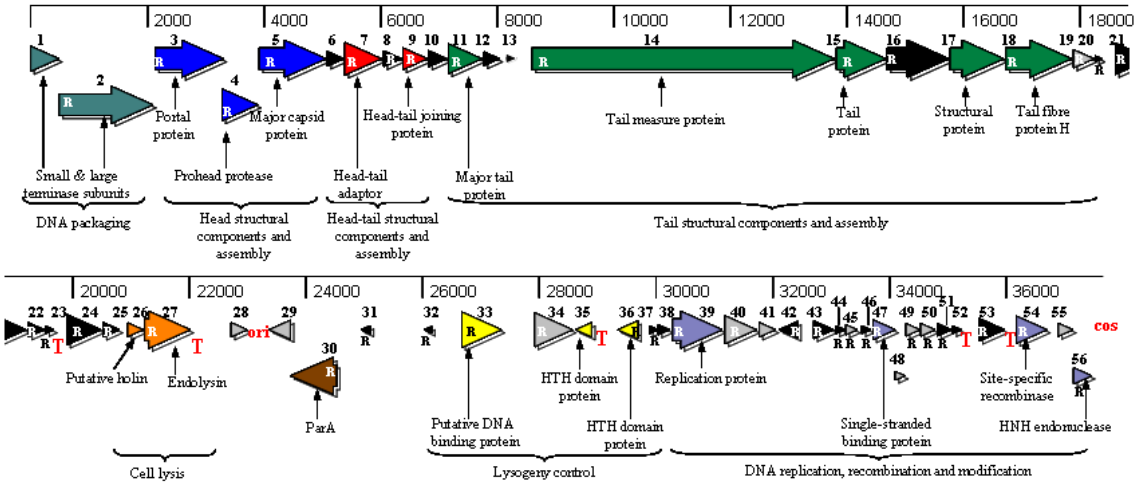
Temperate phages , phiC6356 and phiC6365, induced from clinical *C. difficile* isolated from ulcerative colitis patient

***C.difficile* isolate**	**phiC 6356**	**phiC 6365**
DPC6218	+	
DPC6219	+	+
DPC6220	+	+
DPC6221	+	+
DPC6353		+
DPC6355	+	
DPC6359	+	+
DPC6365	+	
DPC6366	+	+
DPC6534	+	
DPC6538	+	
56	+	+
DPC6506	+	
DPC6508	+	
DPC6511		+
DPC6512		+
DPC6515		+
Total	**13**	**10**

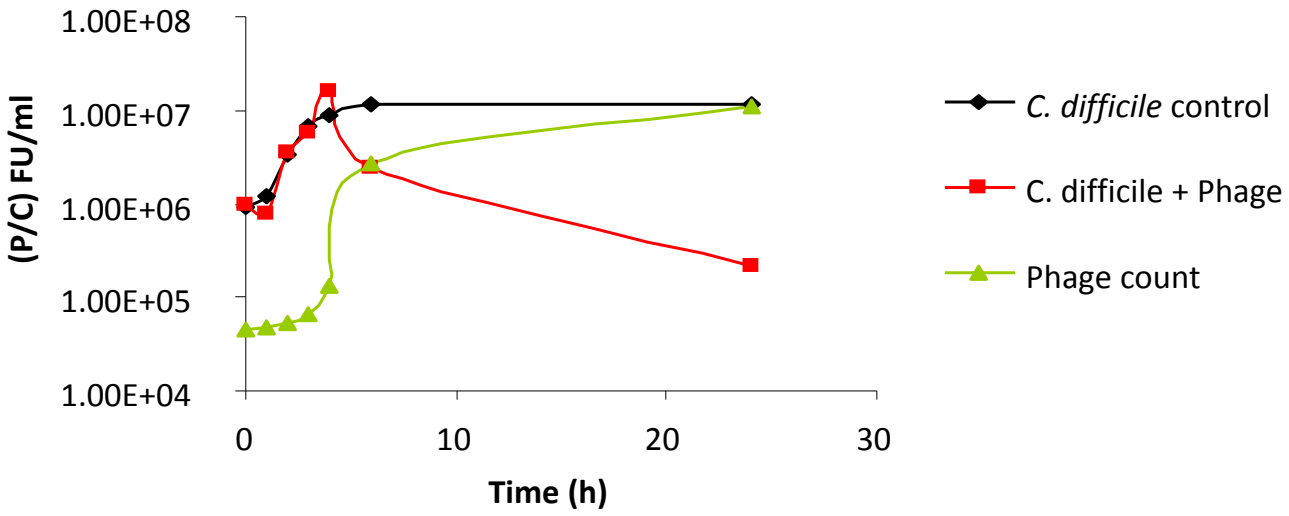
No plaques on *C. spongenes*, *C. histolyticum*, *C. perfringens*, *Lb. paracasei*, *B. cereus* or *S. aureus.*

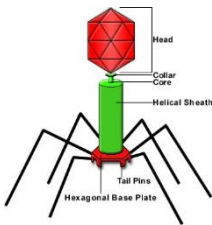
Phage therapy

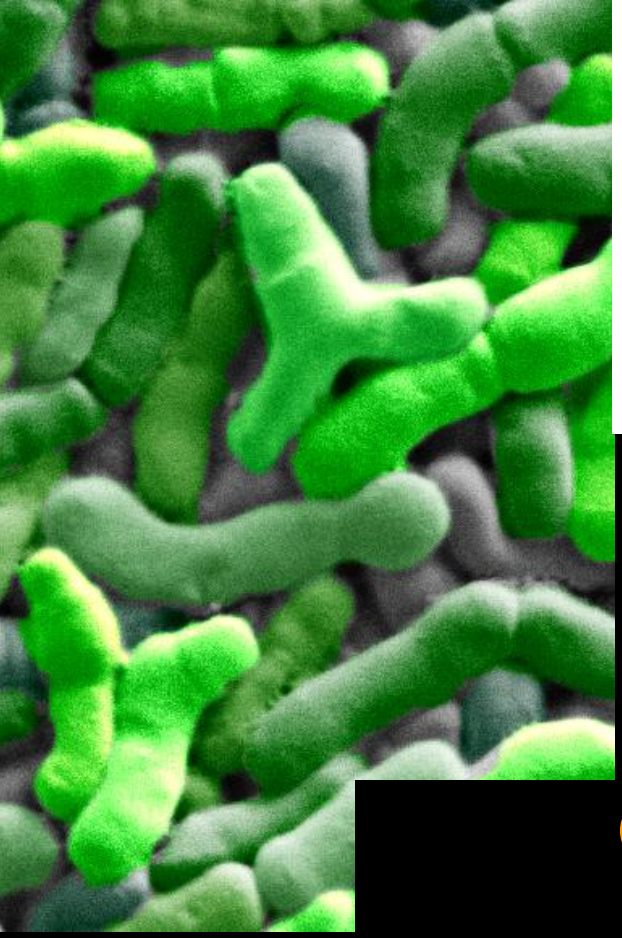




Phage CD6356 in faecal fermentation



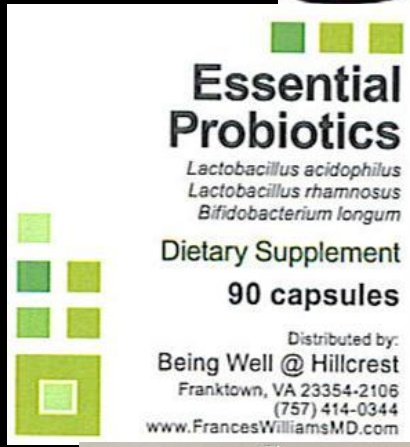




Therapeutic Advances in Gastroenterology

Probiotics in the prevention of antibiotic-associated diarrhoea and *Clostridium difficile* infection

Mary Hickson



Good evidence for AAD

Evidence for CDAD equivocal

Need good clinical studies

May be other strains with better efficacy

Probiotics and CDAD

BMJ

RESEARCH

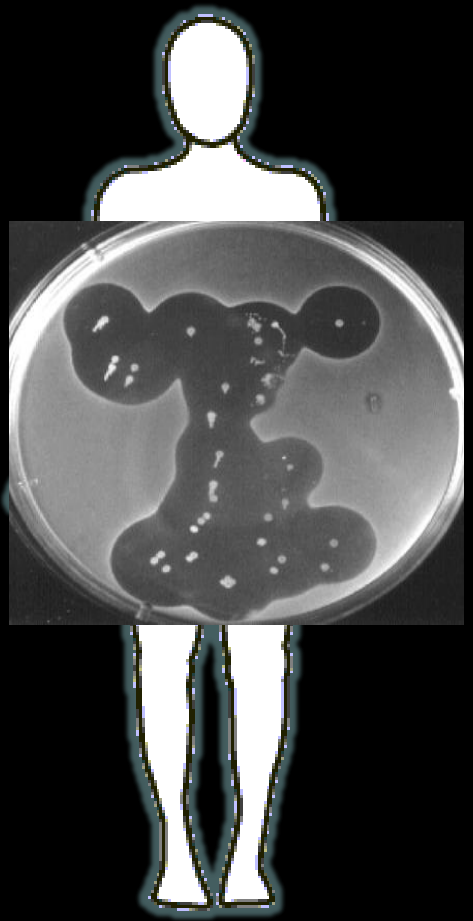
Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial

Mary Hickson, research dietitian,[1] Aloysius L D'Souza, research fellow,[2] Nirmala Muthu, research nurse,[3] Thomas R Rogers, professor of clinical microbiology and honorary consultant (Hammersmith Hospitals NHS Trust),[4] Susan Want, clinical scientist,[5] Chakravarthi Rajkumar, senior lecturer,[2] Christopher J Bulpitt, professor of geriatric medicine[2]

'Prescribed probiotic' in a Dublin Hospital

Hickson *et al.*, BMJ 335:80

Many gut bacteria produce Bacteriocins

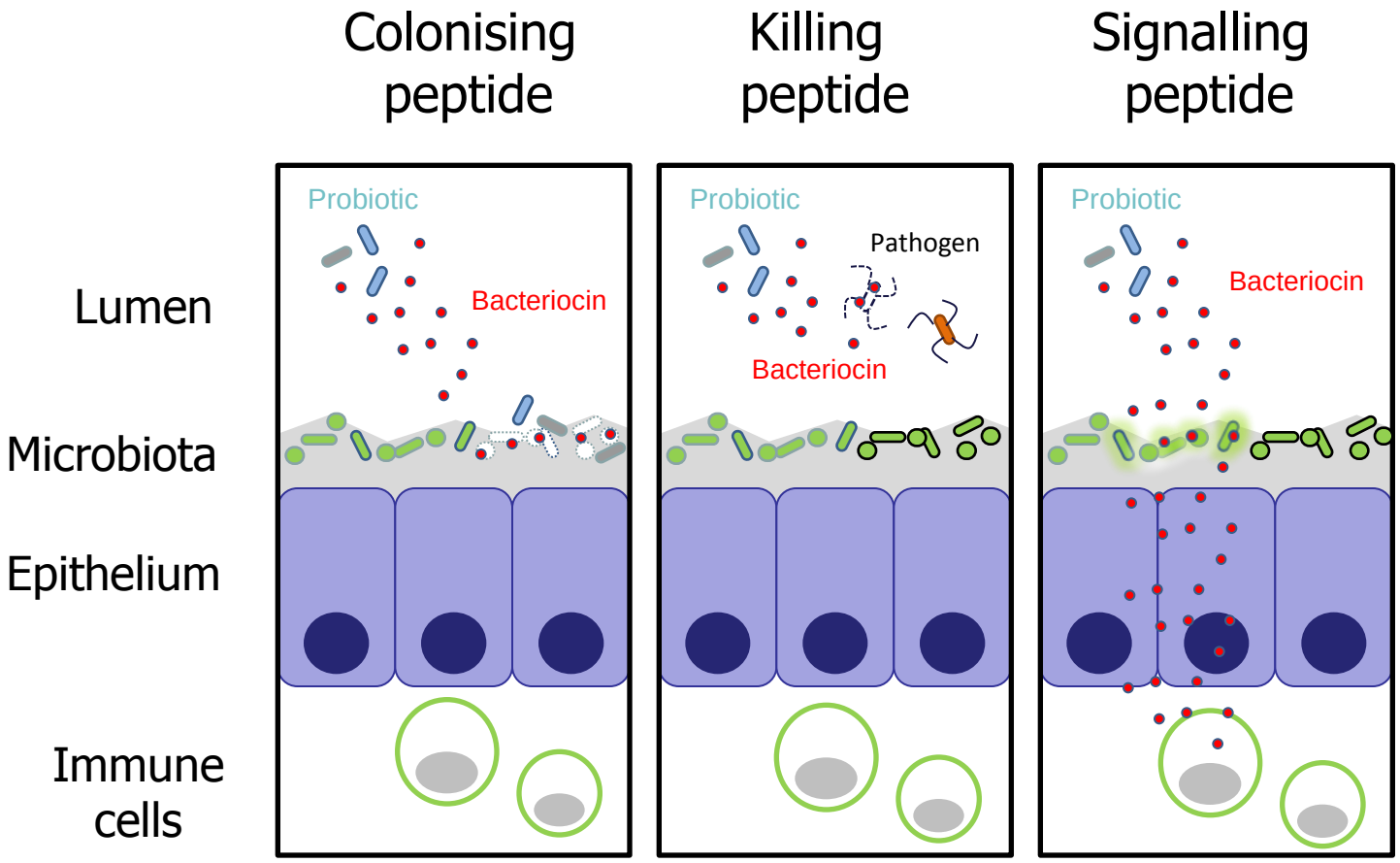


Antimicrobial peptides produced by one bacterium which can kill other bacteria

Bacteriocins are heat stable, active at nanomolar range, producers are immune to their own bacteriocin

Bacteriocin production is widespread among bacteria, including gut bacteria

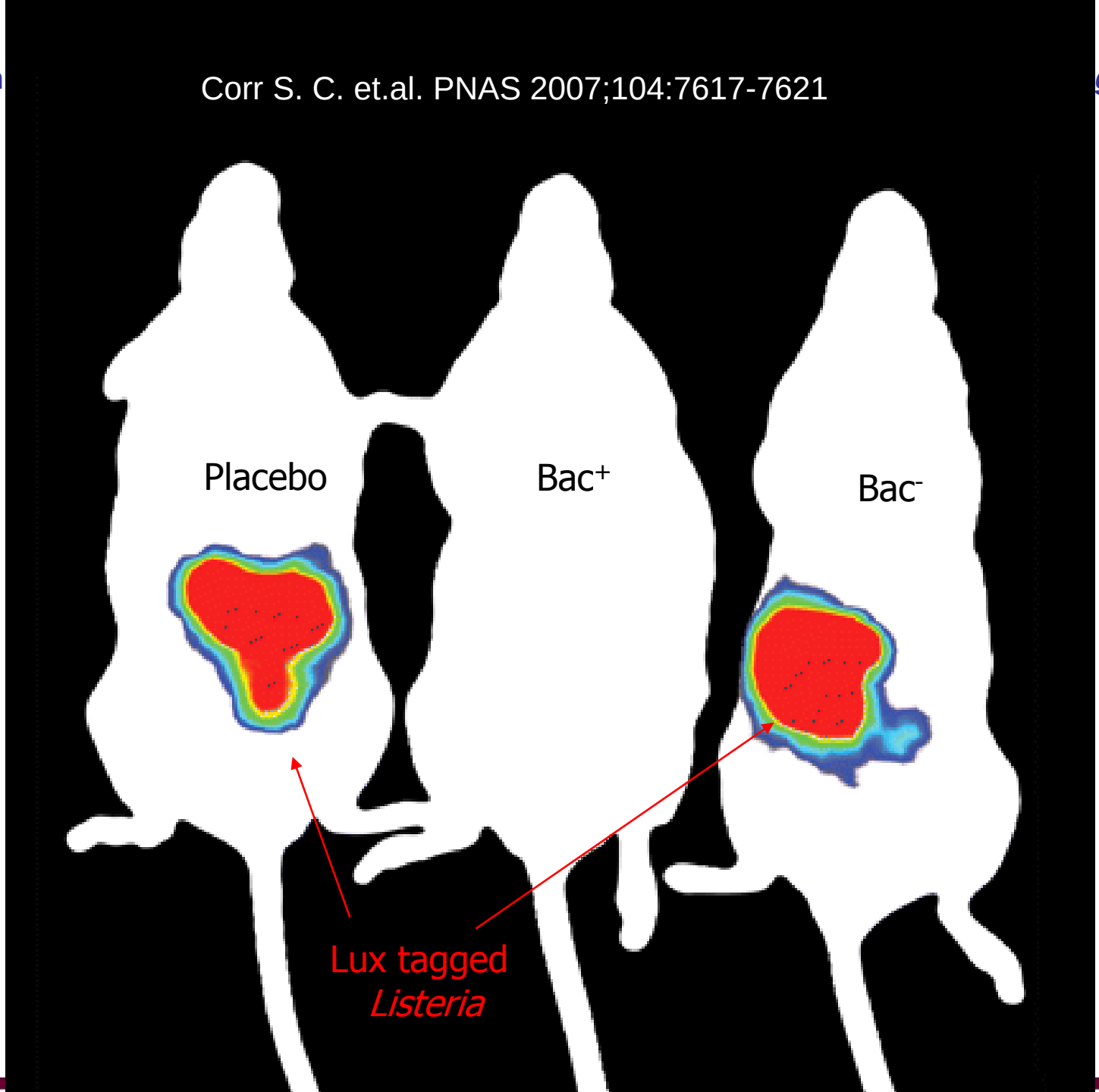
Bacteriocin production as a probiotic trait?



From: Bacteriocin Production as a Probiotic Trait?
Dobson et al, AEM minireview, in Press, 2011

Bacteriocin ***genes***

Corr S. C. et.al. PNAS 2007;104:7617-7621



Thuricin CD Selectively Kills *C. difficile*

Alimentary Pharmabiotic Centre
Mining Microbes for Mankind

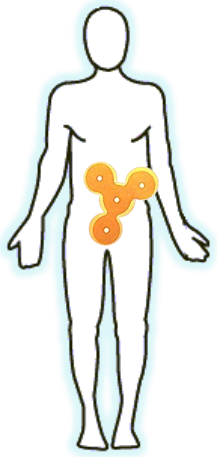
PNAS

Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*

Mary C. Rea[a,b,1], Clarissa S. Sit[c,1], Evelyn Clayton[a,b], Paula M. O'Connor[a,b], Randy M. Whittal[c], Jing Zheng[c], John C. Vederas[c], R. Paul Ross[a,b,2], and Colin Hill[b,d]

[a]Biotechnology Department, Teagasc, Moorepark Food Research Centre, Fermoy, Ireland; [b]Alimentary Pharmabiotic Centre, Cork, Ireland; [c]Department of Chemistry, University of Alberta, Edmonton, AB, Canada T6G 2G2; and [d]Department of Microbiology, University College, Cork, Ireland

Edited* by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved March 30, 2010 (received for review December 1, 2009)



PNAS

Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon

Mary C. Rea[a,b], Alleson Dobson[a,b], Orla O'Sullivan[a], Fiona Crispie[a,b], Fiona Fouhy[a], Paul D. Cotter[a,b], Fergus Shanahan[b,c], Barry Kiely[d], Colin Hill[b,e,1], and R. Paul Ross[a,b]

[a]Teagasc, Moorepark Food Research Centre, Fermoy, County Cork, Ireland; [b]Alimentary Pharmabiotic Centre and Departments of [c]Medicine and [e]Microbiology, University College Cork, Cork, Ireland; and [d]Alimentary Health Ltd., Cork, Ireland

Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved June 8, 2010 (received for review February 2, 2010)

The 3D Structure of Thuricin CD, a Two-Component Bacteriocin with Cysteine Sulfur to α-Carbon Crosslinks

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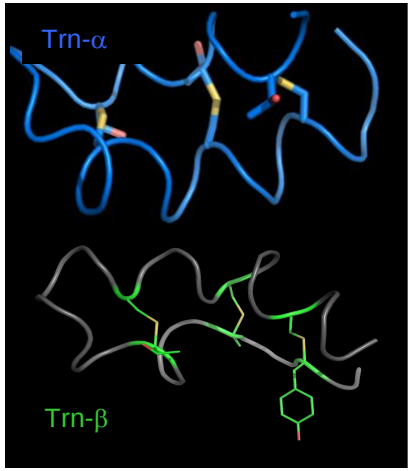
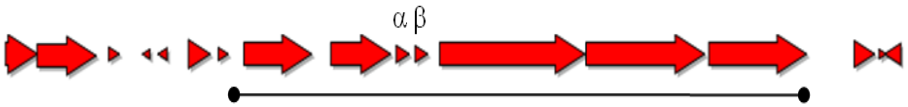
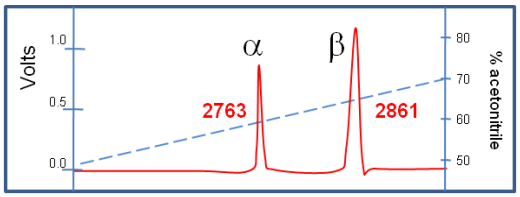
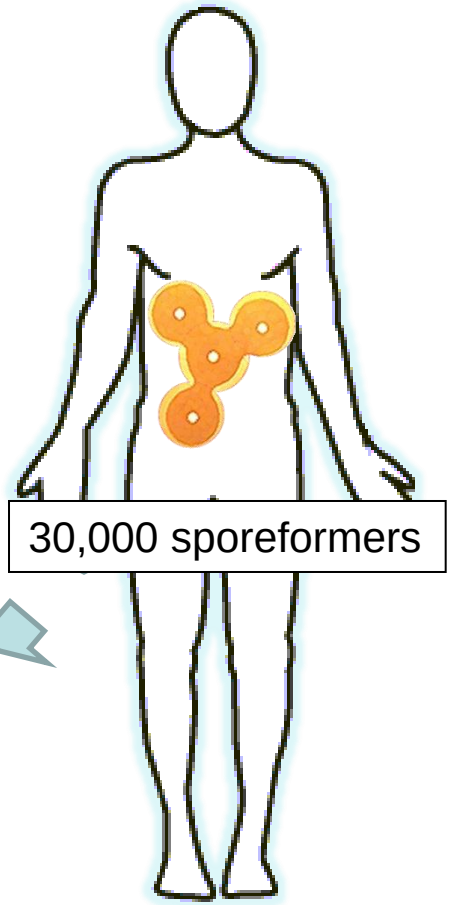


Thuricin CD



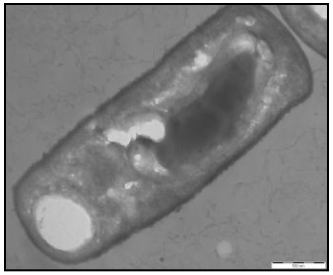
Thuricin CD; a two component bacteriocin

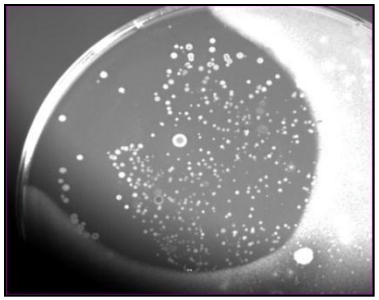




30,000 sporeformers

Bacillus thuringiensis





Overlaid with *Clostridium difficile*

Rea *et al*., unpublished

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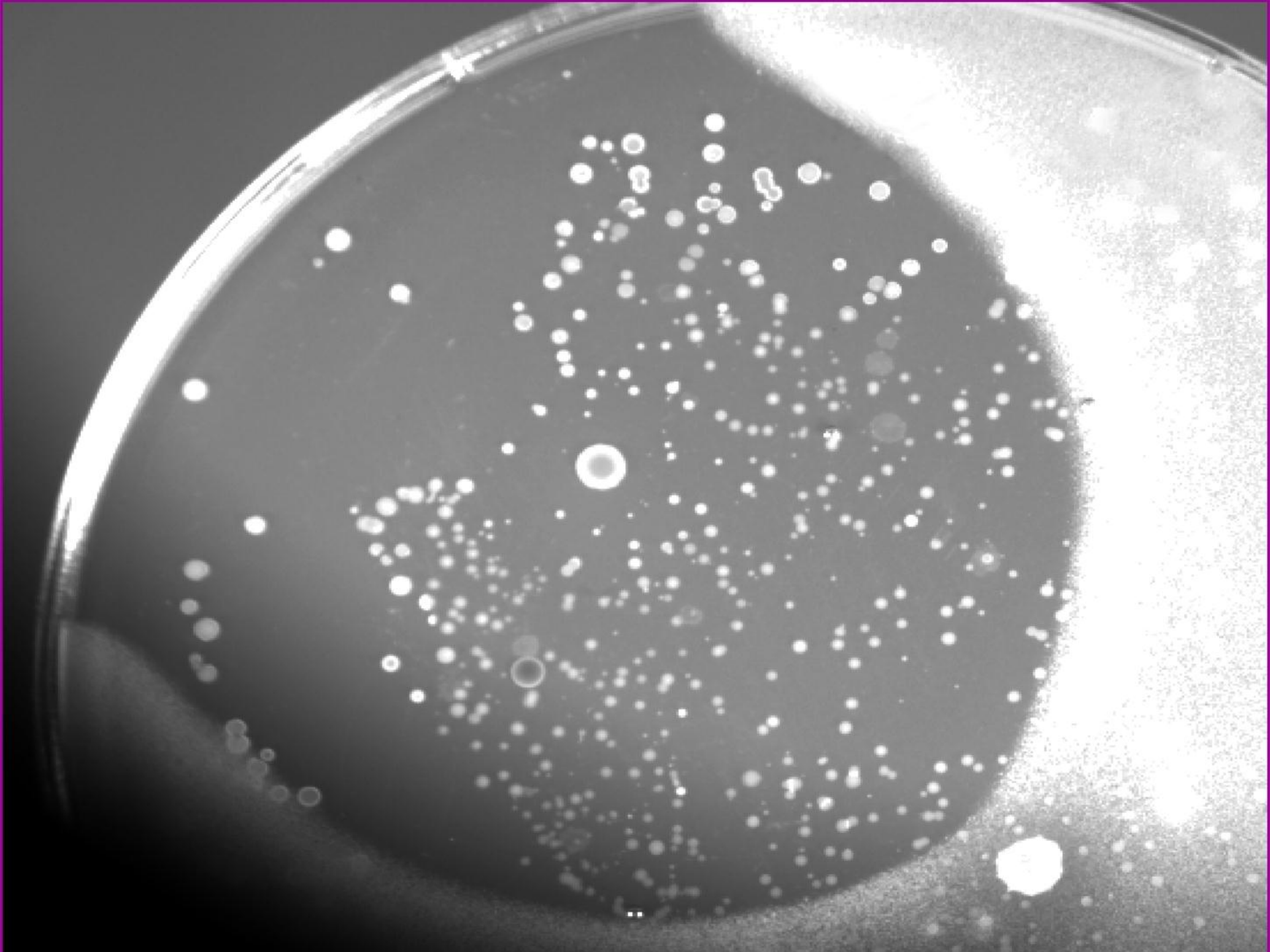

Thuricin Spectrum

Target Strain	Strain No	Growth medium	Incubation Temperature	Sensitivity
Bacillus cereus	NCIMB 700577	BHI[1]	30°C	+++
Bacillus cereus	NCIMB 700578	BHI	30°C	+++
Bacillus coagulans	LMG 6326[T]	BHI	30°C	-
Bacillus firmus	LMG 7125[T]	BHI	30°C	++++
Bacillus mycoides	DPC 6335	BHI	30°C	+++
Bacillus subtilis	LMG 8198	BHI	30°C	-
Bacillus subtilis	DPC 3344	BHI	30°C	-
Bacillus thuringiensis	IBS 14	BHI	37°C	-
Bacillus thuringiensis	LMG 7138	BHI	37°C	+++
Bacteroides fragilis	LMG 10263	BA	37°C	-
Bifidobacterium adolescensis	DPC 6169	mMRS[2]	37°C	-
Bifidobacterium animalis	DPC 5420	mMRS	37°C	-
Bifidobacterium breve	DPC 6166	mMRS	37°C	-
Bifidobacterium breve	BB12	mMRS	37°C	-
Bifidobacterium longum	DSMZ 20097	mMRS	37°C	-
Bifidobacterium mercyicum	DSMZ 6493	mMRS	37°C	-
Bifidobacterium pseudolongum	DSMZ 20092	mMRS	37°C	-
Campylobacter jejuni	CI 120	CA[3]	37°C	-
Clostridium difficile	ATCC 600	RCA[4]	37°C	++++
Clostridium difficile	ATCC 43594	RCA	37°C	++++
Clostridium difficile	R027 NCTC 13366	RCA	37°C	++++
Clostridium difficile	6220	RCA	37°C	++++
Clostridium difficile	6221	RCA	37°C	++++
Clostridium difficile	6219	RCA	37°C	++++
Clostridium difficile	6350	RCA	37°C	++++
Clostridium difficile	6351	RCA	37°C	++++
Clostridium difficile	IBS 16	RCA	37°C	++++
Clostridium difficile	Cr 15	RCA	37°C	++++
Clostridium difficile	UC 38	RCA	37°C	++++
Clostridium difficile	UC 38	RCA	37°C	++++
Clostridium difficile	CR 79	RCA	37°C	++++
Clostridium difficile	UC 47	RCA	37°C	++++
Clostridium difficile	CR11	RCA	37°C	++++
Clostridium difficile	UC 29	RCA	37°C	++++
Clostridium difficile	CR 02	RCA	37°C	++++
Clostridium difficile	UC 59	RCA	37°C	++++
Clostridium difficile	UC 27	RCA	37°C	++++
Clostridium difficile	H 76	RCA	37°C	++++
Clostridium difficile	BS 47	RCA	37°C	++++
Clostridium histolyticum	NCIMB 503	RCA	37°C	+
Clostridium indolis	NCIMB 9731	RCA	37°C	++++
Clostridium limusburense	NCIMB 10637	RCA	37°C	+++
Clostridium sporogenes	NCIMB 9584	RCA	37°C	-
Clostridium probuyricum	NCIMB 8243	RCA	37°C	+++
Enterobacter sakazaki	ATCC 12869	TSA[5]	37°C	-
Enterobacter sakazaki	NCTC 8155	TSA	37°C	-
Enterococcus casseliflavus	LMG 10745[T]	TSA	37°C	-
Enterococcus durans	LMG 10746[T]	TSA	37°C	-
Enterococcus faecalis	LMG 7973[T]	TSA	37°C	-
Enterococcus faecium	LMG 7973[T]	TSA	37°C	-
Enterococcus hirae	LMG 6399[T]	TSA	37°C	-
Escherichia coli	3786	BHI	37°C	-
Lactobacillus acidopuilus	NCDO 1697	MRS	37°C	-
Lactobacillus bulgaricis	LMG 6901[T]	MRS	37°C	-
Lactobacillus casei	ATCC 334	MRS	30°C	-
Lactobacillus crispatus	LMG 9479[T]	MRS	37°C	+
Lactobacillus curvatus	LMG 12009	MRS	37°C	-
Lactobacillus fermentum	LMG 6902	MRS	37°C	+++
Lactobacillus gallinarum	LMG 9435[T]	MRS	37°C	-
Lactobacillus helveticus	LH1	MRS	37°C	-
Lactobacillus johnsonii	DSM 10533[T]	MRS	37°C	+
Lactobacillus paracasei	338	MRS	37°C	-
Lactobacillus reuteri	NCIMB 11951	MRS	37°C	-
Lactobacillus rhamnosus	GG	MRS	37°C	-
Lactobacillus ruminis	DSM 20403[T]	MRS	37°C	-
Lactobacillus salivarius	UCC 118	MRS	37°C	-
Lactococcus lactis	DPC 3157	LM17	30°C	+
Lactococcus lactis	HP	LM17	30°C	-
Leuconostoc	DPC 139	MRS	30°C	-
Leuconostoc	DPC 240	MRS	30°C	-
Listeria innocua	DPC 3572	BHI	37°C	+++
Listeria monocytogenes	DPC 1768	BHI	37°C	+++
Listeria monocytogenes	DPC 3437	BHI	37°C	+++
Listeria monocytogenes	Scott A	BHI	37°C	+++
Micrococcus luteus	DPC 6275	BHI	37°C	-

bacilli

clostridia

listeria

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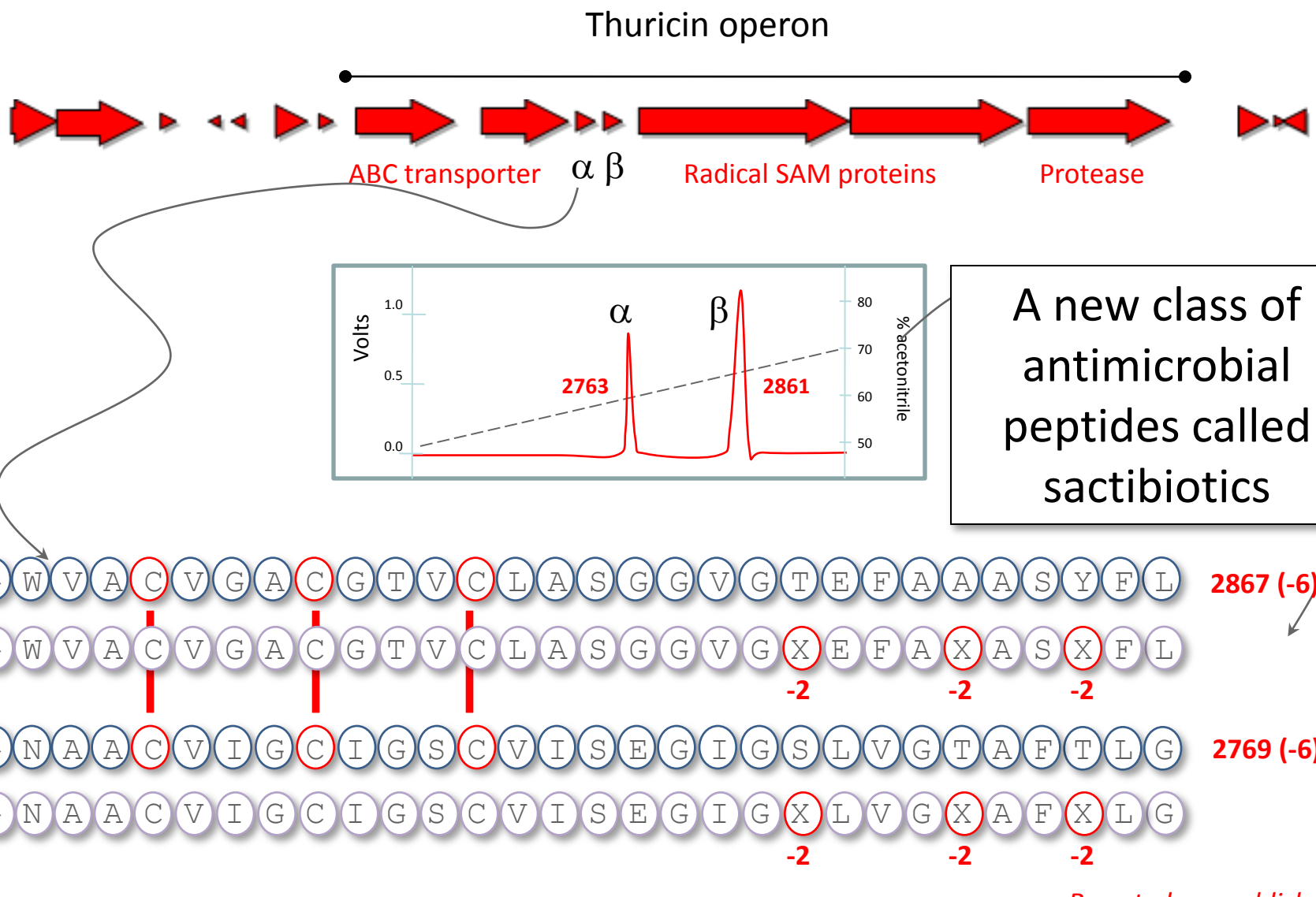
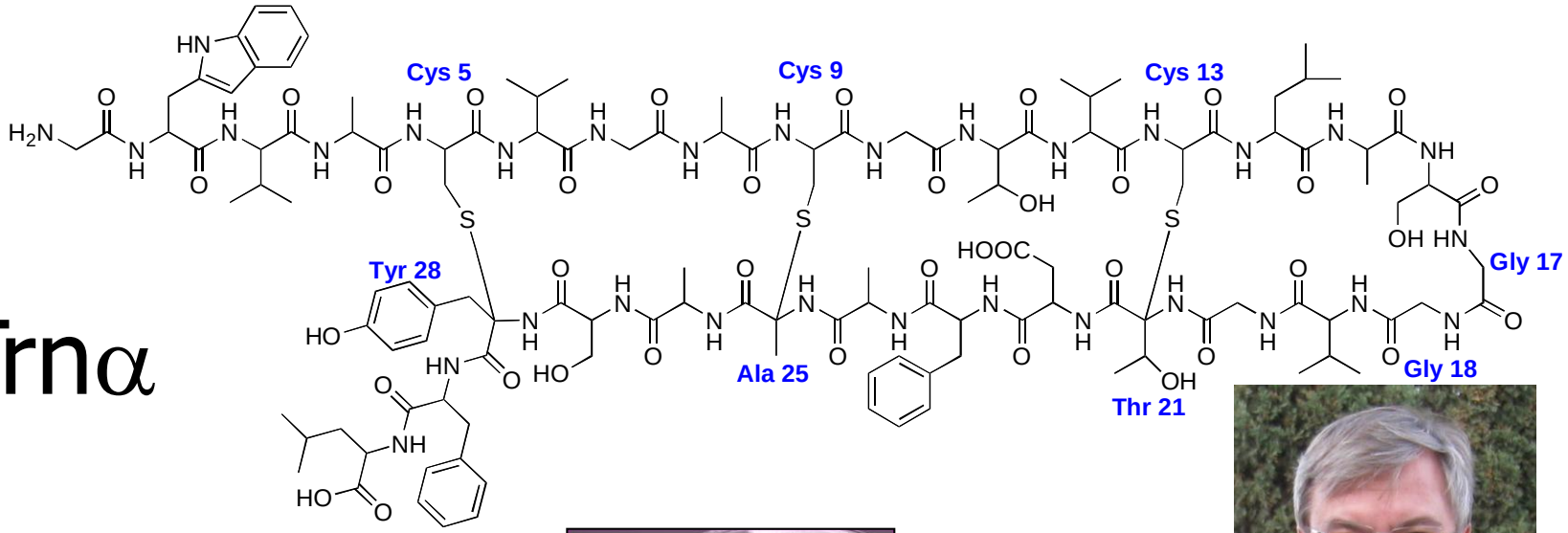
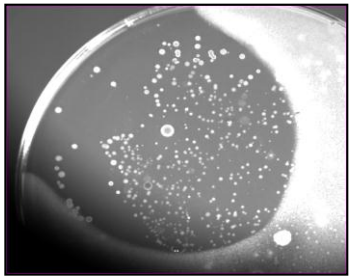
Thuricin contains unusual post-translational modifications



Rea et al., unpublished

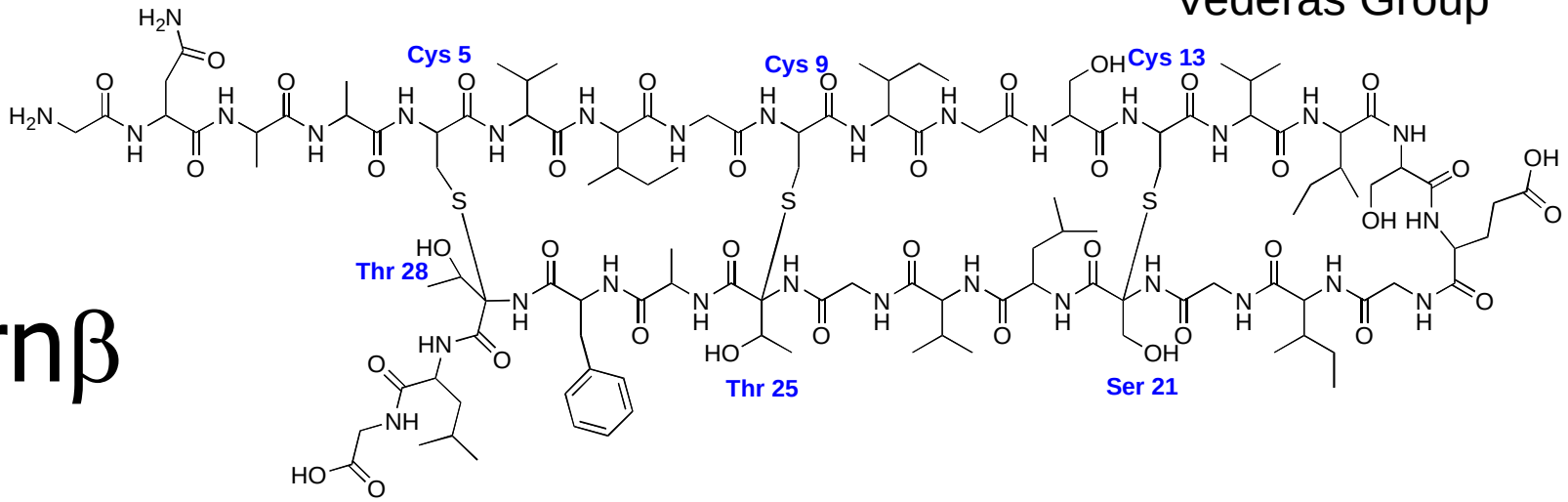
Trnα





Vederas Group

Trnβ



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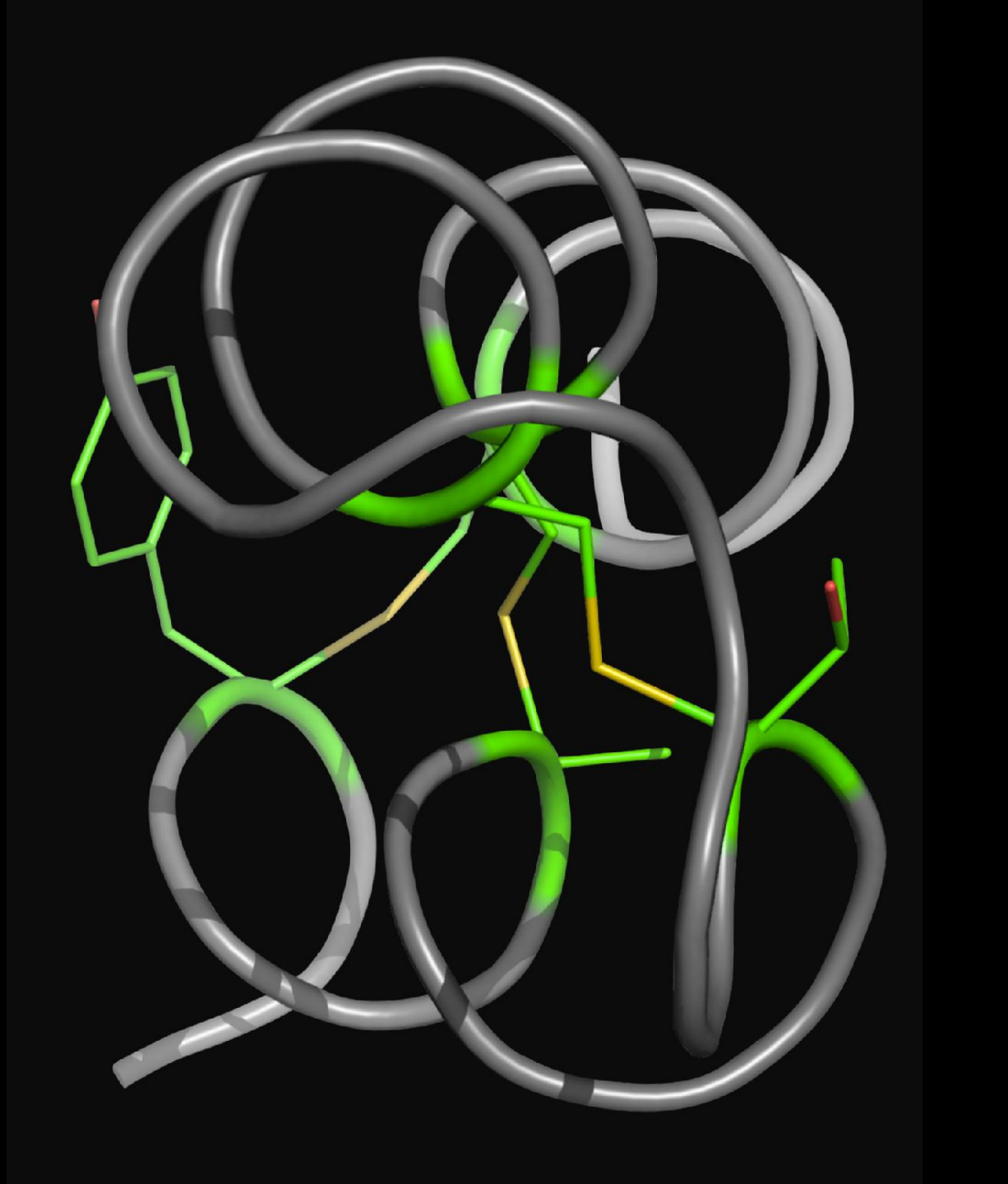
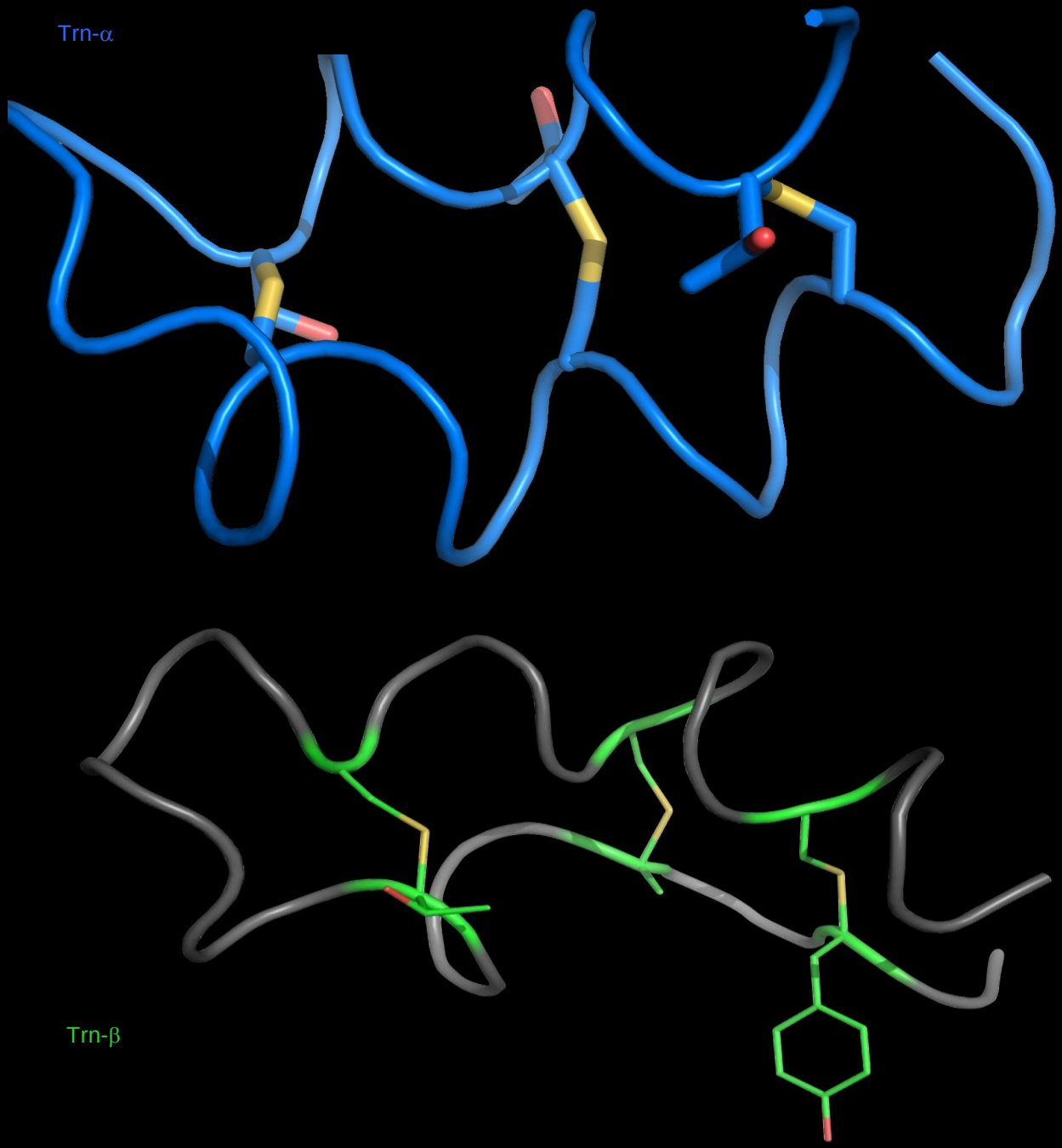


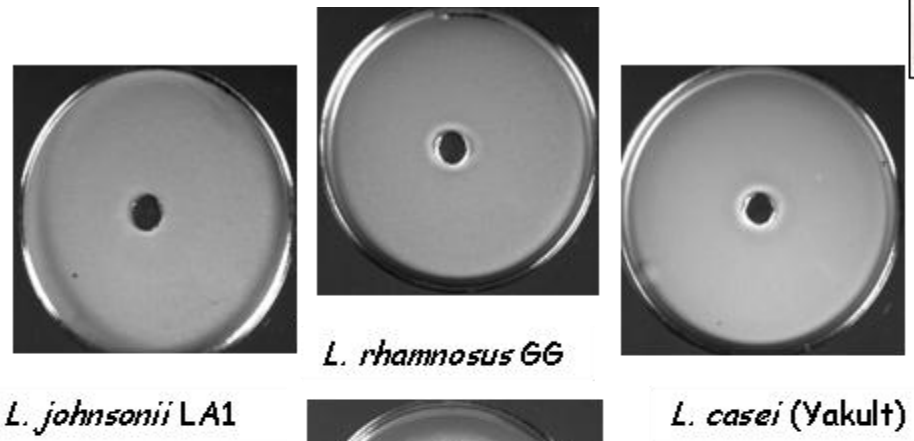

Trn-α
Trn-β

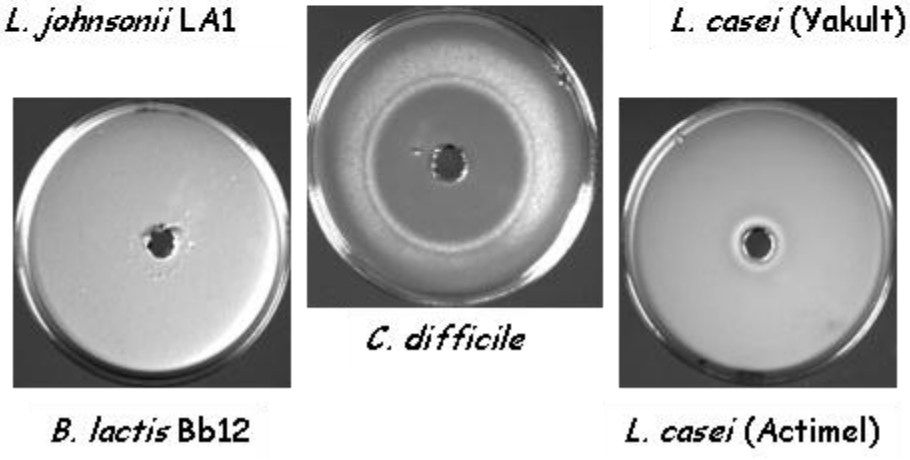
BMJ

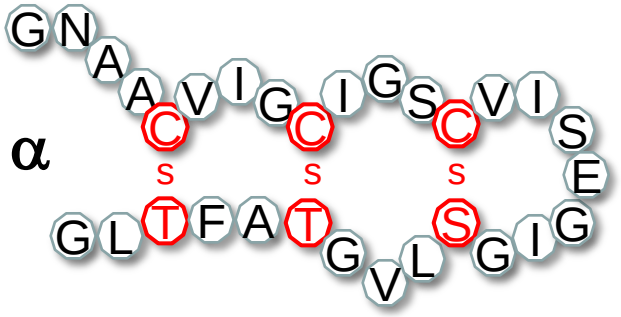
RESEARCH

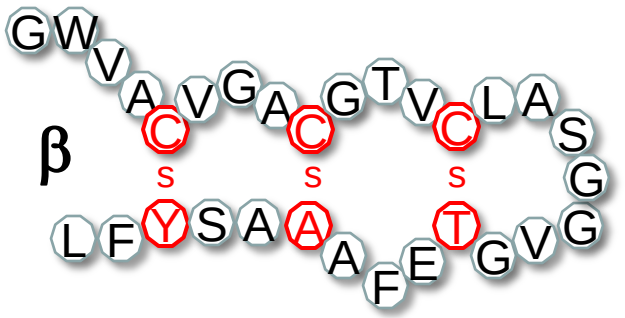
Use of probiotic *Lactobacillus* preparation to prevent diarrhoea associated with antibiotics: randomised double blind placebo controlled trial

Mary Hickson, research dietitian,[1] Aloysius L D'Souza, research fellow,[2] Nirmala Muthu, research nurse,[3] Thomas R Rogers, professor of clinical microbiology and honorary consultant (Hammersmith Hospitals NHS Trust),[4] Susan Want, clinical scientist,[5] Chakravarthi Rajkumar, senior lecturer,[2] Christopher J Bulpitt, professor of geriatric medicine[2]









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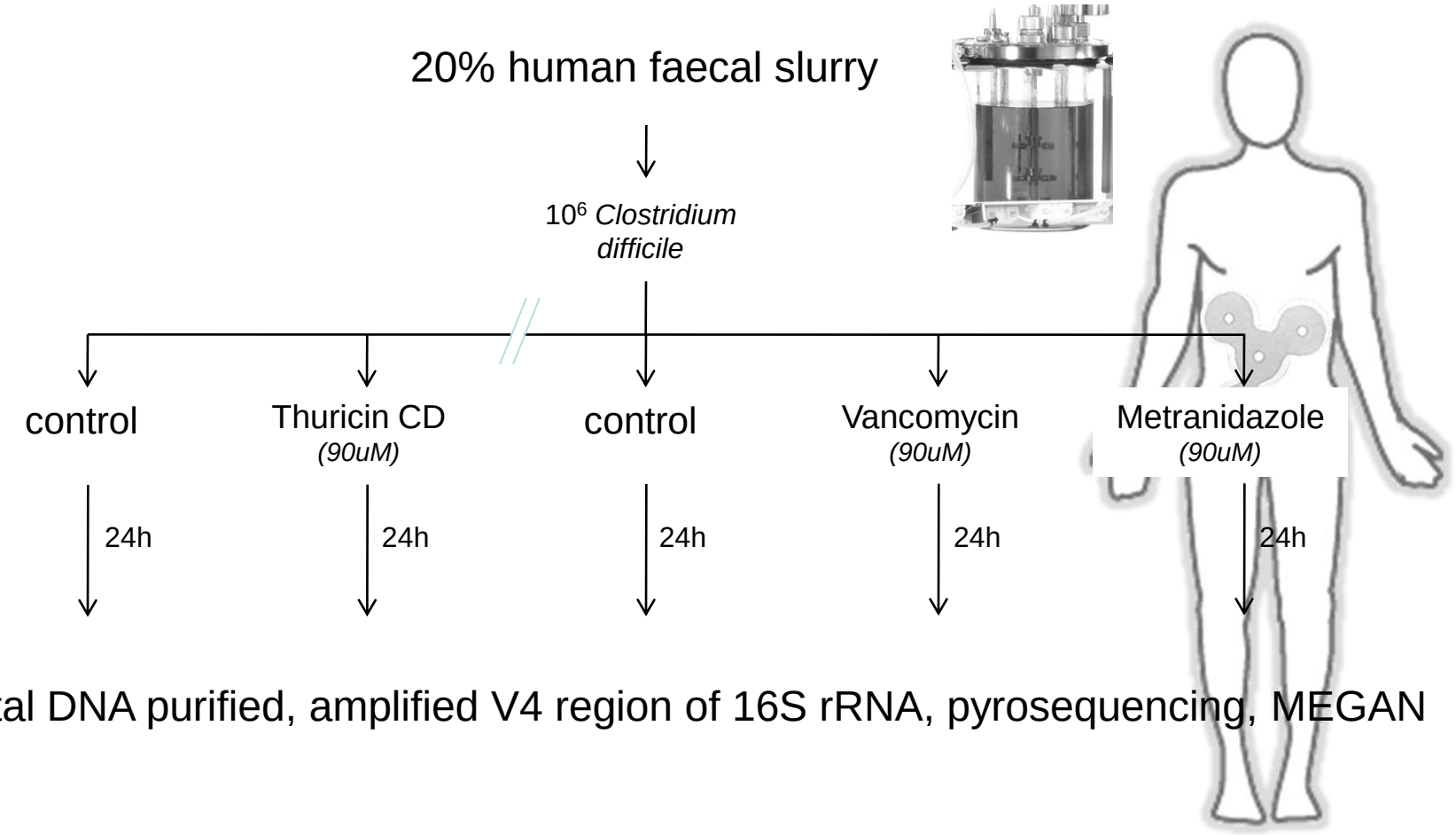
Distal Colon Model



Total DNA purified, amplified V4 region of 16S rRNA, pyrosequencing, MEGAN

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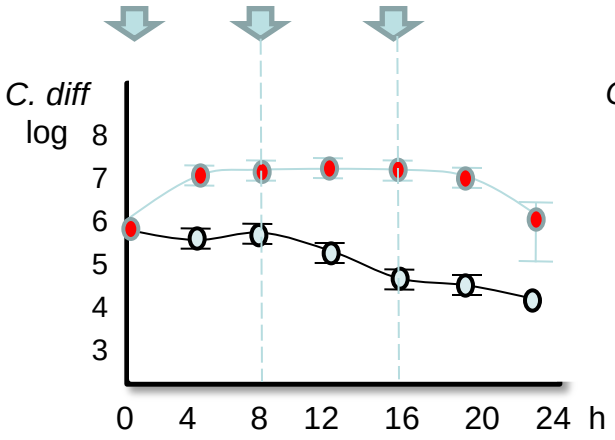
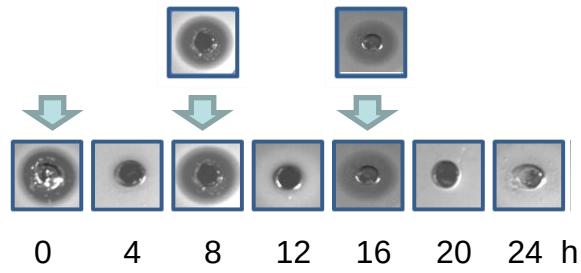
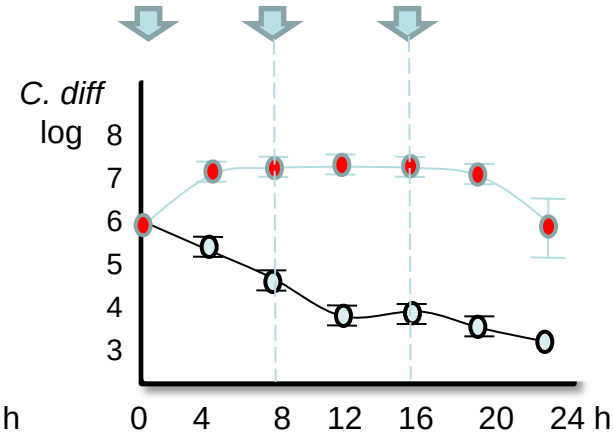
Faecal fermentations

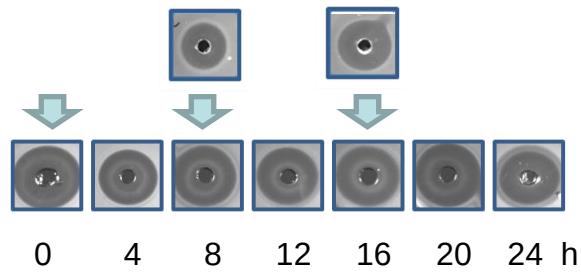
Thuricin CD (90 uM)



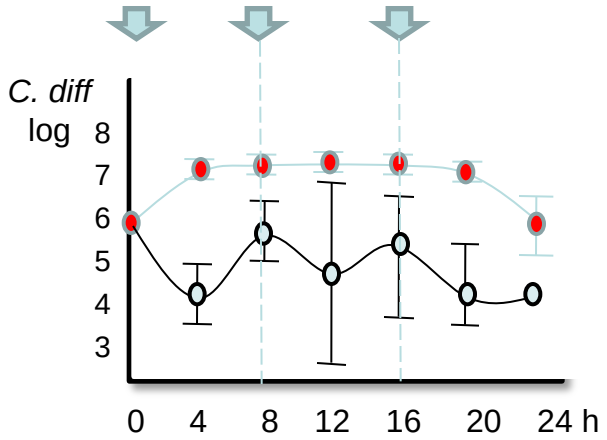


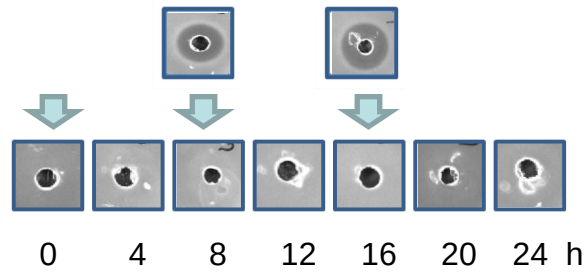
Vancomycin (90 uM)





Metranidazole (90 uM)





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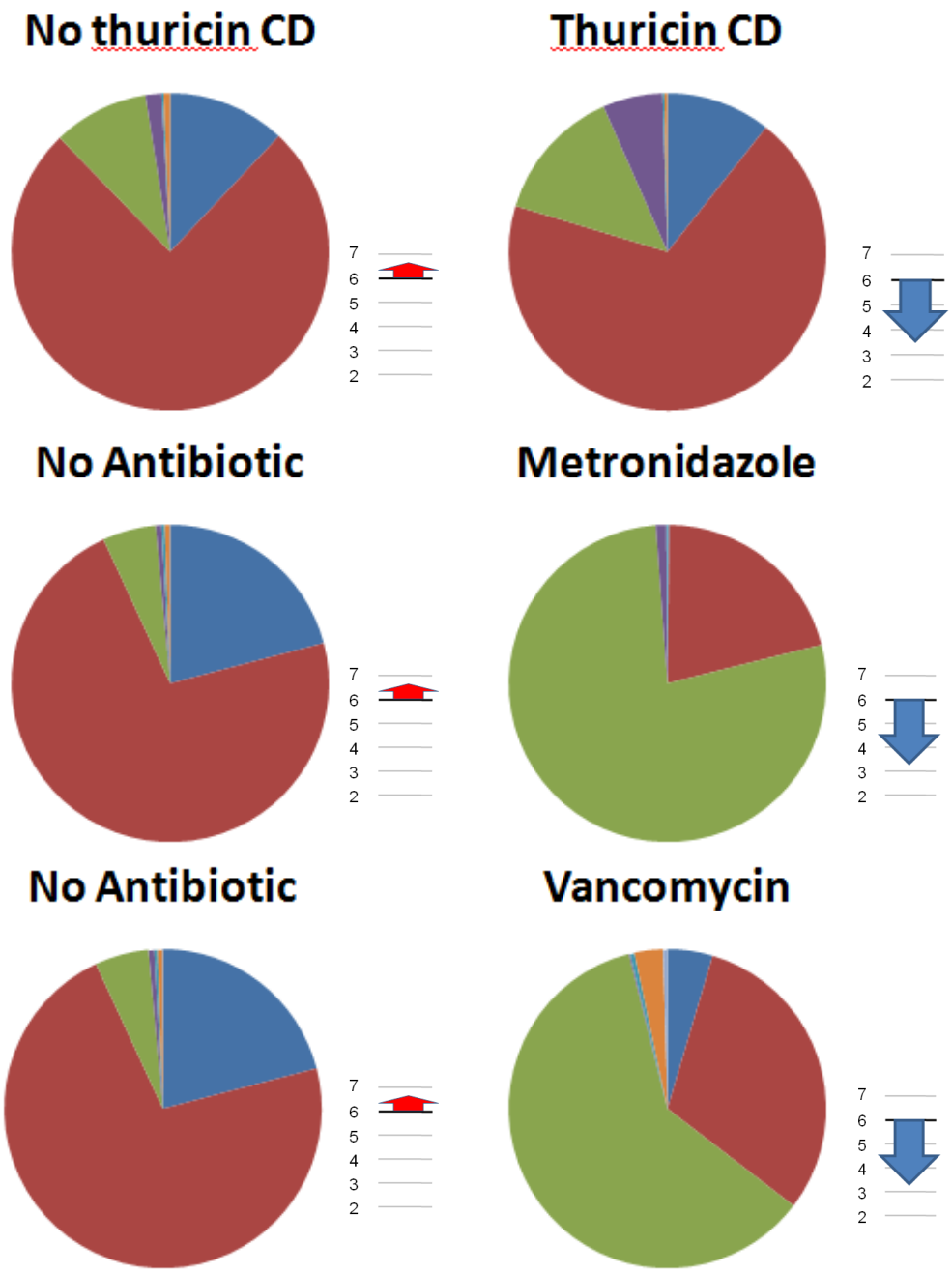
Collateral damage

16S profiling

Phylum

- Bacteriodetes
- Firmicutes
- Proteobacteria
- Actinobacteria
- Spirochaetes
- Lentisphaerae
- Ternicutes



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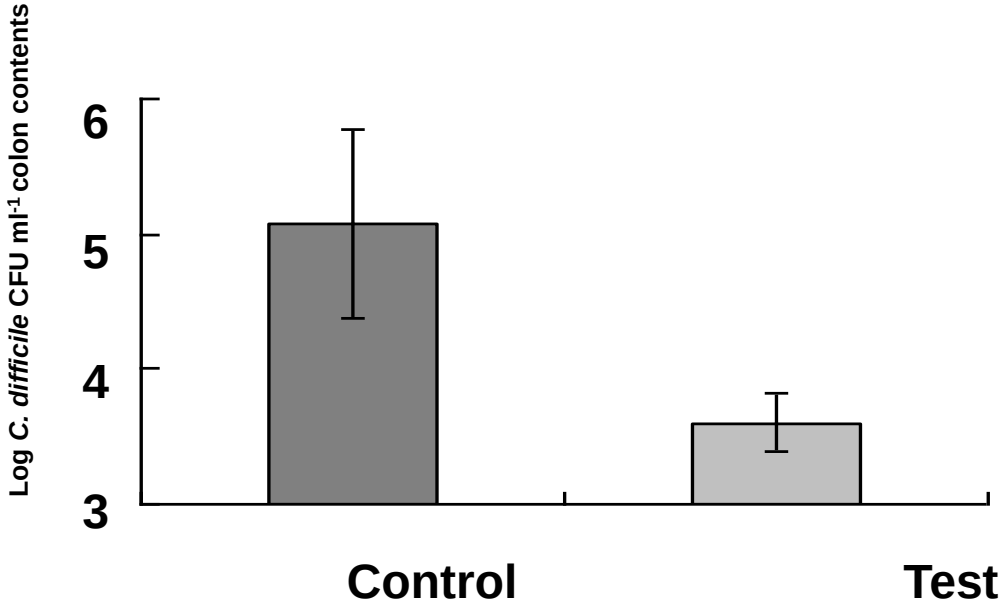
Rectally in conjunction with *C. difficile* ribotype 027



10^5 *C. difficile* 027 introduced rectally to 8 mice

↓

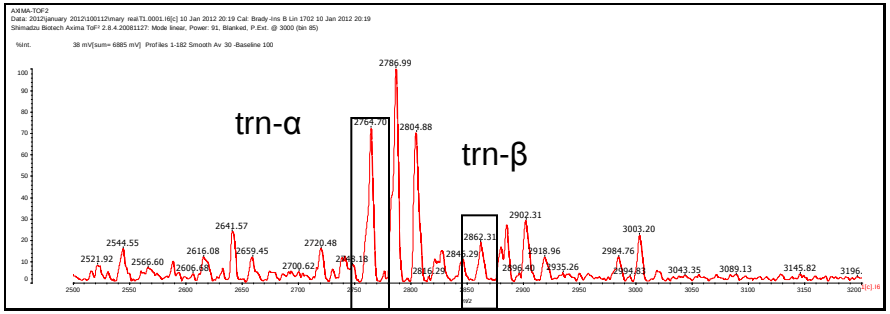
2.5mg thuricin CD introduced rectally 15 min later x 4 mice

↓

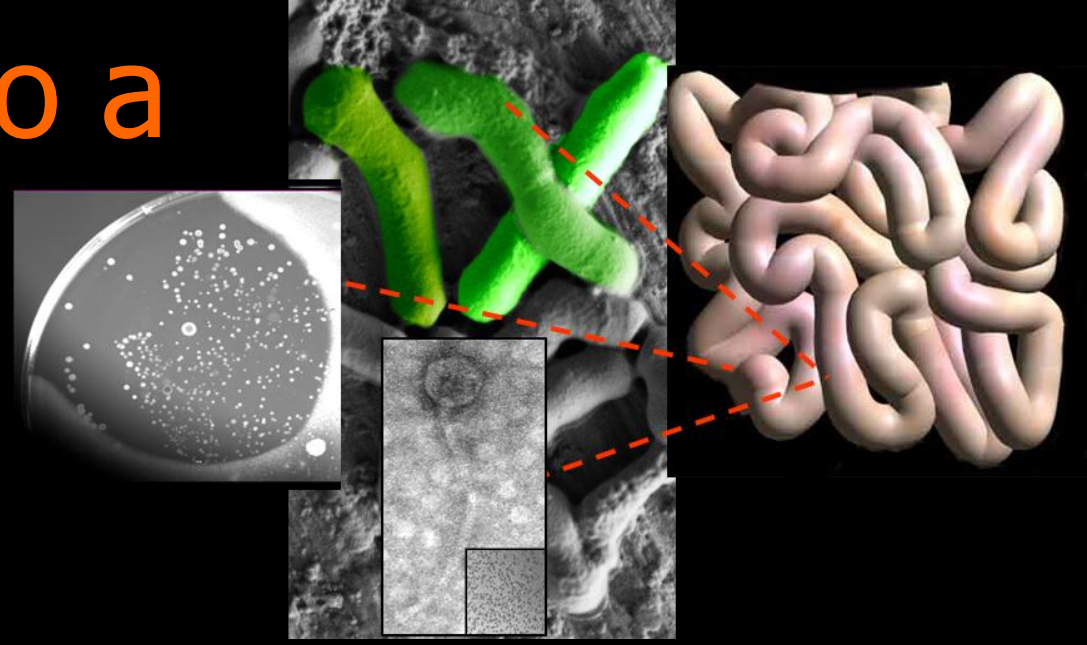
Sacrificed after 1 h

↓

Colon contents plated



Gut Soutions to a Gut Problem



Conclusions:

Carriage up to 10 fold higher in elderly and IBD

Gut microbiota radically changed in active infection

Pharmabiotics: both bacteriocins and bacteriophage hold promise to selective target infection

Probiotics is still at an early stage for C. diff treatment

Thanks

PROEUHEALTH

eagasc

APC
Alimentary Pharmabiotic Centre
Interfacing Food and Medicine

NDP
NATIONAL DEVELOPMENT PLAN

sfi
science foundation ireland

THE DEPARTMENT OF
AGRICULTURE & FOOD
AN ROINN TALMHAÍOCHTA AGUS BIA

MICROBIOME

Paul O'Toole
Catherine Stanton
Ger Fitzgerald
Fergus Shanahan

Mark Wilcox for Ribotyping

arry Kiely

ANTIMICROBIALS

Colin Hill
Paul Cotter
John Vederas

Mary Rea
Eileen O'Shea
Paula O'Connor
Orla O'Sullivan
Gillian Gardiner
Sinead Corr
Evelyn Clayton
Alleson Dobson
Fiona Crispie
Sheila Morgan

Alimentary Health

Fidaxomicin was shown to have a lesser effect on the microbiome as measured by qPCR. Vancomycin treated patients showed a 2-4 log reduction in the Bacteroides/Provetella group which persisted up to 28 days which was absent in the Fidaxomicin treated group. In addition reappearance of the toxin in the culture filtrates was apparent in 28% of the vancomycin treated patients compared to 14% of the Fidaxomicin treated group post treatment and also there was decreased incidences of reoccurrences of CDAD in the Fidaxomicin treated group.